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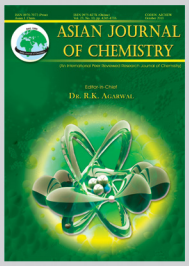

Extraction of Total Triterpenoid Saponins from *Ganoderma lucidum* by Box-Behnken Design

LIANG HE[1], XINGGUO GONG[1,*], JUNWEN CHENG[2], XUEQIAN WU[2], QINGQI WU[2] and HAIBO LI[2]

[1]Institute of Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310027, P.R. China
[2]Zhejiang Provincial Key Laboratory of Biological and Chemical Utilization of Forest Resources, Zhejiang Forestry Academy, Hangzhou 310023, P.R. China

*Corresponding author: Fax: +86 571 8778225; Tel: +86 571 87798225; E-mail: kite006@126.com; violet006@163.com

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A three-factor, three-level Box-Behnken factorial design combined with response surface methodology was conducted to optimize ultrasonic-assisted extraction for triterpenoid-enriched extract from *Ganoderma lucidum*. Three independent variables were extraction time (X_1), ultrasonic power (X_2) and ratio of solution to solid (X_3), respectively. The experimental data obtained were fitted to a second-order polynomial equation with analysis of multiple regressions. The results demonstrated that those three factors significantly affected the yield of total triterpenoid saponins (TPS) from *Ganoderma lucidum*. The optimal processing conditions of the extraction of total triterpenoid saponins by ultrasound treatment were determined: extraction time 27 min, ultrasonic power 135 w and ratio of solution to solid 47 mL/g. At this condition, the actual yield of total triterpenoid saponins was 1.122 %, which increased by a factor of 1.35 compared with that of classical extraction. Scanning electron microscopic (SEM) images of the fungal cells after ultrasound treatment were obtained to provide visual evidence of the disruption effect.

Key Words: Ultrasonic extraction, Triterpenoid saponins, Optimization, *Ganoderma lucidum*, Box-Behnken design, SEM.

INTRODUCTION

Ganoderma lucidum (Leyss. ex Fr.) Karst, regarded as an elixir of life, has been widely used as a medicinal mushroom to promote vitality and longevity in East Asian countries. It has been reported that a number of bioactive ingredients, isolated from the fruiting body of *Ganoderma lucidum*, have received considerable attention owing to their conspicuous pharmacological activities. Among those, triterpenoids identified from *Ganoderma lucidum* have demonstrated inspiring biological activities, including anti-HIV-1[1,2], antihisramine[3], antinociceptive[4], antichloesterol[5], antitumor[6,7], antimicrobial[8], antiandrogenic[9], antihepatitis[10], antioxidant[11], anticomplement[12] and angiotension-converting enzyme-inhibitory activities[13]. Hence, *Ganoderma lucidum* has been developed as a potential prophylactic agent for human health[14].

At present, the total triterpenoids saponins of *Ganoderma lucidum* are mainly extracted from solvent extraction involving methanol, 95 % ethanol, chloroform and saturated sodium carbonate solution[11,15,16]. However, these methods mentioned above are more time-consuming and less efficient in extraction compared with some improved process. In recent years, a variety of novel techniques have been developed in the extraction of triterpenoids from *Ganoderma lucidum* by using supercritical fluid extraction[17-20] and microwave-assisted extraction[21]. To our best of knowledge, there is no report available on the ultrasound extraction from the fruiting body of *Ganoderma lucidum*. Ultrasonic technology, as an inexpensive, simple and efficient alternative, is more desirable in the process of triterpenoids from *Ganoderma lucidum* in view of its own advantages and the characteristics of the raw material. It is known that the high initial lignin extent of the tissue exist in the fruiting body of *Ganoderma lucidum*[22], acoustic cavitation can break the hard cell walls mechanically with shear forces of micro-bubbles and facilitate mass transfer between solid-liquid phases dramatically within the system, which might obtain the maximum of the bioactive constituents with a relatively lower cost[23].

Response surface methodology (RSM) is a collection of mathematical and statistical techniques extensively utilized to optimize the extraction process in the food industry[24-27]. Box-Behnken of response surface methodology, having only three levels and fewer experiments involved, is more efficient and easier to arrange experiments, evaluate the model and determine multiple factors as well as possible interactions between independent variables in comparison with others[20,28-31].

