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Optimization Of Eurycomanone Yield Using Response Surface Methodology By Water Extraction

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Abstract

Keywords:

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Nutraceuticals and phytomedicines are largely used in pharmaceutical industry at this era. Phytomedicines are pharmaceuticals which made from plants which has significant property of treating some illnesses. Tongkat Ali, or eurycoma longifolia, is a traditional herb medicine used as aphrodisiac, general tonic, anti-Malaria, and anti-Pyreti. Nowadays, Tongkat Ali has become known globally due to its ability to treat erectile dysfunction (ED) and to improve sexual desire. Eurycomanon is a very important quassinoids found in Tongkat Ali extract which has a potential to be developed as complementary for anti-cancer therapy. In this research, extraction of eurycomanone from tongkat ali was performed according response surface methodology. Optimization of eurycomanone yield was obtained through central composite design (CCD). The maximum eurycomanone amount which is 0.559% was obtained at solvent to raw material ratio of 28.8:1 g/g, duration of extraction of 3 hour, and 0.054-0.154 mm of raw material.

1. Introduction

The global herbal market of nutraceuticals and phytomedicines with an average annual growth rate between 15% to 20% (Gruenwald J, 2002; Sloan, 2002). This market includes herbal products such as herbal supplements and essential oils, and nutraceutical products such as fortified foods and nutritional supplements. In Malaysia, the herbal product market has been estimated to be worth RM 4.55 billion, of which 80% of the products are imported (Puteh, 1999).

A key emerging global market is phytomedicines, which are pharmaceuticals made from plants. Many allopathic medicines, which are produced synthetically, are also originally derived from plants chemicals, i.e. phytochemicals such as quinine for malaria and quinidine for heart arrhythmia from *Cinchina sp*, and digxin for heart failure from *digitalis spp*. About 25% of drugs prescribed worldwide come from plants, 121 such active compounds being in current use (Rates, 2001). Raskin *et al*. (2002) estimated that over 50% of prescription medicines and over the counter herbal remedies contain phytochemicals with market worth of USD 31 billion in 2002.

Tongkat Ali, or *Eurycoma Longifolia*, is a traditional Malay and Orang Asli herb used as aphrodisiac, general tonic, anti-Malaria, and anti-Pyretic. Scientifically, it has also been found to have anti-tumor and anti-oxidant properties. Tongkat Ali root has various benefits and is taken orally. The tap root is processed traditionally, by decocting the root and is drunk for the benefit. Nowadays, Tongkat Ali has become known globally due to its ability to treat erectile dysfunction (ED) and to improve sexual desire. It has been recognized as a cashcrop by Malaysia due to its high value for the pharmaceutical use.

Due to Tongkat Ali's high market demand as health supplement, these phytochemical products have a high commercial value in local and global market. Market demand of this plant has greatly increased as there are almost 200 products from Tongkat Ali available in health-food market specifically for its aphrodisiac properties. Tongkat Ali product are available are either in the form of capsules mixed with other aphrodisiac herbs, in raw crude powder form especially from roots, as additives mixed with coffee and ginseng, or as health products (Bhat and Karim, 2010).

There are various phytochemicals can be found in this plant such as canthin-6-one alkaloids, β-carboline alkaloids, quassinoids, tirucallane-type triterpenes, squalene derivatives, and biphenylneolignans.

Eurycomanon is a very important quassinoids found in Tongkat Ali extract which has a potential to be developed as complementary for anti-cancer therapy due to its ability to inhibit cancer cells such as lung, liver, breast cancer cells, and, decrease tumorigenic and significant activities against Plasmodium falciparum strains (Chan *et al*., 1986; Kardono *et al*., 1991; Zakaria *et al*., 2009). eurycomanone is usually chosen as a marker phytochemical as it is the most abundant phytochemical in Tongkat Ali(Chan *et al*., 1998).

In this research, conventional extraction of Tongka Ali by water extraction has been performed. The extracts are analyzed by high performance liquid chromatography (HPLC) for the yields of Eurycomanone.

