



Optimization of Solvent Extraction of *Parinari polyandra* Benth Seed Oil Using Response Surface Methodology

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TEO and VOA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors TJA and KRO managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Parinari polyandra B. seed is a potential source of oil with relatively high oil yield. Optimizing the extraction process of the oil will enhance its economic and industrial relevance, providing useful information for would be investors.

Aims: This work is aimed at extracting oil from the seeds of *Parinari polyandra* B. by using solvent extraction method. The process was optimized by using response surface methodology to determine the effect of four sets of parameters corresponding to optimum oil yield.

Methodology: *Parinari* oil was extracted using n-hexane and petroleum ether as solvents, Central Composite Design was used in the design of the 40 extraction experimental runs. The effects of solvent residence time (A), temperature (B), solid/solvent ratio (C) and solvent types (D) on the yield of *Parinari* seed oil was studied using response surface methodology.

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Results: The optimal yield of 64% was obtained at temperature of 60°C, residence time of 4 hours, solid/solvent ratio of 0.05g/ml using n-hexane as solvent. The physicochemical characteristics of the extracted *Parinari polyandra* oil compared well with literature values. Extraction temperature was found to have the most significant effect on the oil yield followed by residence time, solid/solvent ratio and solvent type.

Conclusion: The optimal yield of 64% was obtained at temperature of 60°C, residence time of 4 hours, solid/solvent ratio of 0.05g/ml using n-hexane as solvent. The physicochemical characteristics of the extracted *Parinari polyandra* oil compared well with literature values. Extraction temperature was found to have the most significant effect on the oil yield followed by residence time, solid/solvent ratio and solvent type.

Keywords: *Parinari polyandra* B.; seed oil extraction; optimization; response surface methodology.

1. INTRODUCTION

There is a pressing need to search for more seed oils that can be used as raw materials for industrial application and establish new pathways from potential oil producing seeds [1]. It is pertinent to expand the present vegetable oil feedstock especially in Africa where there is the need to replace industrial seed oils that constitute food sources with non-edible ones [2]. The replacement is necessary to reduce the problem of competition between industrial and food utilization of the same oil (such as soya oil). *Parinari polyandra* Benth is one of the available tropical seed producing plants in West Africa which are potential oil crops to serve as replacement feedstock for the chemical industries waiting to be harnessed [3].

Parinari polyandra B. plant is a savannah plant available in West Africa belonging to the family *Rosaceae* [4], It has a tree of about 8m high, glossy leaves that are elliptical and usually rounded at both ends. The deep red (Fig. 1) or blackish purple colour (based on the variety) smooth fruits are about 2.5cm long. The fruits have yellow white (Fig. 1) endosperm with thick

seed coats containing the oily mass. The variety and season of harvest of fresh seed kernel affects the oil yield giving a range of yields between 31-60% oil [5].

The plant has been mainly used for medicinal purposes [6] for fertility improvement and to relieve painful and inflammatory conditions [7]. Some species of *Parinari* have been used as herbal treatment for diabetics [8]. The coconut water extract of *Parinari polyandra* seeds was reported to possess anti-diabetic property [6]. The phytochemical components of the plant extracts include flavonoids, tannins, saponin and glycosides [7,9] though the active component responsible for the cure of diabetics is yet to be identified [6]. The administration of the ethanolic extract with *Spondias mombin* in alloxan-induced diabetic rats was reported by Emeka et al. [10] to promote desirable reduction on glucose and total protein levels of the rats. Abolaji et al. [9] reported the crude fibre, moisture content, total ash, total fat, total protein, total carbohydrate and phosphorus of the *Parinari polyandra* fruit as 4.21±1.10%, 30.65±5.02%, 2.53±1.20%, 0.53±0.15%, 7.09±0.20%, 54.27±3.20% and 0.690±0.10% respectively.