In this study, the aim of this present work was to optimize ultrasonic technology conditions for the extraction of total

triterpenoid saponins (TPS) from dried fruiting body of *Ganoderma lucidum*. The quantification of total triterpenoid saponins was adopted by a simple, quick and accurate colorimetric method with vanillin-acetic acid system[6,16,32]. Response surface methodology was designed to fulfill the optimal experimental conditions and interpret the mutual interactions between the tested variables. Further, the extraction mechanism on the process of total triterpenoid saponins from *Ganoderma lucidum* powder and advantages of ultrasonic technology was verified by scanning electron microscopy (SEM).

EXPERIMENTAL

The dried raw material (*Ganoderma lucidum* (Leyss. ex Fr.) Karst) was supplied by Zhejiang Essence fungi Co.Ltd (Lishui, South of Zhejiang Province). The dried sample was sliced, then grinded into particle size of 0.29 mm by a miller (DG-120, RuiAn, China).

Ethanol, chloroform, ethylacetate, *n*-buthanol, acetone, acetic acid, perchloric acid, methylene chloride and methanol were purchased from Huadong Medicine Group Co. (Hangzhou, China); Vanillin was obtained from Shanghai Spice Auxiliary Co. (Shanghai, China); oieanolic acid standard was supplied by the China Institute for Drugs and Biological Products Identification (Beijing, China). All the chemicals used in this study were analytical grade.

Ultrasonic-assisted extraction of total triterpenoid saponins from *Ganoderma lucidum*: The process of total triterpenoid saponins extraction from dried fruiting body of *Ganoderma lucidum* by ultrasonic treatment was performed in an ultrasonic cell disintegrator (JY96, Xinzhi Bio-Technology and Science Inc., Ningbo, Zhejiang Province, China). This kind of generator was equipped with a wattmeter allowing the power to be adjusted in the range 0-150 W. The ultrasonic output power could be set to a desired level ranging from 0-100 % of the nominal power by the amplitude controller. The total triterpenoid saponins of *Ganoderma lucidum* was extracted by adding 2 g of ground dried powder into a designed volume of 70 % ethanol in a 250 mL flask. The ultrasonic probe was inserted into the mixture to work at different time and the temperature was regulated at constant desired level to avoid the solvent rise. Each of tested samples was extracted under continuous ultrasonic waves at 20 kHz with different levels of power output. Ice bathing was used to ensure the temperature of solution was below 45 ºC during the whole extraction processing. After extraction, the vessels were kept for at least 15 min to cool down at room temperature. Then each extract was centrifuged at 6000 rpm to separate the liquid and residue. Finally the supernatant was evaporated to a small volume under the vacuum and lyophilized. All the experiments were performed in triplicate.

Classical extraction of total triterpenoid saponins from *Ganoderma lucidum*: Classical extraction was carried out based on the results of preliminary experiments. Two grams of samples were placed in a 250 mL conical flask and 100 mL 70 % ethanol were added for 2 h extraction at 65 ºC with additional stirring. All the extracts were filtered using Whatman No. 1 filter paper. The solution were cooled down to room temperature rapidly and concentrated into a small volume by vaccum and lyophilized to be ready for analysis and SEM.

Detection method

Calibration curve: The measurement of total triterpenoid saponins followed the colorimetric method described by Jiang *et al.*[32] with some minor modification. The mixed stock solution of oieanolic acid (810 g/L) was originally prepared and divided into six parts with 0.5 mL in each 10 mL tube. The solvent was heated to evaporation in a water-bath. Subsequently each sample was added with 0.5 mL of new mixed 5 % (w/v) vanillin-acetic acid solution and 1 mL of perchloric acid. After a thorough mixture, the solution was incubated at 65 ºC for 15 min and transferred into an ice-water bath for 3 min. Finally the whole system was made up for to get a volume of 5 mL with ethyl acetate before it cooled down to room temperature. The absorbance at 552 nm was determined using a spectrophotometer (Shanghai Spectrum instrument Co., Ltd) against a blank solution. Calibration graph was plotted according to the test value for linear regression analysis. The stock solution was stored at 4 ºC.

Quantitative analysis of total triterpenoids saponins: The crude total triterpenoid saponins was dissolved in ethanol and diluted to a certain concentration. Then 0.3 mL extract solution was absorbed to a tube before measurement. The absorbance was measured at 552 nm using the same procedure as above and its quantitative analysis of the ethanolic extract was estimated by extrapolation of the standard curve obtained.