2. literture review

Eurycoma longifolia Jack (*E. longifolia*) is one of the popular medicinal plants in Southeast Asia, including Indonesia and Malaysia. E. longfolia has many local names: in Brunei it is known as tungat ali, langsia siam or pasak bumi; in Cambodia it is known as antoung sar or antong sar; in Thailand it is known as plaalai phuenk, hae phan chan or phiak; in Laos it is known as tho nan; in Vietnam it is known as Cay ba binh; in Indonesia it is known as beseng, bidara laut or pasak bumi; and in Malaysia it is known as bedara merah, bedara putih or tongkat ali (Chan *et al*., 1998).

To determine parameter effects and their interactions in extraction process of Tongkat Ali, response surface methodology has been employed, which allow process optimization of Tongkat Ali to be conducted effectively. Response surface methodology (RSM) is a collection of mathematical and statistical methods based on the fit of a polynomial equation to the experimental data. It is increasingly used for optimization steps and can be well applied when several variables affect the responses of interest. To achieve the greatest performance of the system that involve multiple levels of variables studied and needed to optimize them simultaneously, RSM is very significant application. (Bezerra *et al.*, 2008; Juntachote *et al.*, 2006).

The major advantages of RSM are it reduces the number of experiments required, faster and more economical method, and save the consumption of reagents and materials, when compared with the classic one variable at a time or full factors experimentation. Typically, RSM applies experimental designs like three level factorial, central composite design (CCD), Box-Benhken, and Doehlert designs (DDD) to evaluate the quality of the fitted model (Amaro *et al.*, 2011).

3. Methodology

3.1 Raw Material Preparation

Raw material used for the experiments was purchased from the supplier. The ground Tongkat Ali was stored in a cool and dry environment to prevent fungus growth. Fungus may causes decomposition and changes in the phytochemical in the Tongkat Ali converted to phyto-toxins through bioactivity of bacteria. The raw material was sieved into three size categories: 0.25-0.5 mm, 0.5-1.0 mm and 1.0-2.0 mm. The sieved Tongkat Ali was packed and stored to maintain their quality. Plastic bags were used for sealed packaging and put them in refrigerator.

3.2 Optimaizaiton of Conventional Water Extraction Using Response Surface Methodology (RSM)

A preliminary study was done to determine the appropriate range of the independent variables on the processing parameters of extraction. Eurycoma longifolia extract using aqueous extraction has been patent and standardized (Sambandan, 2006)(Draft Malaysian Standard, 2010). Therefore, in this study water has been used as the solvent in Tongkat Ali extraction. According to Sim *et al*. (2004) and Kumaresan (2008), the optimum temperature for Eurycoma longifolia roots extraction was found at 100 ℃. Hence, in this work the temperature was constant at boiling point of solvent used which is at 100 ℃. In previous study by Mohamad *et al*. (2010), it was shown that the best agitation rate on the extraction of Tongkat Ali to eurycomanone yield is at 400 rpm. In addition, according to Kumaresan (2008), 400 rpm was chosen as the optimal agitation rate as the agitation rate was sufficient to mix the particles and maintain it suspended while not creating a vortex and overcoming the bulk fluid resistance to mass transfer. Thus, the agitation speed was kept at 400 rpm in this study. As a result, the processing parameters of Tongkat Ali extraction that was investigated in this study are solvent to raw material ratio, duration of extraction and particle size of raw material (Kumaresan, 2003) Effect of these parameters was observed on eurycomanone yield. Based on the study, the factor level of each processing parameters are as followed:

Table 1: Processing Parameters for the Extraction of Tongkat Ali

Factor Name	Factor levels
Solvent to raw material ratio (g/g)	10:1 to 40:1 (Kumaresan, 2008; Sim, 2004)
Duration of extraction (hour)	1 to 3 (Kumaresan, 2008; Kumaresan, 2003; Sim, 2004)
Particle size of raw material (mm)	0.054-0.154 mm, 0.154-.0.3 mm and 0.3-0.45 mm (modified from Kumaresan, 2003; Sim, 2004)

The experiment was run according to experimental design that developed by Design Expert (V 8.05b). For the optimization of extraction, central composite design was used to evaluate the effects of processing parameters on response variables and 39 experiments were designed for this research. Processing Parameters were used are solvent to raw material ratio, duration of extraction, and particle size of raw material with three levels. The range of extraction process variable are listed as Table 2 and the experimental matrix of the study is shown in Table 3.

