Analysis of Lavandulyl Flavonoids from *Sophora flavescens* with Anti-inflammatory Activity Based on Molecular Network Technology

Yan LIN, Bo TU, Shanggao LIAO, Minghui HE*

Guizhou Provincial Higher Education Institutions Genuine Medicinal Materials Chronic Disease Prevention and Control Engineering Research Center, College of Pharmacy, Guizhou Medical University, Guiyang 550025, China

Abstract [**Objectives**] This study was conducted to screen lavandulyl flavonoids with anti-inflammatory activity from *Sophora flavescens*. [**Methods**] 35 compounds were screened from traditional Chinese medicine *S. flavescens* using the nitric oxide (NO) anti-inflammatory activity model. [**Results**] Five components, **8** (xanthohumol), **13** (kurarinol), **27** (4-methoxysalicylic acid), **28** (b-resorcic acid) and **30** (b-resorcic acid), exhibited significant anti-inflammatory activity, with IC_{50} values of 5.99, 4.76, 6.96, 3.41 and 5.22 μM, respectively. Especially, **8** (xanthohumol) and **13** (kurarinol) were typical lavandulyl flavonoids in *S. flavescens*, which were worth further exploration. Furthermore, UPLC-Q-Exactive and GNPS molecular networking technique were used for rapid analysis of lavandulyl flavonoids from *S. flavescens*. A total of 15 components were identified. [**Conclusions**] This work lays a theoretical foundation for further separation and analysis of lavandulyl flavonoids with anti-inflammatory activity from *S. flavescens*.

Key words *Sophora flavescens*, Molecular network, Anti-inflammatory activity, Lavandulyl flavonoids

1 Introduction

Sophora flavescens was derived from the roots of *Sophora* species (Leguminosae family), which was widely distributed in various regions of China[1]. *S. flavescens*, as a commonly used traditional Chinese medicine, has a long medicinal history in China, and was strongly bitter in taste, leading to its significant effects of clearing away heat and drying dampness[2]. *S. flavescens* was first recorded in *Sheng Nong's Herbal Classic* and included in the first part of the 2015 edition of *Pharmacopoeia of the People's Republic of China*[3]. Also, in the clinic, *S. flavescens* was often used to treat dysentery, enteritis, oliguria, eczema, skin diseases, hepatitis B and other ailments[4].

In recent years, studies have shown that *S. flavescens* has a wide range of biological activity, mainly including anti-inflammatory, antibacterial, antioxidant, and anticancer pharmacological activity, which were attributed to its main active ingredients including alkaloids and flavonoids[5-6]. Especially lavandulyl flavonoids, which were known as natural defense substances, play an important role in the resistance of natural products to foreign invasion. The previous research for our lab also conducted a systematic review on the composition, pharmacological activity, and pharmaceutical analysis of lavandulyl flavonoids in *S. flavescens*, and found that lavandulyl flavonoids had significant anti-inflammatory activity[7].

Inflammation was the defensive response of living tissues with a vascular system, including symptoms such as redness, swelling, heat and pain caused by stimulation of various injury factors, and it was a complex physiological and pathological reaction phenomenon caused by harmful stimulation in internal and external environment[7]. And, inflammation was a very common and important basic pathological process, and it was a common pathway that causes many major human diseases. It participates in the occurrence and development of many major human diseases, human infection (such as pneumonia, hepatitis, nephritis, *etc.*), tumor, cardiovascular and cerebrovascular diseases, senile dementia, neurodegenerative disorders and psychosis. Like some clinical effects of *S. flavescens*, such as treating hepatitis, eczema and skin diseases, were directly related to its anti-inflammatory activity[8].

Recently, Global Natural Products Social Molecular Networking (GNPS) was a molecular network database (http://gnps.ucsd.edu) created by Watrous *et al.* According to the similarity of MS/MS second-order mass spectrum fragments of related compounds, molecules of the same kind of compounds will be clustered in a molecular network and then visually analyzed[9-10]. Severous studies elucidated that the application of this technique can accelerate the identification of unknown compounds in natural products.

In this study, the anti-inflammatory activity model was used to screening 35 phenolic components in *S. flavescens*, and UPLC-Q-Exactive and GNPS molecular network techniques were then applied to quickly analyze and identified lavandulyl flavonoid. It was laying a theoretical foundation for further in-depth separation and analysis of lavandulyl flavonoids with anti-inflammatory activity in *S. flavescens*.

2 Materials and methods

2.1 Experimental instruments Thermo Vanquish ultra-high performance liquid chromatography (Thermo Fisher, USA);

Received: December 7, 2023 Accepted: March 10, 2024
Supported by Guizhou Provincial Science and Technology (ZK (2022)-362; ZK (2024)-047; [2023]ZK01); The Innovation and Entrepreneurship Training Program for Undergraduates from China [202210660131; 202310660082]; Science Foundation of Guizhou Education Technology (2022-064); University Engineering Research Center for the Prevention and Treatment of Chronic Diseases by Authentic Medicinal Materials in Guizhou Province ([2023]035); Science and Technology Research Project of Guizhou Administration of Traditional Chinese Medicine (QZYY-2024-134).

