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Research Article

Optimization and Modeling of Ultrasound-assisted Extraction of Polysaccharides from *Cynomorium songaricum* and α-glucosidase Inhibitory Activity

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Abstract: In the study, the extraction processing of polysaccharides from *Cynomorium songaricum* was optimized by Response Surface Methodology (RSM) and projected by a computer-stimulated Artificial Neural Network (ANN). The optimal process conditions were obtained as follows: extraction temperature 55°C, solid-liquid ratio 1:10, power 175 W. Under optimized conditions, The R^2 value of 0.99391 and an MSE value of 0.0495 suggested a good generalization of the network and showed a good agreement between the experimental and predicted values. On the other hand, the results also suggested that polysaccharides from *Cynomorium songaricum* had α-glucosidase inhibitory activity with an IC_{50} of 8.316 μg/mL and may be a potential α-glucosidase inhibitory.

Keywords: α-glucosidase, artificial neural network, *Cynomorium songaricum*, polysaccharides, ultrasonic extraction

INTRODUCTION

Cynomorium contains two species: *C. songaricum* Rupr. and *C. coccineum* L. (the family *Cynomoriaceae*). These two species are primarily distributed in dry, rocky, or sandy soils of the northern hemisphere and have been widely utilized in folk medicine in Eastern, North Africa and Europe and Western Asian countries.

C. songaricum is also known as Suo Yang (Chinese: ▪阳), which is found in China, Afghanistan, Mongolia and Iran, it is usually parasitic on the roots of Nitrariaceae, Tamaricaceae and Chenopodiaceous plants. The fleshy stems of *C. songaricum* are used medicinally to treat nocturnal ejaculation and impotence as a tonic (Wan and Chen, 2000). *C. songarcum* is used in traditional Chinese medicinal materials and is generally used to increase sexual capability, as a laxative and to treat lumbar weakness. Besides, it can be used to make tea and gruel. In recent years, extracts and preparations of *C. songaricum* have been patented in China for preventing dizziness and sonitus, treating purpura hemorrhagica and female climacteric syndrome, improving immunity, lowering blood sugar and resisting cancer (Liu, 2009).

Because of the good record of *C. songaricum* as one of the famous herbs and valuable dietary botanical materials, *C. songaricum* can be used in many ways historically. There are many ways to use *C. songaricum*, such as *C. songaricum* tea, *C. songaricum* gruel. The following drinking or eating forms can be easily made in home ordinarily.

Heat reflux is the traditional extraction methods of polysaccharides. Many papers aimed at investigating the influence of extraction parameters, such as extraction time, extraction temperature, solid-liquid ratio, pH value and times of extraction (Zhu *et al*., 2009). Recently, alternative extraction techniques such as Ultrasonic Assisted Extraction (UAE) with lower temperature and enhanced yields had been also reported (Xu and Wei, 2008). Ultrasound-assisted extraction had a high efficiency, due to breakage of the cell wall and enhance of mass transfer through the cell walls.

Response Surface Methodology (RSM) is an effective statistical technique for optimizing complex processes (Li *et al*., 2007). It could reduce times of experimental trials that could evaluate multiple parameters and their interactions. Therefore, it is less laborious and more informational than other approaches.

Artificial Neural Network (ANN) is a highly simplified model of the structure of a biological network. A biological neuron receives inputs from other sources, combines them, performs generally a nonlinear operation on the result and then outputs the final result (Zhong and Wang, 2010). The basic advantage of ANN is that it does not need any mathematical model since an ANN learns from examples and recognizes patterns

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in a series of input and output data without any prior assumptions about their nature and interrelations (Baş and Boyacı, 2007). Applying ANN to a system needs sufficient input and output data instead of a mathematical equation (Mandal *et al*., 2009). ANN is a good alternative to conventional empirical modeling based on polynomial and linear regressions (Ali Akcayol and Cinar, 2005).

α-Glucosidase that located in the brush-border surface membranes of intestinal cells could catalyze the carbohydrate digestion and hydrolyze the α-glucopyranosidic bond to release an α-D-glucose. Hence, α-glucosidase inhibitors can retard the liberation of D-glucose from oligosaccharides and disaccharides from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppressed postprandial hyperglycemia (Kose, 2008). So, many scientists have been seeking for effective α-glucosidase inhibitors from natural sources and developing a physiologically functional food or a compound for use against diabetes.

