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ORIGINAL ARTICLE

Optimization of extraction process of *Dioscorea nipponica* Makino saponins and their UPLC-QTOF-MS profiling, antioxidant, antibacterial and anti- inflammatory activities

Yu Chang [a], Shuqi Wang [b], Jingyi Xu [a], Tong Zhu [a], Siqi Ma [a], Aohua Zhou [a], Yang Song [c], Mingchun Liu [a], Chunlian Tian [a,*]

[a] *Key Laboratory of Livestock Infectious Diseases in Northeast China, Ministry of Education, College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, No 120 Dongling Road Shenhe Dist., 110866 Shenyang, Liaoning Prov., People's Republic of China*
[b] *China Medical University-The Queen's University of Belfast Joint College, China Medical University, No.77 Puhe Road, 110122 Shenyang, Liaoning Prov., People's Republic of China*
[c] *School of Pharmacy, China Medical University, No.77 Puhe Road, 110122 Shenyang, Liaoning Prov., People's Republic of China*

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KEYWORDS

Dioscorea nipponica Makino;
Saponins;
Extraction process;
Effective constituents;
Anti-inflammatory activity

Abstract *Dioscorea nipponica* Makino exhibits many biological activities, including relieving cough, eliminating phlegm and preventing asthma. The present study extensively evaluated the extraction process, major components, antioxidant, antibacterial and anti-inflammatory activities of total saponins extraction from *Dioscorea nipponica* Makino. In this study, the optimal extraction process of total saponins extract was optimized by single-factor test and response surface methodology as follows: extraction time 25 min, ethanol concentration 50 % and liquid to material ratio 55:1 ml/g, and the extraction rate was 1.72 %. Eighteen components were initially analyzed by UPLC-QTOF-MS method. Although total saponins extract exhibited mild antibacterial activities against *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Streptococcus*, and antioxidant activities against ferric-ion, ABTS and DPPH radicals, the perfect anti-inflammatory activity of TSE was demonstrated by significantly reducing the content of NO and the phagocytic activity

* Corresponding author.
E-mail address: chunliantian@syau.edu.cn (C. Tian).
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in LPS induced RAW 264.7 cells, which provided a theoretical basis for the research and development of new anti-inflammatory Chinese medicine.

1. Introduction

With the extensive use of antibiotics, antibiotic resistance is becoming more and more serious. At the same time, the residues of antibiotics in animal food also seriously affect people's quality of life (Ababneh and Alkhazali, 2019; Kumar et al., 2021). Antioxidant plays an important role in medicine and life, which is not only protecting easily oxidized drugs, but also inhibiting aging and immune stress (Agnieszka, 2021; Farid et al., 2020). Inflammation is a beneficial defense response against many stimulate, but an excessive inflammatory response may lead to some inflammatory diseases, such as pneumonia, chronic bronchitis and rheumatoid arthritis (Wang et al., 2014; Wang et al., 2020; Choi et al., 2018). At present, the main treatment is drug therapy from the steroidal and non-steroidal anti-inflammatory drugs in clinic, but there are also many adverse reactions, including gastrointestinal reactions and sticky damage (Baló-Banga et al., 2018; Dona et al., 2016). Therefore, it is an urgent to develop and research antioxidant, antibacterial, anti-inflammatory drugs from natural plants.

Dioscorea nipponica Makino (*D. nipponica*), as a species of the *Dioscorea* genus from the family *Dioscoreaceae*. (Ou-Yang et al., 2018), is a traditional Chinese medicine, and mainly produced in Northeast of China, Hebei and Shanxi provinces. It's main active ingredients are saponins such as dioscin, pseudoprotodioscin and protodioscin, etc. (Ou-Yang et al., 2018; Tang et al., 2013; Tang et al., 2015; Li et al., 2010). Saponins have a variety of pharmacological effects, including anti-inflammatory (Yin et al., 2020; Zhang et al., 2016; Liu et al., 2017), antioxidant (Yang et al., 2019), anti-parasitic (Zhou et al., 2021), cardiac diseases (Li et al., 2021), anti-allergic diarrhea (Huang et al., 2021), treatment of gouty arthritis (Zhou et al., 2020), atherosclerotic cardiovascular (Sun et al., 2020), hepatorenal damages (Li Y et al., 2021), mastitis (Ran et al., 2020), skin cancer (Wang et al., 2020), diabetic nephropathy (Cai et al., 2020).

However, there was no systematic and comprehensive study on preparation process, main components, anti-inflammatory, antimicrobial and antioxidant activities of the total saponins extract (TSE) from *D. nipponica*. Thus, this experiment was conducted to research these gaps.

2. Materials and methods

2.1. Plant collection

D. nipponica was purchased from Shenyang Tasly Pharmacy and identified by licensed Chinese pharmacist Yao Cong. The medicinal materials were crushed and sifted through the screen for the further extraction.

2.2. Chemicals and reagents

2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2′ - azino - bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) (ABTS) were provided from Sigma-Aldrich Chemie (Steinheim, Germany) for the determination of antioxidant activities. Lipopolysaccharide (LPS) from *Escherichia coli* and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nutritional broth and modified Edwards medium were bought form Beijing Solaibao Technology Co., ltd (Beijing, China), peptone soy broth and Salmonella Shigella agar medium from Qingdao Haibo Biotechnology Co., ltd (Qingdao, China). TLR4

Table 1 The Box-Behnken design matrix and response values for TSE yields.