* Corresponding author. E-mail: heminghui@gmc.edu.cn

Q-Exective high-resolution mass spectrometer (Thermo Fisher, USA); Acquity UPLC HSS T3 chromatographic column (2.1 mm × 150 mm, 1.8 μm, Waters, MA, USA); electronic balance (Shanghai Zhuojing Electronic Technology Co., Ltd., BSM-220); carbon dioxide cell incubator (Sanyo, Japan); clean bench (Sujing Antai, Suzhou); Multiskan ELISA (Thermo Fisher Scientific Inc., Finland).

2.2 Experimental reagents and herb

2.2.1 Reagents. Chromatographic grade methanol (Sinopharm Chemical Reagent Co., Ltd.); chromatographic grade acetonitrile (Sigma-Aldrich, MO, USA); tyrosinase (1 ∶ 250, trypsin) (Gibco, Maryland, USA); DMEM culture medium; fetal bovine serum (FBS) (Hyclone, Utah, USA); methanol and ethanol (analytically pure, Beijing Chemical Plant); nitric oxide (NO) detection kit (Biyuntian, Jiangsu); positive drug curcumin (Nanjing Zelang Medical Technology Co., Ltd.).

2.2.2 Medicinal herb and sample preparation. *S. flavescens* was purchased from Dafang County, Bijie City, Guizhou Province in September 2019, and was identified as dried roots of *S. flavescens* Ait., a leguminous plant in the *Sophora*, through DNA barcode analysis using ITS sequences[11]. After drying the medicinal herb, it was crushed, and 100 mg of sample powder was added with 10 mL of 95% ethanol. Ultrasound extraction was performed at 25 ℃ for 30 min. The supernatant was then transferred to a 1.5 mL centrifuge tube and centrifuged at 15 000 rpm for 30 min to obtain a supernatant.

The 37 compounds used in this study were all isolated and prepared from the EtOAc of *S. flavescens* by our research team in the early stage, namely kurarinol A (**1**), kurarinol A (**2**), kurarinol A (**3**), kushenol H (**4**), kushenol L (**5**), kuraridinol (**6**), kuraridine (**7**), xanthohumol (**8**), isoliquiritigenin (**9**), kushenol Q (**10**), sophoraflavanone B (**11**), naringenin (**12**), kurarinol (**13**), kushenol A(**14**), sophoflavescenol (**15**), noranhyoicaritin (16), quercetin (**17**), 7, 3'-di-O-methyl (**18**), genistein (**19**), calycosin (**20**), formononetin (**21**), biochanin A (**22**), 5, 4'-dihydroxyflavone (**23**), luteolin (**24**), 7-hydroxycoumarin (**25**), 7, 8-dihydroxycoumarin (**26**), 4-methoxysalicylic acid (**27**), b-resorcylic acid (**28**), 4-hydroxybenzoic acid (**29**), sophoracarpan A (**30**), trifohrhizin-6'-monoacetate (**31**), trifohrhizin (**32**), maackiain (**33**), (6S,6aS,11aR)-6α-methoxy-pterocarpin (**34**), L-maackiain (**35**), kushenol N (**36**), and neokurarinone (**37**)[11-12].

2.3 Cell culture RAW264.7 (mouse macrophages) was obtained from the American Type Culture Collection (ATCC) using DMEM medium containing 10% fetal bovine serum, maintained at 37 ℃, 5% CO_2, and saturated humidity. And 0.25% trypsin-EDTA was used for digestion and passage.

2.4 Evaluation of nitric oxide (NO)-inhibiting activity RAW264.7 cells were digested and passaged into 96 well plates, according to 1.0×10^6 to 1.2×10^6 cells per well, and medication treatment was performed after 24 h until the cells had adhered to the wall. Medication treatment: Lipopolysaccharide (LPS) was added to each well at 1 μg/mL, and a certain concentration of compounds diluted in serum-free medium was then added. The blank control group was treated with 1 μL/mL of DMSO, and the positive control group was treated with 20 μM of curcumin. There were three parallel wells for each concentration, and the concentration of NO was detected after 24 h. From each well of 96-well plates, 50 μL of culture medium was pipetted into a new 96-well plate, and Griess reagent I and Griess reagent II were added respectively according to the operation instructions of NO detection kit, and the concentration of NO in it was detected on an enzyme-labeled instrument at 540 nm.

