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Optimization of Antibacterial Activity by Gold-Thread (*Coptidis Rhizoma Franch*) Against *Streptococcus mutans* Using Evolutionary Operation-Factorial Design Technique

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Abstract This study was conducted to find the optimum extraction condition of Gold-Thread for antibacterial activity against *Streptococcus mutans* using The evolutionary operation-factorial design technique. Higher antibacterial activity was achieved in a higher extraction temperature (R^2=−0.79) and in a longer extraction time (R^2=−0.71). Antibacterial activity was not affected by differentiation of the ethanol concentration in the extraction solvent (R^2=−0.12). The maximum antibacterial activity of clove against *S. mutans* determined by the EVOP-factorial technique was obtained at 80°C extraction temperature, 26 h extraction time, and 50% ethanol concentration. The population of *S. mutans* decreased from 6.110 logCFU/ml in the initial set to 4.125 logCFU/ml in the third set.

Keywords: Gold-Thread, EVOP-factorial design technique, antibacterial activity, *S. mutans*

Numerous antimicrobials and antibiotics have been used against oral bacteria such as *Streptococcus mutans* (*S. mutans*) to reduce plaque-mediated diseases including dental caries [7, 14]. However, antibiotics have several adverse effects such as diarrhea, vomiting, and teeth staining [19]. Moreover, the development of antibiotics-resistant strains is a growing cause of concern. These disadvantages justify further research and development of natural antimicrobial agents targeting specific oral pathogens while being safe for the host [5].

Natural products have recently been investigated more thoroughly as promising agents to prevent oral diseases, especially plaque-related diseases such as dental caries [1, 9, 10]. Recent studies have demonstrated the antimicrobial activity of natural products against selected oral pathogens [7]. Gold-Thread (*Coptis chinensis Franch*) is a perennial herbaceous plant, which belongs to *Ranunculaceae* taxologically [2]. Antibacterial activity by ethanol extracts of Gold-Thread against food-spoiling bacteria and pathogenic bacteria was prominent and includes antifungal activity [17].

The evolutionary operation (EVOP)-factorial design technique [4, 13, 16] is a more effective approach to optimization of n variable systems, which takes the advantages of the factorial technique including response surface methodology (RSM) [6, 11, 12] and EVOP methodology [3, 18].

Therefore, the combined effects of extraction temperature, extraction time, and the ethanol content on the antibacterial activity of Gold-Thread were investigated in this study using the EVOP-factorial design to maximize the antibacterial activity of *Coptidis Rhizoma* against the dental caries-causing bacteria, *S. mutans*.

Gold-Thread (*Coptis chinensis Franch*) was obtained from Yakrung market (Daegu, Korea) in February 2006, and lyophilized for 48–72 h after storage at −70°C. Freeze dried samples were pulverized with a blender (HJM-7000, Hanil, Korea). Extra-pure grade solvents were purchased from Daemyung Scientific Co. (Daegu, Korea) and chemical reagents from Sigma Co. (St. Louis, MO, U.S.A.), unless otherwise stated.

Streptococcus mutans ATCC 27351 (*S. mutans*) was cultured in Brain Heart Infusion media at 37°C under facultative anaerobic culture.

To design an experiment for establishing proper extraction conditions, 20 g of sample was hydrolyzed and extracted in a reflux extraction apparatus by differentiating the extraction temperature, extraction time, and ethanol concentration, and then freeze-dried. Samples were homogenized for 30 sec and serially diluted with peptone water (Difco, U.S.A.), as needed for the determination of a microbial populations. The colony forming unit (CFU) of particular microbial group was counted in the BHI agar at 37°C, 24–72 h.

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Table 1. Experimental design for the three-inducer system and results of Set I.

