Determining Total Phenolic Content of *Paeonia sinjiangensis* K.Y. Pan and its Antimicrobial Activity Grown in Xinjiang, China

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*Paeonia sinjiangensis* K. Y. Pan is a perennial herb belonging to the family Ranunculaceae which is one of the most important crude drugs in traditional Chinese medicine, used as an anti-inflammatory, analgesic and sedative agent. This paper deals with the detailed total phenolic content of the crude drug *Paeonia sinjiangensis* K.Y. Pan, from Xinjiang, China. Meanwhile, it tested for antimicrobial activity in this study. The total phenolic content of *Paeonia sinjiangensis* K.Y. Pan was 9.58 ± 1.03 mg QE/g dry wt. The microscopic characteristics were investigated, which offer data to differentiate the drug from its other species. It showed strong inhibition against *Blastomyces albicans* and possessed considerable activity against *Staphylococcus aureus* and *Escherichia coli*.

**Key words:** Antimicrobial activity, *Paeonia sinjiangensis* K.Y. Pan, phenolics.
papers have been published about the study of content of *paeoniflorin* in extraction technology of radix paeoniae rubra. (Yu et al., 2008) In recent years, our research group has studied the contents of paeoniflorin by rapid resolution liquid chromatography and polysaccharide with orthogonal test design from *P. sinjiangensis* K.Y. Pan (Zhou. et al., 2011; Tian. et al., 2011). Meanwhile, we have studied the pharmacognostical evaluation of the crude drug *P. sinjiangensis* K. Y. Pan. (Gong. et al., 2012). The other studies concern on the contents of paeoniflorin from Radix paeoniae rubra (Yuanyuan et al., 2008; Quan et al., 2007; Zhenhua et al., 2008; Xu et al., 2008).

In spite of the numerous medicinal uses attribute to this plant, the total phenolic content information and antimicrobial activity about *P. sinjiangensis* K.Y. Pan in Xinjiang of China has not been published.

Hence, the present investigation is an attempt in this direction, determination of total phenolic content by the modified Folin-Ciocalteu method and its antimicrobial activity.

**MATERIALS AND METHODS**

**Plant materials**

The study was conducted with plants were collected in October 2010, locally from the Altai mountain area of Xinjiang, China. The voucher specimen was authenticated as *P. sinjiangensis* K.Y. Pan by Yonghe Li, a chief apothecary of the Traditional Chinese Medicine Hospital of Xinjiang and accessioned into the herbarium of Traditional Chinese Medicine Ethnical Herbs Specimen Museum of Xinjiang Medical University for future reference (the voucher specimen number: 2010-356.)

**Reagents**

**Solvents**

Folin-Ciocalteu phenol reagent, petroleum ether, chloroform, ethanol (95%), methanol; Reagents: ammonia, iodine, ferric chloride, acetic, nitric, sulfuric, silicowolframic, and hydrochloric acid, bromocresol green, β-naphthol, ninhydrin, gelatin, and so on, were purchased from Tianjin Fu-Yu Meticulous Chemical Reagent Company, China.

**Test organisms**

Organisms such as *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) were used for study. The organisms were maintained by serial sub-culturing every month on nutrient agar slants and incubating at 37° for 18-24 hours. The cultures were stored under refrigerated condition. The antifungal activity was tested against Blastomyces albicans (ATCC 10231), Penicillin (Zhongnuo Pharmaceutical Institute Company, H13021634), Gentamycin Sulfate Injection (Zhengzhou Linrui Pharmaceutical Co. Ltd, H41020318), Fluconazole (Tianjin Pharmaceutical Group Xinzheng Co. Ltd, 100108) were served as positive control to determine the sensitivity of *Staphylococcus aureus*, *Escherichia coli*, Blastomyces albicans, respectively.

**Determination of total phenols**

For the total phenols analyses, harvested plant samples were collected in room temperature. Total phenols content in the ethanol extract was determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). An aliquot of extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 1.5 ml of sodium carbonate (20 %). The tubes were vortexed for 20 sec and allowed to stand for 10 min at 75° for color development. Absorbance was then measured at 760 nm using UV-VIS spectrophotometer. The amount of total polyphenols in the extract was calculated from the calibration curve in terms of gallic acid equivalents (y=0.09221+137.25x, R=0.999).

**Test for antibacterial activity**

Antibacterial activity of total phenols from *P. sinjiangensis* K.Y. Pan were studied against two bacterial strains viz. *Staphylococcus aureus*, *Escherichia coli*. A macrodilution broth susceptibility assay was used, as recommended by NCCLS (NCCLS, 1999) and described in Experiment technique of medical microbiology (Guan et al., 2006). The samples were added aseptically to sterile melted Mueller Hinton Broth medium and determined MIC and MBC (Minimum Inhibitory Concentration and maximum bactericidal concentration), standard reference antibiotics (penicillin, gentamycin) were used as positive control.

All tests were performed in Mueller Hinton Broth and performed in triplicate.

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Test for antifungal activity

The antifungal activity of total phenols from *P. sinjiangensis* K.Y. Pan against fungal isolates (Blastomyces albicans) was evaluated using the broth dilution method. The total phenols were added aseptically to sterile melted Sabouraud’s Borth medium and Fluconazole was used as a reference antifungal drug. MIC value was determined as the lowest concentration of total phenols was absence of growth was recorded. Each test in this study was repeated triplicate and performed in Sabouraud’s Borth.

Microscopic studies

Microscopic studies were done by transferring the plants to powder (# 60). Observe powder features of hand sample slides (State pharmacopeia committee of china, 2010).

Data were analysed using SAS software and procedure (Cary, 2005).

RESULTS AND DISCUSSIONS

Total phenolic content was 9.58±1.03 mg QE/g dry wt. It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and also in providing health beneficial effects.

As can be seen in Table 1-2, the total phenolic of *P. sinjiangensis* K.Y. Pan were found to have moderate antimicrobial activity. The results of MIC and MBC values indicated that it has strong inhibition against *Blastomyces albicans* and considerable activity against *Staphylococcus aureus* and *Escherichia coli*, compared with corresponding positive control.

In conclusion, the present study on pharmacognostical characters, total polyphenol content and antimicrobial activity of *P. sinjiangensis* K.Y. Pan may be useful to supplement information in regard to its identification.

The powder microscopy of the plant revealed the presence of fiber, non- glandular hairs, pollen grain, catheter, stomata, glandular scales and hairs, palisade cells (Fig. 1)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Penicillin MIC&lt;sup&gt;a&lt;/sup&gt; / MBC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Gentamycin MIC&lt;sup&gt;a&lt;/sup&gt; / MBC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fluconazole MIC&lt;sup&gt;a&lt;/sup&gt; / MBC&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.03 / 0.06</td>
<td>0.031 / 0.061</td>
<td>25 / 50</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Blastomyces albicans</em></td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Values given as mg·ml<sup>-1</sup>

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total phenolic from <em>P. sinjiangensis</em> K.Y. Pan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sup&gt;a&lt;/sup&gt; / MBC&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>16.254 / 32.508</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>16.736 / 32.647</td>
</tr>
<tr>
<td><em>Blastomyces albicans</em></td>
<td>4.064 / 8.127</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values given as mg·ml<sup>-1</sup>
Fig. 1. Powder microscopy

A) Fiber  B) Non-glandular hairs
C) Pollen grain  D) Catheter
E) stomata  F) palisade cells
ACKNOWLEDGMENTS

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REFERENCES

11. NCCLS (National Committee for Clinical Laboratory Standards), Performance Standards for Antibacterial Susceptibility Testing (9th International Supplement), M 100-S9, 1999.