Fig. 1. Freshly harvested *Parinari polyandra* fruits and seeds

The industrial utilization of *Parinari polyandra* is a relatively new research interest. Earlier works on *Parinari polyandra* B. plant has been predominantly on its medicinal uses [7-10]. Much work has not been done on its industrial utilization. One of the few available works on the seed oil includes Olatunji et al.'s [11] report on the protein and triacylglycerol contents of *Parinari polyandra* seed oil. They were stated as 18.33% and 31.1% respectively. *Parinari* seed oil yield varies between 31 and 60% depending on the season of harvest [11]. The oil was reported as non-edible because of its relatively high concentration of eleostearic acid [6]. Similarly, the oil of *Parinari* seed was extracted and refined using the methods of degumming, neutralization and bleaching by Odetoye et al. [5]. An earlier work also indicated *Parinari* oil as a potential feedstock for alkyd resin production [3].

Parinari seeds oil has not been well-harnessed possibly because of lack of adequate information on the extraction [3,11]. Presently, available information on *Parinari* oil extraction is scanty. There is no published research work on the optimization of the extraction process of *Parinari* seed oil [3]. Such information will be useful in establishing *Parinari* seeds as an industrial feedstock. Therefore, this study is aimed at investigating the effects of some process parameters on the extraction process of *Parinari polyandra* Benth seeds oil providing database for further works on its industrial utilization. The optimization of *Parinari* oil extraction is being reported for the first time.

Response surface methodology has been regarded as an effective tool in the optimization of several chemical processes [12-15]. The choice of central composite design (CCD) was based on its economical advantage of the reduction in the number of required experimental runs to obtain statistically acceptable result [12].

2. MATERIALS AND METHODS

2.1 Materials

The *Parinari* fruits were harvested from trees in Ilorin, Kwara State, Nigeria in the month of November. The seeds of *Parinari* were obtained by cutting fresh but tough fruits with a knife into halves to release the whitish coloured oily seed kernel from the woody endocarp. The *Parinari* seeds were further cleaned by hand-picking the dirt. Then the yield of seeds from the fruits harvested was calculated thus:

$$Y = \frac{X_s}{X_f} * 100 \quad (1)$$

Where

Y = yield (%)
 X_s = weight of seeds obtained (g)
 X_f = weight of fruits harvested (g)

The seeds were dried at room temperature for 10 days. The dried seeds have a light brown colouration. After drying, the dried seeds were crushed by pounding with laboratory mortar and pestle and sieved to obtain a mean particle size of about 2mm. The n-Hexane and the petroleum ether (60 - 80°C) used for the extraction process were commercially obtained from BDH Chemicals.

2.2 Methods

2.2.1 Moisture content determination

The moisture content was determined using the method of Akpan et al. [16]. 50g of the cleaned sample was weighed and dried in an oven at 80°C. Weight was taken after every 2 hours (i.e. the sample was removed from the oven and placed in the desiccator for thirty minutes to cool. It was then removed and re-weighed). The procedure was repeated until a constant weight was obtained (repeated 4 times).

$$M = \frac{W_1 - W_2}{W_1} * 100 \quad (2)$$

Where

w_1 = Original weight of the sample before drying (g)
 w_2 = Constant Weight of the sample after drying (g)
 m = Moisture content.

The moisture content was calculated on a wet basis.

2.2.2 Experimental design

The experiment was designed using Design-Expert version 6.0.8. (Stat-Ease Inc, Minneapolis, U.S.A.). The experimental design was based on a five level, three (numeric) factor CCD with one categorical factor was generated using the parameters presented in Table 1. Time, temperature and solid/solvent ratio were the numeric factors coded as A,B,C respectively. The categorical factor; solvent type, was coded as D.

Table 1. Experimental design summary

Factor	Name	Units	Type	Level				
				- α	-1	0	1	+ α
A	Time	hrs	Numeric	0.64	2	4	6	7.36
B	Temperature solid/solvent	deg C	Numeric	56.21	60	65	70	73.41
C	Ratio solvent	g/ml	Numeric	0.01	0.03	0.05	0.08	0.09
D	Types		Categorical		n- Hexane		Pet ether	

