

Isolation of Flavonoids from the *Potentilla kleiniana* and Evaluation of Their α -Glucosidase Inhibitory Activity and Anti-inflammatory Activity

Qian-Lian Yin^{ID 1,2,*}, Qian-Qian Jin^{ID 1,2}, Li Jiang^{ID 1,2}, Xue Ma^{ID 1,2},
Jia Sun^{ID 1,2}, Yong Huang^{ID 1,2,3*} and Yong-Jun Li^{ID 1,2,3*}

¹State Key Laboratory of Discovery and Utilization of Functional Components in Traditional Chinese Medicine, Engineering Research Center for the Development and Application of Ethnic Medicine and TCM (Ministry of Education), Guizhou Provincial Engineering Research Center for the Development and Application of Ethnic Medicine and TCM, Guizhou Medical University, Gui'an New Area 561113, PR China

²School of Pharmacy, Guizhou Medical University, Gui'an New Area 561113, PR China

³National Engineering Research Center of Miao's Medicines, Guiyang 550004, PR China

(Received December 31, 2024; Revised February 19, 2025; Accepted February 21, 2025)

Abstract: In this study, phytochemical investigation of ethanolic (EtOH 60%, Water 40%) extract from *Potentilla kleiniana* by reflux extraction was carried out for the isolation and characterization of their chemical structures of seventeen known secondary metabolites. Structure determination of the isolated compounds was carried out using 1D NMR data and mass spectrometry techniques. Compounds **3**, **8**, **12** and **13** were isolated from *P. kleiniana* for the first time in this study and were recorded as a new report for the species. Compounds (**2**, **6-7**, **9**, **11** and **14-15**) represent previously unreported constituents within the genus *Potentilla*. As a result of the α -glucosidase inhibitory activity determination studies performed separately on the isolated compounds, it was determined that compounds **10**, **14** and **15** exhibited better inhibition-inhibition percentages of 87.14%, 49.07%, and 86.79%, respectively compared to the positive control acarbose (37.26%). Moreover, compounds **3**, **10**, **14** and **15** were determined to be secondary metabolites with significant nitric oxide (NO). SAR analyses were performed to understand the relationship between α -glucosidase inhibitory activities and the structures of the molecules, and according to the results of these analyses, it was observed that flavonoid derivatives carrying hydroxyl groups at C-3 and C-7 positions showed higher hypoglycemic activity than their counterparts carrying rhamnose groups at these positions.

Keywords: *Potentilla kleiniana*; flavonoids; α -glucosidase; anti-inflammation; structure-activity relationship. © 2025 ACG Publications. All rights reserved.

1. Plant Source

Potentilla kleiniana Wight & Arn. (Rosaceae) was collected in April 2021 from Gaopo Town, Guiyang City, Guizhou Province, China (coordinates: 26°28'N, 106°85'E; altitude: 1400-1600 m above sea level). The botanical identification of the species was carried out by Associate Professor Chunhua Liu from Guizhou Medical University, and the Herbarium specimen of it was preserved in the Herbarium of School Guizhou Medical University (GMB), with Herbarium number No. 20210416.

* Corresponding author: E-Mail: 2283087195@qq.com.

2. Previous Studies

Previous phytochemical investigations of the genus *Potentilla* have led to the isolation and characterization of diverse secondary metabolites, predominantly comprising terpenoids, flavonoids, phenolic acids, tannins, phenylpropanoids, and other bioactive constituents [1]. Previous pharmacological investigations have established that the ethanol extract and its ethyl acetate (EtOAc) fraction of *P. kleiniana* demonstrate a broad spectrum of biological activities, including antimicrobial, antioxidant, cytoprotective, and anti-inflammatory properties [2-3]. Notably, the total flavonoid fraction of *P. kleiniana* has been shown to exhibit specific activity against *Pseudomonas aeruginosa*, along with significant hypoglycemic and antioxidant effects [4-5]. Furthermore, isolated lignans and acidic components from the species have demonstrated multiple pharmacological activities, including cytotoxic, anti-inflammatory, anti-HIV-1 protease, and α -glucosidase inhibitory properties [6-8]. Building upon the documented hypoglycemic potential of *P. kleiniana*'s total flavonoids, the current study aims to systematically investigate individual flavonoid compounds with pronounced α -glucosidase inhibitory and anti-inflammatory activities. Additionally, this research will provide mechanistic insights into the SAR governing their α -glucosidase inhibition efficacy.

