Effects of Different Fractions of *Baeckea frutescens* L. on Proliferation of Human Colon Cancer LOVO Cells *in Vitro*

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Abstract [**Objectives**] This study was conducted to investigate the inhibitory effects of different extraction fractions of *Baeckea frutescens* L. on the proliferation of human colon cancer LOVO cells *in vitro*. [**Methods**] Leaves were separated from the aerial part of *B. frutescens*, immersed in distilled water and treated by ultrasound for half an hour. The volatile oil in *B. frutescens* leaves was extracted by steam distillation. The leaves after removing the volatile oil were extracted by reflux condensation, and then the extract was concentrated into fluid extract. The organic solvent extraction method was applied to extract the fluid extract until the organic phase was nearly colorless, and the extracts were combined, and concentrated into fluid extract, which was then freeze-dried into powder for later use. A cytotoxicity experiment was carried out on human colon cancer LOVO cells with different extraction fractions of *B. frutescens* as test drugs. Cell activity was detected by MTT assay. [**Results**] The petroleum ether fraction and chloroform fraction of *B. frutescens* had no inhibitory effect on the proliferation of human intestinal cancer LOVO cells *in vitro*. The ethyl acetate fraction and n-butanol fraction of *B. frutescens* had inhibitory effects on the proliferation of human colon cancer LOVO cells *in vitro* in a dose-dependent manner. The IC_{50} of ethyl acetate was 398.94 μg/ml, and that of n-butanol was 617.02 μg/ml. [**Conclusions**] The ethyl acetate and n-butanol extraction fractions from *B. frutescens* have inhibitory effects on the proliferation of human colon cancer LOVO cells *in vitro* in a dose-dependent manner.

Key words Extract of *Baeckea frutescens* L.; Human colon cancer LOVO cell; Inhibition; Effect

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Herbal samples were collected from Rong County, Guangxi Province, and identified as a genuine product of *Baeckea frutescens* L. of Myrtaceae by Associate Professor Wang Xiaohua from Pharmacognosy Department of Guilin Medical University. The samples were kept in the Pharmacognosy Department of Guilin Medical University. Its leaves are cold in nature and taste bitter and pungent, and they are traditionally used for treating skin itching, scald and other symptoms, and show modern pharmacological effects such as anti-inflammatory and antibacterial. Studies have shown that phloroglucinol derivatives in the extract of *B. frutescens* can effectively inhibit the proliferation of lung cancer and pancreatic cancer cells. Moreover, cyclopentenone and furanone compounds in *B. frutescens* also showed inhibitory effects on the proliferation of human lung cancer, pancreatic cancer and breast cancer cells. The oil content of *B. frutescens* is low, but the extract has a high content, and demonstrates the value of further research. In this study, the anti-tumor effects of *B. frutescens* extracts on colon cancer LOVO cells were explored, aiming to to find its potential anti-tumor effects.

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Materials and Methods

Materials

Cell line Human colon cancer LOVO cell line (Wuhan Pricella Biotechnology Co., Ltd.).

Materials and instruments The samples were collected from Rong County, Guangxi Province, and identified as a genuine product of *B. frutescens* L. in Myrtaceae by Associate Professor Wang Xiaohua from the Pharmacognosy Department of Guilin Medical University. The samples were kept in Pharmacognosy Department of Guilin Medical University.

Rotary evaporator (N-1300D, Tokyo Rikakikai Co., Ltd.); electric heating and constant-temperature blast drying oven (DHG-9075A, Shanghai Yiheng Technology Instrument Co., Ltd.); cell incubator (Shanghai Eppendorf International Trade Co., Ltd.); clean bench (Suzhou Antai Airtech Co., Ltd.); numerical control constant-temperature water bath (HH-US-A, Shanghai Hetian Scientific Instrument Co., Ltd.); ultra-low temperature refrigerator (MDF-U73V, Matsushita Electric, Japan); precision balance (ML4002, METTLET Company, Germany); desktop ultracentrifuge (Eppendorf, Germany); inverted microscope (Shanghai Jumu Medical Equipment Co., Ltd.); grating type continuous-wavelength microplate reader (TECAN, Switzerland); micropipette (Eppendorf, Germany).