$$\alpha=1.68$$

2.2.3 Oil extraction

Forty (40) extraction experiments with different combinations of time, temperature and solvent types were performed. The extraction of the oils was carried out with soxhlet apparatus of 250cm³ capacity using n-hexane and petroleum ether (fraction 60 – 80°C). The soxhlet thimble was charged with between 5 – 15g ground seeds of 2mm mean particle size and extracted with the solvents according to the experimental design. 200ml of solvent [9] was poured into a round bottom flask. Then, Xg of the sample (as specified in the design) was placed in the thimble and inserted in the soxhlet extractor. The heating mantle was pre-set to the design temperature. As the solvent boiled, the vapour rose through the vertical tube into the condenser at the top. The liquid condensate dripped into the thimble, which contained the solid sample to be extracted. The extract seeped through the pores of the thimble and filled the siphon tube, where it flowed back down into the round bottom flask. This was allowed to continue for the specified time after which the solid sample in the thimble was removed. The solvent was recovered and the oil obtained weighed. The experiment was repeated for other parameters.

2.2.4 Oil yield determination

The oil yield was determined by using the equation 3 [17]:

$$OY = \frac{M_o}{M_s} * 100 \quad (3)$$

Where

OY = oil yield (%)

Mo = mass of oil extracted (g)

M_s = mass of the *Parinari* seed (g)

2.2.5 Physicochemical analysis

The iodine, saponification and the acid values of the extracted oil were determined using the AOCS official method [18].

2.2.6 Optimization of the extraction parameters

The optimization of the extraction parameters was carried out for the maximal extraction of *Parinari* oil using central composite design (CCD) method. Multiple regression analysis of the experimental data was done using Design Expert software 6.0.8. The independent and the dependent variables were fitted to the second-order model equation. The fitted quadratic response model is represented as [19]

$$Y = \beta_0 + \sum_{j=1}^k \beta_j x_j + \sum_{j=1}^k \beta_{jj} x_j^2 + \sum_{i < j}^k \beta_{ij} x_i x_j \quad (4)$$

Y is the response variable (oil yield), x_i, x_j are the coded variables, $\beta_0, \beta_i, \beta_{jj}, \beta_{ij}$ are intercept, linear, interactive and quadratic coefficients respectively, k is the number of factor studied. The goodness of fit of the regression equation Y was evaluated by the coefficient of determination (R^2) and the coefficient of relation (R). Statistical testing of the model was done in the form analysis of variance (ANOVA), which is required to test the significance and adequacy of the model.

3. RESULTS AND DISCUSSION

3.1 Oil Yield

The moisture content of the fresh seed kernel was obtained on a wet basis as 0.96%. The values obtained for the oil yields are as given in Table 2 showing the values obtained for various experimental runs. The maximum oil yield of 64% was obtained for *Parinari* seeds as the yield ranged from 31.7 to 64%. This maximal yield value obtained is slightly higher than that presented by Olatunji et al. [11] and Motojesi et al. [20] (60.7%). The varying oil yield values presented in Table 2 are indications that *Parinari* seed extraction process condition/parameters considerably affect the oil yield.

Table 2. Central composite design layout for *Parinari* oil extraction for coded, uncoded values and response