3. Present Study

15 kg of dry plant consisting of all aerial and underground parts of *P. kleiniana* species was ground in a mill. Then, 15 kg of ground plants were subjected to reflux extraction with ethanol:water (60:40) solvent system under reflux conditions for three cycles. After every three flushes, the reflux extraction process was stopped and the extract obtained after each step was evaporated under reduced pressure in a rotary evaporator and combined in a 5 L beaker. As a result of these operations, 2.1 kg of ethanolic extract was obtained and then, by systematic chromatographic separation and purification procedures, seventeen flavonoid compounds were successfully isolated, and their chemical structures were characterized (Figure 1).

The structural elucidation of these compounds was accomplished through comprehensive 1D NMR analysis, with the corresponding spectral data provided in the supporting information. The isolated compounds were characterized as follows: genistin (**1**) [9], kaempferol-3- α -L-(3,4-di-*O*-acetyl)-rhamnopyranoside-7- α -L-rhamnopyranoside (**2**) [10], quercetin-7-*O*- α -L-rhamnopyranoside (**3**) [11], quercetin-3-*O*- β -D-glucopyranoside (**4**) [12], quercetin-3-*O*- α -L-rhamnopyranoside (**5**) [13], quercetin-3,7-*O*- α -L-dirhamnoside (**6**) [14], quercetin-3-*O*- β -D-glucoside-7-*O*- α -L-rhamnoside (**7**) [15], schaftoside (**8**) [16], kaempferol-3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- α -L-rhamnosyl-7-*O*- α -L-rhamnoside (**9**) [17], quercetin (**10**) [18], quercetin-3,7-di-*O*- β -D-glucoside (**11**) [19], kaempferol-3-*O*- β -glucopyranosyl-(1 $\rightarrow$ 2)- β -glucopyranoside-7-*O*- α -rhamnopyranoside (**12**) [20], kaempferol-3-*O*-neohesperidoside (**13**) [21], 6-methyl-kaempferol (**14**) [22], genistein (**15**) [23], isovitexin-2"-*O*- β -D-glucopyranoside (**16**) [24], and quercetin-7-*O*- α -L-rhamnopyranoside-3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (**17**) [25].

α -Glucosidase Activity Test: α -glucosidase enzymatic activity was assessed through its catalytic action on *p*-nitrophenyl- α -D-glucopyranoside (*p*-NPG), yielding *p*-nitrophenol and glucose as reaction products. The quantification of enzymatic activity was performed by monitoring the absorbance at 405 nm, corresponding to the maximum absorption wavelength of *p*-nitrophenol, following the addition of test samples. The inhibitory potency of each sample against α -glucosidase was subsequently determined by calculating the inhibition rate, with acarbose employed as the positive control for comparative analysis.

