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Research on antibacterial activity of *Schisandra chinensis* extracts by microwave-assisted

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ABSTRACT

This paper investigated the optimum microwave-assisted extraction proeess of Schisandra chinensis by application of orthogonal tests. Taking the antibacterial activity as index, with extraction time, extraction power, solvent and solid-liquid ratio as factors, extraction technology was optimized by L9 (3^4) orthogonal test. By using the mycelium growth rates method, the inhibition of Schisandra chinensis extracts were measured, the lethal concentration EC_{50} were calculated. It was found that the optimum extraction technology were: microwave power at 400W, extraction time at 5 min, solid-liquid ratio is 5:70 and solvent is acetone.

Keywords: Schisandra chinensis; microwave; antibacterial

INTRODUCTION

Schisandra is magnoliaceae[Schisandra chinen-sis (Turcz.) Baill.] ,it's dry fruit acid, sweet, warm[1-3],it has many function as astringe the lung、promote the secretion of saliva or body fluid 、antidiarrheal、kidney impotence, et al.[4-5].Many researches about schisandra on the central nervous system [6-8] had been reported. Schisandra has bacterial effect thus researches were focused on the inhibition of bacteria[9-12], however the study about which inhibited to fungal crops is less .Currently inhibition of fungi pathogens are still mainly rely on chemical fungicides, in order to develop botanical pesticides , this paper discussed Schisandra microwave extracts antibacterial activity through orthogonal disign. This study can provide data for future reference in agriculture for Schisandra.

EXPERIMENTAL SECTION

Instruments and Reagents

FW100-speed grinder (Tianjin Teste Instrument Co.),LDZM-80KCS Vertical Pressure Steam Sterilizer (Shanghai Shen An instrumentarija), 250D constant light incubator (Changzhou City Puda teaching instrument Co., Ltd.), SW-CG-2FD clean bench (Suzhou Antai air Technology Ltd.), WD900B microwave oven (Galanz microwave Shunde City Electric Co., Ltd.), 101C-3 type electric drying oven (Shanghai City Experimental instrument Factory), RE-52A rotary evaporator (Shanghai Ya Rong Biochemical instrument Factory), SHZ-D circulating water pumps (Henan Gongyi City, Henan Yu Ying Hua instrument Factory), electronic analytical balance (America Teller-Toledo Instruments Shanghai Co., Ltd.), MDS-8G microwave extraction instrument (Wuhan Connaught instrument Co., Ltd.)

Agar (AR) (Beijing Soledad Ltd.), glucose (AR) (Tianjin Guangfu Science and Technology Development Co., Ltd.), ethanol (AR) (Tianjin Guangcheng Chemical Reagent Co., Ltd.), acetone (Tianjin City-wide chemical reagent Co., Ltd.), ethyl acetate (Tianjin Guangcheng chemical reagent Co., Ltd.)

Species
Citrus anthracnose fungus, Fusarium oxysporum, Gaeumannomyces bacteria (these three plant pathogenic bacteria were obtained from Shandong Agricultural Bionic Application Engineering Research Center)

Schisandra chinensis dried fruit
purchased in Qingdao City, Hong Jen Tong pharmacy.

Schisandra chinensis microwave extraction method：Weigh some pulverized sample and placed it into a 250 mL three-neck flask, and added into appropriate amount of extraction solvent, then placed the flask in the microwave cavity, with the corresponding extraction power at appropriate times and appropriate extraction temperature. After extraction is completed, cooled, concentrated to a certain concentration by rotary evaporation. $L3^4$ orthogonal experimental design in accordance with the following Table 1

Table 1 Factors and levels

levels	factors			
	Extraction power/W A	Extraction time/min B	solid-liquid ratio C	Solvent D
1	400	3	5:50	Ethanol
2	600	5	5:70	Acetone
3	800	7	5:100	Ethyl acetate

