Quantitative determination of active constituents in compound *Yuping feng* powder and their anti-asthma effects

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To establish a HPLC method for determination of prim-O-glucosylcimifugin and 5-O-methylvisammioside contents in *Yuping feng* powder, and study the therapeutic effect of *Yuping feng* powder on pediatric asthma. Mobile phase is methanol-water (35:65); Alltech C$_{18}$ (5 µm, 250 mm × 4.6 mm); flow rate is 0.8 mL/min; detection wavelength is 254 nm; injection volume is 10 µL; and column temperature is room temperature. Asthmatic children are randomly divided into two groups, all of whom are treated with prescribed dose of inhaled fluticasone propionate at 100~200 µg/d based on their conditions. Treatment group is given additional *Yuping feng* powder (containing 1 gm of crude herbals per gm) 1 bag (5 gm/bag, which contains 3 herbs, *Radix sileris*, *Astragalus* and *Atractylodes*) three times daily. One course of treatment lasts 3 months; and therapeutic effect is evaluated after a course of treatment. Good linearity is observed within a 0.0508 - 0.6096 µg range for prim-O-glucosylcimifugin, and a 0.0516-0.6192 µg range for 5-O-methylvisammioside. Therapeutic effect is evidently improved for asthmatic children in the treatment group after taking *Yuping feng* powder, with cure rate of 47.5%, and total effective rate reaching 90%, which are markedly higher than the control group. FEV (%) and PEFR change evidently before and after treatment in the treatment group; after treatment, both FEV (%) and PEFR increase markedly, and the differences are statistically significant. The method established herein is simple, accurate and specific, which can be used for quantitative determination of the preparation. *Yuping feng* powder is effective in treating pediatric asthma.

**Keywords:** *Yuping feng* powder, HPLC, Prim-O-glucosylcimifugin, 5-O-methylvisammioside, Asthma

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_Yuping feng_ powder has nearly eight hundred years of history as a medicine. The prescription is composed of *Huangqi* [*Astragalus membranaceus* (Fisch.) Bunge.], *Baizhu* [*Saposhnikovia divaricata* (Trucz.) Schischk.] and *Fang feng* (*Atractylodes macrocephala* Koidz.), which is recorded in various editions of China’s pharmacopoeia, with continuously improving quality standards. *Yuping feng* powder has qi-benefiting, exterior-consolidating and antiperspirant effects, which is used for exterior deficiency, spontaneous perspiration, pathogenic wind invasion and other diseases.

Modern TCM practitioners use it for treatment of allergic rhinitis, upper respiratory tract infections due to exterior deficiency and exogenous pathogenic wind, recurrent glomerulonephritis due to susceptibility to colds, chronic urticaria, bronchial asthma and recurrent diseases due to exogenous pathogenic wind, including those relating to urinary and reproductive system, respiratory system, digestive system, gynecology, ophthalmology and otorhinolaryngology, tumors, etc. Owing to its remarkable therapeutic effect, *Yuping feng* powder has been developed to 108 varieties including powders, pills, hard capsules, drop pills, granules, soft capsules, oral liquids, tea, etc., through the joint efforts of TCM pharmaceutical companies, thereby playing a crucial role in promoting the clinical medication. This study optimizes the quality standards of *Yuping feng* powder, and investigates its therapeutic effect on asthma.

**Materials**

**Instruments and reagents**

Agilent 1290 Infinity LC system, with UV detector Prim-O-glucosylcimifugin and 5-O-methylvisammioside reference substances was purchased from National Institutes for Food and Drug Control.

**Herbs**

*Huangqi*, *Fang feng* and *Baizhu* were purchased from the medicine market in Anguo, which were identified as pharmacopoeia species. *Yuping feng* powder was prepared according to the classical formulating method (proportion between *Huangqi*, *Fang feng* and *Baizhu* of...
Each gram of *Yuping feng* powder preparation contained 1 gm of crude herbals.

**Methods**

**Chromatographic conditions**
Mobile phase: methanol-water (35:65); Alltech C18 (5 Lm, 250 mm × 4.6 mm); volumetric flow rate: 0.8 mL/min; detection wavelength: 254 nm; injection volume: 10 µL; column temperature: room temperature.

