Research Article

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

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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: *rhubarb, Scutellaria baicalensis*, and *Outtuynia 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 U5 (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.

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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 research [9, 10]. 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 ($NaOH_4$ percent) were used to dissolve 2 g of powder in aqueous solvents. 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 [2], the extraction rate for each group was calculated by the formula below. Every group of extractions was repeated 3 times and stored in the refrigerator at 4 °C for later use.

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

Table 1: Operation sheet for U_5 (5^3) uniform test design

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

Antimicrobial activity

The concentration of the test bacterium solution was determined using the live bacteria counting method with slight adjustments. Using the appropriate medium, the bacterium liquid was diluted to a concentration of 10^5 CFU/ml. The MIC of SEDQS against Salmonella geese was established using Eloff's broth microdilution technique [11]. In sterile 96-well microplates, serial dilutions of different sets of test medications were made over concentration ranges of 261.67 to 0.5, 204.17 to 0.399, 238.83 to 0.466, 218.33 to 0.426, and 128.33 to 0.25 mg/ml, respectively. The plates were preloaded with 100 µl of nutrient broth in each well, followed by 100 µl of dissolved extract in the first wells of each row, for a total volume of 200 µl in the first wells. Following the mixing, 100 µl was extracted from each of the first row wells and placed in the successive rows of the 9 wells, with the 100 µl drawn being discarded. Then, in wells 1 to 10, 20 µl of test bacterium solution with 10^6 CFU/ml was added, followed by 20 µl of medication solution in wells 11. A control group was employed in wells 10 and 11, while a blank control was used in well 12. For each drug, at least three sets of parallel experiments were conducted. The plates were incubated for 24 hours at 37°C. Using the wire loop, the incubated liquid from the 12 holes of the 96 wells was injected in the corresponding place on the labeled agar plate in wells 1-12 in a row of the plate and incubated at 37°C for 24 hours. The minimal bactericidal concentration (MBC) is the lowest dilution for visually witnessing bacteria-free colonies, while the MIC is the lowest dilution for bacterial growth less than.

Statistical method

The experiment was carried out three times (n=3). Stepwise regression analysis used SPSS 25 software to calculate the regression coefficients and statistical significance of the model terms, as well as to fit the mathematical models of the experimental data. The regression analysis (r^2) and the analysis of variance (ANOVA) (p 0.05) were used to determine the model's adequacy.

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.

Table 2: Comprehensive score of MIC and extraction rate in each group.

No	X1/pH	x2/ Material-to-liquid ratio	x3/ Extraction time (h)	Extraction rate (%)	MIC (mg/ml)	Composite score
1	2	1:50	0	52.33	16.38	34.36
2	4	1:30	2	40.83	25.37	33.09
3	6.8	1:60	1	47.77	24.85	36.31
4	8.3	1:40	0.5	43.67	9.1	26.38
5	10	1:20	6	39.17	13.59	26.37

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.