Inhibition experiment
The mycelium growth rate method was used to determine the antibacterial activity of extracts. Pipette some extracts into 100mL PDA medium with a pipette to prepare the corresponding concentrations.While each test have a controle experiment with equal amount 20% ethanol.The PDA medium was poured inside a diameter of 6 cm or 7.5 cm dish to made of drug-containing tablet , punched at the edge of prepared bacteriathe with 4 mm punch, then inoculated to the prepared dish with inoculation needle at clean benche. Each treatment was repeated three times, wrapped with plastic film, placed into a constant temperature 27 ℃ light incubator about 3 d. When the bacteria pie in the control group grows about 2/3 dish, with a measured cross diameter of each colony, inhibition rate was calculated for each extract. Inhibition rate (%) = (the diameter of blank net growth - the diameter of treatment net growth diameter)/the diameter of blank net growth diameter×100%

Determination of median lethal concentration EC50
Pipette some extracts into 100mL PDA medium with a pipette to prepare the following concentrations:1 mg/mL、2 mg/mL、3 mg/mL、4 mg/mL、5 mg/mL、6 mg/mL、7 mg/mL、8 mg/mL and 10 mg/mL，antibacterial activity was measured using the above mycelial growth rate method.

Data processing
data calculated and processed by EXCEL.

RESULTS AND DISCUSSION

The antibacterial effects of Schisandra microwave extract
Schisandra microwave extracts inhibite citrus anthrax、fusarium、gaeumannomyces three fungi were disigned 3 level 4 factor orthogonal experiment. The extract solvent were ethanol、acetone、ethyl acetate and the concentration of extract was 4.0 mg/mL. The orthogonal experiment results showed in table 2 to table 4

It can be seen from table2~4, Schisandra have a certain inhibitory effect to the three bacteria at 4.0 mg/mL in different extract solvent. Among which Schisandra acetone extracts have the best inhibitory effect, the inhibition rate to citrus anthrax was 77.9%. Schisandra ethanol extracts have better inhibitory effect than ethyl acetate extracts.Throughout the experimental data in table 2~4 and analysis by range R , we can see that the optimal extraction process for Schisandra extract inhibite the three fungi are: acetone, 400W, 5min, solid-liquid ratio is 5:70.

Calculating the lethal concentration EC50 of Schisandra microwave extract
Pipette some extracts into PDA medium with a pipette to prepare the following concentrations:1 mg/mL、2 mg/mL、3 mg/mL、4 mg/mL、5 mg/mL、6 mg/mL、7 mg/mL、8 mg/mL and 10 mg/mL at the optimal extraction process for schisandra(acetone, 400W, 5min,solid-liquid ratio is 5:70). Followed by inoculation,investigated the inhibiton of the three fungi and calculated the EC50 for each species.

Table 2 The orthogonal experiment results of Schisandra microwave extracts inhibite citrus anthrax at 4.0 mg/mL

Test No	A(Power)	B(Time)	C(solid-liquid ratio)	D(solvent)	Inhibition rate
1	1(400W)	1(3min)	1(5:50)	1(Ethanol)	0.343
2	1(400W)	2(5min)	2(5:70)	2(Acetone)	0.779
3	1(400W)	3(7min)	3(5:100)	3(Ethyl	0.236
4	2(600W)	1(3min)	2(5:70)	3(Ethyl	0.200
5	2(600W)	2(5min)	3(5:100)	1(Ethanol)	0.321
6	2(600W)	3(7min)	1(5:50)	2(Acetone)	0.479
7	3(800W)	1(3min)	3(5:100)	2(Acetone)	0.600
8	3(800W)	2(5min)	1(5:50)	3(Ethyl	0.286
9	3(800W)	3(7min)	2(5:70)	1(Ethanol)	0.550
K1	1.357	1.143	1.107	1.214	
K2	1.000	1.386	1.529	1.857	
K3	1.436	1.264	1.157	0.721	
k1	0.452	0.381	0.369	0.405	
k2	0.333	0.462	0.510	0.619	
k3	0.479	0.421	0.386	0.240	
Range (R)	0.145	0.081	0.140	0.379	
Primary and secondary factors	D>A>C>B				
Optimal solution	D2 A3 C2 B2				

Table 3 The orthogonal experiment results of Schisandra microwave extracts inhibite fusarium at 4.0 mg/mL

