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Original Research Article

Extraction optimization of *Eucommia ulmoides* Oliver and its effect on bone quality in OVX rats

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Abstract

Purpose: *To maximize the yield of extract from Eucommia ulmoides Oliver and its effect on bone quality.*
Methods: *Different extraction indices were optimized with response surface methodology (RSM) for maximization of extract yield from Eucommia ulmoides Oliver. Box–Behnken design (BBD) was used to identify the effects of temperature, time, and liquid to solid ratio on extract yield from Eucommia ulmoides Oliver. After 4-week acclimatization, thiry-two rats were randomly assigned to 4 groups (n = 8): group 1 (sham) given vehicle only; group 2 (OVX rats given Eucommia ulmoides Oliver extract at a dose of 4 g/kg; group 3 (OVX + vehicle); group 4 (OVX + EUOE), i.e., OVX rats given Eucommia ulmoides Oliver extract (4 g/kg). Sham rats had intact ovaries. After surgery, the rats received gentamicin intramuscularly for 3 successive days. Two months after surgery, blood and trabecular bones was taken for analysis.*
Results: *Temperature and liquid-to-solid ratio had marked impact on extract yield from Eucommia ulmoides Oliver, with the best conditions being temperature of 88 °C, time of 137 min, and liquid to solid ratio 16:1. Using these optimized conditions, the maximum yield of extract obtained experimentally (2.53 %) was very close to the predicted value of 2.49 %. There was a good fit between the mathematical model evolved and the data on extract yield. The extract significantly ($p < 0.01$) increased the Ca and P and Cr levels in OVX + EUOE group compared to those in OVX control. Moreover, the extract significantly ($p < 0.01$) increased macro-mechanical indices of trabecular bone in OVX+EUOE group, relative to those in OVX control.*
Conclusion: *The yield of Eucommia ulmoides Oliver extract has been successfully optimized using RSM. The extract exhibited strong effects on bone quality.*

Keywords: *Optimization, Eucommia ulmoides, Box–Behnken design, Response surface methodology, Bone loss, Gene*

INTRODUCTION

Eucommia ulmoides Oliver (*Eucommiaceae*) is a medicinal herb in Traditional Chinese medicine TCM) [1]. The medicinal parts of *E. ulmoides* are its stem bark and leaves. Several bioactive compounds with pharmacological effects have been isolated from *E.*

ulmoides [1,2]. Pharmacological research has revealed that the leaves possess anti-hypertensive [3], anti-obesity [4] and anti-inflammatory properties [2].

The effects of variable indices and their interactions are determined using RSM [5]. It is a very popular and reliable method for the optimization of operations involving plant products [6-8]. The technique minimizes the number of trials needed to ascertain the relationships among multiple factors, and it facilitates simultaneous investigations of numerous indices [9–11].

This study was carried out to develop effective response surface models to obtain optimal extract conditions from *Eucommia ulmoides* Oliver, based on temperature, time, and solid: liquid, for efficient extraction of *Eucommia ulmoides*. The effect of *Eucommia ulmoides* Oliver extract on bone quality in OVX rats was also investigated.

EXPERIMENTAL

Preparation of extract from *Eucommia ulmoides* Oliver

The *Eucommia ulmoides* Oliver was first refluxed with 90 % ethanol for 5 h. Then, the resultant residue was extracted with water at different temperatures for various tested times to obtain the aqueous extract. The supernatant fraction was concentrated and dried. Three major extraction parameters: temperature, time, and the liquid: solid ratio were considered in this study.

Study design

Experiments were conducted to optimize extract yield from *E. ulmoides* Oliver with RSM, using a 3- tier, 3-parameter BBD. The BBD permits investigation of factors to at least one at the center point (0) [12]. Variables such as temperature (A), time (B), and liquid: solid ratio (C) were used.

In all, 17 runs were used to measure extract yield, while maintaining the independent variables at levels −1, 0, and +1 (Table 1). Optimum extraction conditions were chosen and validated on the basis of BBD results. Regression coefficients (β) for assessment of the degree of influence of each factor on response were deduced from 3-D response surface plots. For individual responses, model F-value, absence of fit and R^2 were determined.

Induction of osteoporosis in rats

A total of thiry-two rats (mean bwt = 240 ± 20 g) were assigned to 4 groups (8 rats per group): vehicle-treated (sham, group 1); OVX rats treated with *Eucommia ulmoides* Oliver extract (4 g/kg, group 2); OVX + vehicle (group 3), and OVX rats given extract at a dose of 4 g/kg b.w. Each aniaml was fasted for 6 h prior to excision of bilateral ovary under pentobarbitone anaesthesia (50 mg/kg). The ovaries of rats in the sham group were not removed. Post-surgery, gentamicin was administered intramuscularly to the rats for 3 successive days. Two months after surgery, blood and trabecular bones were taken for analysis.