The present research was performed with the following objectives: RSM analysis optimized of the UAE conditions for the extraction of polysaccharides from *C. songaricum* and studied the interaction effects of extraction temperature, solid-liquid ratio and power. Further to design an ANN for process modeling and finally to evaluate and validate the activity against α-glucosidase.

MATERIALS AND METHODS

Materials and chemicals: *C. songaricum* with average weight of 1.0-1.1 kg were purchased from the local farmers' market of Lanzhou, Gansu province, China. Powdered sifted through a 200 meshes and kept in dry environment for this study.

α-Glucosidase and P-Nitrophenly -α-Glucopyranoside (PNP-G) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were obtained from Lanzhou, Gansu province, China and were of analytical grade.

Extraction of polysaccharides: About 10 g of ground powder added with an appropriate amount of distilled water to extract. After extraction, the extracts were centrifuged at 5000 r/min for 10 min and filtered. The supernatant was concentrated with a rotary evaporator under vacuum. The resulting solution was mixed with four volumes of 95% (v/v) ethanol and kept 24 h at 4°C, the remaining solution was centrifuged, washed three times with dehydrated ethanol and was freeze dried at -40°C under vacuum. Finally, the precipitate was collected as polysaccharides. The percentage Yield of Polysaccharides (YP) was calculated by the following equation:

YP (%) = weight of polysaccharides (g) /weight of powder (g)×100

Experimental design:

Box-Behnken Design (BBD): Response Surface Methodology (RSM) was applied to decide the optimized conditions using ultrasound-assisted technique for the extraction of polysaccharides from *C. songaricum*. For describing the polysaccharide extraction process, Box-Behnkeen Design (BBD) in RSM was used to develop a response surface quadratic model. Extraction temperature of 45-65°C, solid-liquid radio of 1/10-1/30 g/mL and power of 125-175 W are three independent variables, the effects of them on the response were investigated and maximized the percent yield of polysaccharides. According to this design, a total 17 experiments were performed.

ANN modeling: An Artificial Neural Network (ANN) is a computational and mathematical model. This model is inspired by the structure of biological neural networks. In this approach weighted sum of inputs arriving at each neuron passed through an activation function (generally nonlinear) to generate an output signal (Lebovitz, 1997). Artificial neural network has been applied successfully for modeling extraction.

ANN model was developed using MATLAB neural network toolbox from the data. In this study, the modeling of extraction of *C. songaricum* developed a three-layer feed forward back propagation neural network. Most ANN has three layers: an input layer (independent variables), a hidden layer (collection of feature detectors) and an output layer (dependent variables). We have chosen solid-liquid radio, extraction temperature and power as independent variables, extraction yield as dependent variables, network model had obviously chosen three neurons in the input layer and one in the output layer. The model's complexity was decided by the number of hidden layers. The best neural network model was generated by a number of training trials. In order to ensure a model with good performance, performance goal and minimum performance gradient were set (Fig. 1).

The ratio of the explained variation to the total variation, R^2, reflects the degree of fit for the mathematical model (Haykyn, 2003). The closer the R^2 value is to 1, the better the model fits to the actual data (Nath and Chattopadhyay, 2007):

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - y_{di})^2}{\sum_{i=1}^{n}(y_{di} - y_m)^2}$$

MSE could also show the degree of fit of the model. It is calculated by Eq. The network having minimum MSE and maximum R^2 is considered as the best neural network model (Sin *et al*., 2006):

