Comparison of antioxidant and anticancer of the extracts from Various Habitats of *Selaginella doederleinii*

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Abstract. In this study, aioxidant and anticancer activities of the extracts were evaluated from various habitats of *Selaginella doederleinii*. The evaluation on antioxidative capacity revealed that the extracts were the highest from Guizhou province using DPPH radical scavenging, ABTS radical scavenging, ferric reducing and FRAP methods, while those of the extracts from Sichuan province were the lowest from different habitats. The evaluation on antitumous effect showed that the extracts from Guizhou province were the highest against A549 cell line and 7721 cell line, while those of the extracts from Sichuan province were the lowest in different habitats. Results obtained revealed that the extracts of *S. doederleinii* were found to be potentially as a readily valuable bioactive source of natural products. Ethyl acetate extraction of *S. doederleinii* exhibited as the most potent fraction will be further in-depth study.

Introduction

Selaginella doederleinii Hieron, as a traditional Chinese medicine, is a well-known perennial pteridophyte plant growing in South and Southwestern China at the low altitude [1]. Anti-tumor effect is an important pharmacological activity of *S. doederleinii*.The herbs was commonly adopted to treat some major neoplastic disease in clinic, including chorionic carcinoma, malignant hydatidiform mole, nasopharyngeal carcinoma, esophageal cancer, gastric cancer, liver cancer, lung cancer, cervical cancer and so on [2].

Modern medicine, meanwhile, means that reactive oxygen species (ROS) that was produced in human body contributes to an array of normal physiology metabolism and clear excess free radicals at the same time under normal condition. But if the reaction mechanism was damaged, the excess free radicals will direct effect on the human body, leading to damage tissues and cells. Therefore, when the free radicals were excessive or human antioxidants and repair function were damaged, it can cause oxidative stress damage, producing various tumor cells [3]. In consequence, it has always been a research hot spot to explore natural antioxidant of plants (Abdille). Some research show that volatile oil, flavonoids and alkaloids all can be used as potential antioxidant [4].

At present, we had evaluated antitumor and antioxidant activities of extracts of *S. doederleinii* from various habits [5]. However, there is no other literatures on antitumor and antioxidant of different exacts of *S.doederleinii*. The aim of this reasearch is further to evaluate antioxidant and anticancer activities of different exacts from various habits, which provide theoretical basis for further comprehensive research of *S. doederleinii*.

Materials and methods

Chemicals and materials

No.1(collected at Guangxi Yulin herbal medicine market on 07/08/2012), No.2(collected at Guangxi Nanning herbal medicine market on 12/08/2012), No.3(collected at Guangxi Guilin herbal medicine market on 03/08/2012), No.4(collected at GuiZhou Simianshan herbal market on 21/08/2012), No.5(collected at GuiZhou Pulaochang herbal market on 25/08/2012), No.6(collected at GuiZhou Chishui herbal market on 26/08/2012), No.7(collected at Chongqing Wusheng herbal

market on 14/08/2012), No.8(collected at Chongqing Tongnan herbal market on 15/08/2012), No.9(collected at Sichuan Hehuachi herbal market on 17/08/2012) and No. 10(collected at Sichuan Chinese crude drμg company on 19/08/2012) were harvested from 10 habitats in China, and identified as *S. doederleinii* by vice-professor Zhang yu-jin from the department of Pharmacy in Zunyi medical college.

Human lung adenocarcinoma cell line (A549) and human liver cancer cell line (7721) were purchased from Shanghai cell bank of Chinese Academy of Sciences. 2,2-diphenyl-1-picrylhydrazyl(DPPH), 2,2'-azinobis- (3-ethylbenzthiazoline-6-sulphonate)(ABTS) and 1,3,5-tri(2- pyridyl)-2,4,6-triazine(TPTZ), 3-(4,5-dimethyl- thiazolyl-2)-2,5-diphenyltetrazolium bromide(MTT), Butylated hydroxytoluene (BHT), ascorbic acid and Cisplatin were purchased from Sigma Pure Chemical Industries (Berlin,Germany). RPMI-1640 culture was purchased from YuanLong biotechnology company (ShangHai, China). Distilled water was used throμghout and All other chemicals and solvents were used of analytical grade.

Preparation of the Extracts

10g of crushed herbs was weighed , grinded respectively from 10 habitats. And then the herbs were placed in the microwave-assisted extraction apparatus (XH-100A, 2450MHz, Xianghao, Microwave Instrument Co., Ltd. Beijing, China) , added 100mL methanol to the round bottom flask and extracted twice continuously under the extraction conditions of radiation for 3 hours and 600W microwave power. The extractive was concentrated with a rotary evaporator (RE-5000A, 1L, Yarong Scientific and Technology Company, Shanghai, China) at 50 ℃ to obtain methanol extracts. The output rate is 12.23%—18.71% .

