Screening for Anti-tumor Activity of Fractions from *Buddleja officinalis* Maxim.

Tong LI[1△], Xin XIE[1], Liyuan LI[1], Aipeng LI[1], Zhidong LEI[2], Tingting HE[2], Sirui MO[2,3△], Shangfeng HUANG[2,3], Yao LIN[2,3], Suoyi HUANG[2,3*], Lixiang LU[4*], Shiyou ZHOU[4*]

1. School of Basic Medical Sciences, Youjiang Medical University for Nationalities, Baise 533000, China; 2. Key Laboratory of Characteristic Ethnic Medicine Research in Youjiang Basin, Youjiang Medical University for Nationalities, Baise 533000, China; 3. School of Pharmacy, Youjiang Medical University for Nationalities, Baise 533000, China; 4. School of Clinical Medicine, Youjiang Medical University for Nationalities, Baise 533000, China

Abstract [**Objectives**] The anti-tumor activity of fractions from *Buddleja officinalis* Maxim. by petroleum ether, ethyl acetate, n-butanol and water solvent was studied. [**Methods**] The ethanol extract from *B. officinalis* Maxim. was extracted and then concentrated with petroleum ether, ethyl acetate, n-butanol and water, respectively, and the extracts were obtained. The inhibitory effects of the four different fractions on the growth of three tumor cell lines in vitro were detected by CCK-8 method, and the median inhibitory concentration (IC_{50} value) was calculated. [**Results**] The four fractions inhibited the growth of the three tumor cell lines *in vitro*, among which the n-butanol fraction had the best anti-tumor activity. The IC_{50} values of the n-butanol fraction on human gastric cancer (SGC-7901), human breast cancer (MCF-7) and human liver cancer (BEL-7404) cell lines were 0.08, 1.58 and 0.12 mg/mL, respectively. [**Conclusions**] Petroleum ether, ethyl acetate, n-butanol and water fractions from the ethanol extract of *B. officinalis* Maxim. had certain anti-tumor effects, and the n-butanol fraction had the best anti-tumor activity.

Key words *Buddleja officinalis* Maxim., Tumor cells, Anti-tumor, Activity screening

1 Introduction

Buddleja officinalis Maxim., a medicinal plant in genus *Buddleja* L. of family Loganiaceae, is known as Jigutouhua, Menghua, Xiaojinhua, and Mitanghua. It likes warm and humid environment and is mainly distributed in southwest China, such as Guangxi, Yunnan and Shaanxi[1]. It is sweet, slightly cold in nature and has extensive pharmacological effects. Ou Chen[2] found that *B. officinalis* Maxim. granules can treat dry eye syndrome by relieving inflammation of lacrimal gland tissue. Jin Tao[3] verified that *B. officinalis* Maxim. capsule can slow down airway remodeling in COPD rats and delay or prevent the occurrence of COPD diseases by establishing a rat model. And modified *B. officinalis* Maxim. can effectively prevent and treat the increase of macular thickness after cataract surgery in diabetic patients[4]. In recent years, it has been found that the chemical constituents of *B. officinalis* Maxim. are mainly flavonoids, phenylethanol, terpenoids, alkaloids and volatile oils, represented by flavonoids and phenylethanol[5-6]. Pharmacological studies have shown that *B. officinalis* Maxim. has anti-inflammatory, anti-oxidant, anti-diabetic, antibacterial, anti-oculopathy and immunomodulatory effects[7]. The main active components of *B. officinalis* Maxim. include flavonoids, phenylethanoid glycosides and other compounds[7-8]. Among them, flavonoids have the functions of reducing blood lipid, protecting liver and resisting inflammation. At present, the anti-tumor effect of *B. officinalis* Maxim. has not been studied at home and abroad, and this research mainly focuses on SGC-7901, MCF-7 and BEL-7404[9].

2 Materials and methods

2.1 Cells, drugs and reagents SGC-7901, MCF-7 and BEL-7404 cell lines were purchased from Kunming Cell Bank, Chinese Academy of Sciences. The sample was purchased from Yulin City, Guangxi Zhuang Autonomous Region, and identified as a shrub plant of the genus Buddleia L. in the family Loganiaceae by Associate Professor Qin Daoguang of Youjiang Medical University for Nationalities. DMEM basal medium, (RPMI) 1640 (Gibco, USA); Cell Counting Kit-8 (CCK-8) Kit (Beyotime Biotechnology, China); fetal bovine serum (FBS) (GEMINI, USA); 95% ethanol, petroleum ether, ethyl acetate, n-butanol, DMSO (dimethyl sulfoxide) (Chengdu Kelong Chemical Co., Ltd., China).