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_i - y_{di})^2$$

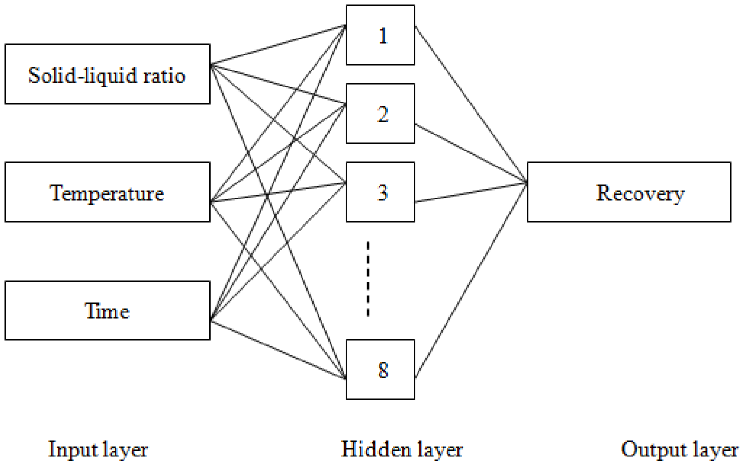


Fig. 1: ANN architecture (3-8-1)

Table 1: Variance analysis of quadratic model on extraction of polysaccharides from *C. songaricum*

Source	S.S.	d	M.S.	F-value	p-value	
Model	17.05	9	1.890	35.84	<0.0001	Significant
Solid-liquid ratio	0.84	1	0.840	15.99	0.0052	
Temperature	3.65	1	3.650	68.96	<0.0001	
Power	5.78	1	5.780	109.35	<0.0001	
Residual	0.37	7	0.053			
Lack of fit	0.37	3	0.120			
Pure error	0.00	4	0.000			

S.S.: Sum of square; M.S.: Mean square

where,

n : The number of points
y_i : The predicted value
y_{di} : The actual value
y_m : The average of the actual values

The training or learning process used a set of data to determine the values of the interconnection weights. The aim is to find the value of the weight and minimizes the error.

Assay for α-glucosidase inhibitory activity: On the basis of a previously described method (Izadifar and Jahromi, 2007), α-glucosidase inhibitory assay was performed with slight modification. Twenty five microliter of α-glucosidase solution (20 mU/mL) was premixed with 25 μL of sample solution at different concentrations (in 2% DMSO) in 200 μL of 0.1 mol/L potassium phosphate buffer (pH 6.8). Following incubation at 37°C for 15 min, 3 μL of p-nitrophenly-α-glucopyranoside (PNPG, 3 mmol/L) as substrate was added to start the reaction. The mixture was incubated at 37°C for 15 min, addition of 100 μL of 0.2 mol/L Na_2CO_3 solution could terminate the reaction. The amount of released product was measured at 405 nm using a UV spectrometer to estimate the enzymatic activity. Inhibitory activity was calculated as the following equation:

$$\alpha\text{-glucosidase inhibitory activity (\%)} = \frac{A-B}{A}\times 100$$

where,

A : The optical density of reaction blank, the reaction blank mixture contained the buffer solution (same volume) instead of the sample
B : The optical density of both α-glucosidase and sample

The relationships between process variables and the response have been expressed in coded parameters units given in Eq. by applying multiple regression analysis on the experimental data:

$$Y = +5.70 - 0.33X_1 + 0.68X_2 + 0.85X_3 - 0.85X_1X_2 - 0.45X_1X_3 + 0.25X_2X_3 - 0.78X_1^2 + 0.025X_2^2 - 0.23X_3^2$$

where,

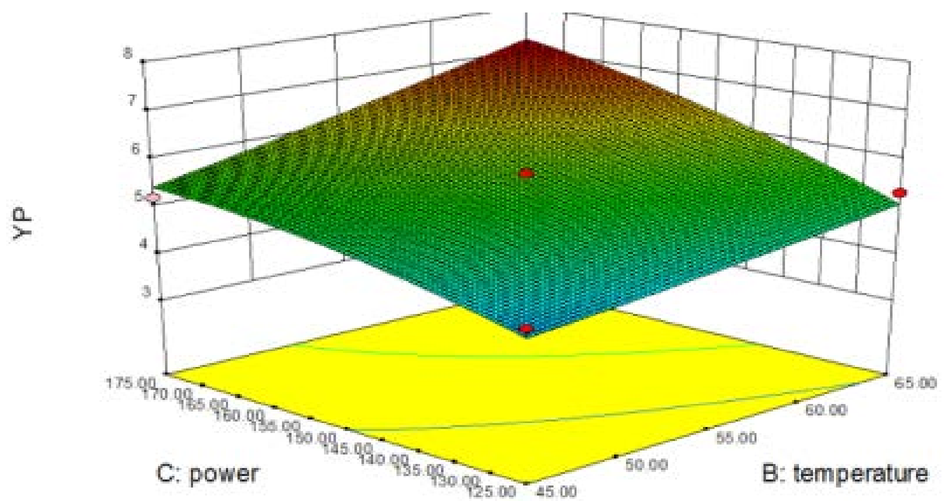
Y = Yield of polysaccharides
X_1 = Solid-liquid ratio
X_2 = Temperature
X_3 = Power are the coded values