Test No	A(power)	B(time)	C(solid-liquid ratio)	D(solvent)	Inhibition rate
1	1(400W)	1(3min)	1(5:50)	1(Ethanol)	0.373
2	1(400W)	2(5min)	2(5:70)	2(Acetone)	0.699
3	1(400W)	3(7min)	3(5:100)	3(Ethyl acetate)	0.196
4	2(600W)	1(3min)	2(5:70)	3(Ethyl acetate)	0.124
5	2(600W)	2(5min)	3(5:100)	1(Ethanol)	0.340
6	2(600W)	3(7min)	1(5:50)	2(Acetone)	0.556
7	3(800W)	1(3min)	3(5:100)	2(Acetone)	0.582
8	3(800W)	2(5min)	1(5:50)	3(Ethyl acetate)	0.222
9	3(800W)	3(7min)	2(5:70)	1(Ethanol)	0.451
K1	1.268	1.078	1.150	1.163	
K2	1.020	1.261	1.275	1.837	
K3	1.255	1.203	1.118	0.542	
k1	0.423	0.359	0.383	0.388	
k2	0.340	0.420	0.425	0.612	
k3	0.418	0.401	0.373	0.181	
Range (R)	0.083	0.061	0.052	0.431	
Primary and secondary factors	D>A>B>C				
Optimal solution	D2 A1 B2 C2				

Table 4 The orthogonal experiment results of Schisandra microwave extracts inhibite gaeumannomyces at 4.0 mg/mL

Test No	A(power)	B(time)	C(solid-liquid ratio)	D(solvent)	Inhibition rate
1	1(400W)	1(3min)	1(5:50)	1(Ethanol)	0.387
2	1(400W)	2(5min)	2(5:70)	2(Acetone)	0.673
3	1(400W)	3(7min)	3(5:100)	3(Ethyl acetate)	0.193
4	2(600W)	1(3min)	2(5:70)	3(Ethyl acetate)	0.160
5	2(600W)	2(5min)	3(5:100)	1(Ethanol)	0.367
6	2(600W)	3(7min)	1(5:50)	2(Acetone)	0.573
7	3(800W)	1(3min)	3(5:100)	2(Acetone)	0.500
8	3(800W)	2(5min)	1(5:50)	3(Ethyl acetate)	0.133
9	3(800W)	3(7min)	2(5:70)	1(Ethanol)	0.420
K1	1.253	1.047	1.093	1.173	
K2	1.100	1.173	1.253	1.747	
K3	1.053	1.187	1.060	0.487	
k1	0.418	0.349	0.364	0.391	
k2	0.367	0.391	0.418	0.582	
k3	0.351	0.396	0.353	0.162	
Range (R)	0.067	0.047	0.064	0.420	
Primary and secondary factors	D>A>C>B				
Optimal solution	D2 A1 C2 B3				

Schisandra chinensis microwave extraction antibacterial activity inhibited citrus anthrax at the optimal extraction process listed in the following table 5

From the linear regression equation Y=3.1594X+3.7772 in table 5, we can seen that when the inhibitory rate probability valueY=5, the logarithmic of concentration X=0.3869，i.e., EC50=2.44 mg/mL. Through statistics and chi-square test(χ^2=*4.68*), degree of freedom df=7, α(significant level)=0.05, $\chi^2_{0.05}(7)=14.07$, $\chi^2<\chi^2_{0.05}$ obviously, so it was accord with chi-square test, 95% confidence interval[1.35,4.40].

Schisandra chinensis microwave extraction antibacterial activity to fusarium at the optimal extraction process listed in the following table 6

Table 5 Schisandra chinensis microwave extraction antibacterial activity to citrus anthrax at the optimal extraction process