Run	Variable levels			Response 1 (R1)
	A Extraction time (min)	B Ethanol concentration (%)	C Ratio of solvent to material (ml/g)	
1	30	40	50	1.23
2	30	30	55	1.07
3	30	50	55	1.35
4	25	50	50	1.64
5	35	40	45	1.51
6	25	40	55	1.48
7	35	30	50	1.65
8	35	40	55	1.52
9	30	50	45	1.07
10	30	30	45	1.18
11	30	40	50	1.27
12	25	30	50	1.25
13	30	40	50	1.29
14	35	50	50	1.52
15	30	40	50	1.33
16	30	40	50	1.27
17	25	40	45	1.35

inhibitor TAK-242 was offered by Wuhan Bode Biological Engineering Co., ltd (Wuhan, China). Other analytical chemicals and reagents were obtained from Beijing Dingguo Changsheng Biotechnology Co., ltd (Beijing, China).

2.3. Cells and microbial strains

RAW 264.7 cells were bought from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

Four standard strains, including *Escherichia coli* (ATCC 25922), *Salmonella* (ATCC 14028), *Staphylococcus aureus* (ATCC 29213) and *Streptococcus* (ATCC 49619) were purchased from American Type Culture Collection for antibacterial test, and stored at College of Animal Science and Veterinary Medicine, Shenyang Agricultural University.

2.4. Instruments

Liquid chromatograph analysis was carried out on Agilent 1290 Infinity II (Palo Alto, CA, USA), and the mass spectrometer analysis with Agilent 6546 QTOF. The data acquisition and processing were performed by MassHunter Quantitative Analysis 10.0 software.

2.5. Preparation method of TSE

A suitable amount (about 0.5 g) of fine powder from *D. nipponica* was accurately weighed and placed in a conical flask. The extraction time, ethanol concentration and ratio of solvent and material were confirmed according to the test scheme. The ultrasonic extraction was performed twice, and the filtrate was combined and concentrated to dryness for subsequent experiments.

2.6. Optimization the extraction process of TSE from D. nipponica

2.6.1. Determination of total saponins content (TSC)

Total saponins content (TSC) was measured by perchloric acid colorimetric method according to the report of Tian et al. (2020). A linear regression equation was drawn with absorbance (Y) of reference substance of diosgenin as ordinate and concentration (X) as abscissa, Y = 0.0236X-0.0634 (r = 0.9991). After the preparation of the extract solution, 0.5 ml of the extraction solution was taken for color reaction, and its absorbance value was determined at 410 nm. The concentration can be calculated when the measured absorbance value was inserted into the standard curve, and TSC was defined as follows: extraction yield of total saponins (w/w, %) = mass of total saponins expressed as g of diosgenin equivalents per g dry weight of *D. nipponica* samples × 100 %.

2.6.2. Optimization the extraction process of TSE by single factor experiments

Three single factors, including extraction time (X_1, min), ethanol concentration (X_2,%) and ratio of solvent and material (X_3, ml/g), were investigated with the yield of TSE as response value. The five different levels of the three single factors were as follows: 20, 30, 40, 50 and 60 min for X_1 factor, 20, 40, 60, 80 and 100 % for X_2 factor, 20:1, 30:1, 40:1, 50:1 and 60:1 ml/g for X_3 factor. When the influence of any single factor was investigated on the yield of TSE, the intermediate level was selected for the other two factors.

2.6.3. Optimization the extraction process of TSE by response surface methodology

According to the optimization results of single factor experiments, a three-factor and three-level response surface analysis experiment was designed by Box-Behnken method using Design Expert 8.0.6 with extraction time (X_1, min), ethanol concentration (X_2,%) and ratio of solvent and material (X_3, ml/g) as independent variables and the yield of TSE as investigation index.

2.7. The compositions of TSE analyzed by UPLC-QTOF-MS

2.7.1. Sample preparation for HPLC-MS analysis

The extraction solution was prepared with 0.5 g of medicinal materials according to the optimal extraction process optimized by response surface experiments. The extraction solution was concentrated up to dryness, and the residue was dissolved in 10 ml of chromatographic methanol and filtered by 0.22 μm microfiltration membrane for UPLC-QTOF-MS analysis.

2.7.2. UPLC-QTOF-MS and LC-DAD conditions and parameters

The samples were separated and analyzed on C_{18} chromatographic column (Poroshell 120 series, 150 mm × 2.1 mm, i.d. 2.7 μm) with the mobile phases (A) water and (B) acetonitrile. The gradient elution procedure of the mobile phases was as follows: 90 %-70 % of A for 1–15 min, keeping 70 % of A for 5 min, 70 %-5% of A for 12 min, keeping 5 % of A for 3 min, and then 5 %-90 % of A for 1 min with a flow rate of 0.3 ml/min at column temperature 30 °C. The detection conditions for UPLC-QTOF-MS were set up with positive ion mode, drying gas flow 7 L/min and 250 °C, nebulizer pressure 35 psi, capillary voltage 3.5 KV, sheath gas flow 11 L/min and 325 °C.