Experimental design: A three level, three variable Box-Behnken design (BBD) (Design Expert Software, Trial version 7.1.3, Stat-Ease Inc., Minneapolis, USA) was employed to determine the best combination of extraction variables for the yield of total triterpenoid saponins from *Ganoderma lucidum*. X_1 (extraction time), X_2 (ultrasonic power) and X_3 (ratio of solution to solid) were selected as the major variables for this research and the proper range of three factors were established on the basis of one-factor-at-a-time trials for total triterpenoid saponins extraction (Table-2). As shown in Table-1, the whole design consisted of 17 experimental trial, five replicates at the centre of the design were used to allow for estimation of a pure error sum of squares. Each experiment was performed in triplicate and the yield of total triterpenoid saponins recovery (%) was taken as the response (Y). For statistical calculation, the experimental variable X_i has been coded as x_i according to the following transformation equation:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (1)$$

where x_i is the dimensionless coded value of the variable X_i, X_0 the value of X_i at the center point and ΔX the step change.

The experimental data were fitted into an empirical second order polynomial model with regression analysis. The mathematical model could be expressed as follows:

TABLE-1
INDEPENDENT VARIABLES AND THEIR LEVELS FOR BOX-BEHNKEN DESIGN

Independent variables	Variable levels		
	-1	0	1
Extraction time (min)	15	25	35
Ultrasonic power (w)	105	120	135
Ratio of solution to solid (mL/g)	25	40	55

TABLE-2
BOX-BEHNKEN EXPERIMENTS AND DATA

Experiments	Coded levels			Yield of TPS (%)	
	X_1	X_2	X_3	Observed	Predicted
1	-1	-1	0	0.607	0.611
2	1	-1	0	0.691	0.678
3	-1	1	0	0.943	0.956
4	1	1	0	1.062	1.061
5	-1	0	-1	0.638	0.626
6	1	0	-1	0.754	0.755
7	-1	0	1	0.835	0.834
8	1	0	1	0.845	0.857
9	0	-1	-1	0.589	0.591
10	0	1	-1	0.905	0.902
11	0	-1	1	0.689	0.690
12	0	1	1	1.109	1.108
13	0	0	0	0.925	0.951
14	0	0	0	0.949	0.951
15	0	0	0	0.969	0.951
16	0	0	0	0.963	0.951
17	0	0	0	0.949	0.951

TPS = Total triterpenoid saponins

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} x_i^2 \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j \qquad (2)$$

where Y is the measured response associated with each factor level combination; β_0 is an intercept; β_i is the regression coefficients computed from the observed experimental values of Y; and X_i is the coded levels of independent variables. The terms X_i, X_j and X_i^2 represent the interaction and quadratic terms, respectively.

Statistical analyses: Data were expressed as means standard error (SE) of three replicated determinations. The multiple regression analysis and analysis of variance (ANOVA) were carried out using a software Design-Expert 7.1.3 Trial to fit quadratic polynomial equations. The quality of the fitted model was expressed by the coefficient determination R^2 and its statistical significant was checked by F-test and *p*-value.

Electron microscopy scanning: A Hitachi S-3400N field scanning electron microscopy (Hitachi, Sanjose, CA, USA) was used to examine the morphological alteration of the dried samples with or without ultrasonic treatment. Samples were mounted on bronze stubs with double-sided adhesive tape allowing surface visualization and then coated with a layer of gold (40-50 nm) in a sputter coater to avoid charging under the electron beam. All samples were employed with SEM under high vacuum condition at an accelerating voltage of 15 kV (20 μm, 2000 × magnification).

RESULTS AND DISCUSSION

Calibration curves and quantitative analysis of total triterpenoids saponins: The standard curve and the linear relationship could be obtained by regression analysis, which was described as follows (Fig. 1):

$$A = 47.505C \qquad (R^2 = 0.9968) \qquad (3)$$

where C (mg/mL) is the concentration of triterpenoids saponins of solution for colorimetric analysis, A is the absorbance at VIS 552 nm. According to eqn. 3, the yield Y was calculated by

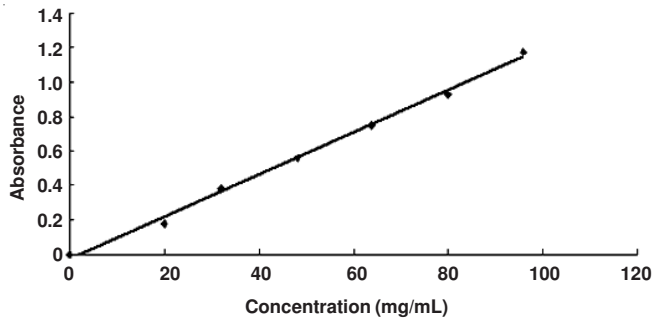

Fig. 1. Standard curve of oieanolic acid

$$Y = 0.105 \times A \times \frac{V}{V_1} \times \frac{1}{m} \times 100\ \%\ (\%;\ m/m) \qquad (4)$$

where V is the total volume of ethanol solution (mL), V_1 is the analysis volume of extraction liquid (mL), m is the mass of *Ganoderma lucidum* sample (g) and A is the absorbance at 552 nm.

