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Optimizing conditions for oleanolic acid extraction from *Lantana camara* roots using response surface methodology

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ABSTRACT

Lantana camara is an ornamental plant used in traditional medicine for the treatment of various diseases. The roots of *L. camara* is a rich source of oleanolic acid which has shown anti-inflammatory, hepatoprotective, antitumor, antioxidant and anti-hyperlipidemic activity. Optimization of various extraction parameters using response surface methodology (RSM) was performed to assess maximum yield of oleanolic acid from *L. camara* roots. Plackett–Burman design criterion was applied to identify the significant effects of various extraction parameters such as temperature, time, mean particle size, solvent–solid ratio, solvent composition and number of extraction steps on extraction of oleanolic acid. Among the six variables tested extraction time, mean particle size, solvent–solid ratio and solvent composition were found to have significant effect on oleanolic acid extraction. Optimum levels of the significant variables were determined by using a central composite design. The most suitable condition for extraction of oleanolic acid was found to be a single step extraction at extraction temperature 35 °C, extraction time 55 min, solvent–solid ratio 55:1, mean particle size 0.5 mm and solvent composition 52.5% methanol in a methanol-ethyl acetate mixture. At these optimum extraction parameters, the maximum yield of oleanolic acid obtained experimentally (1.74% dry weight of root) was found to be very close to its predicted value of 1.69% dry weight of root. The mathematical model developed was found to fit well with the experimental data of oleanolic acid extraction.

1. Introduction

The search for a natural and healthy lifestyle has increased the interest in natural bioactive compounds that could be introduced in our diet or be used as natural drugs. In this context, terpenoids are the most promising group of molecules due to their high medicinal value. *Lantana camara*, is native to tropical America and was introduced in India as an ornamental and hedge plant, which is now completely naturalized and growing throughout India. It has been recorded that different parts of *L. camara* are a rich source of various bioactive principles and has been used in traditional medicine (Sastri, 1962). The presence of the triterpenoid, oleanolic acid, in its roots in high concentrations has been reported (Misra et al., 1997). Oleanolic acid possesses many important biological activities, such as anti-inflammatory (Tsuruga et al., 1991), anti-hyperlipidemic (Ma, 1986; Liu, 1995), antiulcer (Gupta et al., 1981), antioxidant activity (Balanehru and Nagarajan, 1991) and hepatoprotective properties (Ma et al., 1982). Recently, this compound has been noted for its antitumour-promotion effect (Shibata, 2001).

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Table 1 – Level of the extraction parameters for extraction of oleanolic acid from *Lantana camara* root by using Plackett–Burman design criterion

Extraction code	Extraction condition	High level (+)	Low level (−)
X_1	Temperature	70 °C	35 °C
X_2	Time	60 min	30 min
X_3	Solvent:solid ratio	60:1 ml/g	30:1 ml/g
X_4	Particle size	1.2 mm	0.6 mm
X_5	Solvent composition (% methanol in methanol ethyl acetate mixture v/v)	70 (v/v)	35 (v/v)
X_6	Extraction steps	3	1

Table 2 – Yield of oleanolic acid from *Lantana camara* root using the different levels of extraction variables of Plackett–Burman design criterion

Run	X_1	X_2	X_3	X_4	X_5	X_6	Oleanolic acid (% dry weight of root)
1	+	−	+	−	−	−	0.235
2	+	+	−	+	−	−	0.890
3	−	+	+	−	+	−	0.553
4	+	−	+	+	−	+	1.127
5	+	+	−	+	+	−	1.253
6	+	+	+	−	+	+	1.115
7	−	+	+	+	−	+	1.683
8	−	−	+	+	+	−	1.634
9	−	−	−	+	+	−	1.214
10	+	−	−	−	+	+	0.341
11	−	+	−	−	−	+	0.273
12	−	−	−	−	−	+	0.221

Six variables X_1, temperature; X_2, time; X_3, solvent composition; X_4, particle size; X_5, solvent–solid ratio and X_6, extraction steps were screened by conducting 12 experiments.

