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Full Length Research Paper

In vitro antitumor and antioxidant activities of *Belamcanda chinensis* (L.) DC

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The *in vitro* antitumor activities of petroleum ether, ethyl acetate and methanol extracts of *Belamcanda chinensis* (L.) DC root were studied against the PC3, Bcap-37, and BGC-823 cell lines. The 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) free radical scavenging effects of these crude extracts were also tested. The results revealed that ethyl acetate extract and its isolated fractions exhibited significant antitumor activities against the three cell lines. In addition, ethyl acetate extracts exhibited moderate DPPH free radical scavenging effects. The role of these plant extracts in the traditional treatment of tumor in humans is highlighted.

Key words: *Belamcanda chinensis* (L.) DC, ethyl acetate extract, antitumor, 1,1-diphenyl-2-picrylhydrazyl (DPPH,

INTRODUCTION

At present, numerous medicinal plants and related products are used for the treatment of various diseases. Chinese herbal medicines have been used for the treatment of human diseases for centuries. *Belamcanda chinensis* (L.) DC is from the family of *Iridaceae*, which comprises approximately 60 genera and 800 species worldwide. However, only two genera (Iridacea and Belamcanda) are distributed in China. *B. chinensis* shrubs primarily grow in the southwest area of China, especially in the provinces of Guizhou, Yunnan, and Sichuan (Wang et al., 2007; Ji et al., 2001; Meng 2004), and the species was listed as an official drug in the Chinese Pharmacopoeia (2005). The rhizome of *B. chinensis* has been used as a medicinal plant for over 2000 years, and it remains widely used in the treatment of several diseases, such as pulmonary disease, acute and chronic pharyngitis, asthma, and cancer (Wu et al., 2008; Jung and Lee, 2002; Jung et al., 2003; Zhang et al., 2005). A number of researchers (Qin et al., 2003; Jung et al., 2004) also found that isoflavones from *B. chinensis* exhibited antioxidant activities. In our recent work, the rhizomes of *B. chinensis* were extracted using different solvents, and the antitumor activities against the PC3, Bcap-37, and BGC-823 cell lines, as well as the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) free radical scavenging activity of the crude extracts, were tested. The results revealed that ethyl acetate extracts exhibited significant antitumor activities and moderate DPPH free radical scavenging effects.

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MATERIALS AND METHODS

Chemicals and reagents

Sodium dodecyl sulfate (SDS) was purchased from Beijing Dingguo Co., Ltd.; PC3, Bcap-37 and BGC-823 cell lines were purchased from Shanghai Hen Yuan Biotechnology Co., Ltd.; DPPH and vitamin C (Vc) were purchased from Aladdin Reagent Inc.; 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Roche Molecular Biochemicals (1465-007); and Adriamycin (ADM) was purchased from Zhejiang Hisun Pharmaceutical Co., Ltd. Column chromatography was performed using silica gel (200 to 300 mesh) purchased from Qingdao Marine Chemical, Inc.

Plant material

Fresh samples of *B. chinensis* were collected in Bijie, Guizhou,

China, in August 2011. The plant material was identified by Prof. Long Qing-De of the Guiyang Medical University Department of Medicine, and a voucher specimen was deposited at Guiyang Medical University, Guiyang, China.

Extraction and isolation

The dried rhizomes of *B. chinensis* (7.5 kg) were refluxed for 6 h in petroleum ether, ethyl acetate, and methanol, sequentially. Each solvent extraction process was repeated twice, and the extracts were combined and concentrated with a vacuum rotary evaporator at 50°C. Petroleum ether extract (40 g), ethyl acetate extract (289 g), and methanol extract (321 g) were then obtained, respectively. The antitumor and antioxidant activities of these extracts were tested prior to further isolation processes, and ethyl acetate extract was found to exhibit the best *in vitro* antiproliferation activities against the PC3, Bcap-3,7and BGC-823 cell lines. Thus, the ethyl acetate extract was isolated first.

The viscous dark ethyl acetate extract was adsorbed on silica gel (200 to 300 mesh) for column chromatography over a silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixtures of petroleum ether and ethyl acetate (20/1,15/1,10/1,5/1 and 1/1, *V/V*), pure chloroform, and mixtures of chloroform and methanol (10/1, 5/1 and 1/1, V/V, respectively) to yield10 fractions. The fractions were monitored via thin-layer chromatography, and spots were visualized by heating silica gel plates sprayed with 10% phosphomolybdic acid hydrate in EtOH and 1% $FeCl_3$ in EtOH.