Reagents and drugs RPMI-1640 medium (gibco Company, USA); fetal bovine serum (Gemini, USA); trypsin-EDTA digestive juice, dimethyl sulfoxide (DMSO), penicillin streptomycin solution and MTT, all products were from Beijing Solarbio Science & Technology Co., Ltd.; phosphate buffer solution (Wuhan Pricella Biotechnology Co., Ltd.); 96-well cell culture plate and

culture bottle, all products were from JET BIOFIL; anhydrous ethanol (Guilin Bell Experimental Equipment Co., Ltd.); petroleum ether, chloroform, ethyl acetate and n-butanol (all products of Guilin Ruyi Biotechnology Co., Ltd., analytically pure).

Methods

Extraction and separation of drugs[9-11] Fresh aerial part of *B. frutescens* was taken to separate its leaves. The leaves of *B. frutescens* were immersed in distilled water and ultrasonically treated for 30 min. The volatile oil of *B. frutescens* leaves was extracted by steam distillation with the weight ratio of *B. frutescens* leaves to distilled water at 1∶10. After the extraction of volatile oil, the leaves of *B. frutescens* were dried for later use. Next, under the conditions of material-to-liquid ratio of 1∶10, 80% ethanol, and 80 ℃, the leaves were extracted by reflux condensation for three times, 4 h each time. The extracts were combined and concentrated into fluid extract. The liquid extract of *B. frutescens* leaves was dissolved with ultrapure water to obtain a water phase. Using the systematic solvent extraction method, the water phase was sequentially extracted with organic solvents such as petroleum ether, chloroform, ethyl acetate and water-saturated n-butanol with the volume ratio to extraction solvent at 1∶1 until the extraction solvent was nearly colorless. Each extraction solvent was combined and concentrated into fluid extract. Next, each fluid extract was freeze-dried into powder and stored in the refrigerator at 4 ℃ for later use. Fig. 1 shows the extraction, separation and purification process of different fractions of *B. frutescens*.