Table 2: Processing Parameters of the Tongkat Ali Extraction Process

Factor	Factor name	Factor levels
A	Solvent to raw material ratio (g/g)	10:1, 25:1, 40:1
B	Duration of extraction (hour)	1, 2,3
C	Particle size of raw material (mm)	0.054-0.154, 0.154-0.3, 0.3-0.45

Table 3: Central Composite Design Arrangement

Run	Factor 1	Factor 2	Factor 3

	A:solvent to raw material ratio	B:duration	C:partical size (mm)
1	25.00	2.00	0.054-0.154
2	40.00	1.00	0.3-0.45
3	25.00	2.00	0.3-0.45
4	40.00	2.00	0.3-0.45
5	25.00	2.00	0.3-0.45
6	25.00	2.00	0.3-0.45
7	10.00	3.00	0.3-0.45
8	40.00	1.00	0.154-0.3
9	40.00	1.00	0.054-0.154
10	40.00	3.00	0.3-0.45
11	10.00	3.00	0.054-0.154
12	25.00	3.00	0.154-0.3
13	25.00	2.00	0.154-0.3
14	40.00	3.00	0.054-0.154
15	25.00	2.00	0.054-0.154
16	10.00	1.00	0.3-0.45
17	10.00	2.00	0.054-0.154
18	10.00	2.00	0.154-0.3
19	25.00	2.00	0.154-0.3
20	40.00	2.00	0.154-0.3
21	25.00	2.00	0.154-0.3
22	25.00	2.00	0.054-0.154
23	25.00	3.00	0.054-0.154
24	10.00	1.00	0.054-0.154
25	10.00	1.00	0.154-0.3
26	25.00	2.00	0.3-0.45
27	40.00	3.00	0.154-0.3

28	25.00	2.00	0.054-0.154
29	25.00	2.00	0.3-0.45
30	10.00	3.00	0.154-0.3
31	25.00	1.00	0.054-0.154
32	25.00	1.00	0.3-0.45
33	25.00	2.00	0.154-0.3
34	10.00	2.00	0.3-0.45
35	25.00	2.00	0.154-0.3
36	40.00	2.00	0.054-0.154

Extraction was carried out in round bottom flask which connected with a condenser on the top to reduce water loss by evaporation as Fig.4 shows. The extraction was heated at boiling temperature and agitated at 400 rpm on a heating mantel. The solvent used for the extraction is water. After extraction, the samples were filtered to remove gross and suspended solids. The filtered extract was analyzed by HPLC to know the amount of eurycomanone. The result from experiments was analyzed using ANOVA to know the optimum condition for extraction process of Tongkat Ali.

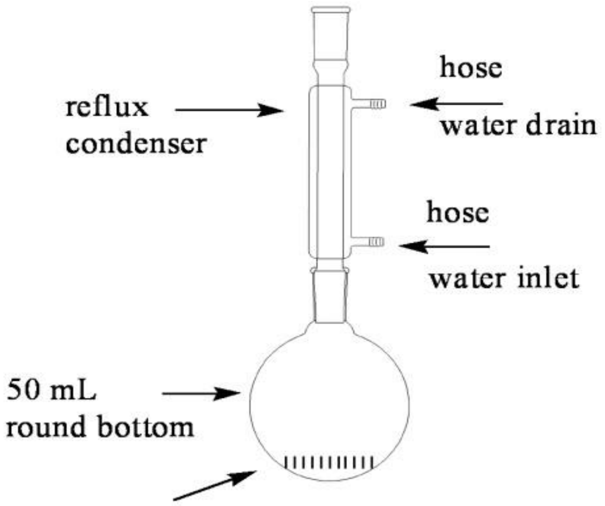

Fig.4 water extraction set

3.3 Amount of Eurycomanone Measurement

To measure the amount of eurycomanone in the Tongkat Ali extract, the High Performance Liquid Chromatography (HPLC) analysis was performed using a Waters 2690 Separation Module auto-sampler and Waters 996 Photodiode-array detector. Separation is achieved using Synerg 4u Fusion-RP80A column with dimension of 150 x 4.60 mm and 4 micron of particle size using a 10 minute water-acetonitrile-ortho-phosphoric acid isocratic system. The mobile phase consisted 85% of 0.05 % phosphoric acid and 15 % of acetonitrile at a flow rate of 1 ml/min (Draft Malaysian Standard, 2010). The low mobile phase flow rate was chosen to allow the peaks to separate more distinctly (Kumaresan, 2003). The UV detector was operated at 254 nm (Draft Malaysian Standard, 2010).