Tests on bone biomechanics

Indices of biomechanics of L5 vertebrae were determined as described earlier [13].

Statistical analysis

Results are presented as mean±SD. The reliability of RSM calculations and equations were determined. Estimation of dissolution was done with 3 formulations chosen at random, using the experimentally-derived models.

RESULTS

Optimized extraction temperature

The influence of temperature on extraction yield of *Eucommia ulmoides* Oliver was studied (Figure 1). The results indicated a sharp rise in the extract yield with temperature up to 90 °C, and a slow decrease subsequently (Figure 1). The highest extract yield (2.42 %) was obtained at 90 °C. Therefore, extraction temperature of 90 °C was chosen for optimization of other parameters.

Optimized extraction time

The extraction yield from *Eucommia ulmoides* Oliver increased rapidly with time for about 140 min, with no increases thereafter (Figure 2). Maximum extract yield (2.39%) was attained after 160 min. Thus, 140 min was selected for optimizing other indices.

Optimized liquid: solid ratio

The optimum liquid: solid ratio was determined using a wide range of 11:1 to -18:1. The optimum liquid-to-solid for maximum extract yield from *Eucommia ulmoides* Oliver extraction yield at solid-to-liquid ratio of 1:16 was 2.51 % (Figure 3).

Further increases in the liquid-to-solid ratio had no marked effect on extract level. Thus, the optimized solid-to-liquid ratio of 1:16 was used in subsequent experiments.

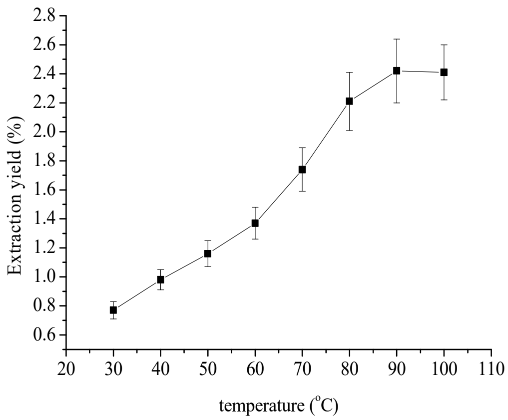


Figure 1: Influence of extraction temperature on extract level of *Eucommia ulmoides* Oliver

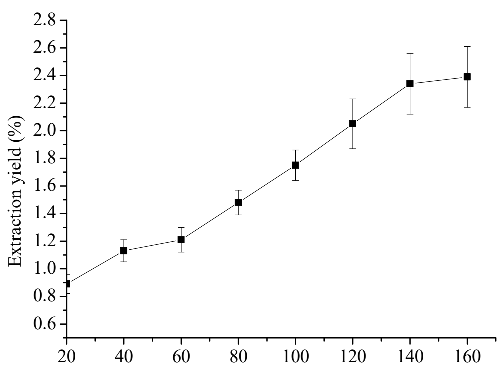


Figure 2: Influence of time on extract yield of *Eucommia ulmoides* Oliver

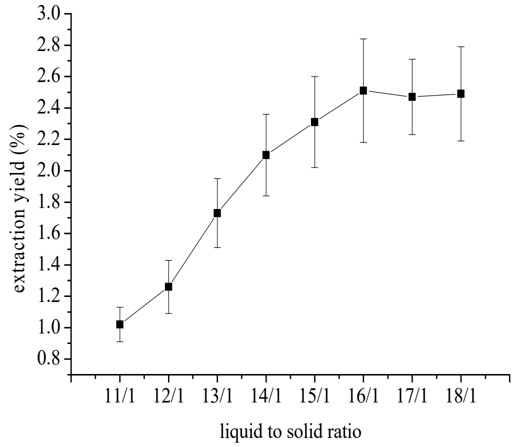


Figure 3: Influence of liquid: solid ratio on extract yield of *Eucommia ulmoides* Oliver

RSM-optimized EUOE

The experimental values of *Eucommia ulmoides* Oliver extraction yield varied from 1.93% to 2.47 % (Table 1).

The quadratic model was chosen as optimized model for EUOE extraction since the linear term was significant ($p < 0.0001$), as inferred from results of regression analysis in Table 3.