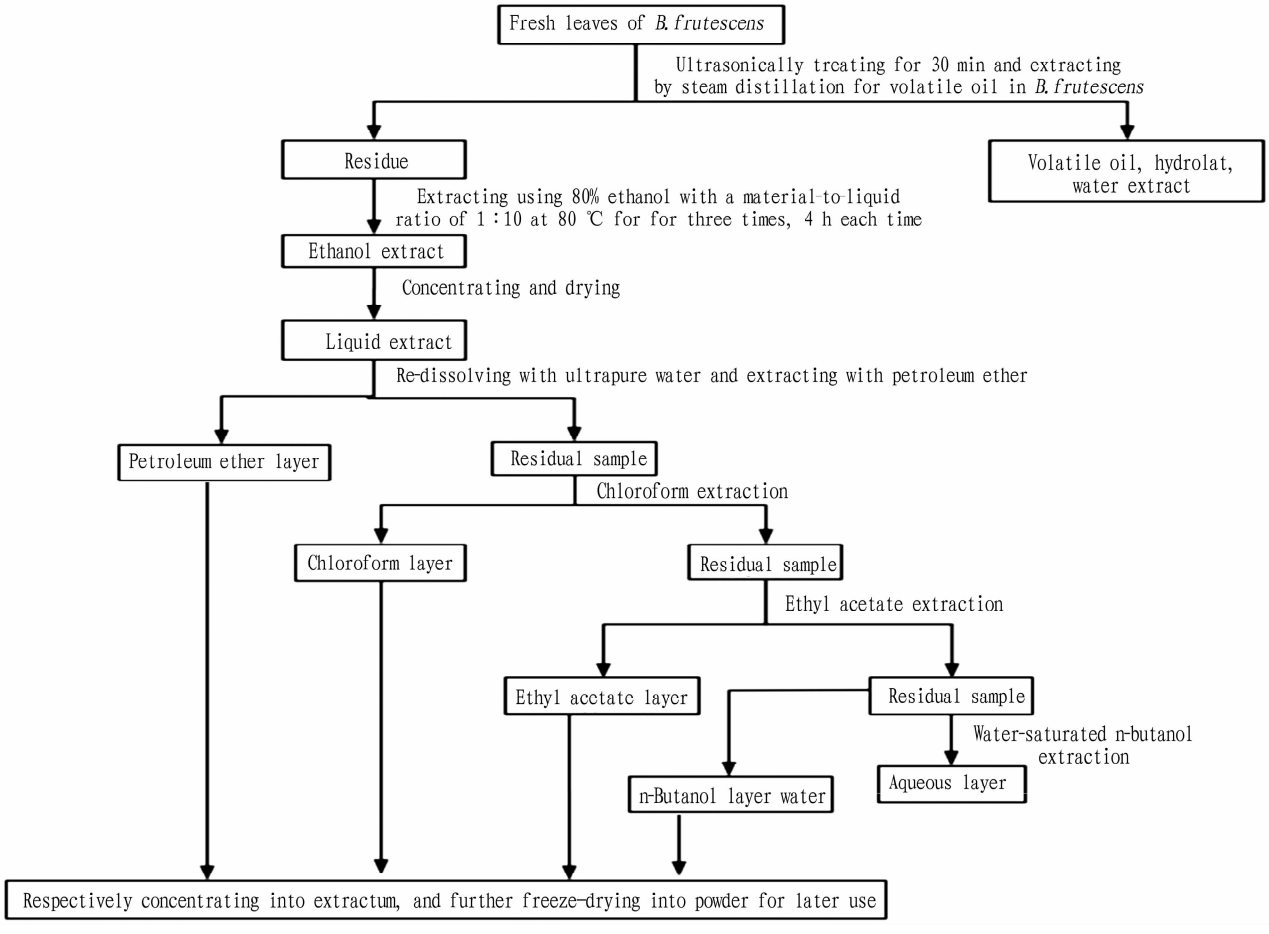

Fig. 1 Flow chart for extraction and separation of different fractions from *B. frutescens*

Preparation of solutions

(1) Preparation of RPMI-1640 complete medium: RPMI-1640 medium + 10% FBS + 1% bispecific antibody.

(2) Preparation of MTT solution: MTT powder (0.5 g/tube) was added with 100 ml of sterile PBS to prepare a 5 mg/ml MTT solution. Then, the solution was added into 1.5 ml brown centrifuge tubes according to 1.2 ml/tube and stored in a refrigerator at −20 ℃ in the dark.

(3) Preparation of cryoprotectant: The cryoprotectant was prepared using fetal bovine serum and DMSO at a ratio of 9∶1.

Cell culture[12-15]

(1) Cell resuscitation: A cell cryopreservation tube was taken out and shaken quickly in a constant temperature water bath at 37 ℃ until the cryopreserved liquid was completely dissolved. It was then centrifuged at 1 200 rpm for 5 min, and the supernatant was discarded. Next, 1 ml of RPMI-1640 complete medium was added to resuspend the cells, and the obtained liquid was mixed evenly by blowing and then transferred to culture bottles. Finally, 5 ml of RPMI-1640 medium was added to each culture bottle, which was put into an incubator for culture.

(2) Cell culture: The cells were cultured in RPMI-1640 complete culture medium and placed in an incubator with 5% CO_2 at 37 ℃. When the cells grew to a density of 50% – 60% of the bottom area of the culture bottle, the liquid was changed. In specific, a culture bottle was taken out, and the old culture medium was drawn out using a sterile pasteur pipette. Next, the cells were washed with 1 ml of sterile PBS for 2 – 3 times, and added with 5 ml of RPMI-1640 complete culture medium. When the cells grew well and reached 80% – 90%, they could be subcultured or inoculated to plates.

(3) Cell passage: When the cell density reached 80% – 90%, the old culture medium was discarded, and the cells were washed with 1 ml of sterile PBS for 2 – 3 times. Next, 1 ml of trypsin was added to the cell, which were then placed in an incubator for digestion for 4 – 5 min. Subsequently, RPMI-1640 complete culture medium was added to stop digestion, and blown with a sterile pasteur pipette to make the cells leave the Petri dish as much as possible. After centrifugation, the cells were re-suspended, and the culture medium was blown by sterile Pasteur pipette to make the adherent cells in a single cell suspension state. Finally, the cells were subcultured in new culture bottles according to the ratio of 1 ∶ 3.