Experimental conditions	E_{10}	E_{11}	E_{12}	E_{13}	E_{14}	E_{20}	E_{21}	E_{22}	E_{23}	E_{24}
Temperature (°C)	50(0)	35(-)	35(-)	65(+)	65(+)	50(0)	65(+)	35(-)	65(+)	35(-)
Time (h)	14(0)	8(-)	20(+)	8(-)	20(+)	14(0)	20(+)	8(-)	8(-)	20(+)
Ethanol concentration (%)	50(0)	25(-)	75(+)	75(+)	25(-)	50(0)	75(+)	75(+)	25(-)	25(-)
Antibacterial activity (cycle I)	6.05	6.52	6.03	5.45	5.29	6.08	4.95	6.38	5.64	6.25
Antibacterial activity (cycle II)	6.17	6.66	6.22	5.23	5.14	6.18	4.81	6.53	5.40	6.10
Difference (cycle I-cycle II)	-0.12	-0.14	-0.19	0.22	0.15	-0.10	0.14	-0.15	0.22	0.15
Average activity	6.110 (a_{10})	6.590 (a_{11})	6.125 (a_{12})	5.340 (a_{13})	5.125 (a_{14})	6.130 (a_{20})	4.880 (a_{21})	6.455 (a_{22})	5.520 (a_{23})	6.175 (a_{24})

Numbers in parentheses are the coded symbols of levels of the extraction conditions.

The EVOP-factorial design technique was applied to select the optimum conditions of three extraction factors in different experiments. Firstly, the control or search level experimental conditions (E_{10}, E_{20}) were selected based on the results of earlier investigation on the effect of individual extraction condition on the antibacterial activity of Gold-Thread. Secondly, the new experimental conditions (E_{be}) were selected with lower and higher levels of inducers compared with the search level (E_{b0}). Antibacterial activities of Gold-Thread were estimated following the given assay procedure and recorded for cycles I and II. Differences in antibacterial activities between cycles I and II, and average antibacterial activities were calculated to estimate the effects and error limits. The magnitudes of effects, error limits, and change in mean effect were examined as the decision-making procedure to arrive at the optimum. When the experimental results of the first set did not satisfy the optimum conditions, a second set of experiments was planned, selecting the best condition of the first set as the new search level for the second set. This procedure was repeated until the optimum condition was obtained.

Table 2. Calculation worksheet of effects of three-variable system and magnitude of effects and error limits of Set I.

Effects		Calculation of effects	
Temperature (°C)		$1/4(a_{13}+a_{14}+a_{21}+a_{23}-a_{11}-a_{12}-a_{22}-a_{24})$	-1.1200
Time (h)		$1/4(a_{12}+a_{14}+a_{21}+a_{24}-a_{11}-a_{13}-a_{22}-a_{23})$	-0.4000
Ethanol concentration (%)		$1/4(a_{12}+a_{13}+a_{21}+a_{22}-a_{11}-a_{14}-a_{23}-a_{24})$	-0.1525
Temperature×Time		$1/4(a_{11}+a_{14}+a_{21}+a_{22}-a_{12}-a_{13}-a_{23}-a_{24})$	-0.0275
Temperature×Ethanol concentration		$1/4(a_{11}+a_{13}+a_{21}+a_{24}-a_{12}-a_{14}-a_{22}-a_{23})$	-0.0600
Time×Ethanol concentration		$1/4(a_{11}+a_{12}+a_{21}+a_{23}-a_{13}-a_{14}-a_{22}-a_{24})$	0.0050
Temperature×Time×Ethanol concentration		$1/4(a_{21}+a_{22}+a_{23}+a_{24}-a_{11}-a_{12}-a_{13}-a_{14})$	-0.0375
Change in mean effect		$1/10(a_{11}+a_{12}+a_{13}+a_{14}+a_{21}+a_{22}+a_{23}+a_{24}-4a_{10}-4a_{20})$	-0.0275
Standard deviation (σ)		$1/2(\sigma_1+\sigma_2)=1/2(R_1\times f_{k,n}+R_2\times f_{k,n})^{(1)}$	0.1200
Error limits:	For average	$\pm 1.414\sigma$ ($\pm 2\sigma/n$)	0.1697
	For effects	$\pm 1.004\sigma$ ($\pm 0.71\times 2\sigma/n$)	0.1205
	For change in mean	$\pm 0.891\sigma$ ($\pm 0.63\times 2\sigma/n$)	0.1069

R_1: (largest difference - smallest difference) in Block 1.
R_2: (largest difference - smallest difference) in Block 2.
$f_{k,n}$=constant depending on number of replications (n) and number of experiments (k) per cycle=0.3 for n=2 and k=5.