The results of the second-order response surface were showed in Table 1 in the form of analysis of variance. *F*-value and p-value determined the significance of each coefficient. If the absolute *F*-value becomes greater and the p-value becomes smaller, the corresponding variables would be more significant (Kim *et al.*, 2004). The model *F* and *p*-value were founded to be 35.84 and <0.0001, it indicated the model was statistically significant. It can be seen that significant terms were the linear terms of power (X_3), extraction temperature (X_2), solid-liquid ratio (X_1). The result indicated that they have significant effects on the yields of polysaccharides. Thus, the response was sufficiently explained by the optimum model.

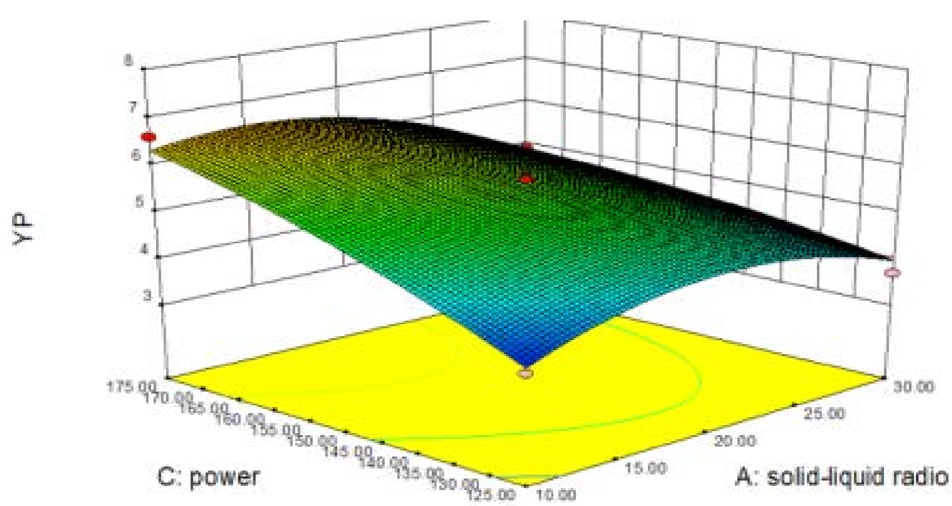
Effect of solid-liquid ratio on the extraction of polysaccharides: The solid-liquid radio is an important factor to the YP. In the study, it indicated that the yield of polysaccharides was increased with the increasing solid-liquid ratio. Too much liquid would not change much of the driving force any more as the limitation to mass transfer is more confined to the solid-liquid ratio (Amin and Anggoro, 2004). However, the more concentrated solution is the follow-up work of trouble, labor-intensive, a waste of time. The conditions of other process variables are as follows: extraction temperature of 55°C power of 150 W and time of 40 min. Taking these factors into consideration, optimal for this present extraction process was solid-liquid ratio of 1/10 g/mL (Fig. 2).

Effect of extraction temperature on the extraction of polysaccharides: From the results, it can be observed that the YP was increased linearly with increasing temperature from 45 to 65°C. The influence of relative greater force ruptured and erupted the formed cavitational nucleus and disrupted the cell tissues during extraction, which would in turn enhance mass transfer (Zhang *et al*., 2008). However, due to a further increase in the temperature beyond 55°C, the yield was increased moderately. Based on the results, 55°C was chosen as the optimum extraction temperature (Fig. 2).