2.2 Instruments DMi8M inverted microscope (Leica, Germany); Mithras LB 943 multifunctional microplate reader (Berthold, Germany); W-O series thermostat water bath (Zhengzhou Greatwall Scientific Industrial and Trade Co, Ltd.); BC-R501 rotary evaporator (Shanghai Beikai Biochemical Equipment Co., Ltd.); Alpha 1 - 2 vacuum freeze dryer (Germany).

2.3 Extraction of different parts of *B. officinalis* Maxim. By conventional method, the dried *B. officinalis* Maxim. was crushed into small granules and prepared into 25 kg samples. Reflux extraction was carried out with 6 times the volume of 95% ethanol according to the following methods, and heating reflux was carried out in a constant temperature water bath at 80 ℃ for 3

Received: December 13, 2023 Accepted: March 31, 2024
Supported by Guangxi Key R&D Project (GuiKeAB18221095); Baise Scientific Research and Technology Development Plan of Baise City (BaiKe20211810); Open Project of Scientific Research in Guangxi Key Laboratory of Molecular Pathology of Hepatobiliary Diseases (GXZDSYS-005); Research Project of High-level Talents in Youjiang Medical University for Nationalities (01002018079).
△These authers contributed equally to this paper.
*Corresponding author. E-mail: huangsuoyi@163.com; 281487859@qq.com; 190023154@qq.com

times, each time for 2 h. After filtration, the filtrates were combined, and the solvent was recovered by rotary evaporator to obtain extract, which was mixed with equal volume of pure water to form suspension. Then it was extracted with petroleum ether, ethyl acetate and n-butanol in turn, and the filtrates were combined with petroleum ether, ethyl acetate, n-butanol and water solvent extracts respectively. The solvent was recovered by rotary evaporator to obtain various solvent extracts, namely petroleum ether extract, ethyl acetate extract, n-butanol extract and water solvent extract, which were dried by vacuum freeze dryer and stored in refrigerator at 4 ℃ for later use. It was dissolved with DMSO (dimethyl sulfoxide) before use, and then adjusted to the concentration required by the experiment with complete culture medium.

2.4 Determination of inhibitory effects of four fractions on the proliferation of SGC-7901, MCF-7 and BEL-7404 cells

2.4.1 Cell culture and plating. The culture conditions of three kinds of cell lines were as follows: 1% penicillin/streptomycin (P/S) and 10% fetal bovine serum (FBS) were added to DMEM or RPMI-1640 medium, and routine static culture was carried out in 5% CO_2 and saturated humidity incubator at 37 ℃. SGC-7901, MCF-7 and BEL-7404 cell lines were collected from normal and logarithmic growth phase. Senescent and dead cells were washed with PBS, and then they were digested with trypsin. The cell density was adjusted to 5×10^4 cell/mL, 100 μL was inoculated in 96-well plate, and the cells were cultured in incubator for 24 h, and the cells were observed before administration.

2.4.2 Drug grouping and administration. Each cell line was divided into blank group, blank control group, negative control group and experimental group, blank group (only adding culture medium), blank control group (including cell fluid and culture medium), negative control group (including cell fluid and culture medium of DMSO at different concentrations in corresponding experimental group) and experimental group (including drugs, cell fluid and culture medium). It was cultured in an incubator in accordance with the conditions of cell growth and culture. After 24 h of culture, the cells were completely adherent and grew well. The cells were cultured with 100 μL medium containing different concentrations of drugs for 48 h. The four extracts were divided into 6 groups (0.05, 0.10, 0.20, 0.40, 0.80, 1.60 mg/mL) from low to high. Three parallel wells were set in blank group, control group and experimental group.

2.4.3 Detection of cell viability by CCK8 method. After 48 h of drug treatment, the growth state was observed first, and the old culture medium was discarded. Complete culture medium containing 90 μL of 5% CCK8 was added to each well, and incubated for 1 – 2 h at 37 ℃, and the *OD* value of each well was read at 450 nm.

Inhibition rate of tumor cell growth (IR%) = 1 – [(*OD* value of experimental group – *OD* value of blank group)/(*OD* value of control group – *OD* value of blank group)] × 100%.

The IC_{50} values of four extracts acting on three cell lines were statistically analyzed and calculated by GraphPad Prism5 software.