(4) Cryopreservation of cells: When cells grew to the logarithmic growth stage, they could be cryopreserved. After taking out the culture bottle, the old culture medium was discarded, and the cells were washed with 1 ml of sterile PBS for 2 – 3 times, and then digested with trypsin for 4 – 5 min. Next, RPMI-1640 complete culture medium was added to stop digestion, and blown with a sterile pasteur pipette to make the cells leave the wall. Subsequently, the cells were transferred to a centrifuge tube, and centrifuged at 1 200 r/min for 5 min. After discarding the supernatant, the cells were added with the prepared cell cryoprotectant, and gently blown to prepare a single cell suspension, which was transferred into a cryopreservation tube, which was refrigerated in a refrigerator at 4 ℃ for 1 h and at – 20 ℃ for 2 h in turn, and finally at – 80 ℃.

(5) Cell counting: The cells in the logarithmic growth phase were selected, and after discarding the old culture medium, the cells was washed with 1 ml of sterile PBS for 2 – 3 times, and digested with trypsin for 4 – 5 min. When most cells fell off from the culture flask, RPMI-1640 medium was added to completely stop digestion. Next, the liquid was transferred into a 10 ml centrifuge, and centrifuged at 1 200 r/min for 5 min. After discarding the supernatant, 1 ml of RPMI-1640 medium was added, and gently blown to form a uniform single cell suspension. Subsequently, 50 μl of single cell suspension was taken out of the centrifuge tube and added to 450 μl of RPMI-1640 complete medium, which was blown to mix well, obtaining a liquid diluted by 10 times. Next, 10 μl of diluted single cell suspension was added into the groove on one side of a blood cell counting plate, which was then covered with a cover glass. Finally, cell counting was performed under an inverted microscope. The calculation formulas are as follows:

Concentration of cell suspension (cells/ml) = Average number of cells in four squares × Dilution times × 10^4

Total cell count = Concentration of cell suspension (cells/ml) × Total volume of cell suspension.

MTT test MTT has a trade name of thiazolyl blue tetrazolium. MTT method, that is, the method of detecting cell survival and growth by MTT, can be used for large-scale anti-tumor drug screening, cytotoxicity test and so on. The detection principle is that succinate dehydrogenase in mitochondria of living cells can reduce exogenous MTT to water-insoluble formazan crystals and deposit them in cells, while dead cells have no such function. Dimethyl sulfoxide (DMSO) can dissolve formazan in cells, and its light absorption value, which can indirectly reflect the number of living cells, can be measured at the wavelength of 490 nm by enzyme-linked immunosorbent assay (ELISA). In a certain range of cell count, the amount of formed MTT crystals is directly proportional to cell count.

(1) Plating: Cells in the logarithmic growth stage were selected and prepared into single cell suspension, which was diluted by 10 times for counting. The volumes of RPMI-1640 complete culture medium and single cell suspension required were calculated, and the cell density was adjusted to 1 × 10^5 cells/ml. Next, the cells were inoculated in 96-well plates according to 100 μl for each well, and 50 μl of sterile PBS was added to peripheral wells to reduce the evaporation of water in the culture medium and reduce the occurrence of edge effect. After the cells were inoculated in 96-well plates evenly, they were cultured in an incubator for 24 h to allow the cells adhere to the wall.

(2) Addition of drugs: A certain amount of various extraction fractions of *B. frutescens* were respectively weighed, and dissolved in DMSO to prepare the mother solutions of the extraction fractions of *B. frutescens* with a concentration of 300 mg/ml. Next, the mother solution of each extraction fraction was prepared into a working solution with a concentration of 1 000 g/ml with RPMI-1640 complete culture medium, and the working solution was diluted into five experimental groups with different gradient concentrations. Specifically, the petroleum ether fraction and chloroform fraction were prepared into concentration gradient groups with concentrations of 100, 300, 500, 700 and 900 μg/ml. The ethyl acetate fraction of *B. frutescens* was prepared into concentration gradient groups with concentrations of 100, 200, 500, 700 and 900 μg/ml, respectively. The n-butanol fraction of *B. frutescens* was prepared into concentration gradient groups with concentrations of 100, 300, 600, 800 and 1 000 μg/ml respectively. Each group was provided with five parallel replicate wells. After 24 h of cell culture, the cell culture plates was taken out, and after discarding the supernatant, 100 μl of culture medium containing corresponding extraction fractions of *B. frutescens* was added to each well. A DMSO control group and a blank group were also set up. The experimental groups were added with RPMI-1640 complete medium plus various extraction fractions of *B. frutescens*. The blank group was added with RPMI-1640 complete medium. The control group was added with DMSO at the same amount as the experimental group with the highest concentration and RPMI-1640 complete medium. The cells in 96-well plates were cultured in an incubator for 24 h to allow the test drugs to interact with the cells.