Extraction is the first important step in the recovery and purification of active ingredients from plant materials. Many techniques have been developed to extract terpenoids from ginseng roots, among which the reflux, cooking (Zhang et al., 2007) and solid–liquid extraction (Herodez et al., 2003) are the most commonly used. Many factors contribute to the efficacy of solvent extraction, such as the type of solvent, pH, temperature, number of steps, liquid-to-solid ratio and particle size of the plant material (Wettasinghe and Shahidi, 1999; Cacace and Mazza, 2003; Pinelo et al., 2005). Extraction of triterpenoid from different medicinal plants by using methanol (Udayama et al., 1998; Zhao et al., 2007) and other organic solvent systems (Hamburger et al., 2003; Vongsangnak et al., 2004; Assimopoulou et al., 2005) have been reported.

Classical optimization studies use one-factor-at-a-time approach, in which only one factor is variable at a time while all others are kept constant. This approach is time-consuming and expensive. In addition, possible interaction effects between variables cannot be evaluated and misleading conclusions may be drawn. The response surface methodology (RSM) can overcome these limitations, since it allows accounting for possible interaction effects between variables (Khuri and Cornell, 1996). If adequately used, this powerful tool can provide the optimal conditions to improve the process (Bas and BoyacI, 2007). The optimization of the extraction process using RSM by establishing a mathematical model would not only serve as a visual aid to have a clearer picture about the effects of various factors on extraction but also help us to locate the region where the extraction is optimized. Response surface methodology has been used successfully to model and optimize biochemical process (Boyacy, 2005; Ibanoglu and Ibanoglu, 2001; Varnalis et al., 2004) including extraction processes, such as extraction of phenolic compounds from *Inga edulis* (Silva et al., 2007) and phenolic compounds from wheat (Pathirana and Shahidi, 2005). Optimization of extraction parameters of the triterpenoid, oleanolic acid from *L. camara* using response surface methodology has not been reported yet.

The objective of this study was to optimize the extraction parameters of oleanolic acid from dried roots of *L. camara*. Response surface methodology was used to optimize the effects of extraction time, particle size, solvent–solid ratio and solvent composition for the extraction of oleanolic acid from dried roots of *L. camara*. The interaction between the factors influencing extraction of oleanolic acid was established and a model describing the effect of the factors on extraction of oleanolic acid from *L. camara* roots was also described.

Table 3 – Regression analysis of Plackett–Burman design criterion data for the prediction of significant extraction parameters

Term	Effect	Coefficient	S.E. coefficient	*T*	*P*
Constant		0.567	0.0529	10.72	0.000
Temperature	0.1567	0.078	0.0530	1.48	0.199
Time	0.3733	0.186	0.0530	3.53	0.017
Solvent composition	0.4467	0.223	0.0530	4.22	0.008
Particle size	−0.6033	−0.302	0.0530	−5.70	0.002
Solvent:solid ratio	0.4200	0.2100	0.0530	3.97	0.011
Extraction steps	0.1133	0.057	0.0530	1.07	0.333

Time, solvent composition, particle size and solvent:solid ratio were significant ($P < 0.05$). *T*, *T*-ratio = coefficient/S.E. coefficient and *P*, probability.

Table 4 – Treatment variables and their coded and actual values used for optimization of oleanolic acid extraction from *Lantana camara* roots by using central composite design

Treatment variables	Coded levels					
	Symbol	−2	−1	0	+1	+2
Extraction time (min)	X_2	15.0	30.0	45.0	60.0	75.0
Solvent–solid ratio (ml/g)	X_3	15.0	30.0	45.0	60.0	75.0
Particle size (mm)	X_4	0.3	0.6	0.9	1.2	1.5
Solvent composition	X_5	17.5	35.0	52.5	70.0	87.5

