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# 叉毛蓬化学成分的体外抗菌及抗氧化活性研究

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**摘 要**:从叉毛蓬全株首次分离出4-羟基苯乙酮(**1**)、紫丁香酸(**2**)、金圣草黄素(**3**)、香草酸(**4**)、4-羟基-3-甲基苯乙醇(**5**)、*N*-[2-(3,4-二羟基苯基)-2-羟基乙基]-3-(4-甲氧基苯基)丙-2-稀酰胺(**6**)和异鼠李素-3-*O*-芸香糖苷(**7**)7个化合物。通过MTT法测定这7个化合物的体外抗菌活性,结果表明多数化合物有较强的抗菌活性,其中化合物**2**对枯草芽孢杆菌,化合物**5**对大肠杆菌,化合物**7**对番茄疮痂病菌和番茄早疫病菌的抑制作用均强于阳性对照硫酸链霉素对同种菌的抑制。用DPPH和FRAP两种方法测定了化合物的抗氧化活性,结果表明在DPPH方法中化合物**6**的抗氧化活性最好,$IC_{50}$值为0.2452 mg/mL,在FRAP方法中化合物**5**有最好的抗氧化活性,FRAP值为9.402 mmol/g,强于阳性对照抗坏血酸。

**关键词**:叉毛蓬;抗菌;抗氧化;MTT;DPPH;FRAP

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# Antibacterial and Antioxidant Properties of Compounds Extracted from *Petrosimonia sibirica* L.

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**Abstract**: 4-Hydroxyacetophenone (**1**), syringic acid (**2**), chrysoeriol (**3**), vanillic acid (**4**), 4-hydroxy-3-methoxy benzene ethanol (**5**), *N*-[2-(3,4-dihydroxyphenyl)-2-hydroxyethyl]-3-(4-methoxyphenyl) prop-2-enamide (**6**) and isorhamnetin-3-*O*-rutinoside (**7**) were isolated from *Petrosimonia sibirica* L. for the first time. *In vitro* antibacterial activity of these compounds was evaluated by MTT assay. The results revealed that most of these compounds exhibited significant activity. The antimicrobial activities of compound **2** against *Bacillus subtilis*, of compound **5** against *Escherichia coli*, and of compound **7** against *Xanthomonas vesicatoria* and *Alternaria solani*, were found to be stronger than those of the positive control, streptomycin sulfate. The antioxidant potential was evaluated by DPPH and FRAP methods. The results demonstrated that compound **6** was the most effective in DPPH method, with $IC_{50}$ value of 0.2452 mg/mL. Compound **5** was relatively active and showed potent antioxidant effect compared to standard ascorbic acid in FRAP assay, with FRAP value of 9.402 mmol/g.

**Key words**: *Petrosimonia sibirica* L.; antibacterial; antioxidant; MTT; DPPH; FRAP

## Introduction

*Petrosimonia sibirica* L. (Genus *Petrosimonia*, Family Chenopodiaceae) is an annual herbaceous plant, which grows mostly in arid and saline areas[1]. In China, it is found only in Xinjiang[2]. The harsh environment imparts some specific biological features to it; therefore, in future it could be used as an important source of therapeutic agent exhibiting significant antibacterial activity. Currently, it is used in desert area as sand fixing plant providing substantial forage yields[3]. The research on it is limited only to its geographical distribution and ecological study[4,5], and its chemical composition and biological activity have not been reported yet.

The results of our preliminary experiments showed that the crude extracts exhibited commendable antibacterial and antioxidant properties[6,7]. Therefore, to better illustrate the biological activity of this plant, extensive research was carried out to study its chemical composi-

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tion and activity. In this study, compounds extracted from *Petrosimonia sibirica* L. were evaluated for antibacterial and antioxidant activities with the objective of obtaining more effective antibacterial agent and an effective potential source of natural antioxidant that might help in preventing various oxidative stresses.

## Materials and Methods

### Materials and reagents

The plant material was collected in September 2013 from Manasi, Xinjiang, China. It was identified by Professor Yan Ping (School of Life Science) of Shihezi University. A voucher specimen was deposited in the laboratory of the College of Chemistry and Chemical Engineering, Shihezi University, China.

Five bacterial species: *Escherichia coli*, *Soil agrobacteria*, *Pseudomonas syringaw pv. tomato*, *Xanthomonas vesicatoria* and *Bacillus subtilis*, and one fungal strain, *Alternaria solani* were used in this study. 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid (AA) and *tert*-butyl hydroquinone (TBHQ) were purchased from Sigma Aldrich; 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) was purchased from Aladdin Technology Corporation; and streptomycin sulfate was purchased from Hubei Xingyinhe Chemical Co. Ltd.. All chemicals were of analytical grade.

