# Screening for Antioxidant Phenolic Compounds from *Polygonum capitatum*

**Yan LIN***

State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 55000, China; University Engineering Research Center for the Prevention and Treatment of Chronic Diseases by Authentic Medicinal Materials in Guizhou Province, School of Pharmaceutical Sciences, Guizhou Medical University, Guiyang 55000, China

**Abstract** [**Objectives**] To discover antioxidant natural products from the famous Hmong medicinal plant *Polygonum capitatum*. [**Methods**] The antioxidant activities of the isolated components were evaluated by ABTS and DPPH assays. [**Results**] A total of 27 free phenolics were isolated form *P. capitatum*. Then the *in vitro* antioxidant potential of these components was evaluated according to the DPPH and ABTS radical scavenging assays. Among them, five compounds (13, 14, 17, 23, and 25) showed most significant ABTS radical-scavenging activity ($IC_{50}$ values of 3.81 - 15.09 μg/mL). And 12 components (1, 2, 6, 7, 9, 12, 13, 14, 16, 17, 23, and 25) showed notable radical scavenging activity against DPPH (inhibition rates >88%). [**Conclusions**] Most of the above bioactive compounds were reported for the first time.

**Key words** *Polygonum capitatum*, Phenolic compounds, Antioxidant activity

## 1 Introduction

*Polygonum capitatum* (Touhualiao in Chinese), a well-known and large-scale Miao medicinal plant in China, was derived from *P. capitatum* (Buch. -Ham. ex D. Don) H. Gross and is widely used for the treatment of urinary tract infections, cystitis, eczema, pyelonephritis, lowering uric acid and anti-gout[1-2]. *P. capitatum* demonstrated a variety of pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, anticancer, analgesic and other bioactive effects[3-4].

The antioxidant activities were attributed to the extracts of *P. capitatum*. The antioxidant activities of *P. capitatum* have been extensively studied using different anti-oxidant models. These models were induced by 2-20-azinobis-3-ethylben-zthia zoline-6-sulphonate (ABTS), 1, 1-diphenyl-2-picrylhydraz-yl (DPPH), hydrogen peroxide ($H_2O_2$). Base on the literatures, *P. capitatum* extract has displayed obvious antioxidant activity *in vitro*. 80% methanol extract of leaves and stems from *P. capitatum* demonstrated strong antioxidant activities against $ABTS^+$/$OH^-$[5]. The ethanol extract revealed stronger antioxidant activities than the aqueous extracts of *P. capitatum*[6]. The same result was shown in another study, the methanol extract of *P. capitatum* revealed higher scavenging activity against DPPH radical and ABTS radical[7]. Additionally, the EtOAc extract of *P. capitatum* exhibited remarkable scavenging activity against DPPH and ABTS radicals. However, little is known about the chemical components from *P. capitatum*[8].

In this study, we intended to discover novel bioactive compounds and expand the diversity and production of bioactive natural products by extracting compounds from the whole herb *P. capitatum*. 27 compounds were isolated from the ethyl acetate extract of *P. capitatum*. And the antioxidant activities of the isolated components were evaluated by ABTS and DPPH assays.

## 2 Results and analysis

**2.1 Compounds structure elucidation** The compounds (**1-27**) were capitaone A (**1**), FR429 (davidiin, **2**), quercetin-3-O-β-D-galactopyranoside (**3**), quercetin-3-O-β-D-glucopyranoside (**4**), quercetin-3-O-α-L-rhamnopyranoside (**5**), 2″-galloylquercitrin (**6**), 3″-galloylquercitrin (**7**), quercetin-3-O-sophoroside (**8**), quercetin-3-O-α-L-rhamnopyranoside (**9**), kaempferol-7-O-β-D-glucopyranoside (**10**), kaempferol-3-O-α-L-rhamnoside (**11**), kaempferol (**12**), quercetin (**13**), 1, 2, 6-trigalloyl-β-D-glucose (**14**), stigmast-5-en-3-O-β-D-glucoside (**15**), catechin (**16**), (-)-epigallocatechin-3-O-gallate (**17**), silybin (**18**), apigenin (**19**), alpine isoflavones (**20**), 3, 3′, 4′-trimethyl ellagic acid (**21**), 5, 7-dihydroxy-4H-4-chromone (**22**), gallic acid (**23**), vanillic acid (**24**), ethyl gallate (**25**), β-sitosterol (**26**) and methyl arachidonate (**27**)[9].