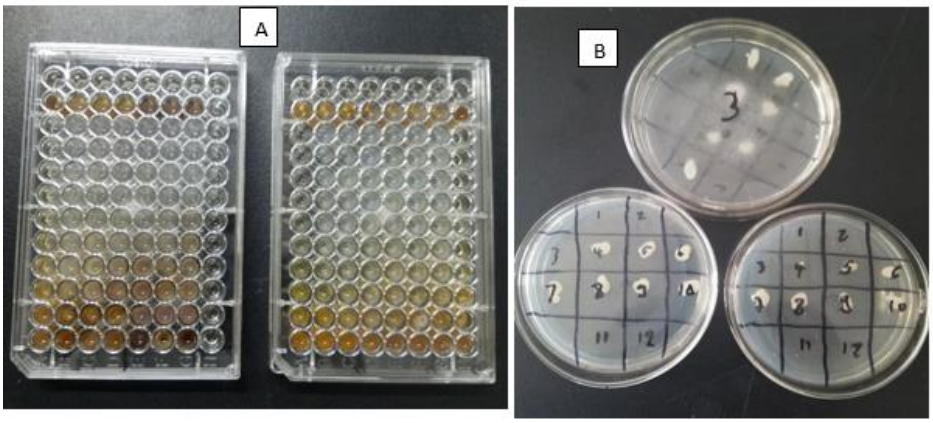

Figure 1: Determination of MIC for ECCD against Salmonella goose. (A) 96 micro plates with plant extracts and test organism, as well as the positive , negative controls and blank control, (B) labeled agar plate with inoculated liquid from the 12 holes of 96 wells after incubation for minimum bactericidal

Table 3: Coefficient of variance of the regression model

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Correlations		
		B	Std. Error	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part
2	(Constant)	29.774	2.965		10.044	0.000	23.315	36.234			
	x2	0.151	0.049	0.512	3.090	0.009	0.044	0.257	0.719	0.666	0.467
	x1	-0.723	0.239	-0.502	-3.028	0.010	-1.243	-0.203	-0.713	-0.658	-0.458

Table 4: Analysis of variance of the regression model (ANOVA)

Model	Sum of square	Degree of freedom	Mean square	F	Significance
regression	188.406	2	94.203	15.9	.000
residual	71.097	12	5.925		
total	259.503	14			

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 [13]. 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 [14, 15].

Optimization of extraction process design method

The method for optimizing the chemical extraction process Chinese medicine can be used to identify extraction factors and select the best settings [16]. 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. We can determine the influence of each component and optimize the optimal level of the factors by studying the findings. However, we must show the reliability of the optimization condition by verification test and the validity of the conclusion by real data using the optimization condition acquired through orthogonal test design and analysis [17]. The mathematical and statistical methodologies used in the star point design-effect surface method are combined. It can simplify the interaction between variables by reducing the number of variables to be evaluated. This method may address the lack of uniform and orthogonal design, ensure test accuracy, and study the interaction between numerous components [18]. Uniform design is a multi-factor optimization experimental design method 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 [19]. 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. [20]. 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 [14]. 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.[21, 22]. 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. [23-25].