2.8. Determination the antioxidant activities of TSE

The antioxidant activities of TSE from *D. nipponica* was evaluated by three methods, including ferric-ion reducing antioxidant power (FRAP) assay, ABTS radical scavenging activity assay and DPPH radical scavenging activity assay. The reducing ability and free radical scavenging ability of the extract were evaluated according to the specific operations reported in literature (Tian et al., 2018). 0.1 ml of different concentration of TSE was mixed with 0.4 ml of FRAP reagent and reacted for 30 min at 37 °C, and then the absorbance was measured at 593 nm. FRAP of TSE was expressed as mmol Fe^{2+} per 100 μg/ml. 0.1 ml of sample solution was added with 0.9 ml of ABTS or DPPH working solution, and the absorbance was measured at 734 nm or 517 nm after incubation for 30 min at room temperature or 37 °C, respectively. The scavenging ability was calculated by the following equation: $[(A_0\text{-}A_1)/A_0] \times 100$ %, where A_0 was the absorbance of ABTS or DPPH, and A_1 for ABTS or DPPH with sample. The free radical scavenging ability can be expressed by IC_{50} (mg/ml), which is TSE concentration of inhibition 50 % free radicals.

2.9. Determination the antimicrobial activities of TSE

2.9.1. Bacterium solution preparation

50 μl of *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Streptococcus* preserved in glycerin were inoculated onto Luria-Bertani nutrient broth, *Salmonella Shigella* agar, peptone soy broth and improved Edward medium, respectively. The plates were incubated at 37 °C for 30 min, and then inverted for further culture for 12–18 h. The typical single colony was selected and enriched in 3 ml of Mueller Hinton broth medium, and then cultured at 37 °C and 170 RPM until the logarithmic growth phase. The bacterial liquid was diluted with Nutrient Broth medium to 1×10^5CFU/ml and stored in a refrigerator at 4 °C for later use.

2.9.2. Samples preparation

The extract was prepared according to the optimized extraction process of response surface test and dissolved in sterile water to the concentration from 1.953 to 1000 μg/ml.

2.9.3. Antibacterial activity assay

The antibacterial activity of TSE was determined with microbroth dilution method according to the method reported by Tian et al.(2019), and the minimal inhibitory concentration (MIC) value was the major evaluation index. After the mixture of the TSE solution and the bacterial liquid with the volume ratio 1:1 incubation at 37 °C for 24 h, the inhibition of bacteria was observed with the antibiotic gentamicin as positive control and sterile water as negative control.

2.10. Determination the anti-inflammatory activities of TSE

2.10.1. Cell culture

RAW 264.7 cells were cultured in DMEM high glucose medium containing 10 % fetal bovine serum under the condition of 5 % CO_2 and 37 °C.

2.10.2. Samples solution preparation

According to the optimized extraction process, TSE was prepared and dissolved with DMEM high glucose medium with the final concentration of 31.25, 62.5, 125 g/ml. After filtered with 0.22 μm of microfiltration membrane, the solution of TSE was preserved at −20 °C for later use.

2.10.3. Cell viability

MTT assay was adopted to evaluate the effect of TSE on cell viability based on the report by Tian et al. (2018). Cells were incubated with 6.25, 12.5, 25 μg/ml of TSE, DEX, TAK-242, LPS and DMEM high glucose medium for 18 h, respectively. After cells cultured with 20 μl of MTT reagent (5 mg/ml) for 4 h, the absorbance value was measured by microplate reader detection at 570 nm.

2.10.4. Measurement of NO content

According to the reported Griess reagent method (Tian et al., 2018), the cell grouping was the same as the assay of cell viability and the absorbance value of the reaction solution was measured at 540 nm after culturing for 18 h. The content of NO in cell supernatant was calculated by the established sodium nitrite standard curve.

2.10.5. Measurement of phagocytic activity

Based on the method reported in the literature (Tian et al., 2020), the effect of TSE on phagocytic activity was observed with neutral red dye. After cultured for 18 h and discarded the cell supernatant, the cells were mixed with 100 μl of neutral red solution (100 mg/ml, dissolved in normal saline) and cultured further in a cell incubator for 1 h. The supernatant was poured out and added with 100 μl of cell lysate for overnight at room temperature in darkness, and then the absorbance value was detected at 540 nm with a microplate analyzer.

2.11. Statistical analysis

The IC_{50} values were calculated by Prime 5 software, and other data was statistically analyzed with SPSS 17.0 software, and $p > 0.05$ expressed no significant difference.

3. Results and discussions

3.1. Optimization of extraction process of TSE from *D. Nipponica*

3.1.1. The optimization of single factor experiments

According to literatures (Hu et al., 2012; Luo et al., 2018; Ren et al., 2015) and preliminary experimental studies (Tian et al., 2020), three main factors, including extraction time, ethanol concentration and ratio of solvent and material, can affect the content of saponins extracted by ultrasonic extraction method. Therefore, this study mainly investigated the effects of the above three factors on the yield of TSE from *D. nipponica*.