Determination of total phenolic content

We adopt Folin-Ciocalteau method to measure total phenolic of different extract of dried *S. doederleinii*. 0.5 mL of extract solution was mixed well with 4mL distilled water, 1.5mL20%Na2CO3 and 0.5 mL Folin-Ciocalteu reagent. The mixed liquor was balanced for 2 h and masured absorbance at 765 nm with a UV-spectrophotometer (Shimadzu UV-Vis 160A, Tokyo, Japan). All determinations were carried out in triplicate. The concentration of phenolic compounds in the extracts was determined from gallic acid calibration curve. The total content of phenolic compounds in the extracts was expressed as gallic acid equivalents (GAE) mg/g of dry extract. Gallic acid (in the 100-600μg·mL^{-1}range) was used as standard substance.

ABTS radical-scavenging activity

The determination method of ABTS radical scavenging ability was subtly improved by reference[6]. 7mmol·L^{-1} ABTS solution was mixed well with 140 mmol·L^{-1} potassium persulfate water solution (ultimate concentration) and left standing overnight in the dark at room temperature to generate $ABTS^{+\cdot}$. The samples from 10 habitats was dissolved with methyl alcohol and was diluted to different concentrations sample (0.4, 0.8, 1.2, 1.6, 2.0 g·L^{-1}). 0.4 mL of each sample was mixed with 2.5 mL $ABTS^{+\cdot}$ solution, left standing for 10 min at room temperature and determined the absorbance of the sample at 734nm in a ultraviolet spectrophotometer. The scavenging rate can be calculated according to the following formula: $I(\%) = [(A_b - A_s)/A_b] \times 100\%$. Where I is the inhibition percentage, A_b is the absorbance of the blank sample and As is the absorbance of the test sample. The regression equation can be done based on the radical scavenging ability to calculate IC_{50} value of radicals scavenging. As positive control, IC_{50} was estimated for the synthetic antioxidant reagent BHT(in the 10-50μg·mL^{-1}range).

DPPH radical-scavenging activity

Determination of antioxidant activity was performed using the method[7]. 0.5 mL of each sample with different concentrations from 10 habitats was mingled with 2 mL DPPH ethanol solution(0.2mmol·L^{-1}) separately. The mixture was shaken hard, produced reaction and left standing at room temperature in the dark for 60 min. The absorbance was measured at 517 nm in the ultraviolet spectrophotometer. IC_{50} was calculated by plotting the inhibition percentage against different concentrations sample. BHT (in the 10-50μg·mL^{-1}range) was used as positive controls.

Evaluation for reducing power

The reducing power of the samples was measured using the method[8] with some modification.

1.2mL of the samples with different concentrations from 10 habitats were migled with 1.0mL phosphate buffer solution (PBS, 0.2mol·L^{-1}, pH6.6) and 1.0mL 1% potassium ferricyanide. The mixture was reacted for 20 min in 50℃ and quickly chilled. Afterwards, the mixture was mixed well with 1.0mL 10% trichloroacetic acid and 5mL distilled water, and then centrifμged at 3000r·min^{-1} for 10 min. 2.5mL of the supernate was mixed with 0.5 mL 0.1% ferric trichloride solution and 2 mL distilled water and left standing for 10 min. Absorbance (A) was measured at 700nm in the ultraviolet spectrophotometer and then the EC_{50} of reducing powers of the volatile oils was calculated. Ascorbic Acid (in the 20−100μg.mL^{-1}range) was used as reference compound.

Evaluation for ferric reducing antioxidant power

Antioxidant activity was evaluated using the method[9]. 0.5mL of the samples with different concentrations from 10 habitats were migled with 1.5 mL TPTZ solution (composed of 25mL acetate buffer solution(0.13 mol·L^{-1}), 2.5 mL TPTZ solution(10mmol·L^{-1}) and 2.5 mL FeCl3 solution(20 mmol·L^{-1}))and reacted at 37 ℃ for 10 min. The mixture was measured at 593 nm in the ultraviolet spectrophotometer. FRAP values were expressed as mmol Fe2+ per g of sample. Ascorbic acid (in the 20-100μg·mL^{-1}range) was used as a positive control.