Coded values				Uncoded values			Experimental predicted				
Std	Run	A	B	C	D	A:Time (hr)	B:Temp (°C)	C:Solid/solvent	D:Solvent type	Oil yield (%)	
24	1	1	1	-1	1	6	70	0.03	Pet Ether	61.7	59.2
3	2	-1	1	-1	-1	2	70	0.03	n-Hexane	31.7	30.8
32	3	0	1.68	0	1	4	73.4	0.05	Pet Ether	52	52.5
27	4	-1	1	1	1	2	70	0.08	Pet Ether	50	48.3
16	5	0	0	0	-1	4	65	0.05	n-Hexane	50	52.0
25	6	-1	-1	1	1	2	60	0.08	Pet Ether	51.4	53.8
11	7	0	-1.68	0	-1	4	56.6	0.05	n-Hexane	61	59.6
12	8	0	1.68	0	-1	4	73.4	0.05	n-Hexane	57	44.5
35	9	0	0	0	1	4	65	0.05	Pet Ether	50	53.1
15	10	0	0	0	-1	4	65	0.05	n-Hexane	47	52.0
9	11	-1.68	0	0	-1	0.6	65	0.05	n-Hexane	30.2	33.5
5	12	-1	-1	1	-1	2	60	0.08	n-Hexane	55.7	53.9
28	13	1	1	1	1	6	70	0.08	Pet Ether	60	59.4
38	14	0	0	0	1	4	65	0.05	Pet Ether	50	53.1
2	15	1	-1	-1	-1	6	60	0.03	n-Hexane	60	61.0
31	16	0	-1.68	0	1	4	56.6	0.05	Pet Ether	61	53.8
23	17	-1	1	-1	1	2	70	0.03	Pet Ether	41.7	38.9
6	18	1	-1	1	-1	6	60	0.08	n-Hexane	45.7	49.5
39	19	0	0	0	1	4	65	0.05	Pet Ether	55	53.1
37	20	0	0	0	1	4	65	0.05	Pet Ether	50	53.1
20	21	0	0	0	-1	4	65	0.05	n-Hexane	57	52.0
33	22	0	0	-1.68	1	4	65	0.01	Pet Ether	53	59.6
14	23	0	0	1.68	-1	4	65	0.09	n-Hexane	58.3	56.7
30	24	1.68	0	0	1	7.36	65	0.05	Pet Ether	51	48.0
7	25	-1	1	1	-1	2	70	0.08	n-Hexane	38	40.1
19	26	0	0	0	-1	4	65	0.05	n-Hexane	52	52.0
4	27	1	1	-1	-1	6	70	0.03	n-Hexane	53.3	58.9
26	28	1	-1	1	1	6	60	0.08	Pet Ether	42.1	43.6
10	29	1.68	0	0	-1	7.36	65	0.05	n-Hexane	54	51.84
13	30	0	0	-1.68	-1	4	65	0.01	n-Hexane	60	58.5
18	31	0	0	0	-1	4	65	0.05	n-Hexane	50	52.0
36	32	0	0	0	1	4	65	0.05	Pet Ether	50	53.1
22	33	1	-1	-1	1	6	60	0.03	Pet Ether	55	55.1
17	34	0	0	0	-1	4	65	0.05	n-Hexane	54	52.2
34	35	0	0	1.68	1	4	65	0.09	Pet Ether	60.5	57.8
21	36	-1	-1	-1	1	2	60	0.03	Pet Ether	60	56.2
1	37	-1	-1	-1	-1	2	60	0.03	n-Hexane	53.3	56.3
8	38	1	1	1	-1	6	70	0.08	n-Hexane	54	57.1
40	39	0	0	0	1	4	65	0.05	Pet Ether	50	53.1
29	40	-1.68	0	0	1	0.64	65	0.05	Pet Ether	40	39.6

3.2 Optimization of the Extraction Process Parameters

Multiple regression analysis of the experimental data using Design Expert 6.0.8. software gave a

second order polynomial model which was then modified to ignore the insignificant terms B² and CD. The reduced quadratic model is given in terms of the coded factors as in equation 5:

$$Y = 52.57 + 3.97A - 2.43B - 0.55C - 3.3A^2 + 1.97C^2 + 5.33AB - 2.29AC + 1.47AD + 2.94BC + 2.05BD \tag{5}$$

Considering the solvent types:

Forn-hexane:

$$Y_h = 315.13 - 22.63 A - 4.4 B - 1926.98 C - 0.83 A^2 + 3890.74 C^2 + 0.53 AB - 50.83 AC + 26.11 BC \quad (6)$$

For Petroleum Ether:

$$Y_p = 268.76 - 24.10 A - 3.58 B - 1926.97C - 0.83 A^2 + 3890.74 C^2 + 0.53 AB - 50.83 AC + 26.11 BC \quad (7)$$

where Y is the response variable in terms of % Parinari oil extraction yield,

Y_h is the response variable in terms of % *Parinari polyandra* oil extraction yield when n-hexane was used, Y_p is the response variable in terms of % *Parinari* oil extraction yield when petroleum ether was used, while A, B, C, D were the coded values of the independent variables i.e. time, temperature, solid/solvent ratio and solvent types respectively.