Using optimized conditions i.e. temperature of 88 °C, time of 137 min and solid: liquid ratio of 1:16 to test the model, the actual extract yield from *Eucommia ulmoides* Oliver was 2.53 % (n = 3), demonstrating that the experimental and predicted values were comparable ($p > 0.05$). Thus, the response model is adequate for use in optimizing extract yield.

Table 1: BBD and responses of variables

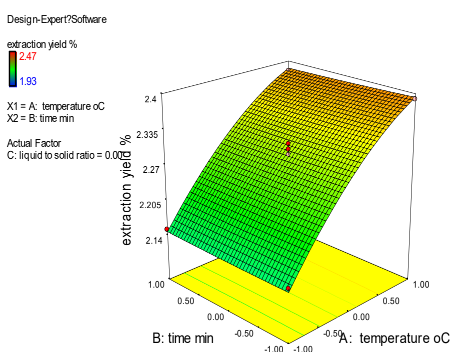
Run	A: Extraction temperature (°C)	B: Extraction time (min)	C: Liquid: solid ratio	R: Extract yield (%)
1	-1.00	-1.00	0.00	2.15
2	1.00	-1.00	0.00	2.39
3	-1.00	1.00	0.00	2.15
4	1.00	1.00	0.00	2.38
5	-1.00	0.00	-1.00	1.93
6	1.00	0.00	-1.00	2.28
7	-1.00	0.00	1.00	2.31
8	1.00	0.00	1.00	2.47
9	0.00	-1.00	-1.00	2.16
10	0.00	1.00	-1.00	2.17
11	0.00	-1.00	1.00	2.4
12	0.00	1.00	1.00	2.39
13	0.00	0.00	0.00	2.31
14	0.00	0.00	0.00	2.3
15	0.00	0.00	0.00	2.29
16	0.00	0.00	0.00	2.3
17	0.00	0.00	0.00	2.29

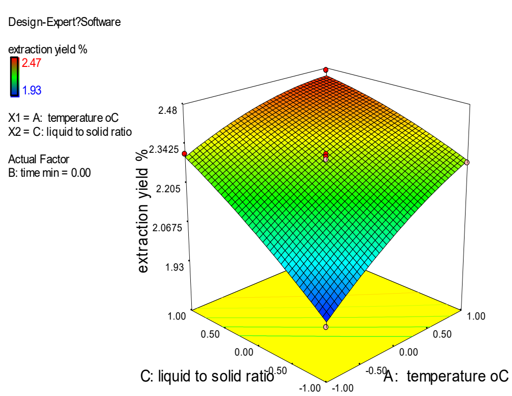
Table 2: Analysis of variances (ANOVA) for RSM-BBD model

Source	Sum of squares	df	Mean square	F Value	*P*-value Prob > F	
Model	0.27	9	0.03	103.89	< 0.0001	Significant
A (temp., ºC	0.12	1	0.12	419.13	< 0.0001	
B (min)	1.25E-05	1	1.25E-05	0.044	0.8405	
C (liquid:solid ratio)	0.13	1	0.13	462.99	< 0.0001	
AB	2.50E-05	1	2.50E-05	0.087	0.7762	
AC	9.03E-03	1	9.03E-03	31.51	0.0008	
BC	1.00E-04	1	1.00E-04	0.35	0.5732	
A^2	4.18E-03	1	4.18E-03	14.59	0.0065	
B^2	4.21E-06	1	4.21E-06	0.015	0.9069	
C^2	1.52E-03	1	1.52E-03	5.31	0.0547	
Residual	2.01E-03	7	2.86E-04			
Lack of Fit	1.73E-03	3	5.75E-04	8.21	0.0348	Significant
Pure Error	2.80E-04	4	7.00E-05			
Cor Total	2.70E-01	16				
SD	0.017		R-Squared		0.9926	
Mean	2.27		Adj R-Squared		0.9830	
C.V. %	0.74		Pred R-Squared		0.8961	
PRESS	0.028		Adeq Precision		38.713	

Effect of EUOE on Ca and P and Cr levels in OVX rats

The experimental analyses revealed that the Ca and P and Cr levels in sham were markedly higher than those in OVX control (Figure 5 and Figure 6). However, there were greater decreases in the Ca and P and Cr levels in OVX + EUOE rats than in OVX control (Figure 5 and Figure 6). There were no marked differences in any of the parameters between the sham, and sham + EUOE groups.