To determine the calibration for eurycomanone, 10 mg standard of eurycomanone was weighed and dissolved into 10 ml deionized water to produce 1000 ppm of stock solution. The stock solution was diluted into another four concentrations, 800 ppm, 600 ppm, 400 ppm, 200 ppm, 100 ppm. 80 ppm, 60 ppm, 40 ppm, 20 ppm to construct a 10 point of calibration curve. The HPLC setting for determination eurycomanone are as following:

Table 4: HPLC Setting

Parameter	Setting
Column	Synerg 4u Fusion-RP80A column with dimension of 150 x 4.60 mm and 4 micron of particle
Detector	Agilent 1100
UV wavelength	254 nm
Flowrate	1 ml/min
Injection volume	20.00 µl
Mobile phase	85% of 0.05 % phosphoric acid and 15 % of acetonitrile

The reference standard solution and sample preparation will be prepared as following: A single injection of an extraction solvent blank was made followed by a single injection of standard preparation. A plot of standard peak areas versus standard concentrations was made with the origin ignored. A single injection of sample preparations. Calculation of the eurycomanone amount in the samples as follow:

$$\text{Amount of eurycomanone } (\%) = \frac{[c][V]}{w} * 100\% \qquad (2)$$

where;

C = concentration of eurycomanone (g/ml) from linear regression analysis

V = volume of extract

W = weight of solid content (g)

4. Result and discussion

This section presents the results of effects of processing parameters on the response variables namely eurycomanone yield in the extraction of Tongkat Ali. The processing parameters considered in the extraction process were solvent to raw material ratio, duration of extraction, and particle size of raw material.

An experimental design with 39 experiments was run on three factorial variables to optimize the extraction of Tongkat Ali process. The three factor variables were solvent to raw material ratio, duration of extraction and particle size of raw material. Design Expert (V 8.05b) was used to carry out the regression analysis and to analyze the points of data. The optimum values for eurycomanone yield in Tongkat Ali extract was simulated from Design Expert using the regression equation and also from the response surface 3D surface graph.

The results for eurycomanone yield from optimization of Tongkat Ali extraction process were gathered in Table 5. The experimental design used was Central Composite Design.

Table 5: Central Composite Design Arrangement and Responses Value for Extraction Process

Run	Factor 1 A:solvent to raw material ratio	Factor 2 B:duration	Factor 3 C:partical size (mm)	Response eurycomanone yield
1	25.00	2.00	0.054-0.154	0.580%
2	40.00	1.00	0.3-0.45	0.069%
3	25.00	2.00	0.3-0.45	0.304%
4	40.00	2.00	0.3-0.45	0.093%
5	25.00	2.00	0.3-0.45	0.270%
6	25.00	2.00	0.3-0.45	0.284%
7	10.00	3.00	0.3-0.45	0.321%
8	40.00	1.00	0.154-0.3	0.151%
9	40.00	1.00	0.054-0.154	0.306%
10	40.00	3.00	0.3-0.45	0.100%

11	10.00	3.00	0.054-0.154	0.309%
12	25.00	3.00	0.154-0.3	0.345%
13	25.00	2.00	0.154-0.3	0.355%
14	40.00	3.00	0.054-0.154	0.528%
15	25.00	2.00	0.054-0.154	0.583%
16	10.00	1.00	0.3-0.45	0.346%
17	10.00	2.00	0.054-0.154	0.300%
18	10.00	2.00	0.154-0.3	0.150%
19	25.00	2.00	0.154-0.3	0.326%
20	40.00	2.00	0.154-0.3	0.165%
21	25.00	2.00	0.154-0.3	0.332%
22	25.00	2.00	0.054-0.154	0.571%
23	25.00	3.00	0.054-0.154	0.513%
24	10.00	1.00	0.054-0.154	0.434%
25	10.00	1.00	0.154-0.3	0.309%
26	25.00	2.00	0.3-0.45	0.274%
27	40.00	3.00	0.154-0.3	0.097%
28	25.00	2.00	0.054-0.154	0.584%
29	25.00	2.00	0.3-0.45	0.269%
30	10.00	3.00	0.154-0.3	0.150%
31	25.00	1.00	0.054-0.154	0.508%
32	25.00	1.00	0.3-0.45	0.254%
33	25.00	2.00	0.154-0.3	0.340%
34	10.00	2.00	0.3-0.45	0.345%
35	25.00	2.00	0.154-0.3	0.330%
36	40.00	2.00	0.054-0.154	0.373%

From Table 5, the best eurycomanone amount of 0.58 % was observed at run 28 which was at a solvent to raw material ratio of 25:1, 2 hour extraction process and 0.054-0.154 mm raw material particle size.