3.3 Statistical Analysis

The independent and the dependent variables were fitted to the second-order model equation (eq 5). Statistical testing of the model was done in the form analysis of variance (ANOVA) which is required to test the significance and adequacy of the model. The analysis indicates that the main linear interaction effects are due to the coded terms A, B, corresponding to time, temperature indicating that time and temperature are significant model terms. The linear interaction effects between the factors time & temperature (AB), time & solid/solvent ratio (AC), temperature & solid/solvent ratio (BC), and time & solvent types (AD) are also significant. The quadratic effects of time (A^2) is also significant. The ANOVA results however, indicated that the terms C, D, CD, B^2 are insignificant.

The model was modified by excluding the insignificant terms while still maintaining the hierarchical order as suggested by the Design Expert software. Therefore the terms B^2 and CD were excluded. The summary of the analysis of variance (ANOVA) obtained from the Design-Expert software corresponding to equation Y for % oil yield oil is shown in Tables 3 and 4. The goodness of fit of the regression equation Y was evaluated by the coefficient of determination (R^2) and the coefficient of relation (R). The coefficient of determination (R^2) and (R^2_{adj}) for *Parinari* oil

extraction was obtained as 0.8707 and 0.8200 respectively indicating the regression model as acceptable [21,22]. The lack of fit value of 1.65 was desirably low for the model indicating adequate representation of the relationship by the model and the lack-of-fit observed is insignificant. The ANOVA results of the regression model corresponding to quadratic equation Y for the % oil yield demonstrates that the model is highly significant, as it is evident from the calculated F-value (=17.15) and a very low probability value (probability (P)>F=0.0001) which is less than 0.05. The adequate precision value is greater than 4 (P_{ad} =17.29) indicating an adequate signal to noise ratio of the model.

For the chosen model equations Y_h and Y_p , the interaction plots illustrate the relationship for the effects of time and temperature respectively on oil yield for the two different solvents. The effects of extraction time on oil yield for both solvents followed the same trend as indicated in Fig. 2A. The oil yield increased with increase in extraction time when both solvents were used then decreased with further increase in time for petroleum ether. From Fig. 2B however, the oil yield was observed to decrease with higher temperature of extraction. This decrease is relatively more prominent in n-hexane than in petroleum ether. Fig. 2B indicates that relatively higher yields were obtained using n-hexane solvent at 60°C compared to petroleum ether in which higher yield was obtained at 70°C. This relatively lower temperature advantage of n-hexane may be indicative of a more economical solvent when considering heating costs among other requirements for the solvent extraction process.

Table 3. Analysis of variance (ANOVA) table for parinari oil extraction

Source	Sum of squares	Degree of freedom	Mean square	F value	Prob > F
Model	1930.51	11	175.50	17.15	< 0.0001 significant
Residual	286.58	28	10.24		
Total	2217.09	39			
A	431.21	1	431.21	42.13	< 0.0001
B	161.59	1	161.59	15.79	0.0005
C	8.34	1	8.34	0.81	0.3745
D	12.32	1	12.32	1.20	0.2819
A2	316.67	1	316.67	30.94	< 0.0001
C2	112.94	1	112.94	11.03	0.0025
AB	453.69	1	453.69	44.33	< 0.0001
AC	83.72	1	83.72	8.18	0.0079
AD	58.95	1	58.95	5.76	0.0233
BC	138.06	1	138.06	13.49	0.0010
BD	115.04	1	115.04	11.24	0.0023
Lack of Fit	214.41	18	11.91	1.65	0.2106not significant
Pure Error	72.17	10	7.22		
Std. Dev. 3.20		R-Squared		0.8707	
Mean 51.66		Adj R-Squared		0.8200	
C.V. 6.19		Pred R-Squared		0.7246	
PRESS 610.68		Adeq Precision		17.294	