2. Material and methods

2.1. *Plant material*

The field study was carried out during the periods of 2005–2006 at the medicinal plant garden, B.H.U., Varanasi, India (25°18″N, 83°50″E). The experimental location experiences a semi-arid tropical climate. The soil of the experimental field was sandy loam texture, organic electrical conductivity 0.42 dS m^{-1}, available carbon 0.38%, available nitrogen 180 kg ha^{-1}, available phosphorus 21 kg ha^{-1} and pH 7.3. Plants used in the study were propagated from a yellow variety *L. camara* stem cuttings. Stem cuttings were initially grown in medium size (20 cm × 20 cm × 20 cm) earthen pots filled with the field soil, kept under partial shade and regularly watered. One cutting was planted in each pot for rooting in the first week of August 2005. Healthy, profusely rooted, 30 days old cuttings were transplanted in the field in defined row spacing of 30 cm between plants. After 1 year, September 2006, 50 plants were harvested from the field. The aerial and root parts of the plants were separated and the roots were washed with tap water, shade dried and kept in cellulose bags for further experiment.

Table 5 – Central composite design criterion of extraction parameters with corresponding experimental and predicted value

Run order	Extraction parameters levels				Oleanolic acid (% dry weight)	
	X_2	X_3	X_4	X_5	Experimental	Predicted
1	60	60	0.6	70	1.550	1.701
2	75	45	0.9	52.5	1.668	1.459
3	30	30	1.2	35	1.110	1.127
4	60	60	1.2	35	1.296	1.378
5	45	45	1.5	52.5	1.092	1.140
6	45	75	0.9	52.5	1.687	1.523
7	60	60	1.2	70	1.320	1.375
8	45	45	0.9	52.5	1.470	1.643
9	60	30	1.2	35	1.121	1.128
10	45	45	0.9	52.5	1.675	1.643
11	30	60	0.6	35	1.412	1.453
12	60	30	0.6	70	1.350	1.399
13	45	45	0.9	52.5	1.675	1.643
14	45	45	0.9	52.5	1.656	1.643
15	45	15	0.9	52.5	1.032	1.097
16	45	45	0.9	52.5	1.687	1.643
17	45	45	0.9	52.5	1.662	1.643
18	30	60	1.2	70	1.110	1.098
19	30	30	1.2	70	1.021	0.976
20	60	30	1.2	70	1.110	1.017
21	60	60	0.6	35	1.543	1.629
22	30	60	0.6	70	1.452	1.485
23	60	30	0.6	35	1.255	1.325
24	45	45	0.9	52.5	1.676	1.643
25	45	45	0.9	17.5	1.186	1.150
26	45	45	0.3	52.5	1.870	1.724
27	15	45	0.9	52.5	1.021	1.320
28	30	30	0.6	35	1.289	1.275
29	45	45	0.9	87.5	1.244	1.181
30	30	60	1.2	35	1.150	1.141
31	30	30	0.6	70	1.332	1.308

X_2, extraction time (min); X_3, solvent:solid ratio (v/w); X_4, particle size (mm) and X_5, solvent composition (% methanol in methanol-ethyl actate mixture).

Table 6 – Regression analysis of central composite design criterion data for oleanolic acid extraction from *Lantana camara* root

Model parameters	Regression coefficient	S.E.s coefficient	*T*	*P*
Intercept	1.64300	0.043	37.647	0.000
Time	0.16358	0.047	3.470	0.003
Solvent–solid ratio	0.21292	0.047	4.517	0.000
Particle size	−0.29175	0.047	−6.189	0.047
Solvent composition	0.01542	0.047	0.327	0.048
Time^2	−0.34763	0.086	−4.025	0.001
Solvent:solid ratio^2	−0.33262	0.086	−3.851	0.001
Particle size^2	−0.21113	0.086	−2.444	0.026
Solvent composition^2	−0.47713	0.086	−5.524	0.000
Time × solvent:solid ratio	0.12525	0.115	1.085	0.294
Time × particle size	0.06075	0.115	0.526	0.606
Time × solvent composition	0.04025	0.115	0.349	0.032
Solvent:solid ratio × particle size	−0.05425	0.115	−0.470	0.645
Solvent:solid ratio × solvent composition	−0.00175	0.115	−0.015	0.988
Particle size × solvent composition	−0.07525	0.115	−0.652	0.524

SE coefficient, Standard error coefficient; *T*, *T*-ratio = regression coefficient/SE regression coefficient; *P*, probability.