2.5 Liquid chromatography conditions Thermo Vanquish ultra performance liquid chromatograph (Thermo Fisher, USA); chromatographic column: Agilent Eclipse Plus C_{18} RRHD (2.10 mm × 150 mm, 1.8 μm); column temperature: 40 ℃; flow rate: 0.3 mL/min; wavelength: 280, 254, 320 nm; sample volume: 2 μL; mobile phase: acetonitrile (A)-0.1% formic acid aqueous solution (B); gradient elution: 5% – 30% (A) from 0 to 2.5 min, 30% – 60% (A) from 2.5 to 5.5 min, 60% – 80% (A) from 5.5 to 8.0 min, and 80% – 100% (A) from 8.0 to 10.0 min.

2.6 Mass spectrometry conditions A Q-Exactive high-resolution mass spectrometer (Thermo Fisher, USA) equipped with heating electrospray ion source (HESI) was connected with a Thermo Vanquish ultra-high performance liquid system. The full scan/ddms2 mode was adopted to collect data under positive and negative ions and following conditions: spray voltage 3.5 kV, pressure of sheath gas N_2 35 arb, pressure of auxiliary gas N_2 10 arb, capillary temperature 350 ℃, and ion source temperature 400 ℃. The resolution of first-order mass spectrometry was 70 000, and the resolution of second-order mass spectrometry was 17 500, and the scanning range was 100 – 1 000 m/z. The top 10 parent ions collected in first-order mass spectrometry in each scanning cycle were selected for collection of second-order data, and the normalized collision energy (NCE) was 35%. The collected MS data were analyzed by XcalibutTM 4.1 software (Thermo Fisher, USA).

2.7 Construction of compound molecular network The original data of high-resolution mass spectrometry were converted to mzXML format (https://github.com/PedrioliLab/ReAdW) by ReAdW program. The generated files were uploaded to the Global NaturalProduct Social Molecular Networking (GNPS, http://gnps.ucsd.edu) platform, and molecular networks were constructed by using the Molecular Networking function[13-14]. The constructed molecular networks were imported into Cytoscape 3.8.1 software (https://cytoscape.org/download.html) for visual analysis.