4.1 Analysis of Variance (ANOVA) for Tongkat Ali Extraction Process

Tables 6 and Table 7 display total, regression, residual, sum of squares and mean squares of eurycomanone yield in Tongkat Ali extract. It was observed that F calculated for eurycomanone yield was 25.21. Degree of freedom for regression and residual of eurycomanone yield were 11 and 27, respectively. Hence, the $F(11,27,0.05)$ tabulated was found to be 2.20.

Table 6: The Results fom Analysis of Variance (ANOVA) for Eurycomanone yield from Tongkat Ali Extraction. The Model Value of 22.69 implied that the Model was Significant.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	7.36E-05	11	6.69E-06	22.69848	< 0.0001	significant
A-solvent to raw material ratio	3.4E-06	1	3.4E-06	11.52655	0.0021	
B-duration	4.18E-07	1	4.18E-07	1.417844	0.2441	
C-partical size	4.1E-05	2	2.05E-05	69.54715	< 0.0001	
AB	2.14E-06	1	2.14E-06	7.257192	0.0120	
AC	7.06E-06	2	3.53E-06	11.96901	0.0002	
BC	1.44E-06	2	7.22E-07	2.44954	0.1053	
A^2	1.55E-05	1	1.55E-05	52.66224	< 0.0001	
B^2	1.9E-11	1	1.9E-11	6.46E-05	0.9936	
Residual	7.96E-06	27	2.95E-07			
Lack of Fit	7.57E-06	15	5.04E-07	15.29689	< 0.0001	significant
Pure Error	3.96E-07	12	3.3E-08			
Cor Total	8.16E-05	38				

Table 7: Coefficient Estimate of the Quadratic Model for Eurycomanone yield

Term	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	3.893E-003	1	1.302E-004	3.626E-003	4.160E-003	
A-solvent to raw material ratio	-4.345E-004	1	1.280E-004	-6.971E-004	-1.719E-004	1.00
B-duration	-1.524E-004	1	1.280E-004	-4.150E-004	1.102E-004	1.00
C[1]	1.440E-003	1	1.230E-004	1.188E-003	1.693E-003	
C[2]	-5.739E-004	1	1.230E-004	-8.262E-004	-3.216E-004	
AB	4.223E-004	1	1.567E-004	1.006E-004	7.439E-004	1.00
AC[1]	7.063E-004	1	1.810E-004	3.350E-004	1.078E-003	
AC[2]	1.094E-004	1	1.810E-004	-2.620E-004	4.808E-004	
BC[1]	3.216E-004	1	1.810E-004	-4.977E-005	6.930E-004	
BC[2]	-3.677E-004	1	1.810E-004	-7.391E-004	3.699E-006	
A^2	-1.369E-003	1	1.886E-004	-1.756E-003	-9.819E-004	1.17

Table 7 shows the coefficients and their confidence interval for quadratic model of eurycomanone yield. The standard error given is the standard deviation associated with coefficient estimates. A full quadratic model was established to express the eurycomanone yield as a function of the chosen variables. The predicted eurycomanone yield model is shown Equation 3.

Eurycomanone yield=+3.893E-003-4.345E-004*A-1.524E-004*B+1.440E-003*C[1]-5.739E-004*C[2]+4.223E-004*AB+7.063E-004*AC[1]+1.094E-004*AC[2]+3.216E-004*BC[1]-3.677E-004*BC[2]-1.369E-003*A^2+1.516E-006*B^2

(3)

From the Equation 3, amount of eurycomanone will be raised when increasing duration to the power of two (B2), interaction between solvent to raw material ratio and duration (AB) and interaction between solvent to patical size (AC[1], AC[2]), interaction between duration and partical size (BC[1]), in conjunction with the decreased of duration (B), solvent to raw material ratio (A),solvent to raw material ratio power two (A^2), and interaction of duration and patical size (BC[2]).