2.2. Extraction procedure

The dried root parts of *L. camara* were milled with the help of a grinder. Solvent extraction of *L. camara* root was carried out in temperature controlled water bath by stirring at the constant speed of 200 rpm. The independent variables were temperature (35–70 °C), mean particle size (0.3–1.5 mm), solvent composition (17.5–87.5% methanol in methanol-ethyl acetate mixture), solvent–solid ratio (15:1–75:1) and extraction steps (1–3). The milled particles were sieved with a sieve shaker of different sizes. *L. camara* root powder of different mean particle size (0.3–1.5 mm) were put into a 150 ml conical flask, then methanol-ethyl acetate solvent mixture (17.5–87.5% methanol in methanol-ethyl acetate mixture) was added in different solvent–solid ratios (15:1–75:1) and put in a temperature controlled water bath at selected temperatures (35–70 °C) for different periods of time (15–75 min). One gram of the root samples was used for each treatment.

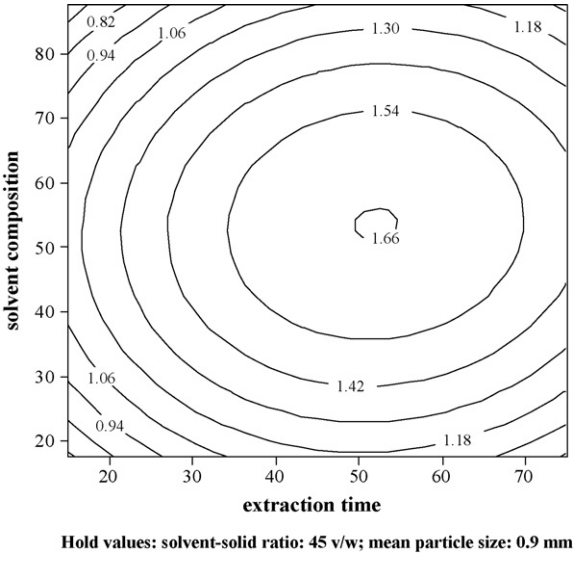

Fig. 1 – Contour plot for oleanolic acid extraction at varying level of time and solvent composition (% methanol in methanol-ethyl acetate mixture).

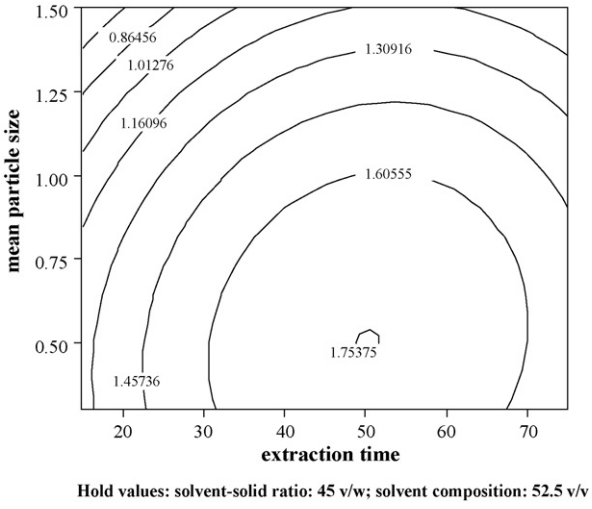

Fig. 2 – Contour plot for oleanolic acid extraction at varying level of mean particle size and time.

2.3. Measurement of oleanolic acid content

The amount of oleanolic acid was determined by the modified method of Kosior et al., 2005 using high performance thin layer chromatography (HPTLC). Standard oleanolic acid and the samples were spotted on precoated silicagel F_{254} aluminium plate (E-Merck grade) as narrow bands 4 mm wide at a constant rate of 10 μl s^{-1} using a Camag Linomat IV model applicator under nitrogen atmosphere. A mixture of chloroform and methanol (95:5 v/v) was used as the mobile phase. The plates were sprayed with anisaldehyde reagent (0.5 ml anisaldehyde, 1 ml H_2SO_4 and 50 ml acetic acid) and heated at 105 °C for 5 min. which gave well resolved spots at R_f 0.5 (Pink colour-visible light). Pink colour developed was quantified using Camag TLC scanner with CATS 4 software at 490 nm.