Fitting the model: For response surface methodology based on the Box-Behnken design that was used for optimizing the ultrasonic-assisted extraction conditions of total triterpenoids saponins, 17 experimental runs with different combinations of three factors, along with their experimental and predicted values, were shown in Table-2. It can be found from Table-2 that there was a considerable variation in the yield of total triterpenoid saponins depending on different ultrasonic-assisted extraction conditions. The maximum total triterpenoid saponins was 1.108 % in run number 16 and the minimum was 0.591 % in run number 5. The centre point in the design was repeated five times to estimate the error.

By applying multiple regression analysis methods, the data obtained were analyzed based on eqn. 2, the predicted response Y for total triterpenoid saponins in shown as follows:

$$Y = 0.95 + 0.041X_1 + 0.18X_2 + 0.074X_3 + 0.00875X_1X_2 - 0.027X_1X_3 + 0.026X_2X_3 - 0.09X_1^2 - 0.035X_2^2 - 0.093X_3^2 \qquad (5)$$

where Y is the predicted response variable, *i.e.*, the yield of total triterpenoids saponins (%); X_1, X_2, X_3 are coded values of the independent variables, *i.e.*, time, ultrasonic power and ratio of solution to solid, respectively.

The statistical significant of eqn. 5 was checked by the Fisher's statistical test for ANOVA and the results were summarized in Table-3. F-test suggested that the second model had a very high model F-value (F = 155.75) and a very low *p*-value ($p < 0.0001$), indicating this model was well adjusted to the experimental data.

TABLE-3
ANALYSIS OF VARIANCE FOR THE FITTED QUADRATIC POLYNOMIAL MODEL OF EXTRACTION OF TOTAL TRITERPENOID SAPONINS

Source	Sum of squares	Degree of freedom	Mean square	F-value	*p*-Value
Model	0.41	9	0.045	155.75	< 0.0001
Residual	0.0023	7	0.00029		
Lack of fit	0.00087 83	3	0.0002928	1.02	0.4733
Pure error	0.00115 2	4	0.000288	–	–
Cor Total	0.41	16	–	–	–

$R^2 = 0.9950$, $R_{Adj}^2 = 0.9886$, CV = 2.01.

The adjusted determination coefficient (R^2_{adj}) implied that the sample variation of 98.86 % for the yield of total triterpenoid saponins was attributed to the independent variables and only about 1.14 % of the total variation could not be demonstrated by the model. The closer value of R to 1, the better is the correlation between the experimental and predicted values. Here, the value of R (0.9975) for eqn. 5 being close to 1 indicated a close agreement between the experimental results and the theoretical values predicted by the quadratic equation. A relatively low value of CV (2.01) in Table-3 illustrated further the experiments were practical with a better precision and reliability.

The corresponding *p*-value along with the parameter estimate was displayed in Table-4. The *p*-value is used as a tool to examine the significant of each coefficient and the mutual interactions between each independent variable. The results shown in Table-4 suggested that all the independent variables (X_1, X_2, X_3) and three quadratic terms (X_1^2, X_2^2, X_3^2) significantly affected the yield of total triterpenoid saponins, the *p*-values being very well. There was significant interaction between time (X_1) and ration of solution to solid (X_3), the interactive term (X_1X_3) had the same features. Meanwhile, the ultrasonic power (X_2) was the major factor in the process of extracting the total triterpenoid saponins from *Ganoderma lucidum* due to the function of cavitation bubbles[33].

TABLE-4
ESTIMATED COEFFICIENTS OF THE SECOND-ORDER REGRESSION MODEL FOR TOTAL TRITERPENOID SAPONINS YIELD

Coefficient	Degree of freedom	Sum of squares	Mean square	F-Value	*p*-Value
X_1	1	0.014	0.014	46.65	0.0002
X_2	1	0.26	0.26	897.41	< 0.0001
X_3	1	0.044	0.044	151.04	< 0.0001
X_{12}	1	3.062×10^{-4}	3.062×10^{-4}	1.06	0.3383
X_{13}	1	2.809×10^{-3}	2.809×10^{-3}	9.69	0.0170
X_{23}	1	2.704×10^{-3}	$2.704*10^{-3}$	9.32	0.0185
X_1X_1	1	0.034	0.034	117.92	< 0.0001
X_2X_2	1	5.195×10^{-3}	5.195×10^{-3}	17.91	0.0039
X_3X_3	1	0.036	0.036	125.22	< 0.0001

Analysis of response surface: The three-dimensional response surface and two-dimensional contour plots are an effective method to visualize the relationship between response and experimental levels of each variable and the type of interactions between two variables. Figs. 2-4 were the fitted response surface plots and their corresponding contour plots for the yield of total triterpenoid saponins generated by the predicted model respectively.