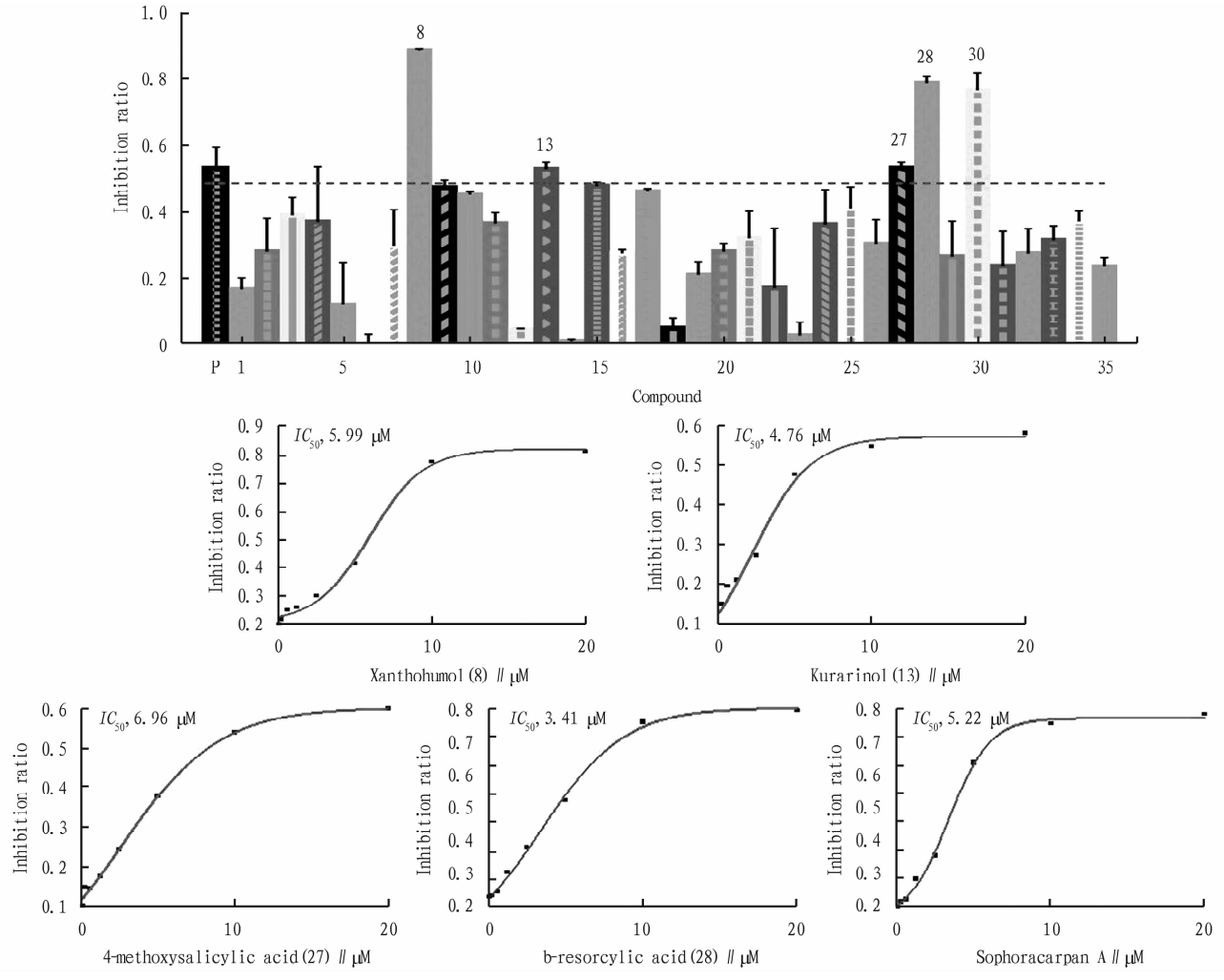
3 Results and analysis

3.1 Screening results of nitric oxide (NO)-inhibiting anti-inflammatory activity According to the literature, the alkaloids of *S. flavescens*, matrine, sophocarpine and sophoridine, have re-

markable anti-inflammatory activity[15-16]. The treatment of chronic liver disease, atopic dermatitis and eczema by *S. flavescens* shows that it is directly related to anti-inflammatory activity.

When inflammation occurs, macrophages produce various inflammatory factors and nitric oxide (NO), which then continue to stimulate inactive macrophages and their neighboring cells, and promote the occurrence of inflammation in a vicious circle[17]. Lipopolysaccharide (LPS) is a commonly used inflammatory inducer, which can stimulate macrophages to produce various inflammatory factors, including NO. The LPS-induced NO production model is a commonly used model for screening anti-inflammatory activity of cells.

In order to elucidate the effective components of anti-inflammatory activity of *S. flavescens*, the inhibitory effect of compounds (10 μM) on NO production of mouse macrophage RAW264.7 was evaluated. The activity screening results are shown in Fig. 1. Among the 35 compounds, five compounds had an inhibition rate of more than 50% at 10 μM (curcumin was used as a positive control in this study, and the inhibition rate was 56% at 10 μM). Compounds **8** (xanthohumol), **13** (kurarinol), **27** (4-methoxysalicylic acid), **28** (b-resorcic acid) and **30** (b-resorcic acid) exhibited the strongest activity, with IC_{50} values of 5.99, 4.76, 6.96, 3.41 and 5.22 μM, respectively.

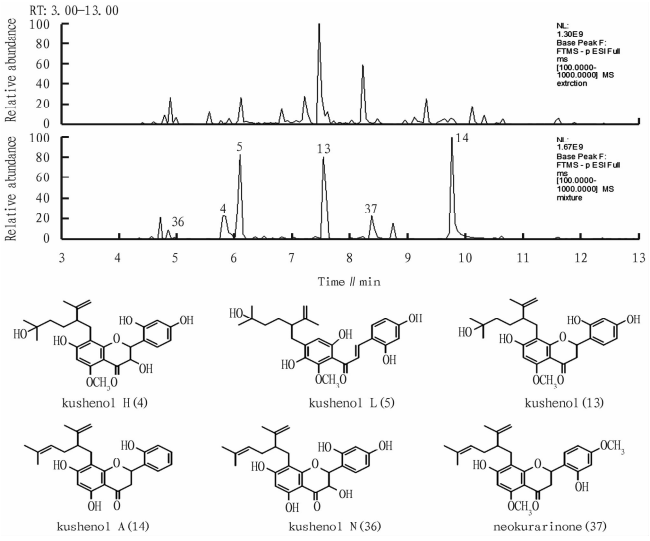


NOTE P. Positive drug, curcumin, 20 μM; 1-35: compounds 1-35.

Fig. 1 Screening results of NO-inhibiting activity of compounds from *Sophora Flavescens* (1-35)

3.2 Molecular network construction and spectral analysis for identification of lavandulyl flavonoids from Sophorae Flavescentis Radix UPLC-MS/MS preliminary analysis was performed on the ethyl acetate fraction of Sophorae Flavescentis Radix rich in lavandulyl flavonoids and six lavandulyl flavonoids isolated from it (**4**, **5**, **13**, **14**, **36**, **37**). The results showed that the first-order mass spectrum scanning detection of the ethyl acetate fraction of Sophorae Flavescentis Radix showed good response of each chromatographic peak in negative ion mode (Fig. 2).

The original data of high-resolution mass spectrometry were converted into mzXML format by MSconvert software. The generated files were uploaded to the Global Natural Product Social Molecular Networking (GNPS, http://gnps.ucsd.edu) platform, and molecular networks were constructed by using the molecular networking function. The constructed molecular networks were imported into Cytoscape 3.8.0 software for visual analysis.