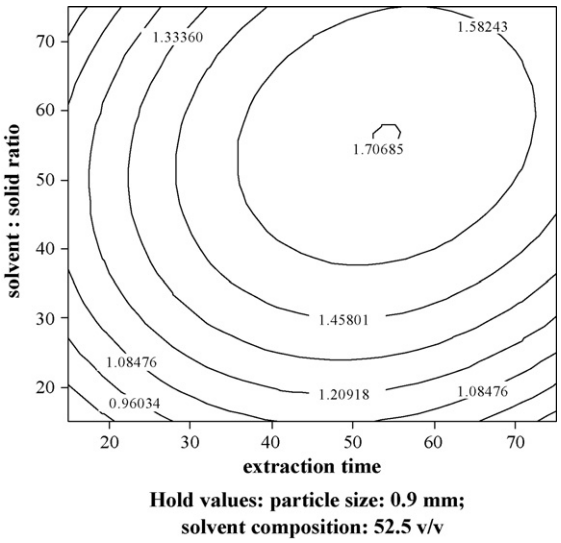

Fig. 3 – Contour plot for oleanolic acid extraction at varying level of solvent–solid ratio and time.

2.4. *Response surface methodology*

Response surface methodology was applied in two stages, first to identify the significant parameters for extraction of oleanolic acid using Plackett–Burman design criterion and later the significant parameters resulted from Plackett–Burman design were optimized by using a central composite design. The experimental design and statistical analysis of the data were done by using Minitab statistical software package (14 version).

2.4.1. *Plackett–Burman design*

Plackett–Burman design criterion were applied to identify the significant variables responsible for extraction of oleanolic acid from *L. camara* roots. This design criterion assumes that there are no interactions between the different extraction parameters and is based on the first-order model:

$$Y = \beta_0 + \sum \beta_i x_i \quad (1)$$

where Y is the estimated target function, β_i the regression coefficients and β_0 is the scaling constant. The effect of six variables (extraction time, solvent composition, mean particle size and solvent:solid ratio, temperature, number of extraction steps) on the extraction of oleanolic acid was tested at two experimental levels high level denoted by (+) and a low level denoted by (−) as listed in Table 1. Six variables were screened by conducting 12 experiments and the experimental design is given in Table 2. All experiments were conducted in duplicate and the average value of extracted oleanolic acid was used for statistical analysis.

The variables which were significant at 5% level ($P < 0.05$) from the regression analysis as given in Table 3 were considered to have greater impact on extraction of oleanolic acid and were further optimized by central composite design.

2.4.2. *Central composite design*

A central composite design was applied to determine the optimum level of four significant extraction parameters screened from Placket–Burman design criterion. As shown in Table 4 the effect of four parameters (extraction time, solvent composition, mean particle size and solvent:solid ratio) on the extraction of oleanolic acid was studied at five experimental levels: $-a$, -1, 0, $+1$, $+a$ where $a = 2^{n/4}$, here n is the number of variables and 0 corresponds to the central point. The experimental levels for these variables were selected from our preliminary work, which indicated that an optimum could be found within the level of parameters studied. The levels of factors used for experimental design are given in Table 4. The actual level of each factor was calculated by the following equation (Paul et al., 1992):

$$\text{Coded value} = \frac{\text{actual level} - (\text{high level} + \text{low level})/2}{(\text{high level} - \text{low level})/2} \quad (2)$$

The experimental design scheme is given in Table 5. Oleanolic acid yield was analyzed by using a second-order polynomial equation and the data were fitted in to the equation by multiple regression procedure. The model equation for analysis is given below:

$$Y = \beta_0 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 + \beta_{44} {X_4}^2 + \beta_{55} {X_5}^2 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{25} X_2 X_5 + \beta_{34} X_3 X_5 + \beta_{35} X_3 X_5 + \beta_{45} X_4 X_5 + \cdots \quad (3)$$

where Y is the predicted response, X_2, …, X_5 are the levels of the factors and β_2, …, β_5 are linear coefficients, β_{22}, …, β_{55} are quadratic coefficients and β_{23}, …, β_{45} are the interactive coefficients with β_0 is a scaling constant. Analysis of variance (ANOVA), regression analysis were done and contour plots were drawn by using Minitab statistical software package.