The effects of five different levels of three factors on the extraction yield of TSE were shown in Fig. 1. When the extraction time reached 30 min, the yield of TSE was the highest, and decreased with the extension of the extraction time. When the ethanol concentration reached 40 %, the content of total saponins was higher than that of other concentration groups, which can be explained by similarity of polarity between saponins and 40 % ethanol. With the increase of ratio of solvent and

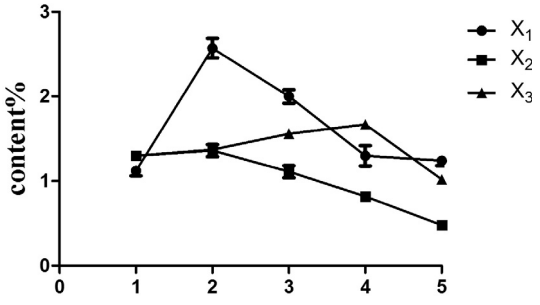

Fig. 1 Effect of extraction time (X_1, min), ethanol concentration (X_2,%), solvent to material (X_3, ml/g) on the extraction yield of total saponins from *D.nipponica*.

material, the extraction ratio of TSE also will increase, and the highest content of total saponins was gained at ratio of solvent and material of 50:1, but TSC showed a trend of decline gradually when ratio of solvent and material than 50:1. This may be due to the increased dissolution of other impurity components.

According to the results of single factor test, three levels were selected for the next step of response surface analysis for the three factors, and the three levels are 25, 30, 35 min for extraction time (X_1), 30, 40, 50 % for ethanol concentration (X_2) and 45:1, 50:1, 55:1 for ratio of solvent and material (X_3, ml/g), respectively.

3.1.2. Optimization of extraction conditions by a BBD

Based on the single-factor design, the Box-Behnken method was used to design a three-factor, three-level response surface analysis test using Design Expert 8.0.6 with extraction time (X_1), ethanol concentration (X_2) and liquid to material ratio (X_3) as independent variables (see Table 1). The final equation of TSE was fitted out by multiple regression analysis as follows:

$$Y(\%) = 1.28 + 0.060X_1 + 0.054X_2 + 0.039X_3\text{-}0.13X_1X_2 + 0.030X_1X_3 + 0.097X_2X_3 + 0.27{X_1}^2 - 0.030{X_2}^2 - 0.080{X_3}^2$$

The results of further analysis of the fitted quadratic regression equation by precision, reliability and accuracy were shown in Table 2, the model had a P-value < 0.0001 ($P < 0.01$) and the Lack of Fit is 0.7101 ($P > 0.05$), which indicated that the model had good fitting accuracy and could be used for subsequent correlation analysis and prediction. The test error was low, which shown that the model can be used for subsequent correlation analysis and prediction; the correlation coefficient $R^2 = 0.9856$ indicated a good correlation between the predicted and actual values; the value of Adj R-Squared and Pred R-Squared were 0.9671 and 0.9221; the coefficient of variation (C.V., 2.37 %) < 10 % showed a better model with perfect accuracy and precision (see Table 3).

Fig. 2 described the effects of three factors on the yield of TSE with 3D response surface curves. As can be seen from Fig. 2a, when the ethanol concentration was a constant value, TSC declined at the begining and rose up in late, and was the highest at extraction time 25 min; while extraction time was constant, TSC increased with the increasing of ethanol concentration, and reached the maximum at 50 % ethanol concentration, and the interaction of extraction time and ethanol concentration had a greater effect on the yield of TSE ($P < 0.0001$).When the extraction time was constant, there was a positive correlation between TSC and the liquid to material ratio, TSC was the maximum value at the the liquid to material ratio 55:1 ml/g (Fig. 2b). TSC was increasing with the improving of ethanol concentration, and reached the maximum at 50 % ethanol concentration; and the interaction exhibited a greater effect on TSC between the ethanol concentration and the liquid to material ratio ($P = 0.0005$, Fig. 2c).

3.2. Chemical components analysis of TSE from D. Nipponica

In order to further reveal the pharmacodynamic substance basis of TSE from *D. nipponica*, the main chemical components of the extract were preliminarily inferred and analyzed by UPLC-QTOF-MS. According to the reported literature (Li and Leng, 2019), there were twenty-three compounds were inferred based on the information of formula, molecular mass, m/z of $[M-H]^-$ and $[M + H]^+$, including thirteen steroidal saponins (protodioscin, protogracillin, methyl protodioscin, methyl protogracillin, pseudoprotogracillin, diosgenin-3-O-β-

Table 2 ANOVA for the response surface quadratic model of TSE yields.

Source	Sum of Squares	DF	Mean Square	F Value	p-value, ProbF	Significant
model	0.49	9	0.055	53.33	< 0.0001	significant
A	0.029	1	0.029	27.98	0.0011	**
B	0.023	1	0.023	22.45	0.0021	**
C	0.012	1	0.012	11.67	0.0112	*
AB	0.068	1	0.068	65.68	< 0.0001	**
AC	0.0036	1	0.0036	3.50	0.1037	
BC	0.038	1	0.038	36.94	0.0005	**
A^2	0.30	1	0.30	292.17	< 0.0001	**
B^2	0.004	1	0.004	3.74	0.0943	
C^2	0.027	1	0.027	26.34	0.0013	**
Residual	0.007	7	0.001			
Lack of Fit	0.002	3	0.0006	0.49	0.7101	not significant
Pure Error	0.005	4	0.001			
Cor Total	0.50	16				
R^2	0. 9856					
Adj R^2	0.9671					
Pred R^2	0.9221					
Adeq Precision	24.232					
C.V.%	2.37					

* $P \leq 0.05$,
** $P \leq 0.01$.

Table 3 The major compounds in TSE identified by UPLC-QTOF-MS method.