The value of determination coefficient (R^2) for eurycomanone yield was 0.9024 which indicates that only 9.76 % of the total variations were not explained by the model and the value of adj-R^2 was 0.8627. F value for eurycomanone model was 22.7 and value of Prob > F was less than 0.0001 which means that the model was significant. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 17.965 on our model indicates an adequate signal. An adequate signal noise to ratio of 17.965 was achieved thus indicating that the model is significant for the process. The "Pred R-Squared" of 0.7290 is in reasonable agreement with the "Adj R Squared" of 0.8627. A “Pre R-Squared” with the values of 0.7290 implies that the overall mean is a better predictor of experiments response than the current model

4.2 Effect of Solvent to Raw Material Ratio, Duration of Extraction and Particle Size of Raw Material on Eurycomanone Yield and Optimization of Eurycomanone Yield by RSM

Effect of solvent to raw material ratio, duration of extraction and particle size of raw material on the eurycomanone yield from Tongkat Ali extraction was investigated. Fig. 5 shows the interaction between solvent to raw material ratio and duration of extraction on the eurycomanone amount. Fig.6 shows the interaction between duration of extraction and particle size of raw material on the eurycomanone amount. Fig. 7 shows the interaction between solvent to raw material ratio and particle size on the eurycomanone amount.

From Fig. 5, the eurycomanone yield increases with the increase of extraction duration. The maximum eurycomanone yield obtained was at solvent to raw material ratio of 28.8:1 g/g in 3 hours extraction. Higher amount of extraction solvent will lead to a higher leaching rate where the desired bioactive compound has higher contact with the solvent. Therefore, higher volume of water usage could leach out the bioactive compounds in higher amount (Ahmad et al., 2013). However if solvent to raw material ratio used is higher, the greater quantity of water used requires the extraction process to be longer. As eurycomanone is the marker chemical for standardization, the raw material needs to be processed in the shortest time possible to preserve its quantity and quality.

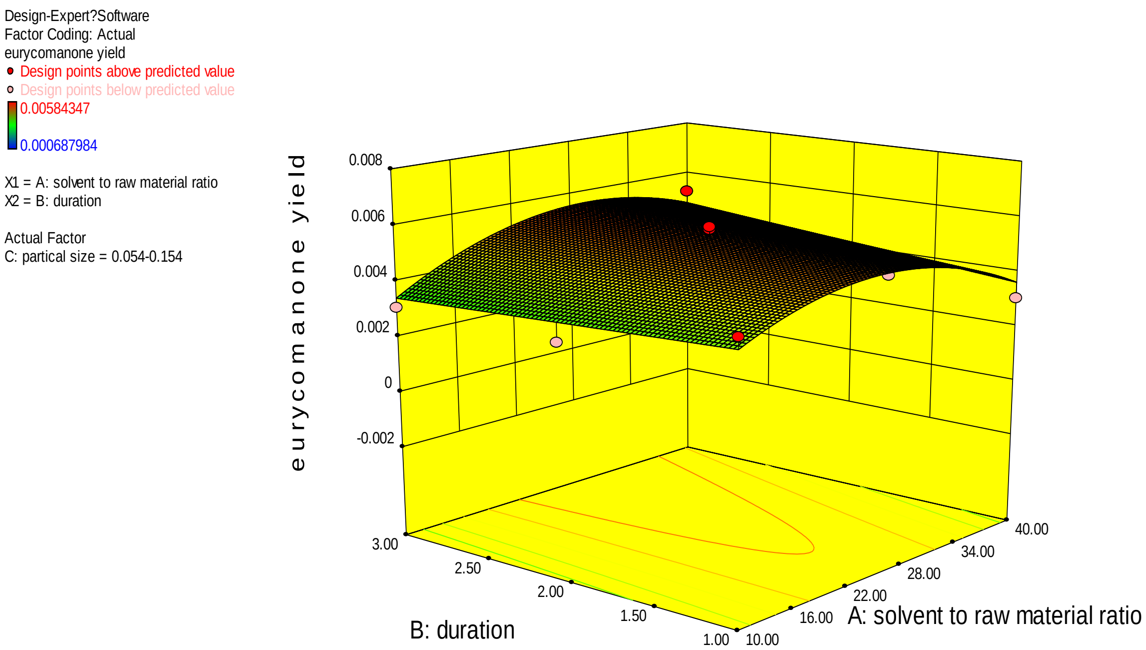

Fig. 5 Tthe interaction between solvent to raw material ratio and duration of extraction on the eurycomanone yield.