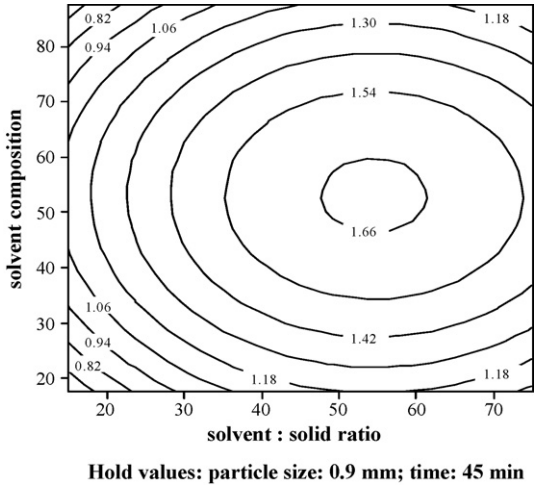

Fig. 4 – Contour plot for oleanolic acid extraction at varying level of solvent–solid ratio and solvent composition (% methanol in methanol-ethyl acetate mixture).

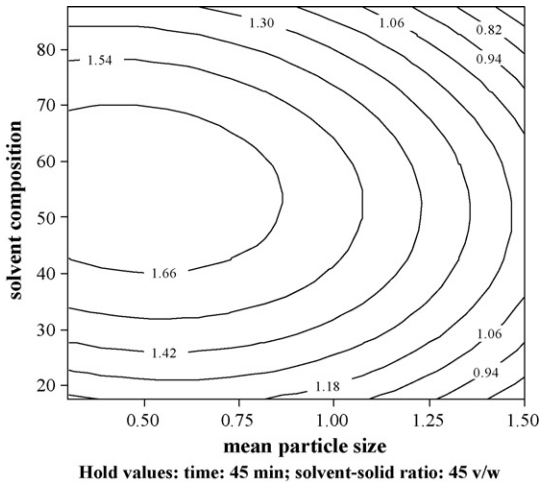

Fig. 5 – Contour plot for oleanolic acid extraction at varying level of mean particle size and solvent composition (% methanol in methanol-ethyl acetate mixture).

3. Results and discussion

3.1. *Screening of extraction parameters using Plackett–Burman design criterion*

A total of six variables were analyzed with regard to their effects on oleanolic acid yield using a Plackett–Burman design (Table 1). The design matrix selected for screening of significant variables for oleanolic acid extraction and the corresponding responses are shown in Table 2. The adequacy of the model was calculated, and the variables evidencing statistically significant effects were screened via regression analysis (Table 3). Among six extraction parameters (temperature, extraction time, solvent composition, mean particle size, solvent:solid ratio and number of extraction steps) studied, four parameters (extraction time, solvent composition, mean particle size, solvent:solid ratio) were found to have significant influence on oleanolic acid extraction as evidenced by their *P* values (<0.05, significant at 5% level) obtained from regression analysis. The coefficient of determination (R^2) of the model was found to be 0.925 which indicates the model can explain up to 92.5% variation of the data. Oleanolic acid yield obtained from Plackett–Burman design experiments showed wide variation (0.221–1.683%), which indicated that further optimization is necessary to get a maximum response.