No.	Retention time (min)	Formula	Experimental *m/z* of $[M-H]^-$	Experimental *m/z* of $[M + H]^+$	Molecular mass	Error (mDa)	Compounds' name
1[a]	1.135	$C_{11}H_{12}O_7$	255.0514		256.0587	−0.39	Tartaric acid
2	1.759	$C_{51}H_{84}O_{22}$	1047.5385		1048.5466	−1.15	Protodioscin
3	1.779	$C_{51}H_{84}O_{23}$	1063.5343		1064.5415	−1.21	Protogracillin
4	4.47	$C_{12}H_{14}N_2O_4$	251.1027		250.0956	−0.19	Cyclo-(Ser-Tyr)
5	7.048	$C_7H_6O_2$	121.0295		122.0368	−0.02	Benzoic acid
6	28.074	$C_{19}H_{18}O_3$	293.1185	295.1331	294.1258	−0.07[b]/-0.21[c]	1,7-bis(4-hydroxyphenyl)hepta-4E,6E-dien-3
7	28.112	$C_{20}H_{20}O_4$	323.1292	325.1437	324.1364	−0.24/-0.27	7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4E,6E-heptadien-3-one
8	30.25	$C_{52}H_{86}O_{22}$	1061.5548		1062.5619	−0.87	Methyl protodioscin
9	30.417	$C_{52}H_{86}O_{23}$	1077.549		1078.5577	−1.14	Methyl protogracillin
10	30.769	$C_{51}H_{82}O_{22}$	1045.5232		1046.5305	−0.77	Pseudo protogracillin
11	31.986	$C_{33}H_{52}O_8$		577.3739	576.3665	−0.33	Diosgenin-3-O-β-D-glucopyranoside
12	32.122	$C_{18}H_{32}O_3$	293.2125		294.2198	−0.29	(5S,6E,8Z)-5-Hydroxy-6,8-octadecadienoic Acid
13	32.526	$C_{27}H_{38}O_3$		411.2895	410.2821	−0.03	25-D-Spirosta −3,5-diene
14	32.985	$C_{51}H_{82}O_{21}$	1029.5281	1031.5421	1030.5421	−0.54/0.25	Protogracellin
15	33.033	$C_{45}H_{72}O_{16}$	867.4752	869.4894	868.484	−0.18/0.04	Dioscin
16	33.123	$C_{45}H_{72}O_{17}$	883.4704	885.4840	884.4844	−0.44/-0.25	Gracillin
17	33.168	$C_{39}H_{62}O_{12}$		723.4318	722.4245	−0.33	Prosapogenin A
18	33.553	$C_{18}H_{30}O_2$	277.2175		278.2247	−0.14	α-Linolenic acid
19	33.975	$C_{18}H_{32}O_2$	279.2331		280.2404	−0.2	Linoleic acid
20	34.309	$C_{27}H_{40}O_3$		413.3054	412.2982	−0.44	Δ3,5-deoxytigogenin
21	34.511	$C_{18}H_{34}O_2$	281.2488		282.2558	0.08	Oleic acid
22	34.835	$C_{27}H_{42}O_3$		415.3208	414.3136	−0.18	Diosgenin
23	35.366	$C_{18}H_{36}O_2$	283.2645		284.2714	0.09	Stearic acid

[a] Peak number as in Fig. 3;
[b] Error of $[M-H]^-$;
[c] Error of $[M + H]^+$.

D-glucopyranoside, protogracellin, dioscin, gracillin, prosapogenin A, Δ3,5-deoxytigogenin, diosgenin, 25-D-Spirosta −3,5-diene), seven organic acid compounds (tartaric acid, benzoic acid, 5S,6E,8Z-5-Hydroxy-6,8-octadecadienoic acid, α-linolenic acid, linoleic acid, oleic acid, stearic acid), two flavonoids (1,7-bis (4-hydroxyphenyl) hepta-4E, 6E-dien-3,7- (4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4E,6E-heptadien-3-one), and one amino acid compound (CycloSer-Tyr). The preliminary inference of the main chemical components in the TSE from *D. nipponica* will provide an important reference for further study of pharmacological mechanism.

3.3. The antioxidant activities of TSE from D. Nipponica

In this study, three antioxidant assay methods, including FRAP, ABTS and DPPH radicals scavenging activity assays, were used to evaluate the antioxidant activity of TSE. TSE exhibited a milder antioxidant activity than BHT ($IC_{50\ DPPH}$ 85.18 μg/ml, $IC_{50\ ABTS}$ 31.90 μg/ml, 0.56 mmol Fe^{2+} for FRAP) with $IC_{50\ DPPH}$ 2906 μg/ml, $IC_{50\ ABTS}$ 74 μg/ml, 0.025 mmol Fe^{2+} for FRAP.

Relevant studies had confirmed the certain antioxidant activity of monomer saponins. Selim S (2016) indicated that the diosgenin showed an effective antioxidant activity against DPPH free radical than VC, and could significantly reduce the content of SOD, LPO and MDA (Kanchan DM, 2016; Kiasalari Z, 2017). Combined with the results of this study, it can be concluded that the antioxidant activity of saponins extract was weaker than that of than their monomeric compounds. However, the price of saponins crude extract is relatively cheap, and it will exhibit a good prospect of application as antioxidant in food and medicine industry.

3.4. The antibacterial activities of TSE from D. Nipponica

Microbroth dilution method was applied for evaluation the antibacterial activities of TSE from *D. nipponica* against *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Streptococcus*. MIC values were 62.5 mg crude drug/ml for *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus,* and 125 mg crude drug/ml for *Salmonella*.