Cell culture

The human prostate cancer cell line PC3, the breast cancer cell line Bcap-37, and the gastric cancer cell line BGC-823 were cultured in an RPMI1640 medium supplemented with 10% heat-inactivated fetal bovine serum. All cell lines were maintained at 37°C in a humidified 5% carbon dioxide and 95% air incubator.

MTT assay

All tested extracts were dissolved in DMSO and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density of 2×10^3 cells/well/ 100 µl of the proper culture medium and treated with the extracts at 50 to 200 µg/ml for 72 h. In parallel, the cells treated with 0.1% DMSO served as the negative control, and the ADM treatment served as the positive control.

Finally, 100 µl of MTT was added, and the cells were incubated for 4 h. The MTT-formazan formed by metabolically viable cells was dissolved in 100 µl of SDS for 12 h. The absorbance was then measured at 595 nm with a microplate reader (BIO-RAD, model 680), a value directly proportional to the number of living cells in culture. The percentage cytotoxicity was calculated using the following formula:

$$\%\text{Cytotoxicity} = \frac{(\text{Control abs} - \text{Blank abs}) - (\text{Test abs} - \text{Blank abs})}{(\text{Control abs} - \text{Blank abs})} \times 100$$

DPPH radical scavenging assay

The radical scavenging activities of plant extracts were measured using the DPPH method (Liu et al., 2004). First, extracts were dissolved in ethanol at 50 and 200 µg/ml, respectively. Then, 1 ml of the extract ethanol solution was added to 1 ml of DPPH solution (10 µg/ml), giving final concentrations of 25 and 100 µg/ml, respectively. The absorbance at 517 nm was then measured after the solution had been allowed to stand in the dark at 37°C for 30 min. All experiments were conducted in triplicates. Ethanol was used as the blank control and Vc served as the positive control. The DPPH radical scavenging activities of the extracts and fractions were calculated according to the following formula:

$$\%\text{DPPH scavenging activity} = \frac{OD_{blank} - OD_{sample}}{OD_{blank}} \times 100\%$$

RESULTS AND DISCUSSION

Antitumor activities of the plant extracts

The antitumor activities of different extracts are presented in Tables 1 and 2. Table 1 indicates that all three extracts of *B. chinensis* exhibited significant antiproliferative activities against the PC3, Bcap-37, and BGC-823 cell lines at 200 µg/mL. However, only the ethyl acetate extract exhibited better antitumor activity at 50 µg/mL than the other two extracts. Thus, the ethyl acetate extract was further fractionated to yield 10 fractions. Table 2 indicates that the 10 isolated fractions of ethyl acetate extract also exhibited significant antitumor activities against the BGC-823 and Bcap-37 cell lines and weak to moderate activities against the PC3 cell line. Fraction 7 showed the best *in vitro* antiproliferation activities against the PC3, BGC-823, and Bcap-37 cell lines at 100 µg/ml, with inhibition rates at 82.2±3.4, 99.3±0.2 and 96.1±0.9%, respectively.

Antioxidant activities of the plant extracts

The antioxidant activities of different extracts are presented in Table 3, which reveals that only the ethyl acetate extract of *B. chinensis* exhibited moderate DPPH free radical scavenging activities. The results revealed that the ethyl acetate extract of the *B. chinensis* exhibited moderate DPPH radical scavenging capability, whereas the other two extracts did not show any significant activities. In addition, the antioxidant activity likely derives from the flavone or isoflavone ingredients contained in the ethyl acetate extracts, as proven via the positive results obtained in the chromogenic experiments with a 1% $FeCl_3$ ethanol solution.

Chinese herbs are typically used in crude extracts for disease treatment. This work demonstrated that the *in vitro* antitumor and antioxidant activities of *B. chinensis* roots against selected cell lines and DPPH radicals primarily derive from the ethyl extracts. To develop and utilize *B. chinensis* for further disease control, discovering the active ingredients of *B. chinensis* is necessary, and future research should focus on the investigation of the phytochemical constituents, as well as the molecular mechanisms, of the antitumor activities.

Table 1. Antitumor activity of different extracts of *B. chinensis*.

Extracts	Concentration (μg/ml)	Inhibition rate (%)		
		PC3	BGC-823	Bcap-37
Petroleum ether	50	27.1±5.2	36.7±7.3	43.1±6.1
	200	93.1±2.6	81.1±1.4	6.3±3,7
Ethyl acetate	50	81.2±3.1	83.1±5.2	76.4±4.6
	200	95.3±1.1	92.2±1.3	90.5±2.5
Methanol	50	65.7±7.8	53.8±3.1	51.6±5.1
	200	95.9±2.1	89.0±2.3	90.8±1.6
ADM	50	96.0±2.3	97.1±1.4	98.7±2.1

ADM, Adriamycin as the positive control; ±, represents the value of standard error.