Table 3. Experimental design for the three-inducer system and results of Set II.

Experimental conditions	E_{10}	E_{11}	E_{12}	E_{13}	E_{14}	E_{20}	E_{21}	E_{22}	E_{23}	E_{24}
Temperature (°C)	65(0)	50(-)	50(-)	80(+)	80(+)	65(0)	80(+)	50(-)	80(+)	50(-)
Time (h)	20(0)	14(-)	26(+)	14(-)	26(+)	20(0)	26(+)	14(-)	14(-)	26(+)
Ethanol concentration (%)	75(0)	50(-)	100(+)	100(+)	50(-)	75(0)	100(+)	100(+)	50(-)	50(-)
Antibacterial activity (cycle I)	4.90	6.25	4.62	4.75	4.18	4.75	4.15	6.01	4.63	4.66
Antibacterial activity (cycle II)	4.79	6.11	4.43	4.88	4.12	4.77	4.27	5.85	4.50	4.86
Difference (cycle I-cycle II)	0.11	0.14	0.19	-0.13	0.06	-0.02	-0.12	0.16	0.13	-0.20
Average activity	4.845 (a_{10})	6.180 (a_{11})	4.525 (a_{12})	4.815 (a_{13})	4.150 (a_{14})	4.760 (a_{20})	4.210 (a_{21})	5.930 (a_{22})	4.565 (a_{23})	4.760 (a_{24})

Numbers in parentheses are the coded symbols of levels of the extraction conditions.

Table 4. Calculation worksheet of effects of the three-variable system and magnitude of effects and error limits of Set II.

Effects		Calculation of effects
Temperature (°C)		-0.9138
Time (h)		-0.9613
Ethanol concentration (%)		-0.0438
Temperature×Time		0.4513
Temperature×Ethanol concentration		0.1988
Time×Ethanol concentration		-0.0438
Temperature×Time×Ethanol concentration		-0.0513
Change in mean effect		0.0715
Standard deviation (σ)		0.1020
Error limits:	For average	0.1442
	For effects	0.1024
	For change in mean	0.0909

R_1: (largest difference - smallest difference) in Block 1.
R_2: (largest difference - smallest difference) in Block 2.
$f_{k,n}$=constant depending on number of replications (n) and number of experiments (k) per cycle=0.3 for n=2 and k=5.

Table 6. Calculation worksheet of effects of the three-variable system and magnitude of effects and error limits of Set III.

Effects of		Calculation of effects
Temperature (°C)		-0.0813
Time (h)		-0.0925
Ethanol concentration (%)		0.0013
Temperature×Time		0.0363
Temperature×Ethanol concentration		-0.0188
Time×Ethanol concentration		-0.0163
Temperature×Time×Ethanol concentration		-0.0338
Change in mean effect		0.2695
Standard deviation (σ)		0.1035
Error limits:	For average	0.1463
	For effects	0.1039
	For change in mean	0.0922

R_1: (largest difference - smallest difference) in Block 1.
R_2: (largest difference - smallest difference) in Block 2.
$f_{k,n}$=constant depending on number of replications (n) and number of experiments (k) per cycle=0.3 for n=2 and k=5.