Fig. 2 depicted the 3D-plot and its respective contour plot showing the effects of X_1 (time) and X_2 (ultrasonic power) on the yield of total triterpenoid saponins, while X_3 (ratio of solution to solid) was fixed at a constant value of 40 (actual). A circular shape of the contour plot indicated that no interaction between X_1 (time) and X_2 (ultrasonic power) was found to contribute to the yield of total triterpenoid saponins at a significant level. This fact could be well accounted by *p*-value (0.3383 > 0.05) in Table-4. From Fig. 2, it was evident that X_2 (ultrasonic power) had a linear positive effect on the yield of total triterpenoid saponins in the range of time (15-35 min), which

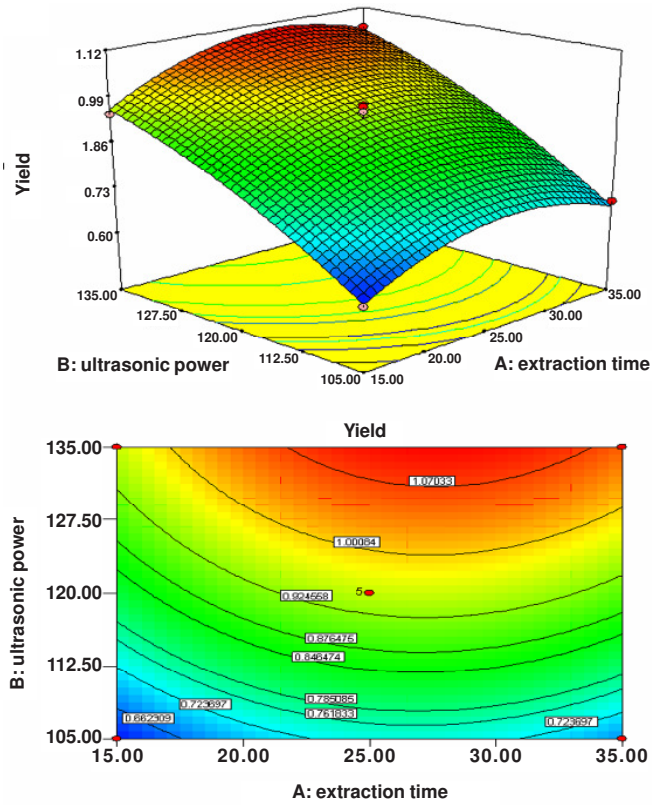

Fig. 2. Response surface plot and contour plot of extraction time and ultrasonic power and their mutual interactions on the yield of total triterpenoid saponins

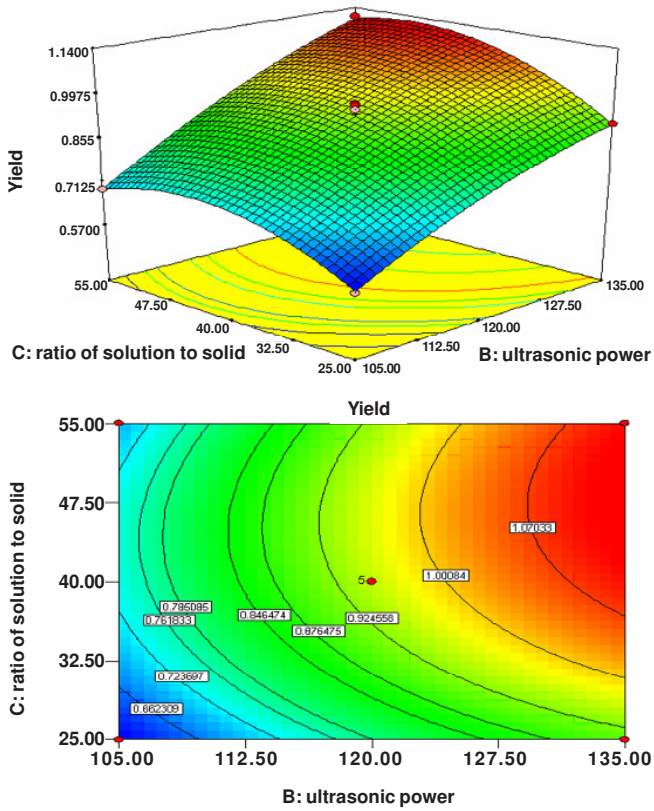

Fig. 3. Response surface plot and contour plot of ultrasonic power and ratio of solution to solid and their mutual interactions on the yield of total triterpenoid saponins