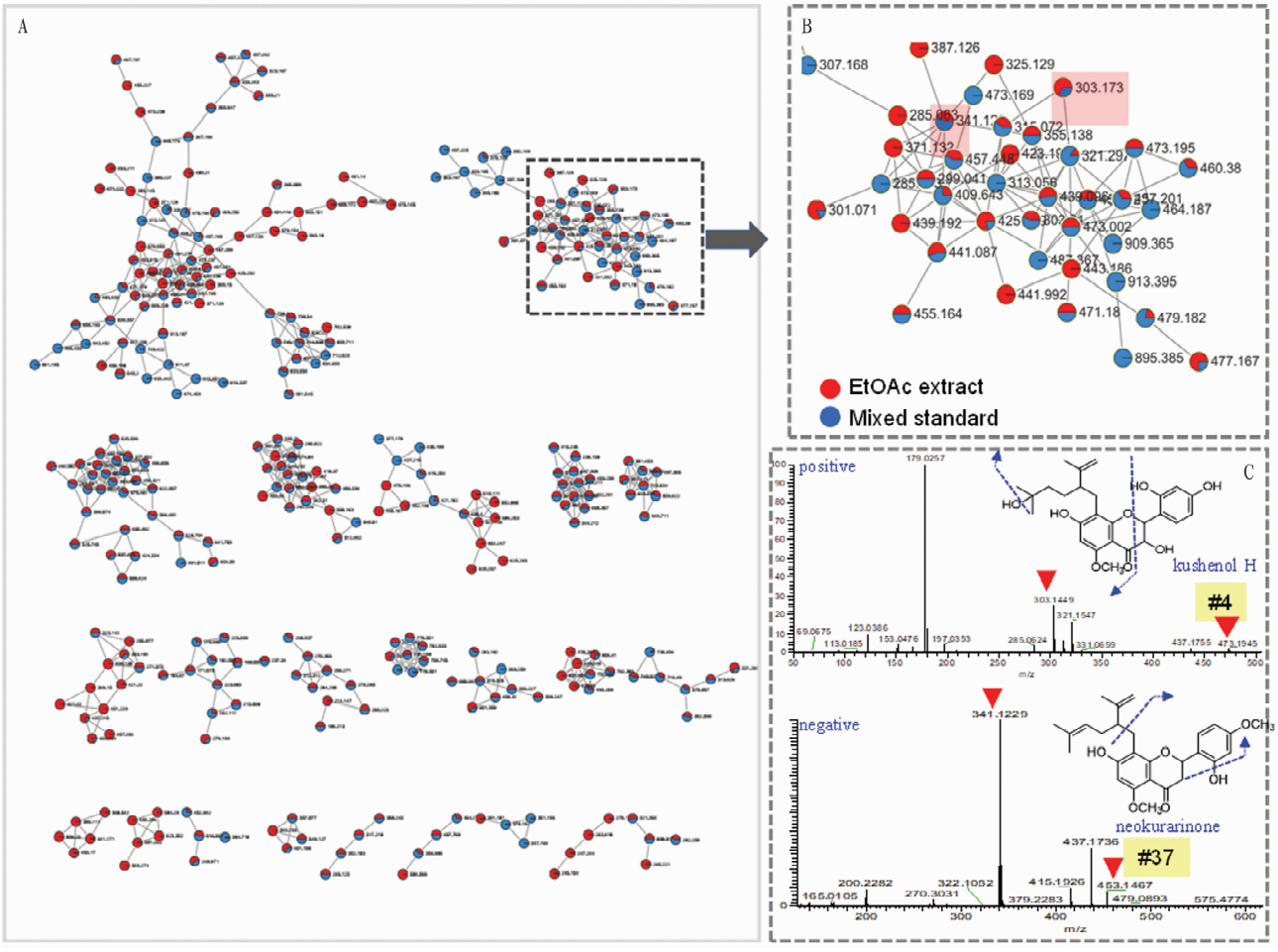


NOTE Standard substances: **4**, kushenol H; **5**, kuraridinol; **13**, kurarinol; **14**, kushenol A; **36**, kushenol N; **37**, neokurarinone.

Fig. 2 Negative ion scanning spectra of first-order mass spectrometry from ethyl acetate fraction of *Sophora flavescens* and mixed standards

Lavandulyl flavonoids have high content and ssignificat activity, and were an important component type in *S. flavescens*. The MS/MS spectra of compounds in the lavandulyl flavonoid cluster (Fig. 3B) were analyzed and compared with standard substances (Fig. 3C), and it was found that fragment ions 303.144 9 $[M+H]^+$, 179.025 7 $[M+H]^+$, 341.122 9 $[M-H]^-$, 313.188 0 $[M-H]^-$ and 161.031 0 $[M-H]^-$ were produced in both spectra, so they could be considered to be lavandulyl flavonoid components in *S. flavescens*. This rule could serve as a basis for quickly determining lavandulyl flavonoid components.