Concentration	Logarithm of the concentration(X)	Colony diameter(r)	Inhibition rate	probabilities of inhibition rate (Y)	logEC50 Standard error	Theoretical probability value	Theoretical inhibition rate(p)	Theoretical colony diameter(np)	Difference(r-np)	(r-np)^2/np/(1-p)
1.00	0.0000	3.80	0.2273	4.2521	0.017	3.7772	0.1107	4.3129	-0.5129	0.0686
2.00	0.3010	3.15	0.3750	4.6814	0.131	4.7282	0.3929	3.0712	0.0788	0.0033
3.00	0.4771	2.80	0.4545	4.8858		5.2846	0.6120	2.1071	0.6929	0.5872
4.00	0.6021	2.10	0.6136	5.2888		5.6793	0.7515	1.4933	0.6067	0.9922
5.00	0.6990	1.55	0.7386	5.6391		5.9855	0.8378	1.1136	0.4364	1.0541
6.00	0.7782	1.00	0.8636	6.0968		6.2357	0.8917	0.8765	0.1235	0.1607
7.00	0.8451	0.65	0.9432	6.5821		6.4472	0.9261	0.7253	-0.0753	0.1057
8.00	0.9031	0.50	0.9773	7.0004		6.6304	0.9485	0.6266	-0.1266	0.4969
10.00	1.0000	0.45	0.9886	7.2780		6.9366	0.9736	0.5162	-0.0662	0.3212
	CK=	4.80							Σ=	3.79
The correlation coefficient	r=	0.9462				EC50 Standard error	SE=	0.74		
The regression equation	Y=	3.7772	+	3.1594	X	95% Upper confidence limit(lg)	0.13	95%Lower confidence limit(lg)	0.64	
	lgEC50=		0.3870			95% Upper confidence limit	1.35	95%Lower confidence limit	4.41	
	EC50=	2.44	Chi-square value(χ2)	χ^2=	3.79	Probability(P)=0.05,N=7	$\chi^2_{0.05}$=	14.07	$\chi^2_{0.05}>\chi^2$	

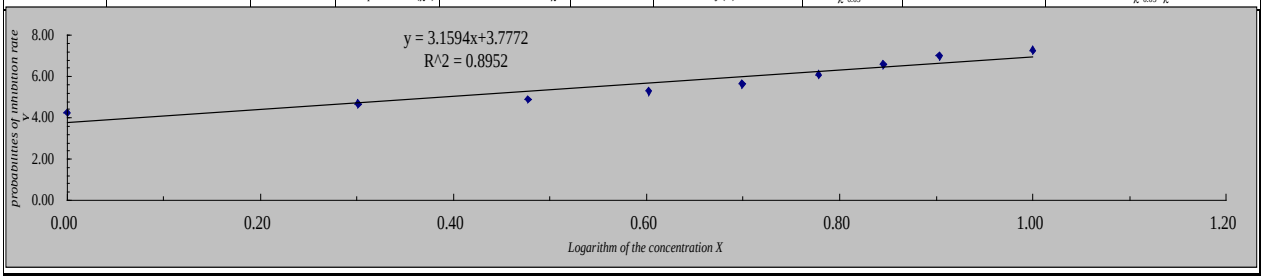
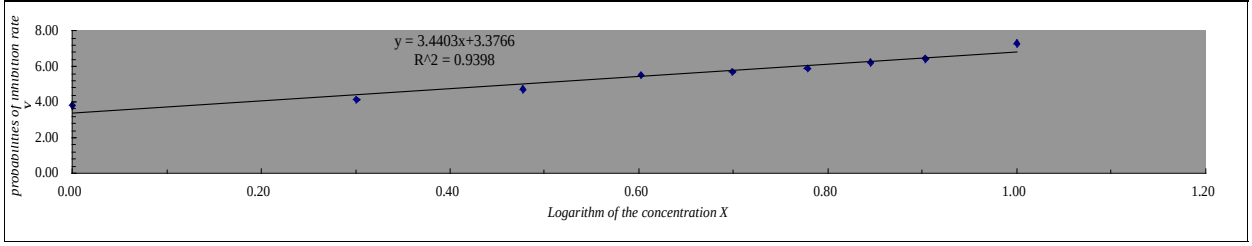


Table 6 Schisandra chinensis microwave extraction antibacterial activity to fusarium at the optimal extraction process