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

1. Wang Y, Jin F, Wang Q, Suo Z. Long-Term Survival of AIDS Patients Treated with Only Traditional Chinese Medicine. AIDS research and human retroviruses. 2017;33(2):90-2.
2. Jiang H, Zhou TZ, Li XG, Zhang H, Liu YZ, Zhen ZG, et al. Antimicrobial Activity of Compound Traditional Chinese Medicine Feizhu Powder. Advanced Materials Research. 2013;781-784:1064-7.
3. Zhaowang Z, Xiumei S. Characteristics and Application of Semi-bionic Extraction (SBE)[J]. WORLD SCIENCE AND TECHNOLOGY. 2000;1.
4. Wang R, Wu G, Du L, Shao J, Liu F, Yang Z, et al. Semi-bionic extraction of compound turmeric protects against dextran sulfate sodium-induced acute enteritis in rats. J Ethnopharmacol. 2016;190:288-300.
5. Lee MH, Kwon HA, Kwon DY, Park H, Sohn DH, Kim YC, et al. Antibacterial activity of medicinal herb extracts against Salmonella. Int J Food Microbiol. 2006;111(3):270-5.
6. Amajoud N, Bouchrif B, El Maadoudi M, Skalli Senhaji N, Karraouan B, El Harsal A, et al. Prevalence, serotype distribution, and antimicrobial resistance of Salmonella isolated from food products in Morocco. Journal of infection in developing countries. 2017;11(2):136-42.
7. Lunguya O, Lejon V, Phoba MF, Bertrand S, Vanhoof R, Glupczynski Y, et al. Antimicrobial resistance in invasive non-typhoid Salmonella from the Democratic Republic of the Congo: emergence of decreased fluoroquinolone susceptibility and extended-spectrum beta lactamases. PLoS Negl Trop Dis. 2013;7(3):e2103.
8. Chang CH, Yu B, Su CH, Chen DS, Hou YC, Chen YS, et al. Coptidis rhizome and Si Jun Zi Tang can prevent Salmonella enterica serovar Typhimurium infection in mice. PLoS One. 2014;9(8):e105362.
9. Zhao Q, Chen XY, Martin C. Scutellaria baicalensis, the golden herb from the garden of Chinese medicinal plants. Science bulletin. 2016;61(18):1391-8.
10. Kumar M, Prasad SK, Hemalatha S. A current update on the phytopharmacological aspects of Houttuynia cordata Thunb. Pharmacogn Rev. 2014;8(15):22-35.
11. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta medica. 1998;64(8):711-3.
12. Mo M, Hu S, Xu X, Ma Z, Ni Y, Wei Y, et al. Optimization of extraction technology of polysaccharide of Tricholoma giganteum. Pharmacology & Pharmacy. 2013;4(5A):1.
13. Zhang Z, Sun X. [Exploration on oral preparations of Chinese materia medica by semi-bionic extraction]. Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica. 1995;20(11):670-3, 703.
14. Tian Jian-hua ZC-yWEIL. Study on the extraction technology and antioxidant activity of total flavonoids from the pomace of sea buckthorn . NATURAL PRODUCT RESEARCH AND DEVELOPMENT. 2021;33(1):65-72.
15. Ni H, Wu Z, Muhammad I, Lu Z, Li J. Optimization of baicalin water extraction process from Scutellaria baicalensis (a traditional Chinese medicine) by using orthogonal test and HPLC. Revista Brasileira de Farmacognosia. 2018;28(2):151-5.
16. Yin Mingyang, Liu Suxiang, Zhang Tiejun, Chen Changqing. Research overview of compound Chinese medicine extraction technology. Chinese herbal medicine. 2018;46(21):3279-83.
17. Guo C-Y, Wang J, Hou Y, Zhao Y-M, Shen L-X, Zhang D-S. Orthogonal test design for optimizing the extraction of total flavonoids from Inula helenium. Pharmacogn Mag. 2013;9(35):192-5.
18. Li Y, Ji S, Gao JJ, Yan JX, Wang HM. [Optimize extraction technology of Fufang Bajitian Shenggu Particles by central composite design and response surface method]. Zhong yao cai = Zhongyaocai = Journal of Chinese medicinal materials. 2014;37(10):1863-7.
19. Fang K-T, Dennis KJL, Winker P, Zhang Y. Uniform Design: Theory and Application. Technometrics. 2000;42(3):237-48.
20. Fang K-T. Uniform Experimental Design. In: Lovric M, editor. International Encyclopedia of Statistical Science. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 1623-5.
21. Deng Youlin TSWMYP. Semi Bionic Extraction and Ultrasonic-Assisted Semi Bionic Extraction of Flavonoids from Eucommia Ulmoides. Journal of Jishou University(Natural Sciences Edition). 2017;38(2):66-70.
22. Yu X, Sun D, Fu X, Zhou S. Ultrasonic-assisted semi-bionic extraction of tannins from wild persimmon leaves. Asian Journal of Chemistry. 2013;25(18):10191-4.
23. McMurray RL, Ball MEE, Tunney MM, Corcionivoschi N, Situ C. Antibacterial Activity of Four Plant Extracts Extracted from Traditional Chinese Medicinal Plants against Listeria monocytogenes, Escherichia coli, and Salmonella enterica subsp. enterica serovar Enteritidis. Microorganisms. 2020;8(6):18-24.
24. Chen K, Wu W, Hou X, Yang Q, Li Z. A review: antimicrobial properties of several medicinal plants widely used in Traditional Chinese Medicine. Food Quality and Safety. 2021;5:81-86.
25. Manandhar S, Luitel S, Dahal RK. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. Journal of Tropical Medicine. 2019;17:1895340.

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