Combined with mass spectrometry database matching of various compounds, the information of main compounds was preliminarily characterized by first-order mass spectrometry and second-order fragment ion information analysis and literature reports (Table 1). Through data analysis, it was found that the molecular weight range of lavandulyl flavonoids from *S. flavescens* was 300 – 600 Da, and the diagnosed ions were 209/114, 231/114, 303/179, 279/161 and 177/149, which lays a foundation for further directional separation of these compounds.



NOTE A. Molecular network drawing; B. cluster results of some lavandulyl flavonoids; C. MS/MS spectra of lavandulyl flavonoids from *Sophorae flavescentis* Radix.

Fig. 3 Molecular network drawing of EtOAc fraction of *Sophora flavescens* and characteristic lavandulyl flavonoid standards

Table 1 Preliminarily-identified lavandulyl flavonoids in EtOAc fraction of *Sophora flavescens*

No.	t_R // min	$[M+H]^+$	Molecular formula	MS^2 // *m/z*	Identified compounds
1	4.60	453.047 5	$C_{26}H_{30}O_7$	435.312 9, 209.155 3, 114.086 5	Sophoraflavanone K
2	4.79	465.233 6	$C_{27}H_{30}O_7$	231.014 0, 164.984 1, 114.086 3	Unknown
3	4.89	396.783 4	$C_{23}H_{24}O_6$	209.155 2, 114.086 5	Unknown
4	4.99	453.322 9	$C_{27}H_{32}O_6$	228.148 9, 209.155 3, 114.086 4	Kushenol N
5	5.38	405.044 6	$C_{25}H_{24}O_5$	317.980 0, 289.949 9, 259.940 8	Unknown
6	5.56	471.180 2	$C_{19}H_{28}O_{11}$	335.134 9, 328.102 5, 179.025 6	Unknown
7	5.76	473.183 5	$C_{19}H_{30}O_{11}$	319.138 9, 179.025 6	Kushenol H
8	5.91	495.176 4	$C_{30}H_{38}O_6$	451.187 1, 343.136 0, 201.006 9	Unknown
9	6.11	457.201 0	$C_{26}H_{32}O_7$	321.154 7, 303.144 9, 179.025 7	Kushenol P
10	6.21	471.222 0	$C_{26}H_{32}O_8$	293.188 0, 177.022 6, 149.030 6	Unknown
11	6.42	523.217 0	$C_{30}H_{34}O_8$	455.218 9, 293.188 2, 161.031 0	Unknown
12	6.82	509.200 4	$C_{30}H_{36}O_7$	441.210 4, 279.177 9, 161.031 0	Kushenol M
13	6.92	471.222 1	$C_{26}H_{30}O_8$	309.184 0, 177.027 1, 161.031 0	Kushenol X
14	7.07	439.194 6	$C_{25}H_{28}O_7$	277.156 0, 259.145 0, 160.031 0	Unknown
15	7.22	473.237 5	$C_{26}H_{34}O_8$	293.188 2, 179.042 3, 137.030 0	Unknown
16	7.47	441.194 6	$C_{25}H_{28}O_7$	303.145 0, 179.025 8	Kushenol L
17	7.61	443.175 5	$C_{25}H_{28}O_{11}$	290.133 4, 165.010 7	Kushenol Q
18	8.03	339.143 2	$C_{20}H_{18}O_5$	253.074 1, 121.023 1	Unknown
19	8.23	453.209 5	$C_{27}H_{32}O_6$	329.086 7, 303.144 5, 179.025 6	2'-Methoxykurarinone
20	8.48	441.170 4	$C_{25}H_{28}O_7$	233.070 0, 195.019 9, 177.010 2	Unknown
21	8.96	439.191 6	$C_{26}H_{30}O_6$	303.144 9, 179.025 7	Kurarinone
22	9.12	439.191 6	$C_{26}H_{30}O_6$	315.071 7, 177.046 5, 165.010 8	Isokurarinone
23	9.33	439.215 1	$C_{26}H_{30}O_6$	275.176 9, 161.013 3, 151.046 2	Kuraridin/isokuraridin
24	9.64	511.264 5	$C_{30}H_{38}O_7$	330.229 6, 177.026 5, 149.030 6	Unknown
25	9.75	409.182 2	$C_{25}H_{28}O_5$	289.129 9, 165.010 7	Kushenol A
26	10.12	381.090 7	$C_{21}H_{16}O_7$	324.027 1, 306.017 3, 343.056 5	Unknown
27	10.33	495.270 5	$C_{30}H_{38}O_6$	205.095 4, 161.031 0	Unknown
28	10.65	355.230 2	$C_{21}H_{22}O_5$	177.099 4, 163.119 6	Isoxanthohumol
29	11.61	341.224 0	$C_{20}H_{20}O_5$	177.119 2, 161.088 7, 149.089 2	Sophoraflavanone B
30	11.89	335.245 1	$C_{20}H_{14}O_5$	275.162 1, 175.123 1	Unknown

4 Conclusions

In this study, 35 phenolic compounds from *S. flavescens* were screened by a nitric oxide (NO) anti-inflammatory activity mode, and the results showed that five compounds (**8**, **13**, **27**, **28** and **30**) had significant anti-inflammatory activity. Furthermore, UPLC-Q-Exactive and GNPS molecular networking technique were used for rapid analysis of lavandulyl flavonoids from *S. flavescens*. As a result, 15 components were identified. It was laying a theoretical foundation for further separation and analysis of lavandulyl flavonoids with anti-inflammatory activity from *S. flavescens*.