### Sample preparation and analysis

Ethanol was used to extract the sample from 10 kg of dried and powdered plant material, and then the enthanol extract was partitioned by liquid-liquid chromatography to obtain 4 fractions: PE, $CHCl_3$, EtOAc, and n-BuOH. 4-hydroxyacetophenone (**1**), syringic acid (**2**), and chrysoeriol (**3**) were isolated from $CHCl_3$ extract; vanillic acid (**4**), 4-hydroxy-3-methoxy benzene ethanol (**5**), *N*-[2-(3,4-dihydroxyphenyl)-2-hydroxyethyl]-3-(4-methoxyphenyl) prop-2-enamide (**6**) and isorhamnetin-3-*O*-rutinoside (**7**) were isolated from EtOAc extract. All the compounds were isolated and purified for the first time by TLC, column chromatography on silica gel, Sephadex LH-20, and identified by NMR and ESI-MS[8].

### Antibacterial assay

The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the median inhibition concentration ($IC_{50}$), using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay[9]. The concentrations of the samples were set at 50, 100, 200, 300, 400, and 500 μg/mL.

Minimum inhibitory concentrations (MIC): The 96 micro well plates were used, and 90 μL of liquid consisting of bacteria containing $10^6$ colony forming unit (CFU) microbial cells and 10 μL of tested samples were added into each well. The cells were incubated for 24 h at 28 ℃, subsequently, 10 μL MTT (5 mg/mL) was added into the wells, and the cells were further incubated at 28 ℃ for 4 h. Sample concentration without violet sediment was MIC. The values obtained from the assay were listed in Table 1.

Median inhibition concentration ($IC_{50}$): Similar to the above mentioned MIC method, the bacterial cells were incubated for 28 h at 28 ℃. The cells were then centrifuged (5,000 rpm, 5 min), clear liquid was removed, 200 μL of dimethyl sulfoxide (DMSO) was added, oscillated until violet substance dissolved completely, and recentrifuged (5,000 rpm, 5 min). The supernatant fluid was drained, 100 μL was transferred to 5 mL centrifuge tube followed by the addition of 1.9 mL DMSO. The contents were shaken well and then monitored by measuring the absorbance at 590 nm. The percentage inhibition by monomer compounds was calculated from mean values by using the following formula (1):

$$\text{Percentage inhibition} = [(A_m - A_n) \div A_m] \times 100\% \quad (1)$$

In which, $A_m$: absorbance values of the solvent compared; An: absorbance values of the sample.

Testing sample concentration logarithm (X), bacteriostatic rate conversion into biological statistical probability values (Y), formed a straight line for virulence regression equation ($Y = aX + b$) and value of $IC_{50}$ was calculated. The values were listed in Table 1.

### Antioxidant assay

*Ferric ion reducing antioxidant power (FRAP) assay*

The FRAP assay was performed according to the procedure reported by Yin[10]. Standard solution of ferrous sulfate ($FeSO_4$, 0.1 mL) with different concentrations, FRAP liquid (3 mL) and distilled water (0.3 mL) were taken in each tube. The reaction mixtures were

**Table 1 *In vitro* antibacterial activities of the isolated compounds and positive control (mg/mL)**

| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | S. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ | MIC | $IC_{50}$ |
| A | 0.4 | 0.307 | 0.3 | 0.259 | 0.4 | 0.321 | 0.1 | 0.059 | 0.05 | 0.034 | – | – | – | – | 0.1 | 0.046 |
| B | – | – | 0.4 | 0.291 | 0.4 | 0.339 | 0.5 | 0.359 | 0.4 | 0.352 | – | – | 0.4 | 0.350 | 0.2 | 0.095 |
| C | 0.4 | 0.202 | 0.2 | 0.155 | 0.4 | 0.227 | 0.4 | 0.296 | – | – | 0.4 | 0.264 | 0.2 | 0.133 | 0.2 | – |
| D | 0.4 | 0.348 | – | – | 0.2 | 0.164 | 0.2 | 0.164 | – | – | 0.3 | 0.209 | 0.2 | 0.105 | 0.2 | 0.159 |
| E | 0.4 | 0.375 | 0.05 | 0.022 | 0.3 | 0.271 | – | – | – | – | – | – | – | – | 0.1 | 0.082 |
| F | 0.5 | 0.519 | 0.2 | 0.071 | – | – | – | – | – | – | 0.2 | 0.110 | 0.1 | 0.055 | 0.2 | 0.185 |