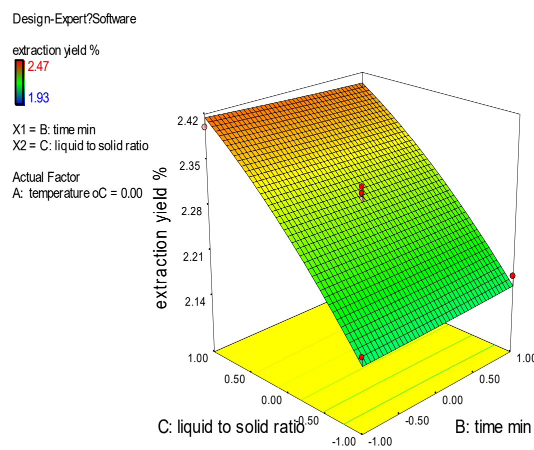


Figure 4: Response surface showing the interaction between three parameters. (A and B) temperature (◦C) and time (min); (A and C) temperature (◦C) and liquid-to-solid ratio; (B and C) time (min) and liquid: solid ratio

Impact of EUOE on biomechanical parameters in OVX rats

Table 3 indicates that macro-mechanical indices were markedly higher in sham rats than in OVX rats. Ultimate load was markedly greater in OVX + EUOE group, relative to OVX groups. The stiffness was significantly greater in the OVX + EUOE group when compared to the OVX groups. The energy to failure was significantly greater in OVX + EUOE group than in OVX groups. The other indices were comparable among the sham, and sham + EUOE groups.

Table 3: Effect of EUOE on ultimate load, stiffness and energy to failure in OVX rats

Group	Ultimate load (N)	Stiffness (N/mm)	Energy to failure (mJ)
Sham	389.71±33.95 **	29.75±2.22 **	215.77±18.45 **
OVX	278.43±23.62	19.41±1.64	164.93±12.58
Sham + EUOE (4g/kg)	390.53±30.14	31.53±26.38	217.95±20.99
OVX + EUOE (4g/kg)	376.21±28.59 ##	25.52±20.51 #	201.39±18.47 ##

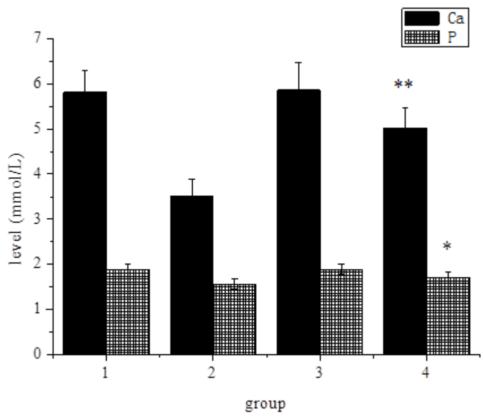


Figure 5: Effect of EUOE on blood Ca and P levels in OVX rats

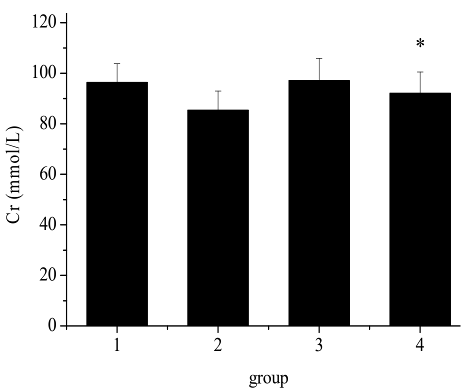


Figure 6: Effect of EUOE on blood Cr levels in OVX rats

DISCUSSION

In this optimization, yields in range of parameters are shown in Table 1. Some specimens were prepared and tested based on these optimized values, and their mean values were obtained. Using linear regression and statistical analysis [14,15], the results demonstrated that all the input parameters had significant impacts on yield of extract from EUOE. Temperature and solid: liquid ratio had the most effect on extract yield. The interaction plot indicated significant interaction between the temperature and liquid: solid ratio with respect to extract yield which is also evident in the output mathematical model of yield response.

The response points are assessed based on 3D response surface and 2D contour plots [16]. There were marked interactions between temperature and liquid: solid ratio. These two factors had the highest influence on extract levels.

In Japan, *Eucommia ulmoides* Oliver is called *Tuchong*, while Chinese call it *Du-zhong* Chinese) [17]. It is a deciduous tree which belongs to the family of *Eucommiaceae* [18]. The present study showed that EUOE treatment improves Ca and P and Cr levels and trabecular bone biomechanical properties in OVX rats

CONCLUSION

The extraction conditions for *Eucommia ulmoides* Oliver have been successfully optimized using RSM. The optimized conditions obtained are temperature of 88 °C, time of 137 min and solid-to-solid ratio of 1:16. The results suggest that EUOE has potential for application in the suppression of bone degeneration in humans.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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