3 Results and analysis

3.1 Inhibitory effect of four fractions on proliferation of SGC-7901, MCF-7 and BEL-7404 cell lines See Table 1 for the inhibitory results of four extracts from *B. officinalis* Maxim. on proliferation of SGC-7901, MCF-7 and BEL-7404 cell lines and IC_{50} values. From Table 1, it can be seen that petroleum ether, ethyl acetate, n-butanol and water solvent fractions from *B. officinalis* Maxim. had inhibitory effects on three tumor cell lines.

Table 1 Effects of four fractions from *Buddleja officinalis* Maxim. on proliferation of SGC-7901, MCF-7 and BEL-7404 cells ($\bar{x} \pm s$)

Group	Drug concentration mg/mL	SGC-7901			MCF-7			BEL-7404		
		OD	Cell inhibition rate//%	IC_{50} mg/mL	*OD*	Cell inhibition rate//%	IC_{50} mg/mL	*OD*	Cell inhibition rate//%	IC_{50} mg/mL
Blank control	–	1.270 ± 0.08	–	–	1.13 ± 0.01	–	–	1.170 ± 0.02	–	–
Petroleum ether	0.05	0.940 ± 0.06	26	0.25	1.05 ± 0.04	7	0.45	0.690 ± 0.03	29	1.32
extract	0.10	0.920 ± 0.02	27		0.98 ± 0.04	13		0.350 ± 0.03**	44	
	0.20	0.550 ± 0.03	56		0.99 ± 0.03	12		0.040 ± 0.02**	50	
	0.40	0.051 ± 0.00	96		0.75 ± 0.04	33		0.033 ± 0.01*	62	
	0.80	0.026 ± 0.00	97		0.14 ± 0.02*	87		0.017 ± 0.00	99	
	1.60	0.010 ± 0.00*	99		0.34 ± 0.04*	69		0.013 ± 0.01	100	
Ethyl acetate	0.05	0.820 ± 0.01	36	0.15	1.02 ± 0.01	9	0.43	0.160 ± 0.04	85	4.12
extract	0.10	0.680 ± 0.02	45		0.99 ± 0.01	12		0.106 ± 0.04	90	
	0.20	0.063 ± 0.05	96		0.84 ± 0.07	25		0.150 ± 0.01**	87	
	0.40	0.020 ± 0.01	98		0.59 ± 0.02*	47		0.260 ± 0.05	77	
	0.80	0.010 ± 0.01	98		0.41 ± 0.40	63		0.360 ± 0.03**	68	
	1.60	0.000 ± 0.00	100		0.20 ± 0.00*	85		0.400 ± 0.00**	54	
N-butanol	0.05	1.190 ± 0.04	6	0.08	0.83 ± 0.04	26	1.58	0.830 ± 0.08	40	0.12
extract	0.10	0.370 ± 0.03	90		0.82 ± 0.07	27		0.650 ± 0.02	69	
	0.20	0.007 ± 0.006	99		0.71 ± 0.06	36		0.580 ± 0.01	96	

(To be continued)

(Continued)

Group	Drug concentration mg/mL	SGC-7901 OD	SGC-7901 Cell inhibition rate// %	SGC-7901 IC_{50} mg/mL	MCF-7 OD	MCF-7 Cell inhibition rate// %	MCF-7 IC_{50} mg/mL	BEL-7404 OD	BEL-7404 Cell inhibition rate// %	BEL-7404 IC_{50} mg/mL
	0.40	0.000 ± 0.00 **	100		0.67 ± 0.03	40		0.440 ± 0.07 *	97	
	0.80	0.000 ± 0.00 **	100		0.41 ± 0.05	63		0.009 ± 0.00 *	98	
	1.60	0.000 ± 0.00 **	100		0.22 ± 0.01	79		0.000 ± 0.00 **	98	
Aqueous solvent	0.05	1.270 ± 0.02 **	0	0.39	0.96 ± 0.06	14	–	0.540 ± 0.05	53	–
extract	0.10	1.240 ± 0.03 **	2		0.99 ± 0.05	12		0.530 ± 0.00	95	
	0.20	1.160 ± 0.03 **	9		0.95 ± 0.01	15		0.006 ± 0.00	99	
	0.40	1.150 ± 0.07 **	9		0.99 ± 0.02	15		0.120 ± 0.02 **	89	
	0.80	0.160 ± 0.04 **	87		1.07 ± 0.10	5		0.250 ± 0.05 **	78	
	1.60	0.000 ± 0.00 **	100		1.10 ± 0.10	2		0.480 ± 0.06 **	58	

3.2 IC_{50} values of four extracts for SGC-7901, MCF-7 and BEL-7404 cell lines The inhibitory effects of four extracts from *B. officinalis* Maxim. on SGC-7901, MCF-7 and BEL-7404 cells are shown in Fig. 1. As can be seen from Fig. 1, petroleum ether, ethyl acetate, n-butanol and water solvent extract from *B. officinalis* Maxim. can inhibit the proliferation of SGC-7901, MCF-7 and BEL-7404 cells *in vitro*. The inhibition of proliferation was proportional to the drug concentration and in a concentration-dependent manner, and the cell survival rate decreased with the increase of drug concentration.