**Preparation of reference solutions**
Appropriate amounts of prim-O-glucosylcimifugin and 5-O-methylvisammioside reference substances, which were dried (in phosphorus pentoxide vacuum desiccator for 12 hrs) to constant weights, were accurately weighed, and diluted to 5 ml with methanol to prepare 25 µg ml⁻¹ solutions.

**Preparation of test solutions**
Five gm of *Yuping feng* powder preparation was accurately weighed, placed in a stoppered conical flask, diluted to 50 ml with methanol, stoppered, weighed, and ultra sonicated; then let cool, stoppered, and weighed again. After the lost weight was replenished with methanol, the solution was shaken well, filtered, and centrifuged; then the supernatant was ultra filtered to give the test solution.

**Linearity range**
Appropriate amounts of prim-O-glucosylcimifugin and 5-O-methylvisammioside reference substances were accurately weighed, and added with methanol to prepare 25.4 µg/mL and 25.8 µg/mL solutions. 2.0, 4.0, 8.0, 12.0, 18.0 and 24.0 µL of the above solutions were injected, respectively, for determination of prim-O-glucosylcimifugin and 5-O-methylvisammioside peak areas. Standard curves were plotted with the injection volume as abscissa and the peak area as ordinate, and regression equations were obtained. Prim-O-glucosylcimifugin: \( Y = 109354.2X - 367.3 \) \( (r = 0.9995) \); 5-O-methylvisammioside: \( Y = 11208.4X + 5635 \) \( (r = 0.9992) \), indicating good linearity of prim-O-glucosylcimifugin and 5-O-methylvisammioside within 0.0508-0.6096 µg and 0.0516-0.6192 µg ranges, respectively.

**System suitability test**
Determination was performed under the above chromatographic conditions. Results showed that the number of theoretical plates was 4530 when calculated based on prim-O-glucosylcimifugin peak, and was 8130 when calculated based on 5-O-methylvisammioside peak.

**Accuracy test**
Each 10 µL of reference solutions was repeatedly injected for 5 consecutive times, and RSD of peak area measured was found to be 2.3%.

**Recovery test**
Five gm of *Yuping feng* powder with known content was accurately weighed, added precisely with prim-O-glucosylcimifugin and 5-O-methylvisammioside reference solutions, respectively, and determined under the sample preparation section. Results showed that the average recovery of prim-O-glucosylcimifugin was 98.42% \( (n = 5) \), with RSD = 1.34%, whereas the average recovery of 5-O-methylvisammioside was 98.82% \( (n = 5) \), with RSD = 1.02%.

**Reproducibility test**
Six aliquots of the same test sample were taken, and tested under the above chromatographic conditions. RSD was found to be 1.14%, indicating good reproducibility of the method.

**Stability test**
The same sample solution was injected at 0, 2, 4, 6, 8, 12 and 24 hrs, respectively, and peak area integrals were recorded. Results showed that RSD = 1.12% for prim-O-glucosylcimifugin content; and RSD = 1.06% for 5-O-methylvisammioside content, indicating stability within 24 hrs.

**Therapeutic effect of *Yuping feng* powder on pediatric asthma**

**General information**
Clinical data of 80 children were all in line with the standards developed by the Third National Academic Conference on Pediatric respiratory Tract Diseases. These patients were aged between 4-14 yrs, with disease course ranging from 3 months to 5 yrs. The patients were randomly divided into observation group and control group \( (n = 40 \) in each group) according to registration order. Age, gender, disease course and severity of patients between the two groups were not statistically significantly different, which were comparable. Children in both groups were treated with prescribed dose of inhaled fluticasone propionate at 100~200 µg/d based on their conditions. Treatment group was given additional *Yuping feng* powder (containing 1 gm of crude herbals per gm) 1 bag three times daily. One course of treatment lasted
3 months; and therapeutic effect was evaluated after a course of treatment.