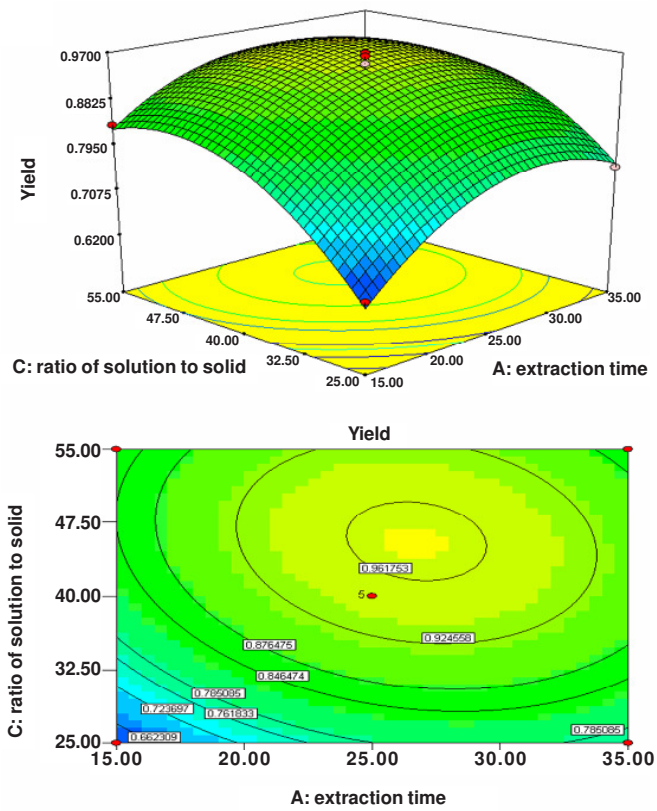

Fig. 4. Response surface plot and contour plot of extraction time and ratio of solution to solid and their mutual interactions on the yield of total triterpenoid saponins

was consistent with the results of steroids and triterpenoids from three *Chresta* spp[34]. They reported that the extraction rate of sterols and triterpenoids under the influence of ultrasound was increased many times compared to the normal extraction. The following reasons are attributed to their findings. One reason is that the increasing number of cavitation bubbles can be formed when the large amplitude ultrasonic wave travel through an alcohol medium of extraction process. Those can put the bubbles into an instantaneous collapse, which effectively enhance the penetration of 70 % ethanol go into *Ganoderma lucidum* cell tissues. The other is of great acceleration of the mass transfer rate made by thoroughly mixture of alcohol with total triterpenoid saponins molecules. To our best of knowledge, the hard cell walls of *Ganoderma lucidum* do exist due to the raw materials' highly lignifications. Herein, the large increase in ultrasonic power may cause the active total triterpenoid saponins of *Ganoderma lucidum* more permeable. It was evident that yield of total triterpenoid saponins increased gradually with the increasing time from 15 to 27 min (actual value), but decreased slowly beyond the range. This indicated that excessive increase of extraction time would get an opposite result in *Ganoderma lucidum* and also highlighted that it was only feasible to make the whole process more economical.

Fig. 3 presented a similar plot at various concentrations of X_2 (ultrasonic power) and X_3 (ratio of solution to solid) at fixed X_1 (time) with middle level. There was an obvious interaction relationship existing between these two test variables, which could be proved by *p*-value (0.0185) in Table-4. It was easily observed from Fig. 3 that the yield of total triterpenoid saponins climbed up continuously when the value of X_3 (ratio of solution to solid) increased from 25 mL/g to an optimum point at all ultrasonic power. Then it went down slightly outside that optimum point. When the liquid to material ratio is at a small value, the effect of ultrasound-assistance is quite significant. However, the decline of the total triterpenoid saponins yield may occupy the most part of the whole process when the ratio is beyond the certain range although much solvent could reduce the viscosity and facilitate the extraction. Those could be similarly observed in ultrasound assisted extraction of saikosaponins from Radix Bupleuri[35].