The IC_{50} values of ethyl acetate, n-butanol, petroleum ether and water solvent extracts from *B. officinalis* Maxim. on SGC-7901 cells were 0.15, 0.08, 0.25 and 0.39 mg/mL, respectively; the IC_{50} values of petroleum ether, ethyl acetate and n-butanol extracts on MCF-7 cells were 0.45, 0.43 and 1.58 mg/mL, respectively; the IC_{50} values of BEL-7404 cells were 1.32, 4.12 and 0.12 mg/mL, respectively. The water extract had poor activity on MCF-7 and BEL-7404, and the IC_{50} value can not be calculated. Compared with the other three fractions, the n-butanol extract had stronger anti-tumor activity on three kinds of tumor cells.

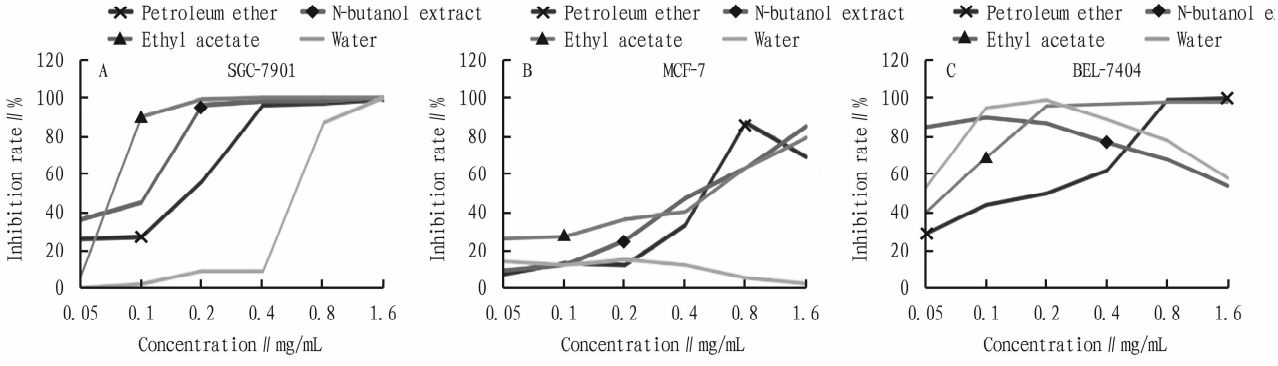

Fig. 1 Effects of four extracts on cell viability of SGC-7901 (A), MCF-7 (B) and BEL-7404 (C)

4 Discussion

The results of this experiment showed that the higher the drug concentration, the higher the lethality to SGC-7901, MCF-7 and BEL-7404 cells, and the lower the survival rate. The proliferation inhibition was proportional to the drug concentration and dose-dependent, and the cell inhibition rate increased with the increase of drug concentration. Compared with the other three kinds of extracts, the three kinds of tumor cells were more sensitive to the n-butanol extract from *B. officinalis* Maxim., which had the best anti-tumor activity. Among the petroleum ether, ethyl acetate, n-butanol and water solvent fractions from ethanol extract of *B. officinalis* Maxim., n-butanol fraction was the one with the best anti-tumor activity. The research group has done a lot of preliminary work on the antioxidant research of Zhuang medicines such as *B. officinalis* Maxim., and now further studies its anti-tumor effect, and screens out its active parts, which lays a solid foundation for further separation of anti-tumor active monomer compounds and research on anti-tumor mechanism. In the later stage, we can refer to the experimental design of Liu Sen[10] and discuss its mechanism.

References

[1] LI Y, LI XJ, ZHANG JL, *et al.* Food additives on the stability of *Buddleja officinalis* Maxim pigment and its application in fruit juice processing [J]. China Food Additives, 2020, 31(7): 76 – 83. (in Chinese).