Note: 'A' *Escherichia coli*; 'B' *Soil agrobacteria*; 'C' *Pseudomonas syringaw pv. tomato*; 'D' *Xanthomonas vesicatoria*; 'E' *Bacillus subtilis*; 'F' *Alternaria solani*; 'S.' Streptomycin sulfate; '-' no inhibitory activity in the respective concentration.

then incubated at 37 ℃ for 30 min, and monitored by measuring the absorbance at 593 nm. The linear regression equation: $y = 0.702 + 0.084x$, $R^2 = 0.9942$ was used to obtain the results. Value of $IC_{50}$ was calculated based on the sample concentration and scavenging rate. AA and TBHQ were used as positive control. The corresponding values were listed in Table 2.

**Table 2 The FRAP (mmol/g) of the isolated compounds**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | AA | TBHQ |
|---|---|---|---|---|---|---|---|---|---|
| FRAP | 0.282 | 4.610 | 1.309 | 1.596 | 9.402 | 4.925 | 1.059 | 7.945 | 17.464 |

*DPPH free radical scavenging assay*

Scavenging effect of seven compounds on DPPH radicals was analyzed by a modified method utilized by Wu[11]. *In vitro* experiments were performed. Samples with different concentration (0.1 mL) and DPPH (3 mL, 0.1 mmol/L) were mixed together and allowed to react for 30 min; the progress was monitored by measuring absorbance at 517 nm. AA and TBHQ were used as positive control. The scavenging ability was calculated by using the formula (1). The values were listed in Table 3.

**Table 3 DPPH radical scavenging activities of the isolated compounds (mg/mL)**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | AA | TBHQ |
|---|---|---|---|---|---|---|---|---|---|
| $IC_{50}$ | 7.2266 | 0.2927 | 1.0005 | 1.1263 | 0.2964 | 0.2452 | 2.5776 | 0.0896 | 0.0660 |

## Results and Discussion

In the antibacterial test, most of the isolated compounds strongly inhibited the growth of microorganisms. The values corresponding to the *in vitro* antibacterial activity of the isolated compounds were listed in Table 1. 4-Hydroxyacetophenone, syringic acid and chrysoeriol exhibited inhibitory activity on most of selected bacterial strains. Syringic acid exhibited the strongest inhibitory effect on *B. subtilis*, with MIC value of 0.05 mg/mL, and $IC_{50}$ value of 0.022 mg/mL; It was found to be more effective than the positive control with the value of MIC as 0.1 mg/mL, and $IC_{50}$ as 0.082 mg/mL. The above mentioned compounds strongly inhibited *E. coli*. *N*-[2-(3, 4-dihydroxyphenyl)-2-hydroxylethyl]-3-(4methoxyphenyl) prop-2-enamide and isorhamnetin-3-*O*-rutinoside did not exhibit significant activity. Moreover, they were not active against all the bacterial strains employed in this study (Table 1). However, *N*-[2-(3, 4-dihydroxyphenyl)-2-hydroxethyl]-3-(4-methoxyphenyl) prop-2-enamide exhibited effective inhibition against the fungal strain *A. solani*, for which the value of MIC was 0.2 mg/mL and $IC_{50}$ was 0.110 mg/mL. Isorhamnetin-3-*O*-rutinoside exhibited the best inhibitory activity against *P. syringaw pv. tomato*, *X. vesicatoria* and *A. solani*. The MIC values were 0.2, 0.2 and 0.1 mg/mL, respectively and the $IC_{50}$ were 0.133, 0.105, and 0.055 mg/mL, respectively, which were stronger

than that of Streptomycin sulphate (0.159 and 0.185 mg/mL, respectively).

In the antioxidant test, most of the compounds exhibited powerful antioxidant potential. The values obtained by the two testing methods were listed in Table 2 and Table 3, respectively. 4-hydroxy-3-methoxy benzene ethanol was relatively more active than standard AA in FRAP assay, with FRAP value of 9.402 mmol/g. *N*-[2-(3, 4-dihydroxyphenyl)-2-hydroxyethyl]-3-(4-meth oxyphenyl) prop-2-enamide was found to be the most effective in DPPH method, with $IC_{50}$ value of 0.2452 mg/mL.

## Conclusions

This study indicated that the compounds isolated from *P. sibirica* can be exploited as ideal, inexpensive and environmentally acceptable agrochemicals for future plant disease management program. Moreover, these compounds can also be used in the treatment of various diseases caused by *E. coli*. The experimental presented in this study provided a scientific support for the further study of *P. sibirica*.

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