DOI: 10.19600/j.cnki.issn2152 –3924.2023.06.004

Activity Screening Study on the Anti-tumor Effects of Extracts from *Mahoniae caulis*

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Abstract [**Objectives**] To explore the anti-tumor activity of the extracts of petroleum ether, ethyl acetate, n-butanol and aqueous solution from *Mahoniae caulis*. [**Methods**] The extracts were extracted with petroleum ether, ethyl acetate, n-butanol and aqueous solution respectively, and then concentrated. The inhibitory effects of these extracts on the growth of three tumor cell lines *in vitro* were detected by CCK-8 method, and the IC_{50} value was calculated. [**Results**] The four extracts inhibited the growth of the three tumor cell lines *in vitro*, among which the n-butanol extract had the best anti-tumor activity. The IC_{50} values of the n-butanol extract on human gastric cancer (SGC-7901), human breast cancer (MCF-7) and human liver cancer (BEL-7404) cell lines were 0.23, 0.25 and 0.58 mg/mL, respectively. [**Conclusions**] The ethanol extract of *Mahoniae caulis* under petroleum ether, ethyl acetate, n-butanol and aqueous solution had certain anti-tumor effect, and n-butanol extract had the best anti-tumor activity.

Key words *Mahoniae caulis*, Tumor cells, Anti-tumor effects, Activity screening

1 Introduction

Mahoniae caulis, first recorded in *An Illustrated Book on Plants*, originated from the dried stems of *Mahonia bealaei* (Fort.) Carr. and *Mahonia fortunei* (Lindl.) Fedde in the Berberidaceae family. There are about 35 species in China, mainly distributed in southwest China (Sichuan, Yunnan, Guizhou, Guangxi and eastern Tibet)[1-2]. Pharmacological effects are diverse, including antioxidant, anti-inflammatory, antibacterial, analgesic, antidiarrheal, liver-protecting, anti-jaundice, anti-tumor, acetylcholinesterase activity-inhibiting, and lipid-lowering effects[3]. Clinically, it is mainly used to treat breast cancer, mammary duct dilatation with inflammation, acne, chronic hepatitis B[4-6] and other diseases. Wang Tianxiao *et al.*[7] summarized the anti-tumor effects, and found that the anti-tumor effects of *M. caulis* are related to alkaloid components, especially berberine. It can inhibit acute T lymphocytic leukemia cell line (MOLT-4), nasopharyngeal carcinoma cell line CNE-2, hepatocellular carcinoma cell line Bel-7402, prostate cancer cell line, human gastric cancer cell line SNU-5, human giant cell lung cancer cell line PG under certain conditions[8-11]. At present, the research on anti-tumor effects of *M. caulis* at home and abroad mainly focuses on the effect of crude extract of *M. caulis* on adriamycin-resistant leukemia and breast cancer, while the research on anti-tumor effects of *M. caulis* monomer components mainly focuses on berberine. However, the research on anti-tumor of other monomer components of *M. caulis* (liver cancer, breast cancer and gastric cancer) is rarely reported, and the research on the material basis and mechanism of anti-tumor effects of *M. caulis* is basically blank. More than 60% of the approved anti-tumor drugs are natural drugs or synthetic and semi-synthetic derivatives with natural products as lead compounds[12-13]. Finding new drugs with anti-tumor activity from natural plants may bring new hope to patients with malignant tumors.

2 Materials and methods

2.1 Materials

2.1.1 Cells. SGC-7901, MCF-7 and BEL-7404 cell lines were purchased from Kunming Cell Bank, Chinese Academy of Sciences.

2.1.2 Drugs and reagents. The drug, purchased from Yulin City, Guangxi Zhuang Autonomous Region, was identified by Associate Professor Qin Daoguang from Youjiang Medical University for Nationalities as the dry stem of *Mahonia bealaei* (Fort.) Carr. and *Mahonia fortunei* (Lindl.) Fedde in the Berberidaceae family. (RPMI) 1640, DMEM basal medium, produced by Gibco, USA; fetal bovine serum (FBS) produced by GEMINI, USA; Cell Counting Kit-8 (CCK-8) produced by Beyotime Biotechnology, China. 95% ethanol, petroleum ether, ethyl acetate, n-butanol, DMSO (dimethyl sulfoxide) and other reagents were all products of AR, produced by Chengdu Kelong Chemical Reagent Factory.