**2.2 Antioxidant activity** According to several studies, the main factor causing degenerative diseases was free radicals, which were actively produced through the human body metabolic process. The increase in the number of free radicals in the body can lead to the disruption of the body's metabolic processes, which can lead to various disease-causing processes. In this study, the antioxidant capacity of compounds 1 - 27 (40 μg/mL) was assessed using two antioxidant assays (ABTS, DPPH). As shown in Fig. 1, 12 compounds (**1**, **2**, **6**, **7**, **9**, **12 - 14**, **16**, **17**, **23**, **25**) exhibited strong antioxidant activity with inhibition rates > 50% for the ABTS test. Among them, 9 (myricetin-3-O-α-L-rhamnopyranoside), 12 (kaempferol) and 17 ((-)-epigallocatechin-

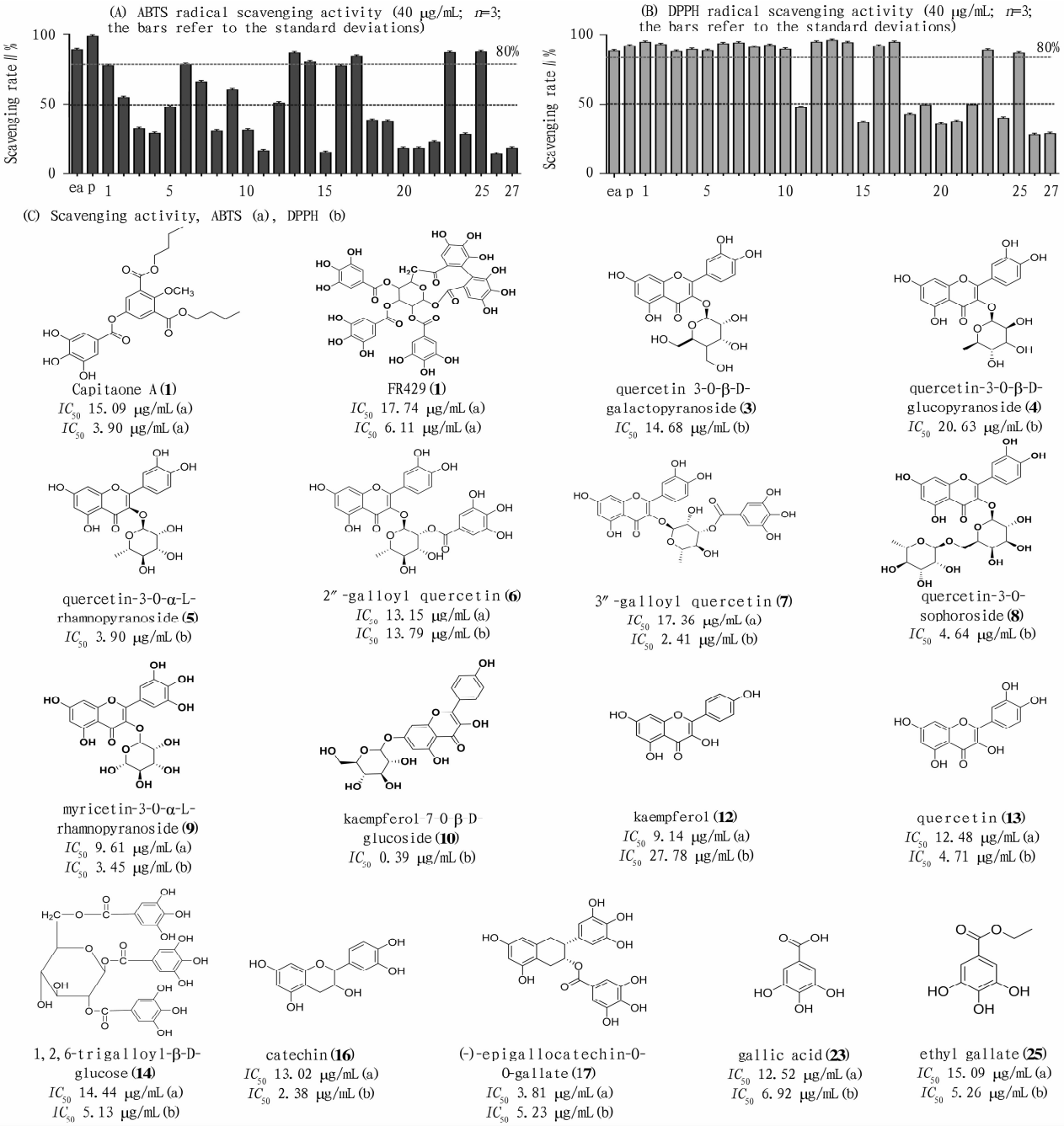
Received: March 2, 2024 Accepted: July 26, 2024