The antibacterial activities of monomer saponins and their new formulations were confirmed by related studies. The antimicrobial activity of diosgenin was demonstrated with a significant zone of inhibition against Gram-positive and Gram-negative bacteria (Khan H, 2015). SevincIlkar Erdagi (2020) found that Genipin crosslinked gelatin-diosgenin-nanocellulose hydrogels exhibited a excellent antibacterial effect towards Gram-positive and Gram-negative bacteria. However, there were few reports on the antibacterial activity of crude saponins. This study revealed the antibacterial activity

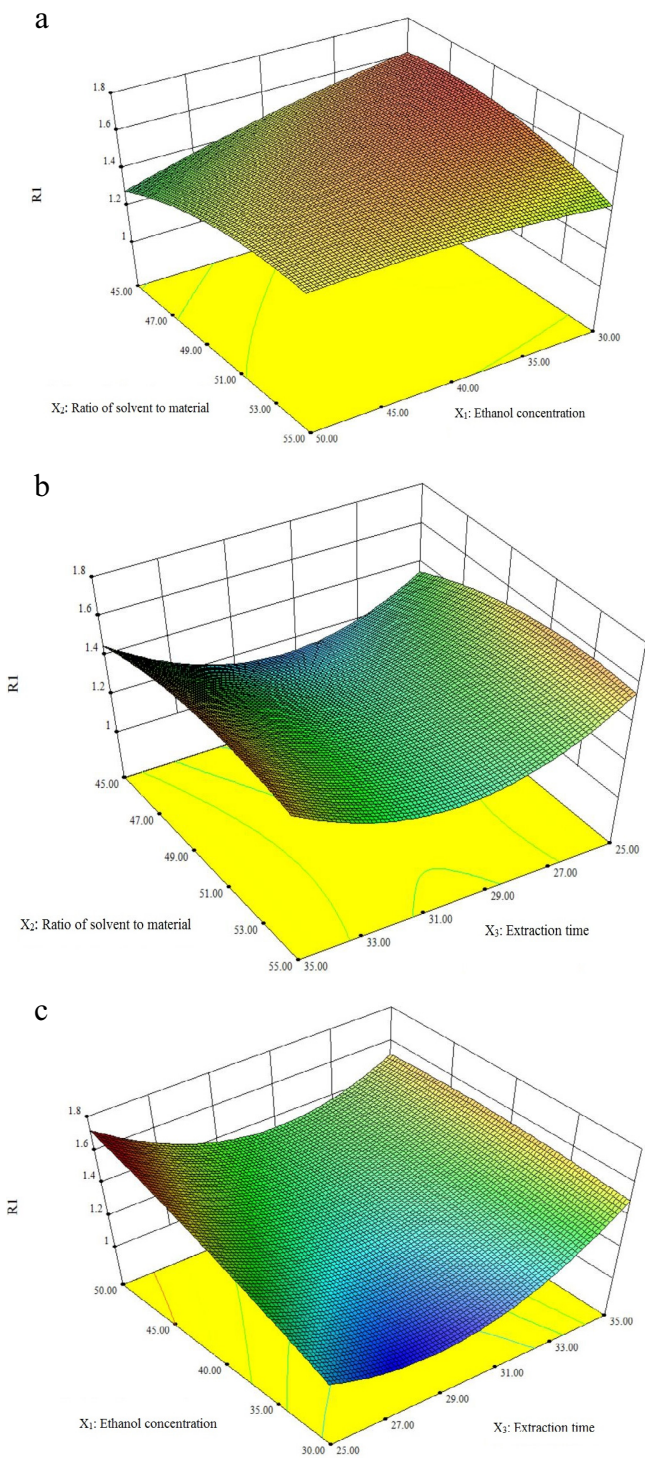

Fig. 2 A Response surface plot of ethanol concentration (X_1,%) and ratio of solvent to material (X_2, ml/g) on the extraction yield of total saponin of *D.nipponica*; b Response surface plot of ratio of solvent to material (X_2, ml/g) and extraction time (X_3, min) on the extraction yield of total saponin of *D.nipponica*; c Response surface plot of ethanol concentration (X_1,%) and extraction time (X_3, min) on the extraction yield of total saponin of *D.nipponica*.

of crude saponins, laying a foundation for the screening of green and healthy antibacterial drugs from Traditional Chinese medicine.

3.5. Evaluation of the anti-inflammatory effects of TSE on LPS induced RAW 264.7 cells

3.5.1. Effect of TSE on the cell viability of LPS induced RAW 264.7 cells

The cytotoxicity of TSE on RAW 264.7 cells was described in Fig. 4. The cell viability ratio was > 98 %, which was indicated that three test concentrations of TSE, LPS, Dex, and TAK-242 all exhibited no cytotoxicity.

3.5.2. Effect of TSE on the NO content in LPS induced RAW 264.7 cells

The effect of TSE from *D. nipponica* on the content of inflammatory mediator NO was described in Fig. 5. It can be seen that 6.25, 12.5 and 25 μg/ml of TSE can significantly reduce the level of NO with dose-dependent, and there was no significant difference between the 25 μg/ml of TSE treatment group with the control group.