**Results**

**Criteria for evaluation of therapeutic effect**

By reference to relevant criteria for therapeutic effect of “bronchial asthma” in the Practices for Prevention and Treatment of Pediatric Asthma (Tentative): 1) those with mild or moderate conditions meeting the above diagnostic criteria; 2) those in the remission stage of bronchial asthma; 3) those aged ≥ 3 yrs, <18 yrs. Exclusion criteria: 1) children in acute exacerbation stage; 2) those whose conditions cannot be mitigated after standard treatment; 3) children complicated by cardiovascular, liver or kidney dysfunction.

**Main observation indices**

Clinical symptoms of children in two groups before and during treatment; and improvement of lung function before and after treatment.

**Comparison of clinical efficacy between two groups**

Experimental results showed that the therapeutic effect was evidently improved for asthmatic children in the treatment group after taking *Yuping feng* powder, with cure rate of 47.5%, and total effective rate reaching 90%, which were markedly higher than the control group. These indicated that *Yuping feng* powder can effectively treat asthma in children (Table 1).

**Comparison of lung function before and after treatment in two groups**

FEV (%) and PEFR increased slightly after treatment in the control group, but the changes were not significant. In the treatment group, FEV (%) and PEFR changed evidently before and after treatment; after treatment, both FEV (%) and PEFR increased markedly, and the differences were statistically significant (Table 2).

**Discussion**

Internal causes of asthma are functional insufficiency of lung, spleen and kidney, as well as phlegm retention. Asthma is mainly located in lungs, and its major pathogenesis is phlegm retention; it occurs and recurs repeatedly upon contacting exogenous factors. When asthma attacks, phlegm ascends with *Qi*, and *Qi* is obstructed due to phlegm, the two intermingle to obstruct the airway, and disturb ascending and descending of *Qi*, resulting in poor breath, rapid respiration, croup and wheezing. Pathogens accumulate in pulmonary collaterals, leading to lung *Qi* congestion and chest stuffiness. Lung *Qi* obstruction causes heart blood stasis, which can lead to acral and facial cyanosis. Pathogen excess and healthy *Qi* deficiency, *Qi* and yang depletion can be present, and symptoms like sweating brow, cold limbs, pale complexion and weak pulse can be shown. External causes of asthma, on the other hand, are sudden change of climate, in coordination between cold and heat, invasion of exogenous pathogens, contacts with foreign substances or odors, overeating of raw or cold foods, hyperactivity, agitation, etc. Internal causes are the pathogenetic bases, while external causes are important conditions for pathogenesis, of which the most common cause is catching cold.

Children have delicate lungs, constantly insufficient spleen and deficient kidney. Normal metabolism of body fluids is managed by lung, spleen and kidney, where lung is the upper source of fluids that dredges and regulates the water passage; spleen is the sea of fluids that transports and transforms the water dampness; and kidney is the organ of fluids that manages and steams the body fluids. Congenital deficiency, acquired disorders or exogenous pathogen invasion can result in dysfunction of lung, spleen and kidney, abnormal body fluid metabolism and endogenous phlegm turbidity. Phlegm is essentially

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**Table 1**—Comparison of therapeutic effect of *Yuping feng* powder on asthmatic children (n = 40)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cure</th>
<th>Improvement</th>
<th>Ineffective</th>
<th>Total effective rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>19</td>
<td>17</td>
<td>4</td>
<td>90</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>16</td>
<td>10</td>
<td>75</td>
</tr>
</tbody>
</table>