Supported by Guizhou Provincial Science and Technology [ZK (2022)-362, ZK (2024)-047], and (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: linyan@gmc.edu.cn

3-O-gallate)) were the most active ones with an $IC_{50}$ value from 3.81 to 9.61 μg/mL. Compounds 2 (FR429, $IC_{50}$ 17.74 μg/mL), **7** (3″-galloyl quercitrin, $IC_{50}$ value 17.38 μg/mL) and **14** (1, 2, 6-trigalloyl-β-D-glucose, $IC_{50}$ value 14.44 μg/mL) were reported here for the first time. The activities of 6 (2″-galloyl quercitrin), 9 (myricetin-3-O-α-L-rhamnopyranoside), **12** (kaempferol), **13** (quercetin), 16 (catechin), **17** (−)-epigallocatechin-3-O-gallate), **23** (gallic acid), **25** (ethyl gallate) were consistent with previous reports.

Interestingly, all of the four active compounds (**5**−**7**, **9**) possess one quercitrin group, and compounds (**6**, **7**, **9**) possessed an extra gallic or hydroxyl group more than the compound 5. For instance, compound 5 showed a scavenging rate of only 48%, whereas its galloyl derivative 6 was 78%. Similar results were also observed for **5** (48%)/**7** (66%), and **5** (48%)/**9** (61%). And then although the only difference between compound **12** and compound **13** is an additional hydroxyl group, inhibition rate was better for compound **13** (87%) than compound **12** (51%), (Fig. 1).




**NOTE** (A) ABTS radical scavenging activity; (B) DPPH radical scavenging activity; (C) The dose-inhibition rate curves for potent compounds and their $IC_{50}$ values.

**Fig. 1 Antioxidant capacity of compounds 1 −27 from *Polygonum capitatum***

For the DPPH radical scavenging assay, the EtOAc extract exhibited stronger inhibitory activities (inhibition rate 88.6%, 40 μg/mL). Among the them, 17 ones (**1**–**10**, **12**–**14**, **16**, **17**, **23**, **25**) showed remarkable DPPH radical scavenging activity, with inhibition rates ranging from 88.2% to 96.3% at 40 μg/mL. Compounds **2**, **6**, and **7** were reported here for the first time, with an $IC_{50}$ value of 6.11, 13.79 and 2.41 μg/mL, respectively (Fig. 2).