Evaluation for anticancer activity

A549 cell line and 7721 cancer cell line were maintained in RPMI-1640 medium supplemented with 100 g·L^{-1} heat-inactivated (56℃) fetal bovine serum, 3 mM·L^{-1}glutamine, 100 mg·mL^{-1} streptomycin and 100 IU·mL^{-1} penicillin, and adjusted to pH 7.2 with bicarbonate solution. Cells were grown in a humidified atmosphere of 95% air/5% CO2 (v/v) at 37℃. 20μL of the samples with different concentrations (20, 40, 60 and 80 μgmL^{-1}) from 10 habitats were migled with PBS , added into each microwell and subcultured in mediums for 44 h. The mixture was mixed with 50 μL MTT solution (1mg·mL^{-1}, dissolved by PBS) in each microwell, then incubated for 4 h again. The supernate in the microwell was carefully sucted and removed from the culture medium. After wards, the mixture was mingled with 150 μL DMSO, shaked for 10 min to dissolve fully the methanol crystal. Absorbance of the sample was measured at 570nm in the enzyme-linked immunosorbent assay tester (DG5033A, Huadong electronics co., Nanjing, China). IC50 of the essential oil of each habitats was calculated by plotting the inhibition percentage against different concentrations sample. Cisplatin(in the 5-30μg.mL^{-1}range) was used as a positive control.

Statistical analyses

In order to verify the statistical significance, all analyses were carried out in triplicate and results were expressed as mean ± SD. In the assessment of the antitumor and antioxidant activity, pearson correlation coefficients (R^2) were adopted. Linear regressions were also calculated.

Results

Total phenolic analysis

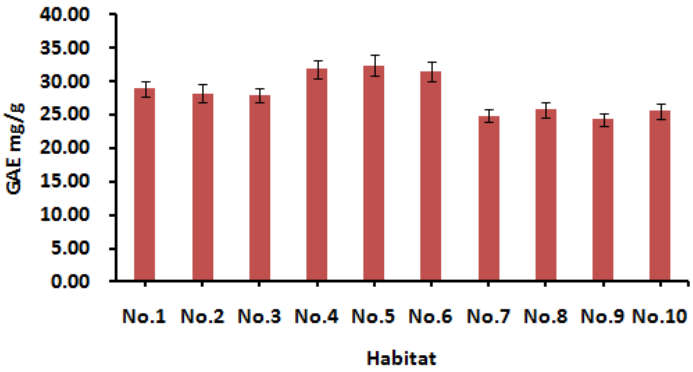

Fig1. Total phenolic content of different extracts from 10 habitatats.

EAE showed a strong presence of phenols in the ferric chloride test among dirrerent extracts, followed BEE and PEE. *S. doederleinii* from No.5 habitat has the most phenolics (32.40±1.49μg·mL^{-1}). While *S. doederleinii* from No.6 habitat has the least phenolics (24.89±0.99μg·mL^{-1}).

Antioxidant activity

It could be showed from Fig.2. that among various habitats via DPPH+·, ABTS+·, reducing power and FRAP the best antioxidant ability was from No.6 habitat(32.24±3.23μg·mL^{-1}), No.5: habitat(53.32±4.16μg·mL^{-1}), No.4 habitat(55.94±4.81μg·mL^{-1}) and No.5 habitat(7836.24±218.52μmol/g), respectively. Morever, The worst antioxidant ability was from No.7 habitat(89.32±6.19μg·mL^{-1}), No.10 habitat(104.56±10.27μg·mL^{-1}), No.10 habitat(116.57±12.93μg·mL^{-1}) and No.8 habitat(6579.62±213.44μmol/g), respectively.

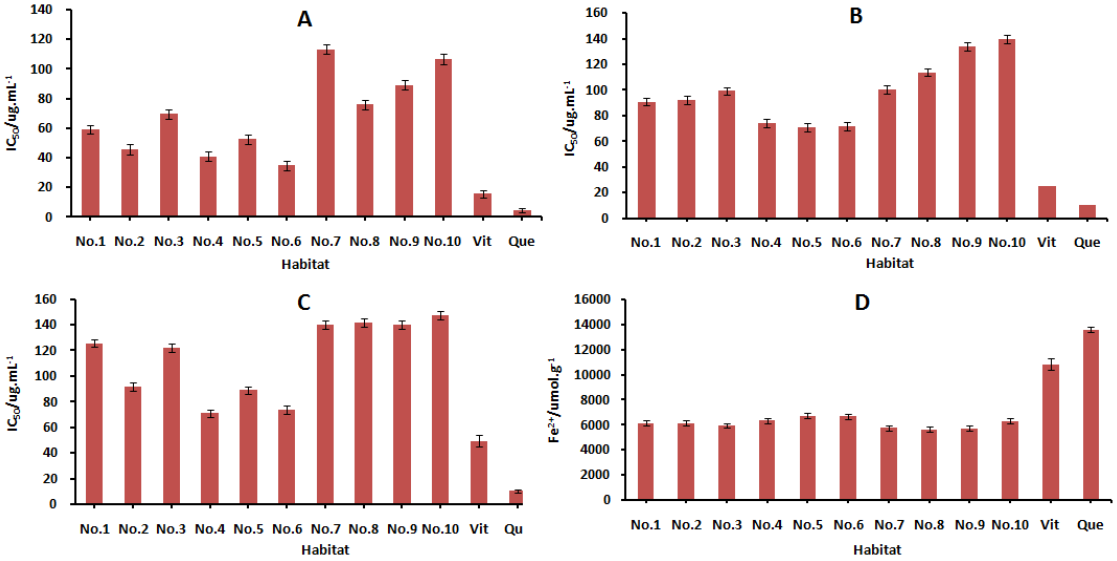

Fig.2. Comparison of antioxidant of the extracts from 10 habitats. A: ABTS radical scavenging ability; B: DPPH radical scavenging ability; C: reducing power; D: ferric reducing antioxidant power.