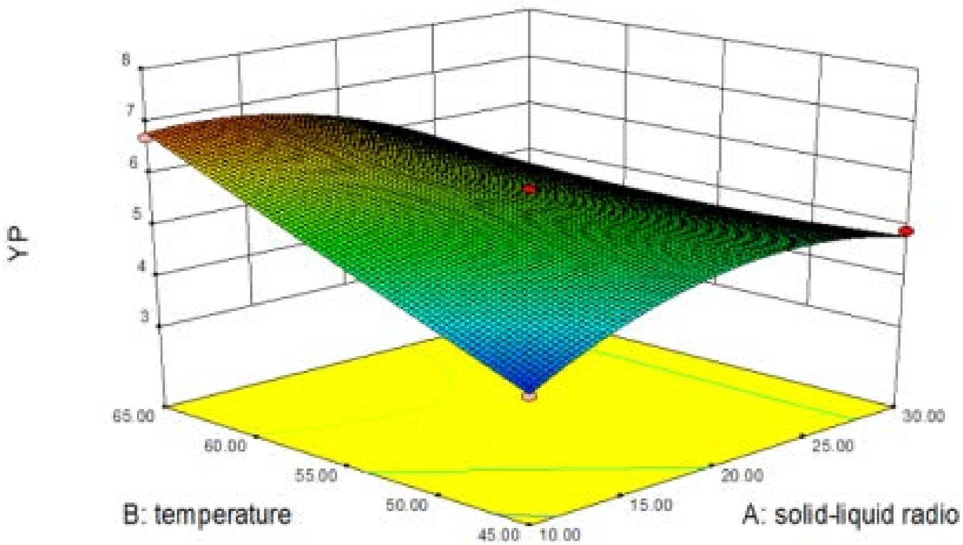
Effect of power on the extraction of polysaccharides: In this study, different power of ultrasound could affect the extraction YP. As the larger amplitude ultrasonic wave passed through the liquid medium, more bubbles were created and collapsed (Toma *et al*., 2001). Since the temperature and pressure were very high inside the bubbles and the collapse of bubbles occurred over a



(a)



(b)



(c)

Fig. 2: Response surface plots representing the effect of process conditions on the extraction yield of polysaccharides

Table 2: Comparative results of the observed and predicted of YP as a response using RSM and ANN model

				YP (%)		
					Predicted values	
Experiments	Solid-liquid radio (g/mL)	Extraction temperature (°C)	Power (W)	Actual values	RSM model	ANN model
1	1/20	55	150	5.70	5.70	5.6980
2	1/30	55	175	4.90	4.77	4.8979
3	1/20	65	125	5.30	5.07	5.4692
4	1/20	55	150	5.70	5.70	5.6980
5	1/30	45	150	4.90	4.80	4.9007
6	1/20	65	175	7.10	7.27	6.7713
7	1/10	45	150	3.70	3.75	3.6476
8	1/20	55	150	5.70	5.70	5.6980
9	1/10	55	175	6.60	6.32	6.5739
10	1/10	65	150	6.70	6.80	6.3832
11	1/20	45	125	4.40	4.22	4.3306
12	1/30	55	125	3.70	3.98	3.7027
13	1/30	65	150	4.50	4.45	4.5036
14	1/20	55	150	5.70	5.70	5.6980
15	1/10	55	125	3.60	3.73	3.6391
16	1/20	45	175	5.20	5.42	5.3304
17	1/20	55	150	5.70	5.70	5.6980

very short time, the violent shock wave and high-speed jet were generated which could enhance the penetration of the solvent into the call tissues and accelerate the intracellular product release into the solvent by disrupting the cell walls (Quan *et al*., 2011). So, the maximum power 175 W was chosen as the optimum output power (Fig. 2).

Optimization on extraction using desirability function: In numerical optimization, three independent variables ranging, maximum for responses and an exact value were the possible goals. A minimum and a maximum level must be considered for each independent variable. A level of solid-liquid ratio within range (1/10-1/30 g/mL), the extraction temperature within the range of 45-65°C and the power within the range of 125-175 W were set for a minimum and a maximum level. The optimal extraction conditions to obtain maximum YP were determined by Derringer's desired function methodology as follows: solid-liquid radio of 1:10, extraction temperature of 55°C and power of 175 W. Under these conditions, the predicted YP was 6.32% with a desirability value of 6.6%.