2.1.3 Instruments. DMi8M inverted microscope, produced by Leica, Germany; Mithras LB 943 multifunctional microplate reader, produced by Berthold, Germany; W-O series thermostatic water bath, produced by Zhengzhou Great Wall Scientific Industrial and Trade Co., Ltd., China; BC-R501 rotary evaporator, produced by Shanghai Beikai Biochemical Equipment Co., Ltd., China; Alpha 1 –2 vacuum freeze dryer, produced by Christ,

Received: August 13, 2023 Accepted: November 18, 2023
Supported by Guangxi Key Research and Development Plan Project (Gui Ke AB18221095); 2023 Innovation Project of Youjiang Medical University for Nationalities Graduate Education (YXCXJH2023009); Scientific Research Project of High-level Talents of Youjiang Medical University for Nationalities (01002018079).
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Germany.

2.2 Methods

2.2.1 Extraction of different parts of *Mahoniae caulis*. The dried *M. caulis* was crushed into small grains by conventional method, and prepared into 25 kg sample. Reflux extraction was carried out with 95% ethanol (1 : 6) according to the following method. After heating and refluxing in a thermostatic water bath at 80 ℃ for 3 times, each time for 2 h, the filtrate was extracted, combined, and the solvent was recovered by rotary evaporator to obtain extract. An equal volume of pure water was added to form suspension, and then it was extracted with petroleum ether, ethyl acetate and n-butanol in turn. The extracts of petroleum ether, ethyl acetate, n-butanol and aqueous solution were filtered and combined, and the solvents were recovered by rotary evaporator to obtain various solvent extracts, namely petroleum ether extract, ethyl acetate extract, n-butanol extract and aqueous solution extract. It was dried in a vacuum freeze dryer and stored in a refrigerator at 4 ℃. It was dissolved with DMSO (dimethyl sulfoxide) before use, and then adjusted to the concentration required by the experiment with complete culture medium.

2.2.2 Inhibitory effect of extracted part on proliferation of SGC-7901, MCF-7 and BEL-7404 cells. (i) Cell culture and plating. Culture conditions of 3 cell lines. RPMI-1640 or DMEM medium containing 10% fetal bovine serum (FBS) and 1 × streptomycin was cultured in a saturated humidity incubator with 5% CO_2 at 37 ℃. Three cell lines in logarithmic growth phase were digested with trypsin. The cell concentration was adjusted to the appropriate density of 5×10^4 cell/mL, the cell (100 μL) was inoculated in 96-well plate, the cells were cultured in incubator for 24 h, and the cells were observed before administration.

(ii) Drug grouping and administration. Each cell was divided into blank group, control group, negative control group and experimental group. Blank group (only adding culture medium), control group (including cell fluid and culture medium), negative control group (including cell fluid and DMSO culture medium in different concentrations in corresponding experimental group) and experimental group (including cell fluid, culture medium and drugs). After 24 h of adherent culture, the cells were replaced with medium containing different drug concentrations (100 μL of medium containing different concentrations of drug was added to each well) and treated for 48 h separately. The extracts were divided into 6 groups (0.05, 0.1, 0.2, 0.4, 0.8, 1.6 mg/mL) from low to high. Three replicates were set in blank group, control group and experimental group.

(iii) Cell viability was detected by CCK-8 method. After 48 h of drug treatment, the growth state was observed first, and the old culture medium was discarded. 90 μL of complete medium containing 5% CCK-8 was added to each well, and the suitable time for incubation in dark place was 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%.

GraphPad Prism5 software was used to calculate the IC_{50} values of drugs in treating three cell lines.

3 Results and analysis

3.1 Inhibitory effect of extracts on proliferation of SGC-7901, MCF-7 and BEL-7404 cell lines The inhibitory effects and IC_{50} values of four extracts of *M. caulis* on the proliferation of SGC-7901, MCF-7 and BEL-7404 cell lines are shown in Table 1.