[2] OU C, SONG HP, LI J, *et al.* Discussion on action mechanism of Mimenghua granule in the treatment of dry eye based on network pharmacology and molecular docking[J]. Journal of Hunan University of Chinese Medicine, 2020, 40(7): 797 – 804. (in Chinese).

[3] JIN T, HU TK, ZHANG JH. Effects of Mimenghua capsule on airway remodeling in rats with chronic obstructive pulmonary disease model[J]. Yunnan Journal of Traditional Chinese Medicine and Materia Medica, 2021, 42(3): 79 – 81. (in Chinese).

(To page 56)

nese).
[30] DONG YS, WEI ZP, ZHAO HX. Observation on the therapeutic effect of Guizhi Fuling capsule combined with azithromycin on patients with pelvic inflammatory disease[J]. Maternal and Child Health Care of China, 2023, 38(1): 30 - 33. (in Chinese).
[31] SUN MZ, HUANG WQ. Research advances on pathogenesis and clinical diagnosis and treatment of endometriosis[J]. International Journal of Laboratory Medicine, 2023, 44(16): 2017 - 2022. (in Chinese).
[32] WU YH, LUO SQ, TONG ZQ. Clinical efficacy and safety of Guizhi Fuling pills combined with dydrogesterone in the treatment of endometriosis[J]. Chinese Journal of Clinical Rational Drug Use, 2023, 16(6): 131 - 133. (in Chinese).
[33] CHEN SX. Clinical effect of Guizhi Fuling pill combined with LNG-IUS and GnRH-a on patients with moderate and severe blood stasis emt[D]. Hefei: Anhui University of Traditional Chinese Medicine, 2023. (in Chinese).
[34] HUANG CS. Application effect of activating blood stasis method in treating endometriosis[J]. Chinese Journal of Urban and Rural Enterprise Hygiene, 2022, 37(2): 156 - 158. (in Chinese).
[35] ZHANG ZY, LIU FY, XU YY, *et al.* GZFLW induces apoptosis of ectopic endometrial stromal cells via promoting VPS53 protein stability [J]. Evidence-based Complementary and Alternative Medicine : eCAM, 2018(2018):1293630.
[36] LI ZY. Factors associated with menstrual irregularities[J]. World Latest Medicine Information, 2019, 19(68): 171. (in Chinese).
[37] REN C. Analysis of the effect of treating menstrual disorders with gynaecological rejuvenation capsule[J]. Contemporary Medical Symposium, 2016, 15(15): 139 - 140. (in Chinese).
[38] ZHOU WW. Clinical effect of Guifu Dihuang pills combined with Guizhi Fuling pills in treating menstrual disorders[J]. Chinese Baby, 2023 (16): 64 - 66. (in Chinese).
[39] CHE J, LIU H. Application effect of Yimucao Keli combined with Guizhi Fuling pills in the treatment of menstrual disorders[J]. Electronic Journal of Practical Gynecological Endocrinology, 2020, 7(26): 58, 63. (in Chinese).
[40] LIU F. Clinical observation of Guifu Dihuang pill combined with Guizhi Fuling pill in treating menstrual disorders of kidney deficiency and blood stasis type[J]. China Health Care & Nutrition, 2021, 31(12): 242. (in Chinese).
[41] GUO MY. Clinical application analysis of Guizhi Fuling pills combined with Embryo capsule in the treatment of menstrual disorders[J]. Chinese Remedies & Clinics, 2018, 18(1): 85 - 86. (in Chinese).
[42] GU JQ, YU SH, XU F, *et al.* Progress of research related to adolescent polycystic ovary syndrome (PCOS) in Chinese and Western medicine [J]. Journal of Practical Traditional Chinese Internal Medicine, 2023, 37(4): 4 - 7. (in Chinese).
[43] LIU M, ZHU HQ, ZHU Y, *et al.* Guizhi Fuling Wan reduces autophagy of granulosa cell in rats with polycystic ovary syndrome via restoring the PI3K/AKT/mTOR signaling pathway[J]. Journal of Ethnopharmacology, 2021(270): 113821.
[44] YE YJ, ZHOU WM, REN YF, *et al.* The ameliorating effects of Guizhi Fuling Wan combined with rosiglitazone in a rat ovarian model of polycystic ovary syndrome by the PI3K/AKT/NF-κB and Nrf2/HO-1 pathways.[J]. Gynecological Endocrinology : The Official Journal of the International Society of Gynecological Endocrinology, 2023, 39(1): 2254848.
[45] LI JN, YU FY, SHEN HH, *et al.* Efficacy of Guizhi Fuling pills combined with western medicine in the treatment of PCOS and its regulatory effect on PI3K/AKT/mTOR pathway[J]. Journal of Molecular Diagnostics and Therapy, 2023, 15(2): 197 - 200, 204. (in Chinese).
[46] WANG Y, WANG JJ. Clinical study on Guizhi Fuling pill and erchen decoction combined with routine Western medicine for polycystic ovary syndrome[J]. New Chinese Medicine, 2023, 55(1): 40 - 43. (in Chinese).
[47] LAN HL, SHI Y, WU S, *et al.* Clinical effect of modified Guizhi Fuling pills in treatment of chronic radiation-induced proctitis with stagnant dampness-heat:An analysis of 33 cases[J]. Hunan Journal of Traditional Chinese Medicine, 2023, 39(9): 1 - 4. (in Chinese).
[48] YAN ZG, CAO H, CHEN J, *et al.* Clinical efficacy of Guizhi poria pills and cadelorol on intracerebral microlesions[J]. Chinese Journal of Integrative Medicine on Cardio-Cerebrovascular Disease, 2023, 21(18): 3444 - 3448. (in Chinese).
[49] LIU Y, YUN B, ZHOU JH. Observation on the clinical efficacy of modified Linggan Wuwei Jiangxin decoction combined with Guizhi Fuling pills in the treatment of acute exacerbation of chronic obstructive pulmonary disease of cold fluid and blood stasis obstruction type[J]. Journal of Guangzhou University of Traditional Chinese Medicine, 2023, 40 (9): 2182 - 2189. (in Chinese).
[50] XU BG, ZHENG JW, TIAN XX, *et al.* Antihepatofibrotic effect of Guizhifuling pill on carbon tetrachloride-induced liver fibrosis in mice [J]. Journal of Traditional Chinese Medicine, 2022, 42(5): 715 - 722.
[51] ZHENG YP, MA CH. Observation on the clinical efficacy of Chaihu Guizhi Ganjiang decoction combined with Guizhi Fuling pills plus and minus on pruritus in elderly diabetes mellitus[J]. The Journal of Medical Theory and Practice, 2023, 36(17): 2959 - 2962. (in Chinese).
[52] LIU YL, HAO XD, CHEN JH. Therapeutic efficacy of Mufangji decoction combined with Guizhi Fuling pills in the treatment of acute exacerbation of pulmonary heart disease (cardiopulmonary and renal Yang deficiency)[J]. Journal of Emergency in Traditional Chinese Medicine, 2023, 32(8): 1411 - 1414. (in Chinese).