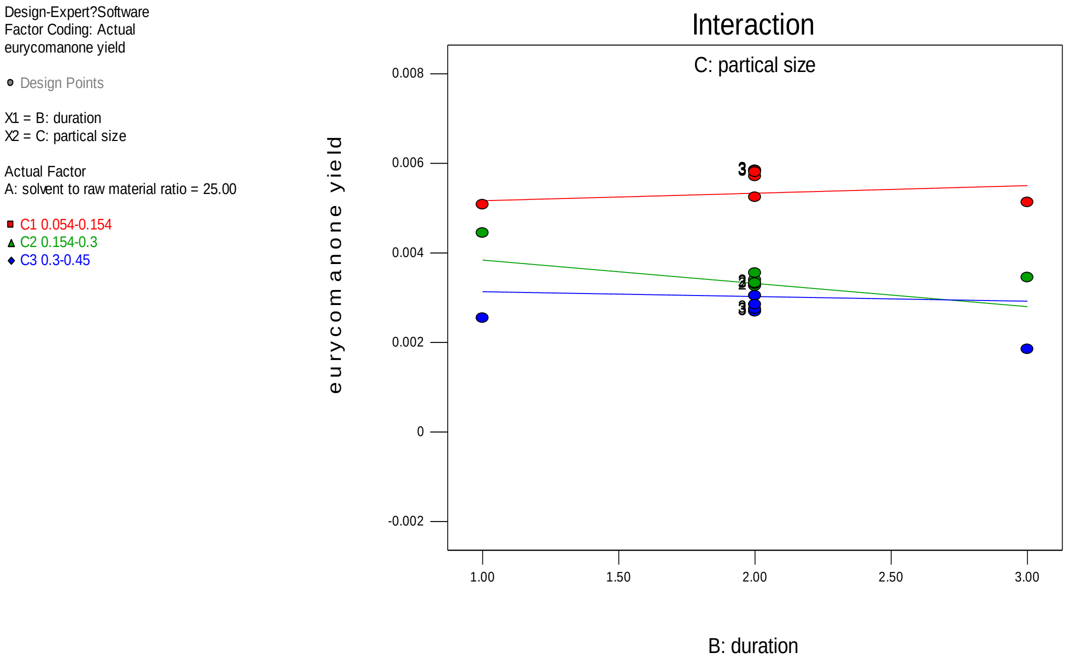

Fig. 6 the interaction between duration of extraction and particle size of raw material on the eurycomanone amount

Fig. 6 illustrates that interaction between duration of extraction and particle size of raw material on eurycomanone yield. The eurycomanone yield highly influenced by partical size. The eurycomanone yield increases with the decrease of raw material particle size. Maximum amount of eurycomanone was observed during 3 hour of extraction when the partical size in the range of 0.054-0.154mm.

For eurycomanone production, the interaction between solvent to raw material ratio and particles size of raw material influenced the responses highly (Fig. 7). Eurycomanone yield increases with the decreasing of particle size of raw material. For example, at 0.3-0.45mm of raw material particle size the eurycomanone yield was 0.364 %. The amount was increased to 0.394 % as particle size decreased to 0.154-0.3mm. When the particle size at 0.054-0.154mm, the yiled of urycomanone increased to 0.559%. Larger particle size has smaller contact surface area which will increase resistance to the water entrance and eurycomanone diffusion towards the water will be lower. Hence compare to the small ones, eurycomanone transferred from inside of the larger particles to the surrounding solution in smaller amount. Therefore, the optimum particle size of raw material to attain the maximum amount of eurycomanone was at 0.054-0.154 mm.

Solvent to raw material ratio affects the eurycomanone yield highly as well. Low solvent to raw material ratio makes lower yield of eurcomanone, and high solvent to raw material ratio dose the same. There is a range of solvent to raw material ratio under different particle size gives the highest eurycomanone yield. For example, When the particle size is 0.3-0.45, the eurycomanone yield could reach 0.364% of its optimum at the solvent to raw material ratio of 15.83:1. And when the particle size is 0.054-0.154mm, the yiled of eurycomanone could reach 0.559% of its optimum at solvent to raw material ratio of 28.8:1.