The experimental conditions used in the first set of experiments, the corresponding antibacterial activities of cycles I and II, their differences, and average values are presented in Table 1. The extraction temperature, extraction time, and ethanol concentration of the central point in the first set (E_{10}, E_{20}) were 50°C, 14 h, and 50%, respectively. The error limits, effects, and change in mean effect were calculated and the results are shown in Table 2. According to the decision-making procedure, after calculating the change in mean effects and error limits, an examination is necessary to determine whether any change in the control (search level) experimental conditions would help to improve the objective function [18]. The optimum condition was achieved when the effect was smaller than the error limit while the change in mean effect is large. Moreover, because the dependent variables are number of *S. mutans* in which growth is suppressed by addition of Gold-Thread, the optimum point was reached when the code of mean effect is negative.

The determination of the magnitude of the change in mean effect, which is negative and large, compared with the error limit, is a requirement in order to confirm the achievement of the optimum condition. Such a situation where some of the effects are large in comparison with the error limit does not ensure that the condition at the search region (E_{10}, E_{20}) of the first set is the actual optimum, and a second set of experiments is called for.

In the second set, the search level (E_{10}, E_{20}) was fixed at the best condition of Set I, at a level of E_{21}, in which the number of *S. mutans* was 4.880 log CFU/ml. The extraction temperature, extraction time, and ethanol concentration of the central point in the second set (E_{10}, E_{20}) were 65°C, 20 h, and 75%, respectively. The experimental conditions and the results of Set II experiments are presented in Table 3 and the effects and error limits are shown in Table 4. The most effective antibacterial activity (4.150 log CFU/ml) was obtained at E_{14}. The extraction temperature, extraction time, and ethanol concentration of the E_{14} point in the second set were 80°C, 26 h, and 50%, respectively. In this case, not all of the effects are smaller than the error limit, even though the change in mean effect is positive and large compared with

Table 5. Experimental design for the three-inducer system and results of Set III.

Experimental conditions	E_{10}	E_{11}	E_{12}	E_{13}	E_{14}	E_{20}	E_{21}	E_{22}	E_{23}	E_{24}
Temperature (°C)	80(0)	65(-)	65(-)	95(+)	95(+)	80(0)	95(+)	65(-)	95(+)	65(-)
Time (h)	26(0)	20(-)	32(+)	20(-)	32(+)	26(0)	32(+)	20(-)	20(-)	32(+)
Ethanol concentration (%)	50(0)	25(-)	75(+)	75(+)	25(-)	50(0)	75(+)	75(+)	25(-)	25(-)
Antibacterial activity (cycle I)	4.23	4.67	4.38	4.40	4.35	4.04	4.46	4.48	4.42	4.37
Antibacterial activity (cycle II)	4.05	4.47	4.49	4.54	4.46	4.21	4.28	4.60	4.39	4.49
Difference (cycle I-cycle II)	0.18	0.20	-0.11	-0.14	0.09	-0.17	0.18	-0.12	0.03	-0.12
Average activity	4.140 (a_{10})	4.570 (a_{11})	4.435 (a_{12})	4.470 (a_{13})	4.405 (a_{14})	4.125 (a_{20})	4.370 (a_{21})	4.540 (a_{22})	4.405 (a_{23})	4.430 (a_{24})

Numbers in parentheses are the coded symbols of levels of the extraction conditions.