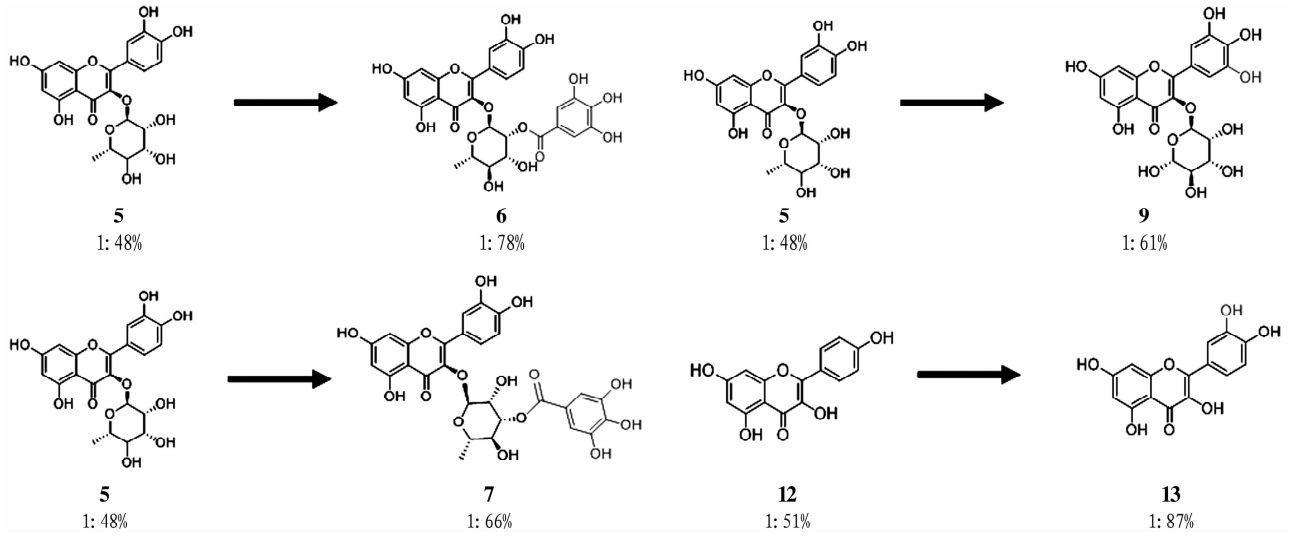



**Fig. 2 Structure-activity relationship analysis of the active compounds for DPPH experiment**

**2.3 ABTS radical scavenging assay** The method ABTS has been commonly used to determine the antioxidant capacity of the biological samples. In brief, the ABTS free radical manucaption solution ($ABTS^+$) was generated by reacting ABTS solution (7.0 mM) with potassium persulfate (2.45 mM) to produce blue-green color solution 24 h[10]. The more obvious the disappearance of blue-green colour solution, the stronger the antioxidant activity. To evaluate the antioxidant activity, the $ABTS^+$ solution was diluted with ethanol until its absorbance reached 0.70 ± 0.02 at the wavelength of 734 nm. Therefore, the $ABTS^+$ solution was obtained. Then, the EtOAc extract and the components (100 μL, 40 μg/mL) with $ABTS^+$ solution (100 μL) were added to the 96-well plates (as the value of $A_i$) and were interacted for 10 min at room temperature in the dark. Immediately followed by the absorbance at 734 nm was measured[11]. Vitamin C (40 μg/mL) as the positive control. The percentage of radical scavenging was calculated using the Formula (1). Herein, $A_i$ represents the absorbance of the sample, $A_j$ denotes the value of 100 μL compounds with 100 μL ethanol, and $A_0$ is the absorbance of the control solution.

$$\text{Radical scavenging activity (\%)} = [1 - (A_i - A_j)/A_0] \times 100\% \quad (1)$$

**2.4 DPPH radical scavenging assay** Antioxidant properties of extracts obtained from *P. capitatum* were determined using the DPPH method[12]. The antioxidant effect on interaction with DPPH, transfers electron or hydrogen atom to DPPH, thus neutralizing the free radical character and converting it to 1, 1-diphenyl-2-picrylhydrazine. Briefly, 4.0 mg DPPH powder was dissolved with 100 mL ethanol to obtain the DPPH solution (0.01 mM) in a volumetric flask. Then, the EtOAc and the compounds (40 μg/mL) with DPPH solution (100 μL) ($A_i$) were added to 96-well plates and incubated for 30 min at room temperature. Subsequently, the activity was measured using the Thermo Scientific Varioskan LUX (Berthold, VL0L00D0, USA), with an absorbance of 570 nm and the data was calculated according to the Formula (1). Also, vitamin C (40 μg/mL) was used as the positive control.

## 3 Conclusions

In this study, tighter with 27 compounds were isolated and identified of the EtOAc extract from *P. capitatum*. 5 compounds (**13**, **14**, **17**, **23**, **25**) exhibited notable radical scavenging activity against ABTS ($IC_{50}$ values 12.48, 14.44, 3.81, 12.52 and 15.09 μg/mL). In particular, 12 components (**1**, **2**, **6**, **7**, **9**, **12**–**14**, **16**, **17**, **23**, and **25**) showed remarkable DPPH radical scavenging activity, and the inhibition rates were in the range of 88.2% to 96.3%. They may be promising natural agents of the antioxidant.