The effect of X_1 (time) and X_3 (ratio of solution to solid) on the yield of total triterpenoid saponins at fixed X_2 (ultrasonic power) with middle value was shown in Fig. 4. It was noticed that there was a response plot went downward, which indicated the maximum predicted value of the yield of total triterpenoid saponins existed. And the significant interactionship of time and ratio of solution to solid could be easily explained by its elliptical shape of the contour plot and *p*-value (0.0170 < 0.05). According to the response curves, the yield of total triterpenoid saponins resulted in a linear increase in ratio of solution to solid and then reduced slightly the designed range of extraction time from 15 to 35 min. An increase of extraction time promoted the yield of total triterpenoid saponins at a constant ratio of solution to solid within 27.09 min. Beyond that range, contradict result would occur.

Optimization of extracting parameters and validation of the model: By applying the software of Design-Expert (7.1.3), the maximum predicted value and the predicted optimum conditions for total triterpenoid saponins extraction could be obtained quietly. Those were as following: extraction time of 27.01 min, ultrasonic power of 135.00 w, ratio of solution to solid 47.64 mL/g and a maximum response of 1.127 % was predicted by the model equation. To confirm the predicted value was not bias toward the practical result, further experiments were carried out under the slightly modified optimal conditions (Table-5). A mean value of 1.122 ± 0.058 % (N = 5) acquired from real experiments, demonstrated the validation of the response surface methodology model. It concluded that the model of eqn. 5 developed was considered to be satisfactory and accurate for predicting the yield of total triterpenoid saponins from *Ganoderma lucidum*. Furthermore, it could be deduced that the maximum yield of total triterpenoid saponins acquired by ultrasonic technology had a close value in supercritical carbon dioxide extraction[36] and microwave-assisted proces, which might furnish another better way to replace those methods with lower cost.

TABLE-5
OPTIMUM CONDITIONS AND THE PREDICTED AND EXPERIMENTAL VALUE OF RESPONSE AT THE OPTIMUM CONDITIONS

	Extraction time (min)	Ultrasonic power (w)	Ratio of solution to solid (mL/g)	Yield of TPS (%)
Optimum conditions	27.01	135.00	47.64	1.127 (Predicted)
Modified conditions	27.00	135.00	47.00	1.122 ± 0.058 (actual)

TPS = Total triterpenoid saponins.

Comparison of classical and ultrasound-assisted extraction: In this study, ultrasound-assisted extraction procedure was compared with the classical extraction technique for the extraction of total triterpenoid saponins obtained from *Ganoderma lucidum* through the experiments. The conditions of different techniques and their results are shown in Table-6. It is noted that all the extraction techniques were used under their optimized conditions. The employment of ultrasonic-assisted extraction improved the yield of total triterpenoid saponins obtained remarkably and it is a production-cost saving technology. A higher yield of 1.122 % could be acquired at lower extraction temperature (45 ºC) and dramatically shorter processing time (27 min). Furthermore, the yield of total triterpenoid saponins extracted by ultrasonic method increased by a factor of 1.35 compared with that by the classical extraction (at a temperature of 65 ºC, a time of 2 h). Therefore, ultrasound method is more suitable for the extraction of total triterpenoid saponins from *Ganoderma lucidum* than those of classical extraction because of its highly extraction efficiency and relatively low cost.

TABLE-6
COMPARISON OF THE YIELD OF CLASSICAL AND ULTRASONICALLY ASSISTED EXTRACTION

Treatment	Extraction time	Temp. (ºC)	Ratio of solution to solid (mL/g)	Yield of TPS (%)
Classical	2 h	65	50	0.832 ± 0.043
Ultrasonic	27 min	45	47	1.122 ± 0.058

TPS = Total triterpenoid saponins.

Scanning electron microscopy (SEM): In order to get further insight into the extraction mechanism on the process of total triterpenoid saponins from *Ganoderma lucidum* powder and to study the structural alteration during the different extraction techniques, scanning electron microscopy was used to investigate the effect of sonication on the physical structure of *Ganoderma lucidum* powder with or without ultrasonic extraction. Fig. 5a-c show a set of SEM images of *Ganoderma lucidum* powder at a magnification factor of 10000×. In Fig. 5a, it reveals the SEM micrograph of the nonsonication-treated samples, the cells of *Ganoderma lucidum* tissues were kept intact, which could be compared with the structures of the sonication-treated sample presented in Fig. 5b-c. From Fig. 5b, it could be noticed that little destruction of the microstructure of the sample occurs under the condition of 60w sonication-treatment. While after a higher ultrasonic power assistance (135w), obvious hollow openings were generated gradually in the surface morphology of *Ganoderma lucidum* tissues (Fig. 5c), which indicated that the ultrasonic power played an important role in breaking up fungal cell walls to enhance extraction yield.