**Table 2**—Comparison of lung function before and after treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>FEV(_1)(L)</th>
<th>FEV (%)</th>
<th>PEFR(L/S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment group</td>
<td>Before treatment</td>
<td>40</td>
<td>2.63±0.24</td>
<td>72.25±5.47</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>40</td>
<td>3.12±0.42</td>
<td>86.54±7.41</td>
</tr>
<tr>
<td>Control group</td>
<td>Before treatment</td>
<td>40</td>
<td>2.36±0.44</td>
<td>72.3±7.93</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>40</td>
<td>2.85±0.27</td>
<td>82.64±0.62</td>
</tr>
</tbody>
</table>
fluid, which originates from the kidney. It is wet in nature, which is mainly managed in spleen. Phlegm's destination is lung, where it is stored. Phlegm retention is an important pathological basis of asthma. In case of recurrent exogenous pathogen invasion and contact with causative factors, deep-lying phlegm can be motived; phlegm and Qi intermingle to obstruct the airway, lung loses its dispersive, descending and clearing functions, phlegm ascends with Qi. Qi is obstructed due to phlegm, and they struggle with each other to disturb the ascending and descending of Qi, resulting in poor breath, rapid respiration, croup and wheezing, thereby causing asthma. Those affected by exogenous wind cold, endogenous injuries and cold, or yang deficiency with endogenous cold phlegm retention have cold asthma; while those affected by exogenous wind heat, wind cold transformation into heat, or yin deficiency with endogenous phlegm heat retention have heat asthma. Those with lung, spleen and kidney insufficiency and phlegm retention constitution have recurrent asthma attacks, which often lead to depletion of lung Qi and yin, impairment of spleen Qi and yang and deficiency of kidney yin and yang, thereby resulting in different syndromes in remission stage such as lung Qi weakness, spleen Qi weakness and kidney Qi weakness.

Due to differences in pathogen affection and constitution, asthma can be differentiated into cold and heat types, which is transformable as well. Recurrent asthma, lung Qi dissipation, spleen and kidney yang impairment due to cold phlegm, and lung and kidney yin consumption due to phlegm heat can be transformed from sthenia into asthenia. Manifestations of lung, spleen and kidney weakness in normal times, healthy Qi recovery, phlegm clarification and favorable turn can gradually reduce attacks and hasten recovery. If phlegm is not removed, and organ weakness is not recovered, asthma has root causes, which can occur and recur repeatedly upon contacting causative factors, leading to weakening of healthy Qi and persistent, refractory illness.

Huangqi, the monarch drug in Yuping feng power prescription, is sweet, warm, which can replenish spleen and lung Qi internally, and consolidate exterior and reduce sweat externally. Baizhu, as the ministerial drug, can invigorate spleen and supplement Qi, which facilitates Huangqi's Qi-invigorating and exterior-consolidating effects. Combined use of the two drugs can invigorate Qi and consolidate exterior, so that sweat does not leak easily, and pathogens cannot invade easily. Accompanied by adjuvant drug Fang feng, which relieves exterior syndromes and dispels wind pathogens, the complete prescription has a primary function of strengthening healthy Qi, and a supplementary function of eliminating pathogens. Compatibility characteristics of the prescription are dominant amount of Qi-invigorating and exterior-consolidating drug and a small amount of wind-dispelling and exogenous syndrome-relieving drug. When Huangqi is combined with Fang feng, exterior is consolidated without leaving pathogens; and when Fang feng is combined with Huangqi, pathogens are eliminated without harming the healthy Qi; the two thus restrain and assist each other. Yuping feng power has Qi-invigorating, exterior-consolidating, healthy Qi-strengthening and pathogen-eliminating effects for those with exterior deficiency, spontaneous perspiration, or susceptibility to cold.

In this study, clinical asthmatic children are used as subjects, who are randomly divided into control and treatment groups. Children in both groups are treated with prescribed dose of inhaled fluticasone propionate at 100–200 ug/d based on their conditions. Treatment group is given additional Yuping feng powder (containing 1 gm of crude herbals per gm) 1 bag three times daily. One course of treatment lasts 3 months. Results show that the therapeutic effect is evidently improved for asthmatic children in the treatment group after taking Yuping feng powder, with cure rate of 47.5%, and total effective rate reaching 90%, which are markedly higher than the control group. FEV (%) and PEFR change evidently before and after treatment in the treatment group; after treatment, both FEV (%) and PEFR increase markedly, and the differences are statistically significant. FEV (%) and PEFR increase slightly after treatment in the control group, but the changes are not significant. In the treatment group, FEV (%) and PEFR change evidently before and after treatment; after treatment, both FEV (%) and PEFR increase markedly, and the differences are statistically significant. It can thus be seen that the Yuping feng powder has anti-asthma effect. Its mechanism of action will be explored further in the future.

References
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