## References

[1] HUANG Y, ZHOU Z, YANG W, *et al.* Comparative pharmacokinetics of gallic acid, protocatechuic acid, and quercitrin in normal and pyelonephritis rats after oral administration of a *Polygonum capitatum* extract[J]. Molecules, 2019, 24(21): 3873.

[2] CHEN H, YUAN L, MA X, *et al.* Herb-drug interaction: The effect of *Polygonum capitatum* extract on pharmacokinetics of levofloxacin in rats [J]. Journal of Pharmaceutical and Biomedical Analysis, 2021(195): 113832.

[3] YANG Y, YANG YB, LU WQ, *et al.* A new hydroxylamine acid derivative from *Polygonum capitatum*[J]. Chemistry of Natural Compounds, 2017(53): 417–421.

[4] LIN Y, HE L, CHEN XJ, *et al.* *Polygonum capitatum*, the Hmong medicinal flora: A comprehensive review of its phytochemical, pharmacological and pharmacokinetic characteristics[J]. Molecules, 2022, 27(19): 6407.

[5] HUANG WY, CAI YZ, XING J Comparative analysis of bioactivities of four *Polygonum* species[J]. Planta Medica, 2008(74): 43–49.

(To page 10)

col for a randomized controlled and double-blinding trail[J]. Medicine (Baltimore), 2018, 97(13): e0265.
[3] HE Y, TU ZW, ZOU AY, *et al.* Identification of chemical constituents in Gancao Xiexin Decoction by HPLC-QTOF/MS[J]. Drugs & Clinic, 2021, 36(11): 2246 - 2254. (in Chinese).
[4] SONG MM. Modulatory effects of Scutellaria baicalensis-Coptis chinensis herb pair and its main active constituents on AhR-CYP1A axis[D]. Nanjing: Nanjing University of Traditional Chinese Medicine, 2022. (in Chinese).
[5] SHEN LN, LIU J, QIAN YD, *et al.* Efficacy of Gancao Xiexin Decoction combined with mesalazine on patients with ulcerative colitis, and the influence of intestinal flora and serum inflammatory factors[J]. Chinese Journal of Integrated Traditional and Western Medicine on Digestion, 2021, 29(7): 474 - 478. (in Chinese).
[6] ZHU MX, LIN YN, ZHOU X. Research progress on the prevention and treatment of Chinese medicine on transmissible gastroenteritis[J]. Chinese Journal of Veterinary Medicine, 2018, 38(6): 1255 - 1259. (in Chinese).
[7] ZHAO JM, LI XR, GAO FF, *et al.* Roles of NLRP3 in mediating skin wound repair in mice[J]. Chinese Journal of Veterinary Medicine, 2022, 42(4): 733 - 739. (in Chinese).
[8] LIU S, WANG PL, HU YJ, *et al.* Study on HPLC fingerprint of *Rhodiola crenulata*[J]. Journal of Southwest University (Natural Science Edition), 2014, 36(12): 215 - 221. (in Chinese).
[9] LI J, NIE K, SU L, *et al.* HPLC fingerprint of the "Puheyin" preparation [J]. Journal of Southwest University(Natural Science Edition), 2014, 36(3): 14 - 20. (in Chinese).
[10] ZHAO X, LIU XL, LAN XJ, *et al.* Study on the fingerprints chromatogram of *Polygonatum sibiricun* Red. by HPLC[J]. Acta Agriculturae Boreali-occidentalis Sinica, 2011, 20(2): 114 - 119. (in Chinese).
[11] FAN SM, YANG YX, ZHANG CL, *et al.* Changes of HPLC fingerprint and multi-index components of Anemarrhenae Rhizoma-Phellodendri Chinensis herb pair before and after salt-processing[J]. Chinese Traditional Patent Medicine, 2023, 45(3): 820 - 828. (in Chinese).
[12] LUO ZJ, XU Y, WU JY, *et al.* Spectrum-effect relationship between HPLC fingerprints and antioxidant activity of *Polygonum cospidatum*[J]. Journal of Southwest University (Natural Science Edition), 2012, 34(1): 138 - 142. (in Chinese).
[13] TANG HM, ZHAI SQ, CHEN CL, *et al.* Simultaneous determination of eight constituents in Gancao Xiexin Decoction by QAMS[J]. Chinese Traditional Patent Medicine, 2022, 44(5): 1410 - 1415. (in Chinese).
[14] ZHU MX, YU CC, ZHU ZR, *et al.* The effect of compound Zhuqin extract resisting porcine epidemic diarrhea virus infecting PK-15 cells *in vitro*[J]. Chinese Journal of Veterinary Medicine, 2014, 34(3): 485 - 488. (in Chinese).
[15] HUANG GW, CHEN HC, LIU Y, *et al.* Research on spectrum-effect relationship of bacteriostasis in Qinsu Capsule based on grey correlation degree and partial least squares regression analysis[J]. Drug Evaluation Research, 2022, 45(6): 1060 - 1069. (in Chinese).
[16] YANG LF, GAO Y, ZHAO BN. Grey relational analysis and mathematical model for the fingerprint-efficacy study of Danhong Injection[J]. China Journal of Traditional Chinese Medicine and Pharmacy, 2018, 33(3): 878 - 881. (in Chinese).
[17] GAO XJ, BU TS. Explorating the mechanism of compound ingredients of the Gancao Xiexin decoction in treating oral ulcer based on network pharmacology[J]. Clinical Journal of Chinese Medicine, 2022, 14(36): 9 - 15. (in Chinese).
[18] ZHU MX, TANG HM, CHEN CL, *et al.* Effect of coptis rhizoma extract on inhibition of TGEV *in vitro*[J]. Acta Agriculturae Boreali-occidentalis Sinica, 2020, 29(5): 801 - 807. (in Chinese).
[19] DU W, HUANG WT, LUO JP, *et al.* Effect of Ilicischinensis folium water extract on the inflammatory bodies of AIM2/Caspase-1 in rats with chronic allergic contact dermatitis[J]. Journal of Southwest University (Natural Science Edition), 2021, 43(2): 63 - 69. (in Chinese).
[20] MAN SM. Inflammasomes in the gastrointestinal tract: Infection, cancer and gut microbiota homeostasis[J]. Nat Rev Gastroenterol Hepatol, 2018, 15(12): 721 - 737.
[21] WEN JH, LI DY, LIANG S, *et al.* Macrophage autophagy in macrophage polarization, chronic inflammation and organ fibrosis[J]. Front Immunol, 2022(13): 946832.
[22] ZHAO Y, XU GL, LIU HN, *et al.* Research progress of traditional Chinese medicine compounds in regulating macrophage differentiation[J]. China Journal of Traditional Chinese Medicine and Pharmacy, 2022, 37(1): 297 - 301. (in Chinese).