Neural network training: The range of four independent variables for building the neural network was set. Table 2 listed the input matrix, the yield of polysaccharides and Predicted values. Seventeen input values were divided into three sets, 11 values for the

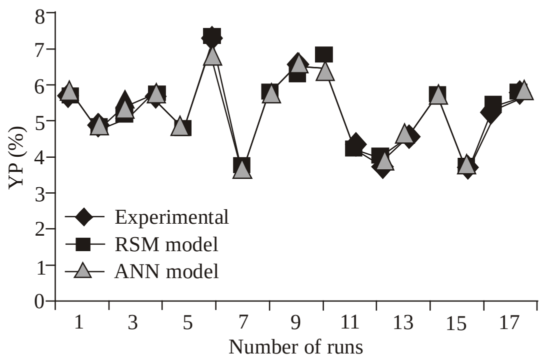


Fig. 3: Plot of experiment and theoretical results of RSM and ANN model

Table 3: Inhibitory effect of the polysaccharides and acarbose on α-glucosidase

Compound	α-glucosidase inhibitory activity (IC_{50})
Sample	8.316 μg/mL
Acarbose	9.475 μg/mL

training set and 3 values each for the validation set and the testing set. Computing environment (MATLAB 7) was used to design the neural network model. After testing, 8 neurons produced minimum value of error of the training and validation sets. The training data was used to compute the network parameters. To ensure robustness of the network parameters, the validation data was used. The testing stage was used to control error to avoid this “overfitting” phenomenon (Song *et al*., 2011).

The network gave a coefficient of determination (R^2) between the model prediction and experimental results, R^2 near to 1 considered to be a perfect and selected model. R^2 and MSE were determined as 0.99391 and 0.0495 for all data sets, 0.99955 for training set, 0.99917 for validation set, 1 for testing set, respectively. These results showed that the predictive accuracy of the model is high.

A comparison was made between the experimental values and the predicted values of extraction yield to examine the ANN model and RSM model. As shown in Fig. 3, it demonstrated the comparison of predictive responses of the two models (RSM model and ANN model) and the observed responses. The results were very close to the real experimental values. It means that the two models were able to successfully predict the extraction yield.

α-glucosidase inhibitory activity: α-Glucosidase inhibitory activities of the samples were shown in Table 3. Polysaccharides from *Cynomorium songaricum* had α-glucosidase inhibitory activity with an IC_{50} of 8.316 μg/mL and acarbose (positive control) had α-glucosidase inhibitory activity with an IC_{50} of 9.475 μg/mL. The inhibition of the polysaccharides was less compared with the reference inhibitor acarbose, but this may provide further scope for utilization of the plant for the treatment of diabetic complications. These studies indicated that polysaccharides are attractive as a new class of α-Glucosidase inhibitors. Nevertheless, further study will be focused on the chemical structures.

CONCLUSION

Experimentation has been done to simulate the polysaccharides extraction from *C. songaricum* using ultrasound-assisted extraction technology. Using response surface methodology and artificial neural network approach generate the model on yield of polysaccharides. Three factors of solid-liquid radio, extraction temperature and power markedly influenced the polysaccharides extraction from *C. songaricum*. Response surface methodology optimized conditions of the three independent variables, solid-liquid radio of 1/10 and extraction temperature of 55°C and power of 175 W have been selected to obtain the maximum yield of polysaccharides of 6.32% and the experimental extraction was 6.6%. Response surface plots gave a good interaction between the three variables and the response.

In this study, application of ultrasound-assisted extraction method in the extraction from *C. songaricum* reduced extraction time as well as improved the yield of polysaccharides. A three layered neural network with 10 neurons in the hidden layer successfully predicted the extraction of polysaccharides. The R^2 (0.99391) and MSE (0.0495) values of model suggested good fitness and generalization of the ANN. Between the predictive responses of the RSM model and ANN model, the optimization procedure showed a close agreement.

Polysaccharides from *Cynomorium songaricum* had α-glucosidase inhibitory activity with an IC_{50} of 8.316 μg/mL and acarbose (positive control) had α-glucosidase inhibitory activity with an IC_{50} of 9.475 μg/mL, respectively. This study has indicated that polysaccharides are attractive as a new class of α-glucosidase inhibitor.

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