References

[1] PARK JS, SEONG ZK, KIM MS, *et al.* Production of flavonoids in callus cultures of *Sophora flavescens* aiton[J]. Plants, 2020(9): 688 – 701.

[2] HE XR, FANG JC, HUANG LH, *et al.* *Sophora flavescens* Ait: Traditional usage, phytochemistry and pharmacology of an important traditional Chinese medicine[J]. Journal of Ethnopharmacology, 2015(172): 10 – 29.

[3] Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China, 1 [M]. Beijing: Chinese Medical Science and Technology Press, 2015: 202 – 203. (in Chinese).

[4] LIN Y, CHEN XJ, HE L, *et al.* Systematic elucidation of the bioactive alkaloids and potential mechanismfrom *Sophora flavescens* for the treatment of eczema via network pharmacology[J]. Journal of Ethnopharmacology, 2023(301): 115799.

[5] LU W, ZHANG R, ZHU J, *et al.* Oxymatrine and cancer therapy[J]. European Journal of Inflammation, 2015, 13(3): 148 – 153.

[6] LAN X, ZHAO JN, ZHANG Y, *et al.* Oxymatrine exerts organ- and tissue-protective effects by regulating inflammation, oxidative stress, apoptosis, and fibrosis: From bench to bedside[J]. Pharmacological Research, 2019, 151(5):104541.

[7] LI JJ, ZHANG X, SHEN XC, *et al.* Phytochemistry and biological properties of isoprenoid favonoids from *Sophora favescens* Ait[J]. Fitoterapia, 2020(143): 104556.

[8] LIN Y, CHEN XJ, LI JJ, *et al.* A novel type lavandulyl flavonoid from *Sophora flavescens* as potential anti-hepatic injury agent that inhibit TLR2/NF-κB signaling pathway[J]. Journal of Ethnopharmacology, 2023(307): 116163.

[9] ZHANG YT, WANG CG, DENG XQ. Rapid identification of chemical constituents in Erigeron breviscapus injection based on UHPLC-LTQ-orbitrap MSn and molecular network technology[J]. Journal of Chinese Mass Spectrometry Society, 2022(43): 287 – 299. (in Chinese).

[10] CHENG TF, JIN HZ, LIU CX. LC-MS/MS-based molecular networking producing enlighten study of Chinese materia medica[J]. Chinese Traditional and Herbal Drugs, 2018(49): 265 – 273. (in Chinese).

(To page 14)

ly ethanol concentration (35%, 55%, 75%), material-to-liquid ratio (1∶10, 1∶20, 1∶30), extraction time (1.5, 2.0, 2.5 h) and extraction times (once, twice and thrice), were selected to carried out an orthogonal experiment. The optimum extraction conditions of total flavonoids from *P. chinense* Pursh obtained through the orthogonal experiment were as follows: 55% ethanol, material-to-liquid ratio 1∶20 (g/mL), twice, and 2.0 h each time. With the optimum conditions, the contents of total flavonoids in the stems, leaves, flowers and whole grass of *P. chinense* Pursh were 3.63%, 8.90%, 11.28% and 4.36%, respectively. The content of total flavonoids was quite different in different parts of *P. chinense* Pursh, showing the highest content in flowers of *P. chinense* Pursh, followed by leaves and whole grass, and the lowest value was found in stems. Therefore, it is suggested that the proportions of leaves and flowers should be paid attention to in the industrial processing of *P. chinense* Pursh. Therefore, it is suggested that the ratio of leaves to flowers should be paid attention to in the industrial processing of *P. chinense* Pursh.

References

[1] DING QC, JIN Z, DONG JH, *et al.* Bioactivity evaluation of pinocembrin derivatives from *Penthorum chinense* Pursh stems[J]. Natural Product Communications, 2019, 14(9): 1-9.

[2] YANG LL, LI T, YANG DD, *et al.* Constructing a dummy-template adsorbent using magnetic molecular-imprinted chitosan for rapid enrichment of flavonoids compounds from *Penthorum chinense* Pursh[J]. Microchemical Journal, 2023(191): 108833.

[3] WANG AQ, LI MX, HUANG H, *et al.* A review of *Penthorum chinense* Pursh for hepatoprotection: Traditional use, phytochemistry, pharmacology, toxicology and clinical trials[J]. Journal of Ethnopharmacology, 2020, 251(6): 26-32.