The solvent extraction of solid dried materials normally involves two processes: first is soaking the raw materials into solvent to facilitate the swelling and the hydration process; and second is the mass transfer of soluble ingredients from the cell to solvent environment by osmotic and diffusion process[37]. With the assistance of ultrasonic treatment on the process of total triterpenoid saponins from *Ganoderma lucidum*, it enhanced

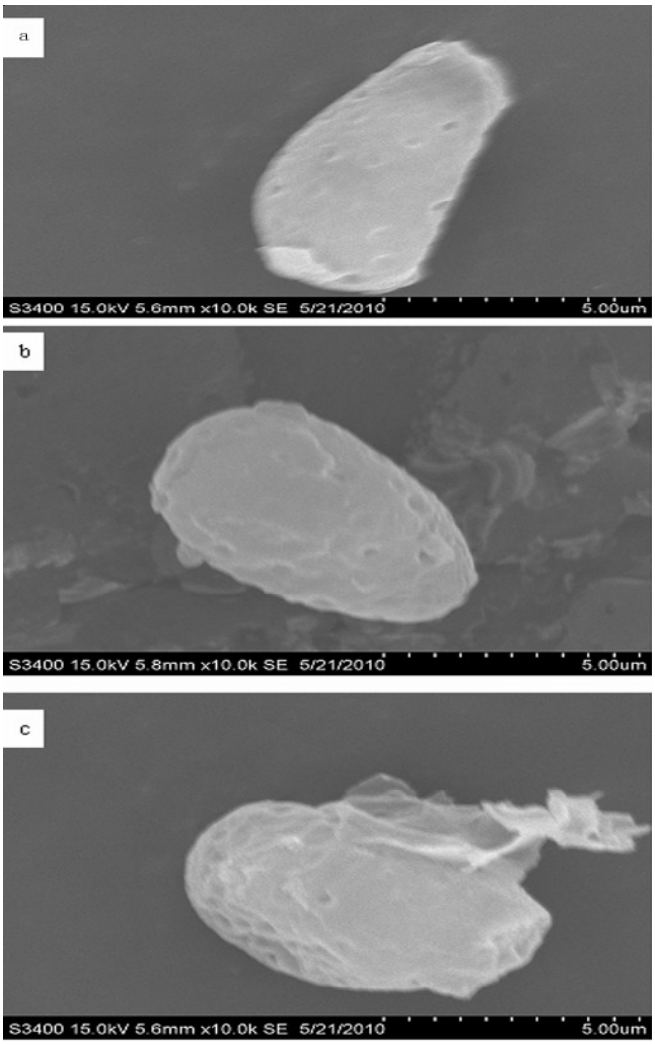

Fig. 5. Scanning electron micrographs of *Ganoderma lucidum* surface after different extraction techniques

the swelling process and provided a greater penetration of solvent into cellular materials by cavitation energy, which greatly increased the avaible surface area between ethanol solvent and the cells, improved the yield of total triterpenoid saponins and reduced extraction time.

Conclusion

To date, no reports are available in literature regarding the optimization of ultrasound extraction conditions for the yield of total triterpenoid saponins from *Ganoderma lucidum*. Conventional process studies are usually of low-yield and time-consumption. Ultrasonic technology was then performed for the total triterpenoid saponins extraction with aim to enhance the yield value and response surface methodology based on one-factor-at a-time was used to estimate and optimize the three experimental variables-time (min), ultrasonic power (w) and ratio of solution to solid (mL g^{-1}). Through the regression analysis, all the independent variables, quadratic terms and interactive terms had positive influence on the response values regardless of the interactionship of time and ratio of solution to solid ($p < 0.05$). The ultrasonic power was the leading factor during the whole extraction process due to its unique advantages. The high regression coefficient of the second polynomial model equation by Box-Behnken Design greatly help optimize

the extraction condition of *Ganoderma lucidum* in the process-optimizing exercise. The optimal ultrasonic-assisted extraction conditions for the yield of total triterpenoid saponins were obtained as follows: time 27 min, ultrasonic power 135 w, ratio of solution to solid 47 mL/g. Under these conditions, the experimental yield of total triterpenoid saponins was 1.122 ± 0.058 %, which favoured the predicted yield value very well. From the SEM microscopic images, ultrasound-assisted total triterpenoid saponins extraction from *Ganoderma lucidum* have shown more hollow openings on the surface structure as compared to that without ultrasound treatment. This observation confirmed that ultrasonic caused the fungal cell walls to swell and rupture, thereby facilitating the extraction of total triterpenoid saponins from *Ganoderma lucidum*.

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