Semi-bionic Extraction Process and Antibacterial Activity Against Salmonella goose of Compound Chinese Medicine Dahuang Qinyu San

Hatungimana Françoise1 2*, Hongjun Wang1

1 College of Animal Husband & Veterinary Medicine, Jinhzhou Medical University, Jinhzhou, Liaoing, 121001, China.
2 Ministry of Education and Scientific Research, Burundi, China.
*Corresponding author's E-mail: hatufrancoise2019@gmail.com

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ABSTRACT
Various classical Chinese medicines have shown their efficiency in curing various infectious diseases. Among them, Dahuang Qinyu San (DQS) found in the Chinese Veterinary Pharmacopoeia is composed of three kinds of Chinese herbs: rubarb, Scutellaria baicalensis, and Houttuynia cordata. Due to its urgent need in human health and its effectiveness, a semi-bionic extract of Dahuang Qinyu San (SEDQS) was studied to evaluate its optimal extraction conditions and investigate its antibacterial activity against Salmonella goose. The US (53) uniform design method was used to investigate the effects of three independent variables, including pH value (X1), solid-to-liquid ratio (X2), and extraction time (X3), on the composite score (Y) of the extract rate and the MIC, using the semi-bionic extraction process. The broth microdilution method was also used to determine the minimum inhibitory concentration (MIC) against Salmonella goose. After 30 minutes of extraction, the optimal conditions for SEDQS were found to be pH 8.3 and a solid-to-liquid ratio of 1: 40. Under these optimal conditions, the extraction rate was 43.66 % and the MIC was 9.10 mg/ml, which indicates antibacterial efficacy against Salmonella goose.

Keywords: Traditional Chinese medicine; Dahuang Qinyu San; extraction process, antibacterial activity, semi-bionic extraction.

INTRODUCTION
Traditional Chinese medicines (TCM) have been used to treat a wide variety of infectious diseases in China for over five thousand years 1. TCM has been found to have a variety of pharmacological activities, including clearing heat, detoxification, sterilization, improving the body's immunity, and nutritional growth as an organic whole, and has shown promising results in the prevention and control of animal diseases, as well as promoting animal growth 2. Although some traditional compounds have minimal physiological activity, as they are metabolized, they become active or enhanced. Semi-bionic extraction (SBE) has been presented as a new technology for making oral TCM preparations based on the primary challenges encountered in the extraction of TCM components 3. This extraction method’s process parameters must be acceptable for industrial production, satisfy traditional Chinese medicine usage guidelines, and reflect the features of the human gastrointestinal environment 4.

Furthermore, where biological processing precedes the complex chemical components in a multi-herb prescription, SBE is a preferable choice and offers various advantages. Organic solvents are not employed in SBE, which is known as the perfect incorporation of inheritance and invention based on core principles of traditional Chinese medicine. As a result, there is no organic solvent residue in the extraction, and the extraction temperature is lower, allowing heat-sensitive components to escape damage. Furthermore, SBE is consistent with Chinese medicine theory, as it considers pharmacodynamics responses when designing the extraction procedure. More than 20 traditional Chinese medications have been thoroughly researched and proven effective 4.

Several therapeutic Chinese herbs have been proven to have antibacterial power against Salmonella 5, a common pathogenic intestinal bacterium that causes a variety of diseases. Salmonella continues to be a major threat to human and animal health in China and around the world because it causes diseases such as salmonellosis, gastroenteritis, and food poisoning, all of which cause significant morbidity, death, and economic losses 6. Salmonella, like other bacteria, has developed resistance against antimicrobial agents 7. Therefore, new innovative antibacterial medications are urgently needed to combat the growth of these threats. Previous research has shown that Chinese medicine components have high antibacterial efficacy against pathogenic microorganisms. Because of its low toxicity and non-toxic side effects, TCM has become a hotspot of study for highly effective antimicrobial therapy in order to discover alternative antibiotics 2, 8. Among these, Dahuang Qinyu powder San, a traditional Chinese medicine prescription found in the Chinese Veterinary Pharmacopoeia contains three Chinese herbs: rhubarb, Scutellaria baicalensis, and Houttuynia cordata. It is a type of traditional herbal medication that is used to get rid of...
heat, cleanse, and treat fish and shrimp with Gill Rot. *Rhubarb, scutellaria,* and *houttuynia cordata* have all been found to have antibacterial properties in numerous researches. However, there is no information about these three compounds' antibacterial properties. Thus, the goal of this research is to optimize SEDQS and evaluate its antibacterial effectiveness against Salmonella goose.