(From page 3)

[6] LIU ZJ, QI J, ZHU DN. Study on the chemical components and antioxidant activity of *Polygonum capitatum*[J]. China Journal of Chinese Materia Medica, 2008: 995 - 998.
[7] YAN XL, LI CQ, LIU YX. Study on antioxidant activity of *Polygonum capitatum*[J]. China Pharmacy, 2010(21): 3659 - 3661. (in Chinese).
[8] YUN CY, LI XB, ZHENG KL. Antioxidative activity of polyphenol extracts from *Polygonum capitatum* with different polarities[J]. Science and Technology of Food Industry, 2018(39): 61 - 64.
[9] HE L, ZHONG F, CHEN XJ, *et al.* A new phenolic compound from Persicaria capitata[J]. Natural Product Research, 2024.
[10] RANNEH Y, BAKAR MFA, AMALINA N, *et al.* Anti-aging and antioxidant of four traditional malaysian plants using simplex centroid mixture design approach[J]. Saudi Journal of Biological Sciences, 2021, 28(12): 6711 - 6720.
[11] ALI A, WU H, PONNAMPALAM EN, *et al.* Comprehensive profiling of most widely used spices for their phenolic compounds through LC-ESI-QTOF-MS2 and their antioxidant potential[J]. Antioxidants, 2021, 10(5): 721.
[12] GHIMIRE BK, YU CY, KIM SH, *et al.* Diversity in accessions of *Panicum miliaceum* L. based on agro-morphological, antioxidative, and genetic traits[J]. Molecules, 2019, 24(6): 1012.