Table 1 Effects of four extracted part of *Mahoniae caulis* on the 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	–	0.79 ± 0.01	–	–	0.71 ± 0.02	–	–	0.88 ± 0.01	–	–
Petroleum ether extract	0.05	0.82 ± 0.02	0.00	–	0.92 ± 0.07**	0.00	3.55	0.84 ± 0.02	2.90	42 422.70
	0.1	0.80 ± 0.04	0.00		0.84 ± 0.05**	0.00		0.82 ± 0.03**	2.77	
	0.2	0.86 ± 0.01	0.00		0.77 ± 0.02	0.00		0.78 ± 0.02**	10.63	
	0.4	0.82 ± 0.02	0.00		0.73 ± 0.01	0.00		0.73 ± 0.01**	10.94	
	0.8	0.74 ± 0.01	0.00		0.60 ± 0.01**	2.43		0.71 ± 0.04**	9.44	
	1.6	0.62 ± 0.01*	0.22		0.38 ± 0.05**	30.89		0.72 ± 0.02**	5.71	
Ethyl acetate extract	0.05	0.70 ± 0.01**	5.47	0.33	0.70 ± 0.03	0.00	0.37	0.74 ± 0.04	13.95	0.44
	0.1	0.64 ± 0.01**	12.79		0.63 ± 0.01	0.00		0.78 ± 0.04	7.45	
	0.2	0.52 ± 0.01**	37.63		0.44 ± 0.02**	33.42		0.67 ± 0.01**	23.37	
	0.4	0.28 ± 0.02**	61.35		0.24 ± 0.01**	62.51		0.51 ± 0.00**	38.43	
	0.8	0.08 ± 0.00**	87.28		0.06 ± 0.00**	90.61		0.30 ± 0.03**	61.44	
	1.6	0.14 ± 0.01**	76.94		0.13 ± 0.00**	76.54		0.01 ± 0.00**	98.81	
N-butanol extract	0.05	0.65 ± 0.01*	13.14	0.23	0.65 ± 0.02	2.80	0.25	0.84 ± 0.06	2.40	0.58
	0.1	0.57 ± 0.03	21.47		0.48 ± 0.03*	19.82		0.80 ± 0.02	4.87	

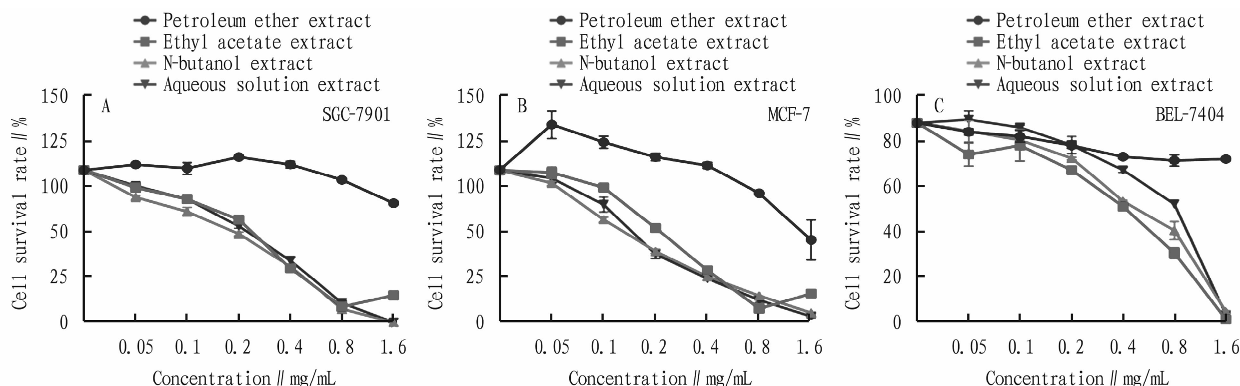
(To be continued)

(Continued)

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
	0.2	0.46 ± 0.01**	44.56		0.33 ± 0.01**	50.15		0.72 ± 0.02**	17.27	
	0.4	0.29 ± 0.01**	60.71		0.21 ± 0.00**	68.18		0.53 ± 0.03*	35.83	
	0.8	0.07 ± 0.01**	88.61		0.12 ± 0.00**	81.18		0.40 ± 0.04*	49.06	
	1.6	0.00 ± 0.00**	100.00		0.04 ± 0.00**	92.26		0.04 ± 0.00**	94.38	
Aqueous solution extract	0.05	0.71 ± 0.02**	4.98	0.29	0.67 ± 0.08	0.00	0.26	0.91 ± 0.03	0.00	0.79
	0.1	0.64 ± 0.02**	12.06		0.55 ± 0.03*	9.61		0.86 ± 0.01	0.00	
	0.2	0.50 ± 0.01**	39.51		0.32 ± 0.03**	51.07		0.80 ± 0.03**	8.37	
	0.4	0.32 ± 0.03**	55.69		0.20 ± 0.01**	69.06		0.67 ± 0.02**	18.28	
	0.8	0.10 ± 0.01**	85.17		0.10 ± 0.01**	85.28		0.52 ± 0.00**	33.09	
	1.6	0.00 ± 0.00**	100.00		0.02 ± 0.00**	96.61		0.03 ± 0.03**	96.18	

Note: Compared with the control group, ** $P < 0.01$, * $P < 0.05$.