(3) Testing: After the cells were cultured for 24 h, the 96-well plates were taken out, and 20 μl of 5 mg/ml MTT solution was added to each well in the dark. The 96-well plates were

incubated in an incubator for 4 h. The culture medium was gently drawn from each well, and 150 μl of DMSO was added. After the formazan crystals were fully dissolved, the absorbance (OD value) of each well was measured at a wavelength of 490 nm using a microplate reader, and the original data were recorded. The survival rate (SR%) and the median inhibitory concentration (IC_{50}) of different extraction fractions of *B. frutescens* on the growth of human colon cancer LOVO cells were calculated using appropriate formulas.

SR% = (Average *OD* value of dosing group/Average *OD* value of blank group) × 100 %

Statistical method SPSS17.00 software was employed for statistical analysis of data. The experimental data of each group were expressed by $\bar{x} \pm s$. The comparison between groups was made by one-way ANOVA, and pairwise comparison was made by *t*-test, with $P < 0.05$ indicating statistical significance.

Results and Analysis

Extraction, separation and purification of *B. frutescens*

From 2 kg of fresh *B. frutescens* leaves, 8.082 9 g of volatile oil was extracted by steam distillation, and 780 g of alcohol extract was obtained. Next, 1.646 5 g of petroleum ether extract, 2.153 6 g of chloroform extract, 2.865 6 g of ethyl acetate extract, 2.740 1 g of n-butanol phase extract and 96.285 3 g of aqueous extract of *B. frutescens* were obtained from 120 g of alcohol extract of *B. frutescens* after extraction.

Effects of different fractions of *B. frutescens* on human colon cancer LOVO cells

After 24 h of treatment on human colon cancer LOVO cells, the dose-effect relationship of survival rate with various extraction fractions from *B. frutescens* at different concentrations are shown in Table 1, Table 2, Table 3 and Fig. 2. As shown in Table 1 and Fig. 2, the order of cytotoxicity of various extraction fractions from *B. frutescens* was ethyl acetate extraction fraction > n-butanol extraction fraction > chloroform extraction fraction > petroleum ether extraction fraction of *B. frutescens*, among which ethyl acetate and n-butanol extraction fractions of *B. frutescens* had cytotoxicity, but chloroform extraction fraction and petroleum ether extraction fraction of *B. frutescens* had no cytotoxicity. SPSS17.0 software was employed for statistical processing. The IC_{50} of the ethyl acetate fraction from *B. frutescens* was 398.94 μg/ml, and that of the n-butanol fraction from *B. frutescens* was 617.02 μg/ml.

Table 1 Cytotoxicity of petroleum ether and chloroform fractions of *B. frutescens* to LoVo ($\bar{x} \pm s$, $n = 5$)

Concentration	Extraction fraction		
μg/ml	Petroleum ether fraction	Chloroform fraction	IC_{50}
100	1.160 6 ± 0.023 9	1.052 0 ± 0.061 7	
300	1.214 0 ± 0.051 0	1.109 4 ± 0.086 0	
500	1.183 8 ± 0.069 0	1.109 0 ± 0.035 4	–
700	1.208 0 ± 0.059 9	1.105 8 ± 0.065 6	
900	1.174 0 ± 0.063 4	1.164 0 ± 0.082 4	
Blank	1.188 6 ± 0.047 3	1.195 8 ± 0.099 5	
DMSO	1.188 6 ± 0.047 3	1.195 8 ± 0.099 5	

Compared with the blank group, $P > 0.05$.

Table 2 Cytotoxicity of various ethyl acetate fractions from *B. frutescens* to LoVo ($\bar{x} \pm s$, $n = 5$)

Concentration // μg/ml	Extraction fraction	
	Ethyl acetate fraction	IC_{50}
100	1.050 8 ± 0.067 7	
200	0.875 0 ± 0.080 4 *	
500	0.474 5 ± 0.058 1 *	
700	0.033 9 ± 0.023 8 *	398.94
900	0.232 7 ± 0.021 0 *	
Blank	1.160 6 ± 0.023 9	
DMSO	1.160 6 ± 0.023 9	