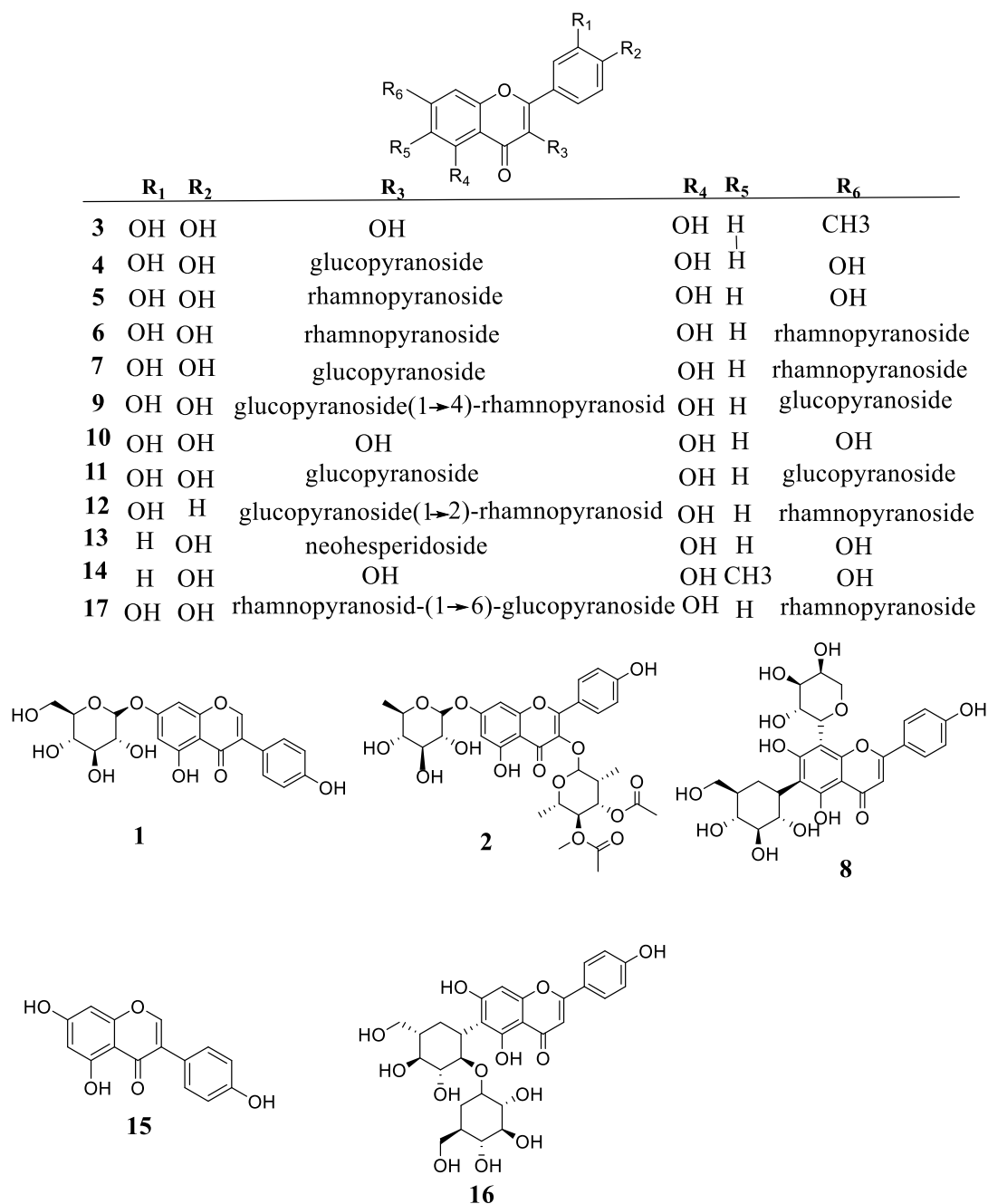


Figure 1. Structures of isolated compounds **1-17** from the *P. kleiniana*

The preliminary screening results revealed the inhibitory potential of **17** flavonoid compounds isolated from the crude extracts of *P. kleiniana* against α -glucosidase activity (Figure 2). These findings provide valuable insights into the structure-activity relationships of flavonoid derivatives as potential α -glucosidase inhibitors.

Anti-inflammatory Activity of flavonoid compounds: The anti-inflammatory potentials of the crude extract and the **17** isolated flavonoid compounds were evaluated using an *in vitro* inflammation model. LPS was employed to stimulate excessive NO production in RAW264.7 macrophages, thereby establishing the inflammatory response. DEX was utilized as a positive control in this assay. RAW264.7 cells in the logarithmic growth phase were systematically divided into four experimental groups: the control group, cultured in complete DMEM medium, the model group, exposed to 0.25 μ g/mL LPS in DMEM medium,

Flavonoids from the *Potentilla kleiniana*

the positive control group, treated with DEX in combination with LPS, and the treatment groups, which were incubated with varying concentrations of the test compounds in the presence of LPS. Following the respective treatments, the concentration of NO in the culture supernatant was quantified using a commercially available NO detection kit, according to the manufacturer's protocol. The anti-inflammatory efficacy was expressed as percentage inhibition of NO production, calculated using the following formula (Figure 3).

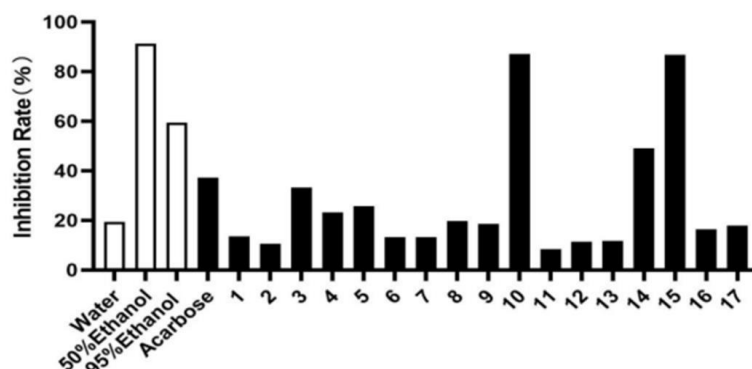


Figure 2. The primary screening inhibition rate of α -glucosidase by 17 flavonoids isolated from crude extracts of *P. kleiniana* (n=3)