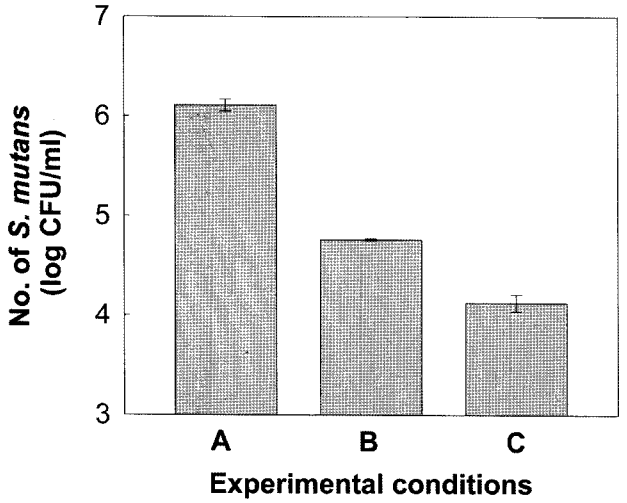

Fig. 1. Comparison of the antibacterial activity of Gold-Thread against *S. mutans* at the central point of each set.
A. Central point of Set I (Extraction temperature, 50°C; extraction time, 14 h; ethanol concentration, 50%). **B.** Central point of Set II (Extraction temperature, 65°C; extraction time, 20 h; ethanol concentration, 75%). **C.** Central point of Set III (Extraction temperature, 80°C; extraction time, 26 h; ethanol concentration, 50%).

the error limit. If all or any of the effects are larger than the error limits, the change in the experimental conditions may yield better results [3].

Under the above situation, the third set of experiments was designed, in which the best condition of Set II (E_{14}) was selected as the search level (E_{10}, E_{20}) for Set III. The experimental conditions and the results of Set III are shown in Table 5 and the calculated effects and error limits are presented in Table 6. In the EVOP-factorial design, if the effect is smaller than the error limit while the change in mean effect is large, then the minimum has been reached if the change in mean effect is positive. Thus, in the third experiment, we were able to arrive at the proper optimum condition, in which all the effects are smaller than the error limit and the change in mean effect is large and positive. Furthermore, the population of *S. mutans* decreased from 6.110 logCFU/ml in the first set to 4.125 logCFU/ml in the third set, as shown in Fig. 1.

Moreover, it was shown that a higher antibacterial activity was achieved in a higher extraction temperature of 80°C (R^2=−0.79) and in a longer extraction time of 26 h (R^2= −0.71). However, antibacterial activity against *S. mutans* was not affected by differentiation of ethanol concentration in the extraction solvent (R^2=−0.12), as shown in Fig. 2.

Therefore, the maximum antibacterial activity of clove against *S. mutans* determined by the EVOP-factorial technique was obtained at 80°C extraction temperature, 26 h extraction time, and 50% ethanol concentration.

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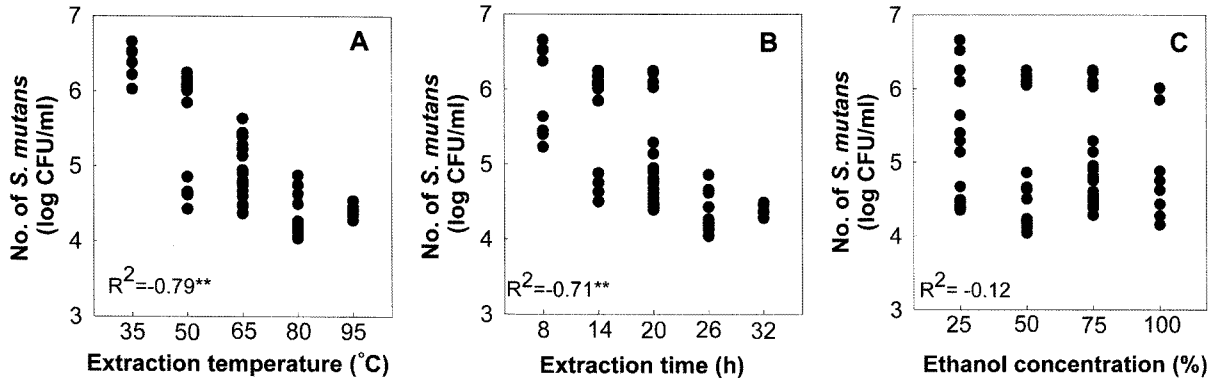

Fig. 2. Main effects plot for responses against independent variables in EVOP.
** p<0.05. **A.** Extraction temperature (°C); **B.** Extraction time (h); **C.** Ethanol concentration (%).

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