Table 3 Cytotoxicity of n-butanol extract from *B. frutescens* to LoVo ($\bar{x} \pm s$, $n = 5$)

Concentration // μg/ml	Extraction fraction	
	n-Butanol extraction fraction	IC_{50}
100	1.007 8 ± 0.053 8	
300	0.886 0 ± 0.036 2 *	
600	0.647 6 ± 0.033 5 *	
800	0.486 2 ± 0.064 2 *	617.02
1 000	0.028 6 ± 0.031 9 *	
Blank	1.139 0 ± 0.069 0	
DMSO	1.139 0 ± 0.069 0	

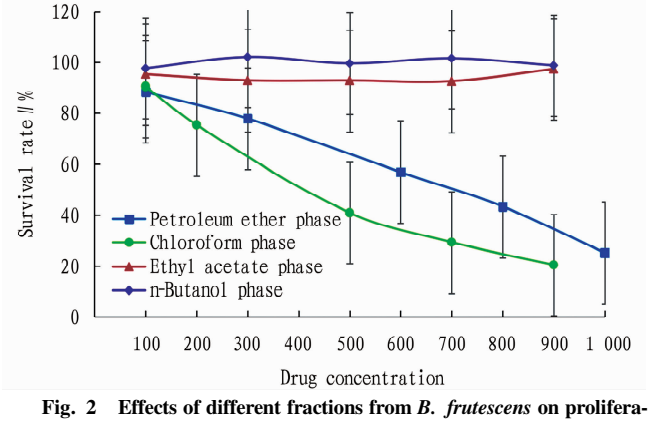

Fig. 2 Effects of different fractions from *B. frutescens* on proliferation of LoVo cells

Conclusions and Discussion

In this study, *B. frutescens* leaves were first extracted for volatile oil and then for alcohol extract, which was further extracted to get different extraction fractions as the tested drugs. The experiment of Shen *et al.* [8] showed that the branches and leaves of *B. frutescens* contained *B. frutescens* oil at a low content in the range of 0.9% – 1.8%. The branches and leaves of *B. frutescens* after extracting volatile oil or the water extract after extracting volatile oil by steam distillation have a large content, but are discarded, resulting in a waste of resources. This study proved that the ethyl acetate fraction and n-butanol fraction of *B. frutescens* had inhibitory effects on the growth of human colon cancer LOVO cells, which provides a basis for the development and utilization of the extract of *B. frutescens* after extracting volatile oil, which supports the rational use of natural resources. Ito *et al.* [5-7] isolated cyclopentenone and furanone derivatives and phloroglucinol derivatives

from B. frutescens and verified that they could inhibit the proliferation of tumor cells such as human lung cancer cells. According to the polarity table of organic solvents, the polarity of extraction solvents ranks as petroleum ether > chloroform > ethyl acetate > n-butanol. However, ethyl acetate and n-butanol are lipophilic organic solvents with low polarity, which are easy to dissolve flavonoids such as cyclopentenone and furanone derivatives and phloroglucinol derivatives, suggesting that the substances that inhibit the proliferation of human colon cancer LOVO cells in this study may be cyclopentenone and furanone derivatives and phloroglucinol derivatives.

In this study, it was found that ethyl acetate fraction and n-butanol fraction of *B. frutescens* could inhibit the proliferation of colon cancer cells, which provides a scientific basis for further research *in vivo*.

References

[1] Health Department of Guangxi Zhuang Autonomous Region. Standards of Chinese herbal medicines in Guangxi[M]. Nanning: Guangxi Science & Technology Publishing House, 1996. (in Chinese).

[2] State Administration of Traditional Chinese Medicine. Chinese materia medica[M]. Shanghai: Shanghai Scientific and Technical Literature Publishing House, 1998. (in Chinese).

[3] YU LF, ZHEN DD, QIU Q, *et al.* Research progress of *Baeckea frutescens* L. of Yao medicine[J]. Chinese Journal of Ethnomedicine and Ethnopharmacy, 2018, 27(14): 36 – 39. (in Chinese).

[4] LU WJ, LIU BM, YA QK, *et al.* General situation of research on traditional herbal medicine *Baeckea frutescens* L.[J]. Guangxi Medical Journal, 2008(10): 1517 – 1519. (in Chinese).

[5] ITO T, AKAINSA SK, NISA K, *et al.* Three new abietanetype diterpenoids from the leaves of Indonesian *Plectranthus scutellarioides*[J]. Fitoterapia, 2018 (127): 146 – 150.