Nitric oxide, as an important inflammatory mediator, is a major reference index to evaluate the anti-inflammatory effect of drugs. A large number of studies had revealed that saponins can reduce the amount of nitric oxide produced in inflammatory response. The total saponins from *Dioscorea nipponica* Makino can decrease the content of NO in carbon tetrachloride-induced liver injury in mice (Yu et al., 2014). Diosgenin glucoside, as a relatively common monomer compound of saponins, can also suppress the synthesis of promote inflammation molecule NO in regulating microglial M1 polarization (Wang et al., 2017). Sapogenin diosgenin can exhibit anti-inflammatory properties by inhibiting the production of NO induced by IL-1β (Wang et al., 2015). This study revealed that the extract can reduce the content of NO, which was consistent with literature reports. However, which signaling pathways play a regulatory role needs to be further revealed in future studies.

3.5.3. Effect of TSE on the phagocytic activity of LPS induced RAW 264.7 cells

The phagocytic activity of RAW 264.7 cells after treatment with 6.25, 12.5 and 25 μg/ml of TSE from *D. nipponica* was shown in Fig. 6. The phagocytic capacity of the LPS model group was very significantly ($P < 0.01$) up-regulated compared to the control group, and significantly ($0.05 < P < 0.01$) down-regulated in three drugs groups compared to the LPS model group with a dose-dependent trend. The difference between the 25 μg/ml of TSE and the control group was not significant ($P > 0.05$).

Cytophagocytosis is the function of certain cells in organism to recognize foreign body and swallow it and destroy it, which is the basic defense mechanism of organism. Phagocytosis plays a major role in the genesis and development of inflammation. Therefore, it is often used as an important index to measure the anti-inflammatory effect of drugs. In a certain concentration range, the phagocytosis activity increased with the increasing concentration of diosgenin 3-*O*-α-L-rhamnopyranosyl(1 → 4)-α-L-rhamnopyranosyl (1 → 4)-[α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranoside, diosgenin 3-*O*-α-L-rhamnopyranosyl (1 → 2)-α-L-arabino furanosyl-β-D-glucopyranoside and diosgenin, but with the further increasing of dosage, the phagocytosis activity began to decrease (Zhang et al., 2007). Studies had also been reported that dioscin and

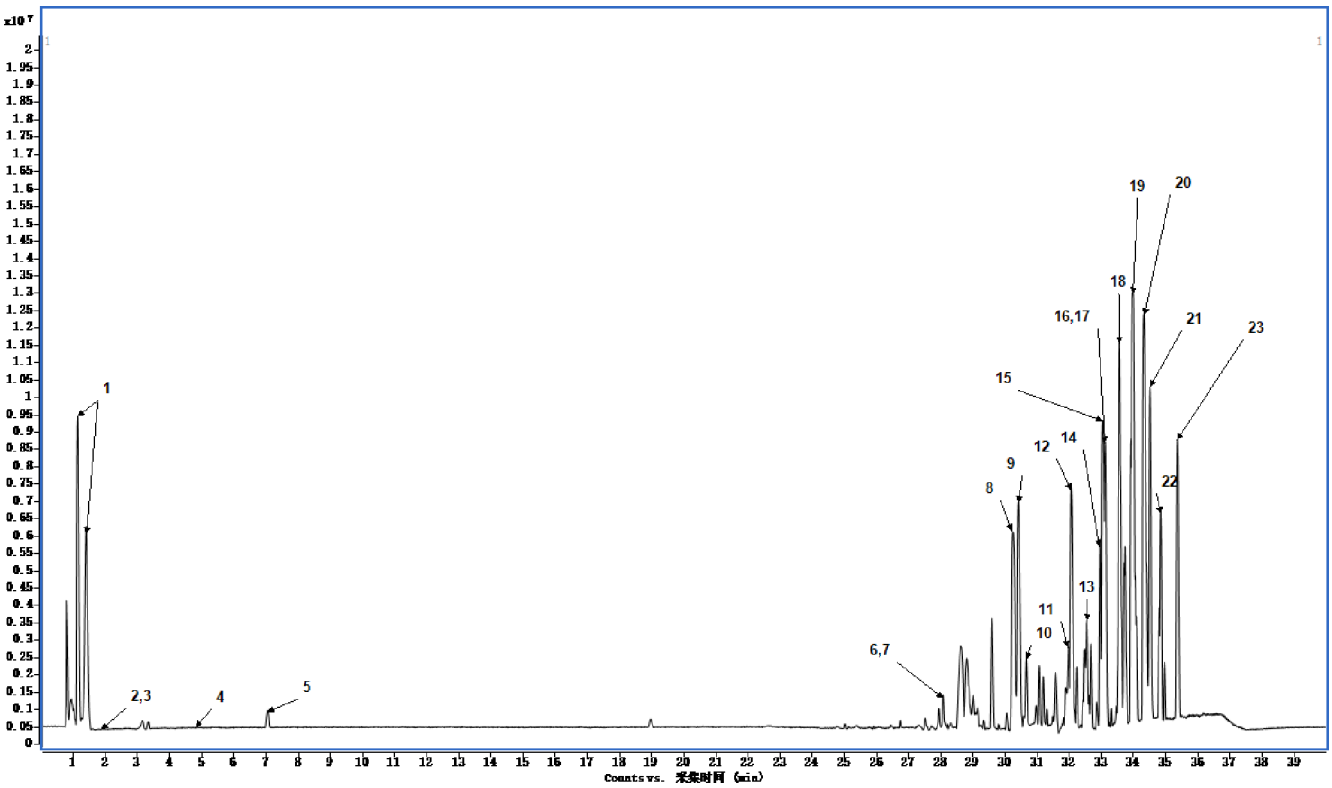

Fig. 3 Chromatographic profile of the chemical components from TSE of *D.nipponica* at 204 nm.