NO content (mol/L) = (OD measurement of sample - OD measurement of blank) / (OD measurement of standard - OD measurement of blank) * concentration of standard (20 mol/L) * dilution factor (4-fold)

NO inhibition rate (%) = (NO content in LPS - NO content in sample) / (NO content in LPS - NO content in blank) * 100%

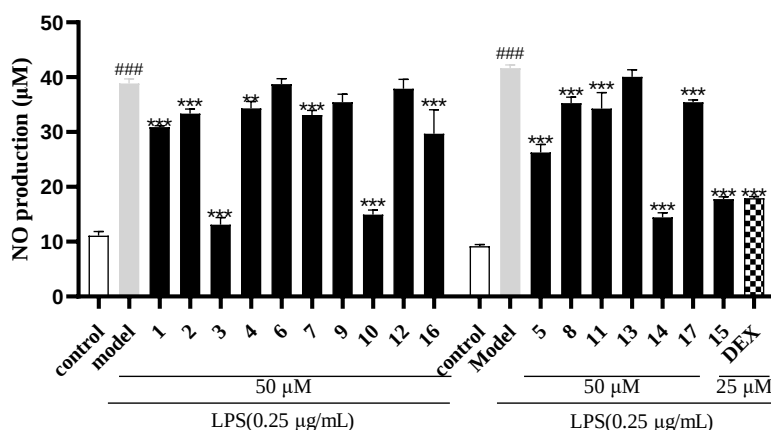


Figure 3. Inhibition rate of compounds from *P. kleiniana* LPS-induced NO release from RAW264.7 cells (n = 3, mean $\pm$ SD). ###, $p < 0.001$ vs. control group without LPS group; **, $p < 0.01$; ***, $p < 0.001$ vs. LPS group

The α -glucosidase inhibitory activities of compounds 1-17 were evaluated at a concentration of 100 μ g/mL, with acarbose serving as the positive control. Among the tested compounds, only the compounds 10, 14, and 15 demonstrated significant inhibitory activities, exhibiting inhibition percentages of 87.14%, 49.07%, and 86.79%, respectively, which were substantially higher than that of acarbose (37.26%). In the anti-inflammatory assessment, compounds 3, 10, 14 and 15 showed marked inhibitory effects on NO production in inflammatory cells, with inhibition rates of $71.99 \pm 0.61\%$, $86.28 \pm 0.82\%$, $65.02 \pm 0.61\%$, and $70.45 \pm 0.41\%$, respectively.

Through comprehensive evaluation of the α -glucosidase inhibitory activity of 17 flavonoid compounds, it was demonstrated that the observed differences in inhibitory efficacy were predominantly

attributed to variations in their aglycone structures. The comparative analysis of experimental data reveals that the substituent types at C-3 and C-7 positions of the flavonoid nucleus significantly influence α -glucosidase inhibitory activity. When they substituted with hydroxyl, rhamnosyl, and glucosyl groups respectively, the inhibition efficacy demonstrates a distinct gradient pattern with the following potency order: -OH > Rha-O- > Glc-O-. Notably, the hypoglycemic activity exhibits an inverse correlation with the number of glycanic fragments incorporated at C-3 and C-7, showing progressive attenuation as glycosylation density increases. It remains to be validated whether the observed attenuation could be attributed to steric hindrance effects, whereby increased glycosylation at these critical positions may potentially compromise the compound's binding affinity to α -glucosidase.

In contrast to previous studies that primarily focused on examining other chemical constituents, total flavonoid fractions, and solely evaluating the α -glucosidase inhibitory activity of flavonoids, our research has comprehensively assessed an expanded flavonoid library. Through systematic analysis of SAR, we have delineated the critical structural determinants governing inhibitory efficacy. This SAR-driven approach facilitates the rational optimization of lead compounds through enhanced α -glucosidase targeting capability, thereby establishing a foundation for the development of next-generation hypoglycemic agents.