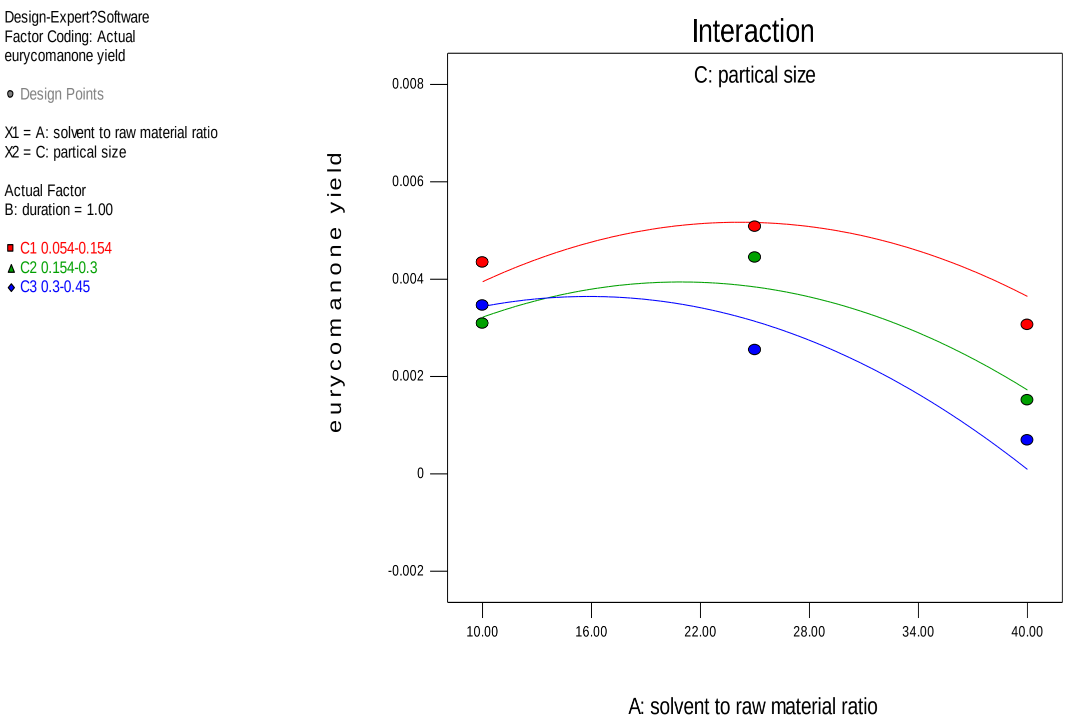

Fig. 7 shows the interaction between solvent to raw material ratio and particle size on the eurycomanone amount

The optimization of solvent to raw material ratio, duration of extraction and particle size of raw material in Tongkat Ali extraction was carried out to identify the optimum condition for the extraction process in order to obtain maximum eurycomanone yield.

The maximum eurycomanone amount which is 0.559% was obtained at solvent to raw material ratio of 28.8:1 g/g, duration of extraction of 3 hour, and 0.054-0.154 mm of raw material.

Table 8: Optimum Condition for Tongkat Ali Extraction Process

Variables			Response
Solvent to raw material ratio (g/g)	Duration of extraction (hour)	Particle size of raw material (mm)	eurycomanone yield (%)
28.8:1	3	0.054-0.154	0.559 %.

5. Conclusion

Optimization process on the Tongkat Ali extract was investigated. The optimized operating conditions of extraction was successfully identified using response surface methodology (RSM). In Tongkat Ali extraction process, optimization of processing parameters; solvent to raw material ratio, duration of extraction, and raw material particle size on eurycomanone yield was successfully performed using Central Composite Design (CCD) of Design Expert (V 8.05b) software. The experiment values were analysed using Analysis of Variance (ANOVA), and results showed that the operating parameters have significant effect on Tongkat Ali extraction. The optimum condition obtained was at solvent to raw material ratio of 28.8:1 g/g, 3 hours of extraction duration, and 0.054-0.154 mm of raw material particle size, with the values of eurycomanone yield of 0.559 %.

Acknowledgments

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