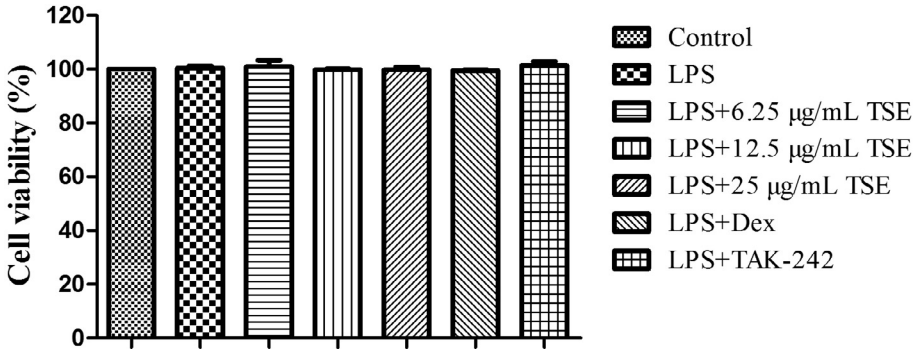

Fig. 4 Effect of TSE from *D.nipponica* on cell viability in LPS-induced RAW 264.7 cells.

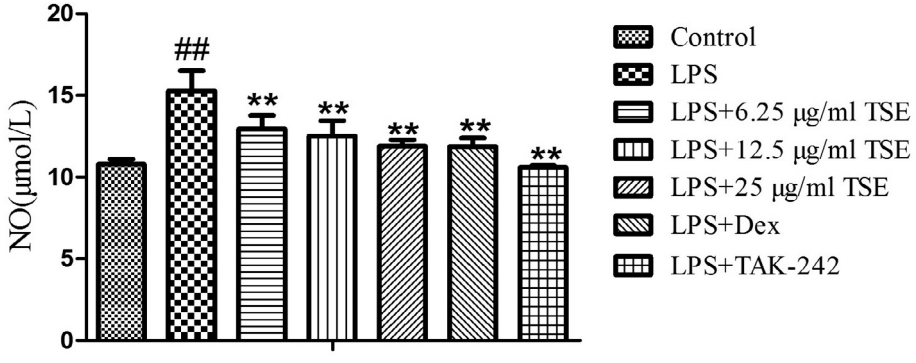

Fig. 5 Effects of TSE from *D.nipponica* on the NO content in LPS induced RAW 264.7 cells ($^{\#}$compared with the control, *compared with LPS, **/ $^{\#\#}$ $P < 0.01$).

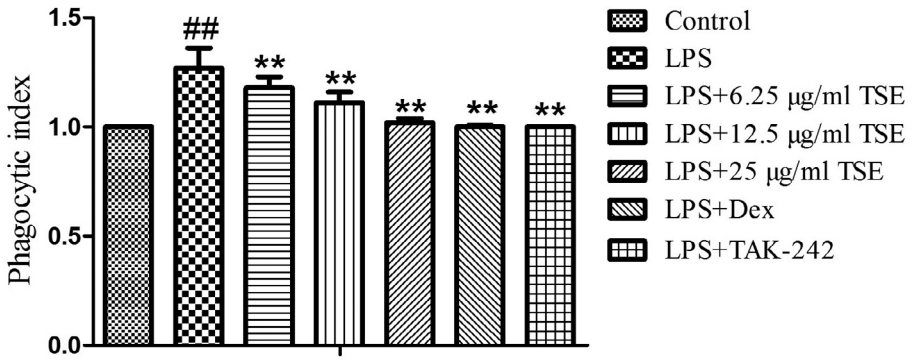

Fig. 6 Effects of TSE from *D.nipponica* on phagocytic index in LPS induced RAW 264.7 cells ($^{\#}$compared with the control, *compared with LPS, **/ $^{\#\#}$ $P < 0.01$).

diosgenin all can induce macrophage M2-to-M1 phenotype or lymphocyte transformation, and enhance phagocytic capability of macrophages *in vitro* (Cui et al., 2020; He et al., 2012). TSE can exert anti-inflammatory effects by directly affecting phagocytosis activity, but the mechanism of anti-inflammatory effects needs to be further explored in the future.

4. Conclusion

This research comprehensively explored the extraction process, main active ingredients, and the biological activities of TSE from *D. nipponica in vitro*. The optimal extraction process of TSE was extraction time 25 min, 50 % ethanol solution, and the ratio of solvent to material 55:1 (ml/g); eighteen compounds were preliminarily inferred with UPLC-QTOF-MS method, including thirteen steroidal saponins, two organic acids, two flavonoids, and one amino acid. Although TSE showed mild antibacterial activities against *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Streptococcus*, and antioxidant activities against ferric-ion, ABTS and DPPH radicals, the stronger anti-inflammatory activity of TSE was demonstrated by significantly reducing the content of NO and the phagocytic activity.The above research content laid a preliminary foundation for further research on the anti-inflammatory effect and mechanism of TSE from *D. nipponica*, and provided an important reference for the screening of new anti-inflammatory Chinese medicines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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