[6] ITO T, NISA K, KODAMA T, *et al.* Two new cyclopentenones and a new furanone from *Baeckea frutescens* and their cytotoxicities[J]. Fitoterapia, 2016(112): 132 – 135.

[7] FUJIMOTO Y, USUI S, MAKINO M, *et al.* Phloroglucinols from *Baeckea frutescens*[J]. Phytochemistry, 1996, 41(3): 923 – 925.

[8] SHEN MY, HE ZH, LIU H. Study on chemical constituents of volatile oil from *Baeckea frutescens*[J]. Guangxi Forestry Science, 1993, 22(4): 157 – 158. (in Chinese).

[9] LI JJ, CHEN WG, LI XY, *et al.* Analysis on the quality and extraction efficiency of volatile oils obtained from dried and fresh *Baeckea frutescens* leaves by different treatments[J]. Flavour Fragrance Cosmetics, 2020 (6): 7 – 11. (in Chinese).

[10] ZHAO XH, SHENG JR, CHEN JH. Study on extraction of volatile oil from leaves and branches of Yao medicine *Baeckea frutescens* L. and β-cyclodextrin inclusion techniques[J]. Chinese Traditional Patent Medicine, 2009, 31(11): 1776 – 1778. (in Chinese).

[11] ZHOU JN, HOU JQ, FAN SY. Chemical constituents of the n-butanol extracts from the aerial parts of *Baeckea frutescents*[J]. Pharmaceutical and Clinical Research, 2017, 25(5): 389 – 392. (in Chinese).

[12] XU J, PAN YL. Adiponectin inhibits the proliferation and invasion of colorectal cancer LoVo cells[J]. Journal of Practical Oncology, 2021, 36(1): 33 – 36. (in Chinese).

[13] ZHANG MF, SHEN YQ. Research advances in pharmacologic effects of matrine and oxymatrine against large intestine carcinoma[J]. Drug Evaluation Research, 2020, 43(6): 1189 – 1196. (in Chinese).

[14] WANG ML. Preliminary study on chemical constituents of *Scutellaria barbata* and its anti-tumor mechanism[D]. Nanjing: Nanjing University of Chinese Medicine, 2020. (in Chinese).

[15] LIN LQ, LIN XH, HUANG LY, *et al.* Anti-tumor activity of extracts of *Bidens bipinnata* L. *in vitro*[J]. Journal of Fujian Medical University, 2010, 44(2): 83 – 85. (in Chinese).

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(Continued from page 88)

ecological environment management. The study aimed to provide reference and inspiration for comprehensive river management and landscape design, representing an active attempt to promote the healthy development of river construction in China.

References

[1] WANG ZC, SUN ZY. Ecological design of Beijing Chuanbei River landscape restoration[J]. Fengjing Yuanlin Gongcheng, 2014(10): 120 – 124. (in Chinese).

[2] YU KJ, ZHANG L, LIU YJ. The multifunctional approach toward waterfront landscape design: A case study of Cixi in Zhejiang Province[J]. Chinese Landscape Architecture, 2004(5): 28 – 32. (in Chinese).

[3] YI C. Restoration of urban riverside ecological landscape and its application[J]. Resources Economization & Environmental Protection, 2019 (6): 19. (in Chinese).

[4] YU ZJ. Exploration of low impact development: Landscape design of Daihe River waterfront green space in Qinhuangdao City, Hebei Province [J]. Xiandai Horticulture, 2019(12): 74 – 75. (in Chinese).

[5] SHAO CY. Study on river landscape design strategy under the concept of urban doble repair: A case study of Qihe River in Sishui[D]. Qingdao: Qingdao University of Technology, 2019. (in Chinese).

[6] CHEN J, HUANG L. Urban river comprehensive treatment and river ecological landscape[J]. Hydropower and New Energy, 2012(3): 75 – 78. (in Chinese).

[7] XU F. Study on river planning and design of integration of ecology, landscape and hydraulic engineering[D]. Fuzhou: Fujian Agriculture and Forestry University, 2011. (in Chinese).

[8] SONG W, ZHANG YP. Analysis on ecological landscape design and management of urban landscape rivers[J]. Science Technology Association Forum, 2011(10): 132. (in Chinese).

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