Compounds (**2**, **6,7**, **9**, **11,14** and **15**) represent previously unreported constituents in the genus *Potentilla*. The detection of these compounds in *Eriobotrya*, *Amygdalus L.*, and *Spiraea*, all members of the Rosaceae family, suggests a potential phylogenetic proximity between these genera and *Potentilla*. However, it should be noted that these compounds have been identified across diverse plant families, including Rosaceae, Brassicaceae, Vitaceae, and Tiliaceae, thereby limiting their utility as taxonomic markers for plant classification.

Acknowledgments

This work was supported by National Natural Science Foundation of China (U1812403-5), Guizhou Science and Technology Department (grant numbers ([2023]006/GCC [2022] 031-1).

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

ORCID

Qian Lian Yin: [0009-0002-6516-8739](https://orcid.org/0009-0002-6516-8739)

Qian Qian Jin: [0009-0006-5936-7773](https://orcid.org/0009-0006-5936-7773)

Li Jiang: [0000-0002-1553-2136](https://orcid.org/0000-0002-1553-2136)

Xue Ma: [0000-0001-8636-7599](https://orcid.org/0000-0001-8636-7599)

Jia Sun: [0000-0001-7205-9579](https://orcid.org/0000-0001-7205-9579)

Yong Huang: [0000-0002-4519-1192](https://orcid.org/0000-0002-4519-1192)

Yong Jun Li: [0000-0002-0479-6259](https://orcid.org/0000-0002-0479-6259)

References

- [1] J. Wu, Z. Q. Zhang, H. H. Yu, F. B. Huang, Z. L. Chen, L. L. Chu, B. Li and W. Wang (2022). Research progress on chemical constituents and pharmacological activities of *Potentilla*, *J.Chin. Mater. Med.* **47**, 1509-1538.
- [2] C. G. Zhang, Z. J. Wang and J. J. Liu (2018). Anti-inflammatory and antibacterial activities of *Potentilla kleiniana* Wight et Arn ethanol extract, *J. Cent. South. Univ.* **16**, 1547-1552.
- [3] S. H. Xuan, I. K. Hong, Y. J. Lee, J. W. Kim and S. N. Park (2019). Biological activities and chemical components of *Potentilla kleiniana* Wight & Arn, *Nat. Pro. Res.* **34**, 3262–3266.
- [4] J. Y. Tao, S. L. Yan, C. Zhou, Q. H. Wang, L. Q. Zhao, H. Zhu and Z. Wen (2021). Total flavonoids from *Potentilla kleiniana* Wight et Arn inhibits biofilm formation and virulence factors production in methicillin-resistant *Staphylococcus aureus* (MRSA), *J. Ethnopharmacol.* **279**, 114383.