[4] ZHANG J, WU SM, YANG X, *et al.* Advances in chemical constituents of *Penthorum chinense*[J]. Chinese Traditional and Herbal Drugs, 2017, 48(21): 4571-4577. (in Chinese).

[5] ZHAO WW, GUO WW, GUO JF, *et al.* Three new flavonoids from *Penthorum chinense* Pursh and their docking studies[J]. Natural Product Research, 2021, 35(1): 49-56.

[6] YIN J, REN W, WEI B, *et al.* Characterization of chemical composition and prebiotic effect of a dietary medicinal plant *Penthorum chinense* Pursh[J]. Food Chemistry, 2020(319): 126568.

[7] SUN XL, WU AG, LAW BYK, *et al.* The active components derived from *Penthorum chinense* Pursh protect against oxidative-stress-induced vascular injury via autophagy induction[J]. Free Radical Biology and Medicine, 2020(146): 160-180.

[8] SUN Y, HE LB, WANG W, *et al.* Polyphenols from *Penthorum chinense* Pursh. attenuates high glucose-induced vascular inflammation through directly interacting with Keap1 protein[J]. Journal of Ethnopharmacology: An Interdisciplinary Journal Devoted to Bioscientific Research on Indigenous Drugs, 2021(321): 117513.

[9] XU XQ, YU Q, XU Y, *et al.* Optimization of Ultrasonic-assisted extraction technology for total flavonoids from *Penthorum chinense* by response surface methodology and evaluation its *in vitro* antioxidant activity[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2012, 18(18): 38-41. (in Chinese).

[10] LU MQ, RUAN JY, YU R, *et al.* Neolignan derivatives from *Penthorum chinense* with antitumor activity in human colorectal cancer cells by regulating Wnt/β-catenin signaling pathway[J]. Phytochemistry, 2023(214): 113827.

[11] CHEN Y, CHEN P, LIU H, *et al.* *Penthorum chinense* Pursh polysaccharide induces a mitochondrial-dependent apoptosis of H_{22} cells and activation of immunoregulation in H_{22} tumor-bearing mice[J]. International Journal of Biological Macromolecules, 2023(224): 510-522. (in Chinese).

[12] FU ML, JIU H, YUAN YF. Protective effect of total flavonoids of *Penthorum chinense* Pursh on acute chemical liver injury induced by carbon tetrachloride in mice[J]. Modern Food Science and Technology, 2021, 37(6): 50-56. (in Chinese).

[13] JIU H, FU ML, LIU HS, *et al.* Comparative study on hepatoprotective effects of alcohol extract and total flavonoids of *Penthorum chinense* Pursh against alcohol-induced subacute liver injury in mice[J]. Science and Technology of Food Industry, 2023, 44(3): 391-397. (in Chinese).

[14] FU ML, YAO HY, HOU XF, *et al.* Protective effect of total flavonoids of *Penthorum chinense* Pursh on acute drug-induced liver injury induced by acetaminophen in mice[J]. Journal of Southwest Medical University, 2023, 46(1): 70-74. (in Chinese).

[15] SHI X, ZHUO J. Experimental study on the anti-alcoholic hepatic fibrosis effect of total flavonoids of *Penthorum chinense* Pursh in rats[J]. Journal of Chinese Medicinal Materials, 2015, 38(7):1485-1487. (in Chinese).

[16] QIN YR, ZHANG YS, ZHANG K, *et al.* Comparative study of determination methods of total flavonoids in vine tea (*Ampelopsis grossedentata*)[J]. Modern Food Science and Technology, 2019, 35(12): 302-310. (in Chinese).

(From page 5)

[11] LI JJ, LIN Y, HE L, *et al.* Two new isoprenoid flavonoids from *Sophora flavescens* with antioxidant and cytotoxic activities[J]. Molecules, 2021(26): 7228.

[12] LIN Y, LI JJ, HE L, *et al.* A new modified pterocarpan glycoside from *Sophora flavescens*[J]. Natural Product Research, 2022, DOI: 10.1080/14786419.2022.2075861.

[13] WANG M, CARVER JJ, PHELAN VV. Sharing and community curation of mass spectrometry data with global natural products social molecular networking[J]. Nature Biotechnology, 2016(34): 828-837.

[14] NOTHIAS LF, PETRAS D, SCHMID R. Feature-based molecular networking in the GNPS analysis environment[J]. Nature Methods, 2020(17): 905-918.

[15] HUANG JL, XU H. Matrine: bioactivities and structural modifications[J]. Current Topics in Medicinal Chemistry, 2016, 16(28): 3365-3378.

[16] CHOI AJ, BUISSON N, KIM CT. Digestion characteristics and kinetic analysis of bio-molecules in a simulated human intestinal system[J]. Proceedings of Spie the International Society for Optical Engineering, 2015, 2(3): 189-192.

[17] AKIRA S, UEMATSU S, TAKEUCHI O. Pathogen recognition and innate immunity[J]. Cell, 2006(124): 783-801.