3.2. *Effects of extraction time, solvent:solid ratio, particle size and solvent composition on oleanolic acid extraction*

Response surface methodology using central composite design was applied to optimize the levels of significant extraction parameters resulting from Plackett–Burman design experiments. Thirty-one experiments were carried out from the design and the experimental values are given in Table 5 along with predicted values obtained from the model equation. All the experiments were carried out in duplicate and the mean value of oleanolic acid yield was taken for statistical analysis. By applying multiple regression analysis on the experimental data, the following second-order polynomial equation was developed:

$$Y = 1.64300 + 0.16358X_2 + 0.21292X_3 - 0.29175X_4 + 0.01542X_5 - 0.34763X_2^2 - 0.33263X_3^2 - 0.21113X_4^2 - 0.47713X_5^2 + 0.04025X_2X_5 \quad (4)$$

The effects of extraction time (X_2), solid–liquid ratio (X_2), mean particle size (X_4) and solvent composition (X_5) on oleanolic acid extraction are reported in Table 6 by the coefficient of the second-order polynomials. Response surfaces for oleanolic acid yield are shown in Figs. 1–6 which give the contour maps for the effect of extraction time, solid–liquid ratio, mean particle size and solvent composition on the oleanolic acid yield. Regression analysis of the experimental data (Table 6) showed that time, solvent–solid ratio and solvent composition had significant positive linear effects on oleanolic acid yield while mean particle size has negative linear effect on oleanolic acid yield. This was evident from the low *P* value obtained from the regression analysis. Among the four parameters, solvent–solid ratio, was found to have the highest impact on oleanolic acid yield as given by the highest linear coefficient (0.213) followed by time (0.163) and solvent composition (0.015), while mean particle size has negative linear effect (−0.292). These extraction parameters also showed significant negative quadratic effects on oleanolic acid yield indicating that oleanolic acid extraction increased as the level of these factors increased and decreased as the level of these parameters increased above certain values. Table 6 also indicate that the interaction between time and solvent composition has significant effect on oleanolic acid extraction and all other interactive variables are insignificant. Hence, only the term indicating interaction between time–solvent composition was included in the model regression Eq. (4).

Analysis of variance for the oleanolic acid extracted from *L. camara* roots obtained from this design were given in Table 7.

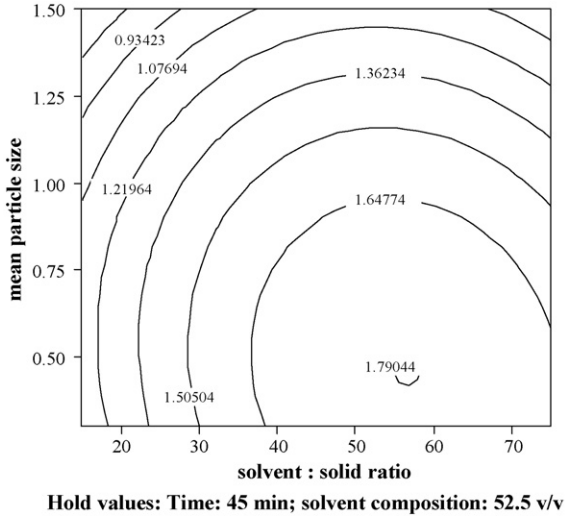

Fig. 6 – Contour plot for oleanolic acid extraction at varying level of solvent–solid ratio and mean particle size.

Table 7 – Analysis of variance for oleanolic acid extraction from *Lantana camara* root using central composite design criterion

Source	d.f.	Sequential SS	Adjusted SS	Adjusted MS	*F*	*P*
Regression	14	1.679	1.679	0.1199	9.00	0.000
Linear	4	0.944	0.944	0.2361	17.71	0.000
Square	4	0.705	0.705	0.1763	13.23	0.000
Interaction	6	0.029	0.029	0.0049	0.37	0.887
Residual error	16	0.213	0.213	0.0133		
Lack of fit	10	0.177	0.177	0.0177	3.00	0.096
Pure error	6	0.035	0.035	0.0059		
Total	30	1.892				

d.f., degree of freedom; sequential SS, sequential sum of square; adjusted SS, adjusted sum of square; *F*, *F*-statistics test to determine significance and *P*, probability.