Flavonoids from the *Potentilla kleiniana*

- [5] S. H. Li, J. Tao, J. Y. Zeng, X. J. Wu and J. Zhang (2017). Antihyperglycemic and antioxidant effect of the total flavones of *Potentilla kleiniana* Wight et Arn. in streptozotocin induced diabetic rats, *Pak. J. Pharm. Sci.* **1**, 171-178.
- [6] B. Zhang, H. Yang, W. M. Kuang, T. T. Chen, Q. Q. Jin, Y. J. Li and Y. Li (2024). Lignans from *Potentilla kleiniana* and their cytotoxicity, *Guihaia*. **6**, 1082-1090.
- [7] Y. Q. Zhou, S. M. Li, X. Wei, X. Yang, J. W. Xiao, B. W. Pan and Y. Wei (2022). Identification and quantitative analysis of bioactive components from *Potentilla kleiniana* Wight et Arn with anti HIV-1 proteases activity, *Nat. Pro. Res.* **23**, 4028–4031.
- [8] M. Z. Liu, X. Q. Huang, Q. Liu, X. J. Li, M. Chen, Y. Q. Zhu and X. Q. Chen (2019). Separation of α -glucosidase inhibitors from *Potentilla kleiniana* Wight et Arn using solvent and flow-rate gradient high-speed counter-current chromatography target-guided by ultrafiltration HPLC-MS screening, *Phytochem. Anal.* **6**, 661-668.
- [9] Z. R. Peng, Z. X. Wang, J. P. Zhao, Q. M. Xu and S. L. Yang (2020). Chemical constituents of isoflavones in the stem of wisteria, *Chin. Tradit. Herb. Drugs.* **51**, 3850-3855.
- [10] B. S. Min, M. Tomiyama, C. M. Ma, N. Nakamura and M. Hattori (2001). Kaempferol acetylramnosides from the rhizome of *Dryopteris crassirhizoma* and their inhibitory effects on three different activities of human immunodeficiency virus-1 reverse transcriptase, *Chem. Pharm. Bull.* **49**, 546-50.
- [11] k. Song, H. Q. Wang, C. Liu, J. Kang, B. Li and R.Y. Chen (2014). Chemical constituents from *Nepeta*, *Chin. J. Chin. Mater. Med.* **39**, 254-257.
- [12] J. Li, S. B. Pan, Y. Miao, Y. Li and Y. Q. Zhang (2017). Chemical constituents of n-butanol fraction from fruit of *Lonicera japonica* thunb, *Chin. Arch. Tradit. Chin. Med.* **35**, 1097-1099.
- [13] B. P. Zhou, C. L. Zhang, X. H. Zou, X. J. Li, X. Y. Yan, C. Z. Li and Z. Y. Cao (2016). Chemical constituents from the Aerial Parts of *Ribes diacanthum* Pall, *Chin. Pharm. J.* **51**, 1918-05.
- [14] X.Y. Shang, S. Li, S. J. Wang, Y. C. Yang and J. G. Shi (2009). Flavonoids from *Bauhinia aurea*, *Chin. Tradit. Herb. Drugs.* **40**, 96-199.
- [15] Y. Li, B. P. Zhou, W. J. Zhang, G. L. Yang, C. L. Zhang and Z. Y. Cao (2018). Chemical Composition from aerial parts of *Ribes mandshuricum*, *Chin. Tradit. Herb. Drugs.* **49**, 772-779.
- [16] C. P. Liu, W. Y. Sun, R. Q. Wang, Z. B. Huang, C. B. Huang, M. Y. Cao, X. B. Zhang, L. Dong and G. Y. Li (2022). Isolation and identification of chemical constituents of *Scoparia dulcis* L, *J. Shenyang Pharm. Univ.* **39**, 780-785.
- [17] N. Li, X. Li Z. G. Feng and S. L. Yang (2007). Studies on flavonoidal components from *Camptosorus sibiricus* Rupr, *Chin. Pharm. J.* **14**, 1049-1052.
- [18] J. Yang, T. J. Huang, J. F. Luo, H. Li and Y. H. Wang (2018). Chemical constituents from *Leea asiatica*, *Nat. Pro. Res. Dev.* **30**, 1382-1386.
- [19] D. D. Mu, Y. X. Zhao, G. Y. Sun, K. M. Bobakulov, H. A. Aisa and T. Wu (2021). Chemical composition of flowers of *Gossypium hirsutum*, *Chem. Nat. Compd.* **57**, 939-941.
- [20] G. C. Kite, N. C. Veitch, M. E. Boalch, G. P. Lewis, C. J. Leon and M. S. J. Simmonds (2009). Flavonol tetraglycosides from fruits of *Styphnolobium japonicum* (Leguminosae) and the authentication of *Fructus Sophorae* and *Flos Sophorae*, *Phytochem.* **70**, 785-79.
- [21] J. Liu, S. C. Yang, C. K. Yang, L. Zhang, Q. B. Luo and Y. Shen (2020). Non-alkaloid Composition in the aerial parts of *Aconitum straightatus*, *Chin. Tradit. Pat. Med.* **42**, 3193-3198.
- [22] P. Song, Y. R. Ma, Y. Z. Wen, X. L. Hong, X. D. Zhou, Q. X. Lin and X. Z. Yang (2021). Chemical constituents from the underground part of *Sophora davidii* (Franch), *J. Yunnan Univ. Nat. Sci. Ed.* **43**, 1228-1234.
- [23] J. T. Yi, Y. Zhong, J. Y. Sun, L. Liu and B. Wang (2006). Chemical constituents from *Pueraria lobata*, *Chin. Tradit. Herb. Drugs.* **37**, 350-352.
- [24] L. Xu, Q. Ren, K. H. Wan and H. Z. Fu (2019). Chemical constituents from stem of *Gordonia chrysandra*, *J. Chin. Med. Mater.* **42**, 2045-2048.
- [25] H. Yoshimitsu, M. Nishida, F. Hashimoto, M. Tanaka, Y. Sakata and M. Okawa and T. Nohara (2007). Chromone and flavonol glycosides from *Delphinium hybridum* cv. "Belladonna Casablanca", *J. Nat. Med.* **61**, 334-338.