3.2 IC_{50} values of extracts for SGC-7901, MCF-7 and BEL-7404 cell lines It can be seen from Fig. 1 that petroleum ether, ethyl acetate, n-butanol and aqueous solution extract of *M. caulis* can inhibit the proliferation of SGC-7901, MCF-7 and BEL-7404 cells *in vitro*. The cell survival rate decreased with the increase of drug concentration, and the inhibition of proliferation was proportional to and dependent on drug concentration. The survival rate of SGC-7901 cells was not significantly decreased after 48 h intervention with petroleum ether extract.



Note: A. SGC-7901; B. MCF-7; C. BEL-7404.

Fig. 1 Effects of four extracts on the viability of SGC-7901, MCF-7 and BEL-7404 cells

The IC_{50} values of ethyl acetate, n-butanol and aqueous solution extracts of *M. caulis* on SGC-7901 cells were 0.33, 0.23 and 0.29 mg/mL, respectively; the IC_{50} values of petroleum ether, ethyl acetate, n-butanol and aqueous solution extracts on MCF-7 cells were 3.55, 0.37, 0.25 and 0.26 mg/mL, respectively; the IC_{50} values of petroleum ether, ethyl acetate, n-butanol and aqueous solution extracts on BEL-7404 cells were 42 422.70, 0.44, 0.58 and 0.79 mg/mL, respectively. And petroleum ether extract had poor activity against SGC-7901, and the IC_{50} value can not be calculated. The anti-proliferation effect of n-butanol extract on three kinds of tumor cells was relatively strong.

4 Discussion

The results showed that petroleum ether, ethyl acetate, n-butanol and aqueous solution extracts of *M. caulis* had inhibitory effects on three tumor cell lines. The cell survival rate decreased with the increase of drug concentration, and the inhibition rate increased with the increase of drug concentration, which was dose-dependent. The IC_{50} value of n-butanol extract of *M. caulis* to SGC-7901, MCF-7 and BEL-7404 was the smallest, which indicated that ethyl acetate extract was relatively sensitive to three kinds of tumor cells and had the best anti-tumor activity. The results of this study showed that among petroleum ether, ethyl acetate, n-butanol and aqueous solution extracts from *M. caulis*, the n-butanol extract had the best anti-tumor effect.

The research group has done a lot of preliminary research work on the oxidation resistance of Zhuang medicines such as *M. caulis*, 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 *et al.* [14] and discuss its mechanism.

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creases in the activity of MGC-803 and AGS gastric adenocarcinoma cells, the number of cell colonies, and the migration ability of the cells. Moreover, after TRAIP knockdown, the expression of proliferation-related protein CyclinD1, migration-related protein MMP2 and key proteins of Notch signaling pathway Notch1, Jagged1 and Hes1 significantly reduced. Previous experiments confirmed that TRAIP was involved in the occurrence and development of gastric adenocarcinoma, and promoted the proliferation and migration of gastric adenocarcinoma cells through Notch signaling pathway.

To further confirm that TRAIP promoted the proliferation and migration of gastric adenocarcinoma through Notch signaling pathway, Notch signaling pathway agonist (JAG-1) was added on the basis of TRAIP knockdown. From CCK-8 assay, it is found that the activity of gastric adenocarcinoma cells significantly enhanced. Plate colony-forming assay showed that the colony-forming ability of gastric adenocarcinoma cells significantly improved. Through Transwell assay, the migration ability of gastric adenocarcinoma cells increased significantly. Western blot assay revealed that the expression of proliferation-related protein CyclinD1, migration-related protein MMP2, key proteins of Notch signaling pathway Notch1, Hes1 and Jaggde1 increased significantly. It was confirmed that after TRAIP knockout, JAG-1 not only increased the proliferation and migration ability of gastric adenocarcinoma cells, but also enhanced the expression of key proteins of Notch signaling pathway Notch1, Hes1 and Jagged1.

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