Concentration	Logarithm of the concentration(X)	Colony diameter(r)	Inhibition rate	probabilities of inhibition rate (Y)	logEC50 Standard error	Theoretical probability value	Theoretical inhibition rate(p)	Theoretical colony diameter(np)	Difference(r-np)	(r-np)^2/np/(1-p)
1.00	0.0000	4.45	0.1131	3.7900	0.012	3.3766	0.0523	4.7280	-0.2780	0.0173
2.00	0.3010	4.10	0.1898	4.1213	0.109	4.4123	0.2784	3.6955	0.4045	0.0614
3.00	0.4771	3.20	0.3869	4.7125		5.0181	0.5072	2.6504	0.5496	0.2313
4.00	0.6021	1.80	0.6934	5.5056		5.4479	0.6729	1.8938	-0.0938	0.0142
5.00	0.6990	1.50	0.7591	5.7035		5.7813	0.7827	1.3924	0.1076	0.0383
6.00	0.7782	1.25	0.8139	5.8922		6.0537	0.8540	1.0668	0.1832	0.2156
7.00	0.8451	0.90	0.8905	6.2292		6.2840	0.9004	0.8547	0.0453	0.0241
8.00	0.9031	0.75	0.9234	6.4280		6.4836	0.9310	0.7149	0.0351	0.0249
10.00	1.0000	0.45	0.9891	7.2921		6.8170	0.9654	0.5581	-0.1081	0.6046
	CK=	4.97							Σ=	1.23
The correlation coefficient	r=	0.9694				EC50 Standard error	SE=	0.74		
The regression equation	Y=	3.3766	+	3.4403	X	95% Upper confidence limit(lg)	0.26	95%Lower confidence limit(lg)	0.69	
	lgEC50=		0.4719			95% Upper confidence limit	1.81	95%Lower confidence limit	4.85	
	EC50=	2.96	Chi-square value(χ2)	χ^2=	1.23	Probability(P)=0.05,N=7	$\chi^2_{0.05}$=	14.07	$\chi^2_{0.05}>\chi^2$	



From the linear regression equation Y=3.4403X+3.3766 in table 6, we can seen that when the inhibitory rate probability valueY=5, the logarithmic of concentration X=0.4719，i.e., EC50=2.96 mg/mL. Through statistics and chi-square test(χ^2=*1.23*), degree of freedom df=7, α(significant level)=0.05, $\chi^2_{0.05}(7)$=14.07, $\chi^2<\chi^2_{0.05}$ obviously, so it was accord with chi-square test, 95% confidence interval[1.81,4.85].

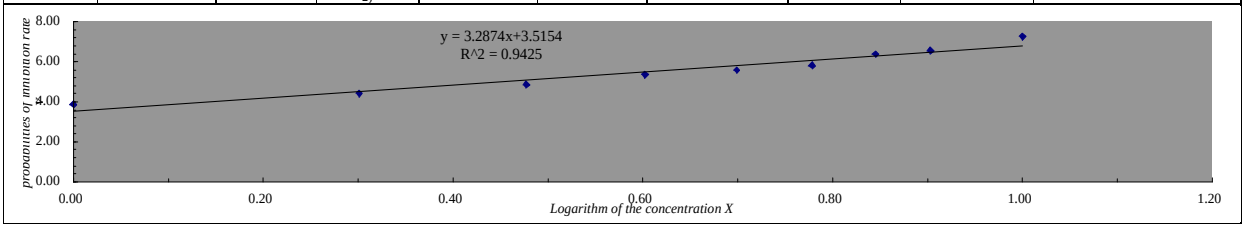
Schisandra chinensis microwave extraction antibacterial activity to gaeumannomyces at the optimal extraction process listed in the following table 7.

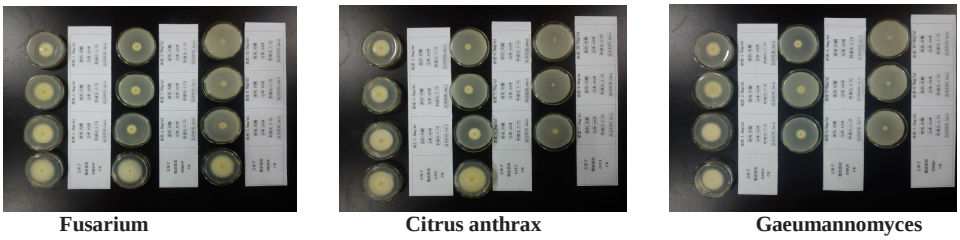
From the linear regression equation Y=3.2874X+3.5154 in table 7, we can seen that when the inhibitory rate probability valueY=5, the logarithmic of concentration X=0.4516，i.e., EC50=2.83 mg/mL. Through statistics and chi-square test(χ^2=1.62), degree of freedom df=7, α(significant level)=0.05, χ20.05(7)=14.07, $\chi^2<\chi^2_{0.05}$ obviously, so it was accord with chi-square test, 95% confidence interval[1.65,4.85].