(From page 50)

[4] CHEN YL, XIA W, ZHU JF, *et al.* Clinical observation on panretinal laser photocoagulation combined with Mimenghua recipe in the treatment of diabetic retinopathy[J]. Guangming Journal of Chinese Medicine, 2021, 36(10): 1683 - 1685. (in Chinese).
[5] LIU F, GUO YP, LIU B, *et al.* Isolation and identification of chemical constituents of ethyl acetate extraction layer from *Buddleja officinalis* Maxim[J]. Journal of Kunming Medical University, 2021, 42(8): 31 - 34. (in Chinese).
[6] LONG ZH, LI BY, WANG QY, *et al.* A new hexenol glycoside from *Buddleja officinalis*[J]. China Journal of Chinese Materia Medica, 2021, 46(23): 6178 - 6184. (in Chinese).
[7] XU C,SONG JT. Effect of modified Mimenghua decoction on the retinal thickness in macular area after cataract surgery in diabetic patients[J]. China Journal of Chinese Ophthalmology, 2020, 30(10): 719 - 722. (in Chinese).
[8] CHEN YL,XIA W,ZHU JF,*et al.* Clinical observation on panretinal laser photocoagulation combined with Mimenghua recipe in the treatment of diabetic retinopathy[J]. Guangming Journal of Chinese Medicine,2021, 36(10): 1683 - 1685. (in Chinese).
[9] DONG SQ, YAN WW, XIAO CW. CBX2 regulates the proliferation and apoptosis of cervical cancer cells by Wnt/β-catenin signaling pathway [J]. Journal of Youjiang Medical University for Nationalities,2023, 45 (5): 731 - 735. (in Chinese).
[10] LIU S, PENG LY, ZHU MY, *et al.* Screening of anti-tumor active components of *Solanum torvum* Swartz extract in vitro and its mechanism of action[J]. Journal of Youjiang Medical University of Nationalities, 2016, 38(2):157 - 159,167. (in Chinese).