Anticancer activity

It could be found from Fig.2. that the most effective essential oil against A-549 cell line and 7721 cell line was from No.6 habitat (46.81±3.23μg·mL^{-1}) and No.5 habitat (34.02±2.58μg·mL^{-1}) , respectively. In addition, the most ineffective essential oil was from No.10 habitat (143.31±5.62μg·mL^{-1}) and No.9 habitat (106.64±3.97μg·mL^{-1}) , respectively. But they were less potent than the pure compounds used as positive controls, namely the antitumor of wide spectrum Cisplatin against A-549 cell line and 7721 cell line (20.89±2.67 μg·mL^{-1}, 6.27±1.53μg·mL^{-1}, respectively).

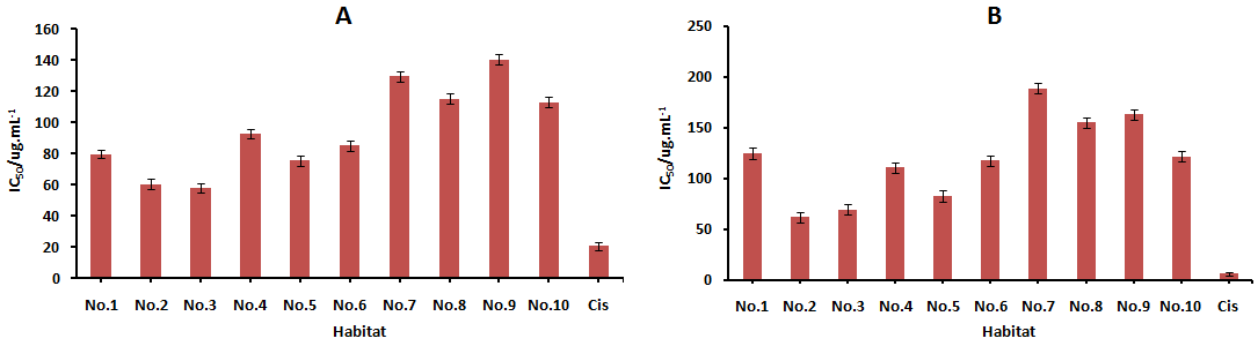

Fig.3. Comparison of anticancer of the extracts from 10 habitats. A: human lung cancer cell line (A-549); B: human liver cancer cell line (7721).

Discussions

As shown in Fig.2., the extracts from *S. doederleinii* in Guizhou province had relatively stronger anti-oxidative capacity, while the extracts from *S. doederleinii* in Sichuan province and Chongqing municipality had relatively weaker anti-oxidative capacity. This activity of the extracts has been attributed likely to their content of phenolic, especially amentoflavone, hinokiflavone and

robustaflavone, and the strong antioxidant activity of these compounds is well established. The high content of total hydroxybenzene in Guizhou province therefore likely explains their strong antioxidant activity compared with extracts from other habitats.

Similarly, the extracts from *S. doederleinii* in Guizhou province had relatively stronger anticancer capacity, and the extracts from *S. doederleinii* in Sichuan province and Chongqing municipality had relatively weaker anticancer capacity against 7721 cells and A549 cells. The main bioactive substances of the extracts were mostly phenolic in *S. doederleinii*. Amentoflavone can not only effectively inhibit a wide variety of tumor cell proliferation but also induced tumor cell apoptosis and differentiation. Hinokiflavone can strongly induced the activity of glutathione peptide transferase in mice liver and small intestine[10]. Moreover, the high content of total hydroxybenzene in Guizhou province therefore likely explains their strong ancancer activity compared with the extracts from other habitats.

The antioxidant and antineoplastic activity of the extracts was great differences between different habitats. It is conjectured that such difference maight be put down to harvest seasons of the herb ,different areas, different processing technology of Chinese herbal pieces and so on.

Conclusions

S. doederleinii is extensively growing in South China and is of very high medical value. However the researches on *S. doederleinii* is currently very limited, especially there has been no systematic pharmacological studies, resulting in lack of theoretical supports to its clinical application. The study in this paper is of certain reference values for further development about utilization of S. doederleinii resources and its application in food and medical domains.

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