ANOVA gives the value of the model and can explain whether this model adequately fits the variation observed in oleanolic acid extracted with the designed extraction level. If the *F*-test for the model is significant at the 5% level ($P < 0.05$), then the model is fit and can adequately explain the variation observed. If the *F*-test for lack of fit is significant ($P < 0.05$), then a more complicated model is required to accommodate the data. The closer the value of *R* (multiple correlation coefficient) to 1, the better the correlation between the observed and predicted values. Here the value of *R* (0.8814) indicates that the model can explain up to 88.14% variation of oleanolic acid extracted. The *P* value for lack of fit (0.096) reveals that the experimental data obtained fit well with the model and explains the effect of extraction time, solvent–solid ratio, particle size and solvent composition on oleanolic acid extracted from the *L. camara* roots. Figs. 1–6 shows the contour plots of oleanolic acid extracted for each pair of extraction parameters by keeping the other two parameters constant at its middle level. The effect of extraction time and solvent composition on the extraction of oleanolic acid are shown in Fig. 1. Maximum oleanolic acid (1.66%) was obtained at extraction time 55 min and solvent composition 52.5%. Further increase in solvent composition leads to deceleration in extraction of oleanolic acid. The results presented here on the effect of solvent composition (% methanol in methanol-ethyl acetate mixture) are in good agreement with those of Cacace and Mazza (2003) for total phenolic compounds extraction from black currant, where total phenolic content increased with ethanol concentration up to a maximum of about 60% and then decreased with further increase in solvent concentration. Fig. 2 indicates that maximum oleanolic acid was extracted (1.75%) when particle size was 0.5 mm, further increase in the mean particle size leads to decrease in extraction of oleanolic acid. Decrease in particle size leads to increase in exchange surface and decrease the path length of the solute to reach the surface, thus extraction of oleanolic acid from *L. camara* roots was increased. On the contrary, very small particles may lead to technical difficulties related to the permeability of the solid bed, during the mixing of the plant material with solvent, as well as during the filtration. The contour plot of Fig. 3 indicates that maximum oleanolic acid (1.7%) extraction occurred at the solvent:solid ratio of 55:1 and extraction time of 55 min. The extraction of oleanolic acid increases with increase in solvent:solid ratio up to 55:1 and further increase in the solvent:solid ratio leads to decelerate in the extraction of oleanolic acid. Fig. 4 indicates that maximum oleanolic acid extracted at the solvent–solid ratio 55:1 and solvent composition around 52.5% methanol in methanol-ethyl acetate mixture. Maximum oleanolic acid was extracted when particle size was 0.5 mm and solvent composition 52.5% methanol in methanol-ethyl acetate mixture (Fig. 5). Further increase in both the parameters leads to deceleration of oleanolic acid extraction. Fig. 6 contour plot shows that maximum oleanolic acid was extracted at solvent–solid ratio (55:1) and mean particle size (0.5 mm).

3.3. *Validation of the model*

The experimental data were fitted in to the model Eq. (4) and the optimum values were found to be: extraction time (55 min), solvent–solid ratio (55:1), mean particle size (0.5 mm) and solvent composition (52.5% methanol in methanol-ethyl acetate mixture). At these optimum levels of extraction parameters oleanolic acid extracted from *L. camara* roots was 1.74%, which is very close to the predicted value of 1.686%.

4. Conclusion

Response surface methodology was successfully used to optimize the extraction parameters for extraction of oleanolic acid from *L. camara* roots. To optimize various parameters for extraction of oleanolic acid from *L. camara* roots six parameters (temperature, time, solvent–solid ratio, solvent composition, mean particle size and extraction steps) were tested by using Plackett–Burman design criteria and four parameters time, solvent–solid ratio, mean particle size and solvent composition showed significant effects on extraction of oleanolic acid. The existence of interactions between the parameters was studied and the interaction between time and solvent composition showed significant effects on extraction of oleanolic acid. The extraction parameters were optimized by applying central composite design and the parameters for best extraction of oleanolic acid from *L. camara* root was found to be extraction time (55 min), solvent–solid ratio (55:1), mean particle size (0.5 mm) and solvent composition (52.5% methanol

in methanol-ethyl acetate mixture).The maximum oleanolic acid yield from *L. camara* root was 1.74% dry weight. The second-order polynomial model developed was found to be satisfactory in describing the experimental data. This is the first report of the optimization of extraction of oleanolic acid from *L. camara* root using response surface methodology.

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