**EXPERIMENTAL METHODS**

**Dahuang Qinyu San extraction process**

A semi-bionic extraction technique was used to extract DQS. To make 2.0 g of the chemical, 1.08 g of *rhubarb*, 0.65 g of *scutellaria*, and 0.27 g of *houttuynia cordata* were weighed and combined. Artificial gastric juice and artificial intestinal juice were added to groups 1 and 3, respectively, during the extraction procedure. Each experimental group's stock solutions of hydrochloric acid (HCl 36-38 %) and sodium hydroxide (NaOH 70% percent) were used to dissolve 2 g of powder in aqueous solutions. Table 1 shows how the three independent parameters of pH, material-to-liquid-ratio, and extraction time were adjusted. In addition, the mixture was incubated for a specific amount of time at 37°C (table 1). The supernatant was collected and centrifuged for 5 minutes at 2500 rpm and dried by vacuum drying at 60°C until it was completely dry. Following the extraction process, each group of extractions was repeated 3 times and stored in the refrigerator at 4 °C for later use.

\[
\text{Extract rate} = \frac{\text{Weight of extract (g)}}{\text{Weight of Chinese medicinal materials (g)}} \times 100\%
\]

<table>
<thead>
<tr>
<th>No.</th>
<th>pH value (X1)</th>
<th>Material-to-liquid ratio (X2)</th>
<th>Extraction time/h (X3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1:50</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>1:30</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>6.8</td>
<td>1:60</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>8.3</td>
<td>1:40</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>1:20</td>
<td>6</td>
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</table>

**RESULTS**

**Results of extraction**

The SBE technique was utilized to extract DQS in this investigation. As demonstrated in Table 2, aqueous solvents were utilized to determine the extract rate of SEDQS at various concentrations.
Antimicrobial activity results

SEDQS antibacterial tests against Salmonella geese were conducted out using the broth microdilution method. SEDQS was diluted in 96 microplate wells using a two-fold serial dilution procedure, which included plant extracts, test organisms, as well as positive, negative, and blank controls. For the MIC experiments, each hole was inoculated on a corresponding labeled agar plate and analyzed under the same conditions (figure 1). Table 2 shows the findings of the MIC values (mg/ml). The extract rate was 43.67 % and the lowest concentration of SEDQS, MIC, which inhibited salmonella goose, was 9.1 mg/ml. SEDQS has a very good antibacterial performance, according to these findings.

Statistical analysis results

As shown in table 2, we employed the uniform design (UD) method to progress the SEDQS optimization by assessing the effect of several independent factors on the comprehensive score of the extract rate and MIC (Y), including pH value (X1), solid-liquid ratio (X2), and extraction duration (X3). Using the stepwise linear regression method, the multiple linear regression equation Y= 29.77–0.502 X1+ 0.512 X2, R = 0.944, F = 15.9 (P 0.05) was generated (table 3 and 4). When the p-value is less than 0.05, the factor was shown to have a significant impact. When the p-value is less than 0.01, on the other hand, it indicates that the factor is highly significant and has a bigger impact than other factors 12. The results of the analysis of variance indicated that the model correlation was good and that it could be used to match the test data. Aside from that, linear regression revealed significant differences, demonstrating that the model was correct. Further analysis of the equation findings revealed that the solid-liquid ratio (X2) and pH value (X1) both had a significant effect on the Y value (P<0.009<0.01), whereas extraction time had no significant effect (P>0.05). Based on the measured findings of the uniform design experiment, the actual antibacterial effect, and the projected outcomes, the optimal conditions for SEDQS were X1/pH is 8.3, X2/solid-to-liquid ratio is 1: 40, and X3/extraction time is 30 minutes.

![Figure 1](image_url): Determination of MIC for ECCD against Salmonella goose. (A) 96 micro plates with plant extracts and test organism, as well as the positive, negative controls and blank control, (B) labeled agar plate with inoculated liquid from the 12 holes of 96 wells after incubation for minimum bactericidal

![Table 3](table_url): Coefficient of variance of the regression model

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95.0% Confidence Interval for B</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td>Lower Bound</td>
</tr>
<tr>
<td>2 (Constant)</td>
<td>29.774</td>
<td>2.965</td>
<td>10.044</td>
<td>0.000</td>
</tr>
<tr>
<td>x2</td>
<td>0.151</td>
<td>0.049</td>
<td>0.512</td>
<td>3.090</td>
</tr>
<tr>
<td>x1</td>
<td>-0.723</td>
<td>0.239</td>
<td>-0.502</td>
<td>-3.028</td>
</tr>
</tbody>
</table>