The following are physical map of the optimal solution antibacterial activity of Schisandra chinensis extracts by microwave-assisted

Table 7 Schisandra chinensis microwave extraction antibacterial activity to gaeumannomyces at the optimal extraction process

Concentration	Logarithm of the concentration(X)	Colony diameter(r)	Inhibition rate	probabilities of inhibition rate (Y)	logEC50 Standard error	Theoretical probability value	Theoretical inhibition rate(p)	Theoretical colony diameter(np)	Difference(r-np)	(r-np)^2/np/(1-p)
1.00	0.0000	4.00	0.1290	3.8690	0.017	3.5154	0.0688	4.2489	-0.2489	0.0157
2.00	0.3010	3.40	0.2742	4.3998	0.131	4.5050	0.3103	3.2507	0.1493	0.0099
3.00	0.4771	2.70	0.4435	4.8580		5.0839	0.5334	2.3285	0.3715	0.1271
4.00	0.6021	1.90	0.6371	5.3507		5.4946	0.6896	1.6831	0.2169	0.0900
5.00	0.6990	1.55	0.7218	5.5881		5.8132	0.7920	1.2599	0.2901	0.3210
6.00	0.7782	1.25	0.7944	5.8216		6.0735	0.8585	0.9849	0.2651	0.5040
7.00	0.8451	0.75	0.9153	6.3743		6.2936	0.9021	0.8047	-0.0547	0.0379
8.00	0.9031	0.65	0.9395	6.5507		6.4842	0.9311	0.6847	-0.0347	0.0255
10.00	1.0000	0.45	0.9879	7.2540		6.8028	0.9643	0.5476	-0.0976	0.4871
	CK=	4.53							Σ=	1.62
The correlation coefficient	r=	0.9708				EC50 Standard error	SE=	0.85		
The regression equation	Y=	3.5154	+	3.2874	X	95% Upper confidence limit(lg)	0.19	95%Lower confidence limit(lg)	0.71	
	lgEC50=		0.4516			95% Upper confidence limit	1.57	95%Lower confidence limit	5.11	
	EC50=	2.83	Chi-square value(χ 2)	χ2=	1.62	Probability(P)=0.05,N=7	$\chi^2_{0.05}$=	14.07	因为$\chi^2_{0.05}$>χ^2，符合	





Fusarium **Citrus anthrax** **Gaeumannomyces**

Fig 1 Physical map of the optimal solution antibacterial activity of Schisandra chinensis extracts by microwave-assisted

Through the above comparative analysis we can know that the lethal concentration of Schisandra acetone extract to citrus anthrax fungi was 2.44 mg/mL , to fusarium was 2.96 mg/mL, and to gaeumannomyces was 2.83 mg/mL at the optimal process. These results were in line with the chi-square test, it have a better inhibition to citrus anthrax fungi.

CONCLUSION

In this study, schisandra were extracted by microwave through Orthogonal way, and its extract were conducted antifungal activity tested , the bacteria are Fusarium,Citrus anthrax and Gaeumannomyces. The antibacterial activity were obtained from each of experiments , through inhibition data we can be seen that optimal extraction process is: acetone, 400W, 5min, solid-liquid ratio is 5:70. And we calculated the lethal concentration EC50. It can be seen that Schisandra microwave extracts have antibacterial activity to fungi, and it's effect is higher than shichangpu discribed in reference [13]. This study provide some guidance for Schisandra used in botanical pesticides in the future agriculture. In the same time microwave extract has high dissolution rate, shorten the extraction time, and maintained the biological activity of the original substance, so the microwave extraction has an advantage in herbal extracts.

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