Table 2. Antitumor activity of 10 isolated fractions of ethyl acetate extract.

Fractions	PC3 (%)	BGC-823 (%)	Bcap-37 (%)
1	65.8±5.3	57.6±6.7	49.1±4.7
2	65.8±6.1	94.1±2.1	98.4±1.3
3	51.5±2.5	94.9±1.6	95.0±1.0
4	6.6±7.1	92.5±2.1	87.4±1.9
5	30.7±7.3	94.8±1.2	78.4±4.1
6	63.6±8.2	98.5±0.5	86.7±4.4
7	82.2±3.4	99.3±0.2	96.1±0.9
8	5.2±4.1	30.4±5.9	52.6±3.5
9	25.5±6.2	97.8±0.7	98.6±0.4
10	49.3±5.5	67.9±8.3	42.4±6.7
ADM	96.4±1.3	97.0±1.3	98.1±0.5
DMSO	-	-	-

Results are expressed as inhibition rates at 100μg/ml; ADM as the positive control; DMSO as the negative control;-, no activity; ±,standard error.

Table 3. Antioxidant activities of different extracts of *B. chinensis*.

Extracts	Concentration (μg/ml)	Zone of scavenging rate (%)
Petroleum ether	25	14
	100	17
Ethyl acetate	25	75
	100	84
Methanol	25	28
	100	41
Vc	25	95

Vc: vitamin C, positive control.

Conclusion

This work provided preliminary information on the different extracts of *B. chinensis*. The petroleum ether, ethyl acetate, and methanol extracts exhibited high inhibition activities at 200 μg/ml against the PC3, BGC-823, and Bcap-37 cell lines, and fractions isolated from the ethyl acetate extract also exhibited high antitumor activity. Fraction 7, in particular, displayed an inhibition rate of 99.3±0.2 and 96.1±0.9% at 100 μg/ml against the proliferation of the BGC-823 and Bcap-37 cell lines. In addition, the ethyl acetate extract also exhibited moderate DPPH free radical scavenging capability, likely attributed to the flavone or isoflavone ingredients in the extracts. Further studies, which include the constituent isolation and purification of the extracts, as well as the bioassay and mechanism study of the constituents, are currently underway.

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REFERENCES

Ji WL, Qin MJ, Wang ZT (2001). Studies on the constituents of *Bealmacada chinensis* (I). J. Chin. Pharm. Univ. 32(3):197-200.

Jung SH, Lee S (2002). Isoflavonoids from the rhizomes of *Belamcanda chinensis* and their effects on aldose reductase and sorbitol accumuiation in streptozotocin induced diabetic rat tissues. Arch. Pharm. Res. 25(3):306-312.

Jung SH, Lee YS, Lee S (2003). Anti-angiogenic and anti-tumor activities of isoflavonoids from rhizomes of *Belamcanda chinensis*. Planta Med. 69(7):89-93.

Jung SH, Lee YS, Lim SS, Lee S, Shin KH, Kim YS (2004). Antioxidant activities of isoflavones from the rhizomes of *Belamcanda chinesis* on carbon tetra chloride induced hepatic injury rats. Arch. Pharm. Res. 27(2):184-188.

Liu JK, Hu L, Dong ZJ (2004). DPPH radical scavenging activity of ten natural *p*-terphenyl derivatives obtained from three edible mushrooms indigenous to China. Chem. Biodivers. 1:601-605.

Meng JH (2004). Research progress of *Bealmacada chinensis*. J. Hubei. Univ. TCM. 6(3):49-53.

Qin MJ, Ji WL, Liu J, Zhao J, Yu GD (2003). Scavenging effects on radicals of isflavones from *Belamcanda chinensis*. Chin. Tradit. Herbal. Drugs, 34(7):640-645.

Wang J, Liu XJ, Zhong Y (2007). General research of Blackberrylily Rhizome. Qilu. Pharm. Affairs 26(5):168-172.

Wu B, Zhu JS, Zhang Y, Shen WM, Zhang Q (2008). Predictive value of MTT assay as an *in vivo* chemosensitivity testing for gastric cancer: One institution's experience. World Gastroenterol. 14:3064-3068.

Zhang CZ, Wang SX, Zhang Y (2005). *In vitro* estrogenic activities of Chinese medical plants traditionally used for the management of menopausal symptoms. J. Ethnopharmacol. 98(3):295-300.