![Table 4](table_url): Analysis of variance of the regression model (ANOVA)

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of square</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>F</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
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<td>94.203</td>
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<td>.000</td>
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<tr>
<td>residual</td>
<td>71.097</td>
<td>12</td>
<td>5.925</td>
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<td></td>
</tr>
<tr>
<td>total</td>
<td>259.503</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Characteristics of semi-bionic extraction

TCM preparations were prepared using the SBE method, which was based on traditional Chinese medicine theory as well as principles of current scientific analysis in extraction processes. In the process, the acidic water and alkaline water of the selected pH are used as the extraction solvent, and extraction can be full of acidic components and basic components, even to the extent that some enzymes and surfactants in the extraction solvent can help to improve the efficiency of the composition. The most important point was the effective substance obtained by SBE, which can be better absorbed in the gastrointestinal tract. Previous research found that the local injury of SBE to the gastrointestinal tract was relatively rare. The method for optimizing the chemical extraction process Chinese medicine can be used to identify extraction factors and select the best settings. Orthogonal design, star point design-effect surface approach, and uniform design method are three typical experimental design optimization strategies. In the study of Chinese medicine extraction, the orthogonal test design and analytic approach are the most commonly utilized. To start, we first determine the major and secondary status of each element, as well as their interactions, and then choose the optimum combination design strategy for each level of the factors. The statistical processing, which includes range analysis and variance analysis, can discover the optimal process parameters based on the results of the orthogonal selection test. The beneficial components of traditional Chinese medicine can then be isolated in greater depth. The most noticeable quality of orthogonal tests is uniformity and comparability.

Optimization of extraction process design method

The method for optimizing the chemical extraction process was developed by Fang Kaitai and Wang Yuan, two well-known Chinese mathematicians. The test points can be fully "dispersed equally" within the experimental range using this design strategy, and each point is more representative. The regression equation, which can be utilized in multi-factor and multi-level test design, is used to find the best experimental conditions. The advantage of a uniform design is that it achieves the experiment's goal in a short amount of time, which is especially evident in the research of traditional Chinese medicine compounds, which considerably reduces the length of the experiment, the number of experiments, and the cost. The extraction rate and the comprehensive score of the MIC were utilized as the response values to optimize the semi-bionic extraction process parameters of Dahuang Qinyu powder in this study, and the uniform design approach was employed to optimize the extraction process. The uniform test revealed that the extraction process conditions were stable and reliable.

Influencing factors and response values

Extraction is a necessary step in the production of Chinese medicinal materials and products, but a number of factors can influence the outcome. The results showed that two independent parameters, such as the material to liquid ratio (X2) and pH value (X1), had an effect on the SEDQS. However, the extraction duration (X3) had no significant effect on the composite score. In compared to other variables, the solid-to-liquid ratio (X2) was shown to have the largest effect on the composite score (Y), which could be attributable to the high P-value of 0.009. The solid-to-liquid ratio and pH value were previously thought to be critical criteria for the SEDQS based on previous research. Furthermore, the extract with the highest extraction rate of 43.67 % had the best activity and the lowest MIC value of 9.10 mg/ml among the extracts examined. Similarly, antibacterial activity against salmonella species has been found by multiple investigations using various TCM at the lowest MIC.

CONCLUSION

Semi-bionic extraction was used to optimize DQS in this study. The best extraction conditions, according to the U5 (53) uniform design method, are pH 8.3, a solid-to-liquid ratio of 1: 40, and a 30-minute extraction time. Salmonella goose growth was inhibited at a 43.66 % extract rate with a minimum concentration of 9.10 mg/ml under these optimal conditions. With the antibacterial tests, SEDQS has a very good antibacterial performance against Salmonella goose.

Abbreviations

MIC: Minimum Inhibitory Concentration
MBC: Minimum Bactericidal Concentration
SBE: Semi-Bionic Extraction
TCM: Traditional Chinese Medicines
HCl: Hydrochloric acid
NaOH: Sodium Hydroxide
DQS: Dahung Qinyu San
SEDQS: Semi-Bionic Extract of Dahung Qinyu San
ANOVA: Analysis Of Variance
CFU: Colony Forming Unit
SPSS: Statistical Package for the Social Sciences
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REFERENCES


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