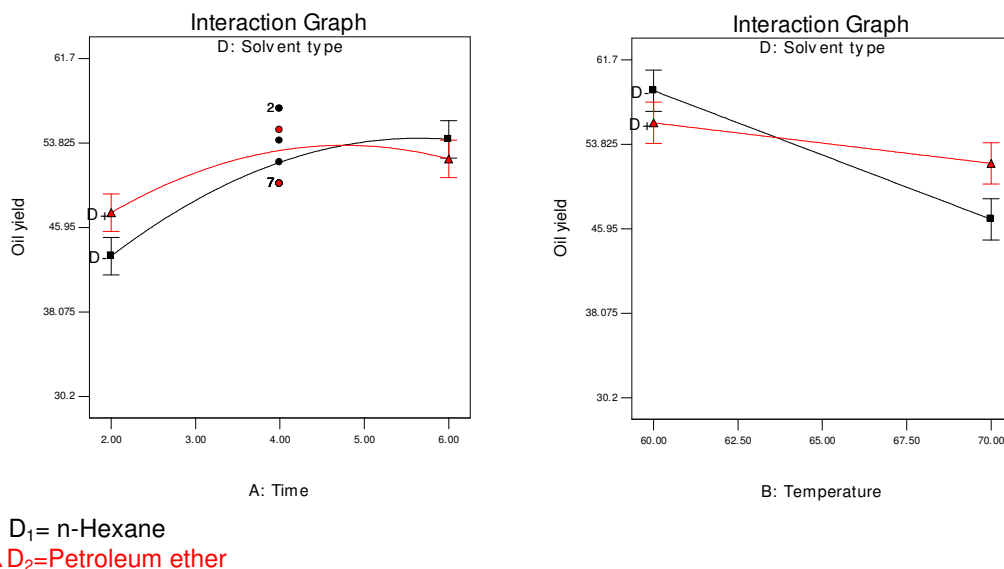


Fig. 2. Interaction of solvent types (n-hexane and petroleum ether) with time and temperature respectively

The 3D response surface plots of interactions between independent variables are shown in Figs. 3 to 8. From Fig. 3, it is observed that while oil yield increased gradually as extraction time increased, oil yield decreased with temperature increase for n-hexane and petroleum ether solvent. The interaction between

time and temperature is significant as indicated already by ANOVA results. In Fig. 4, the trend is shown to be similar to the interactions observed for n-hexane, although increasing extraction time had more declining effect on the oil yield obtained using petroleum ether as solvent.

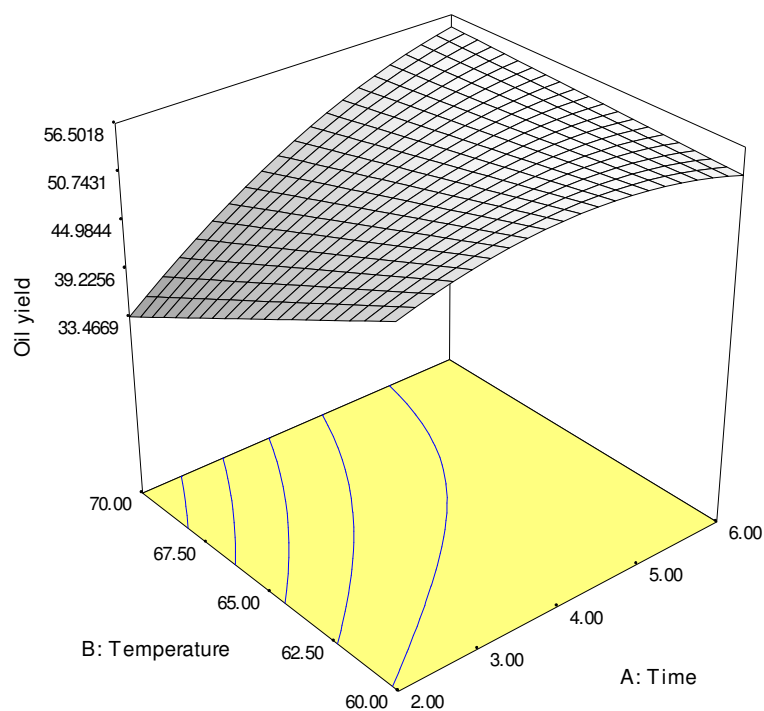


Fig. 3. 3D response surface plots showing effect of temperature, time and their interactive effect on oil yield for n-hexane

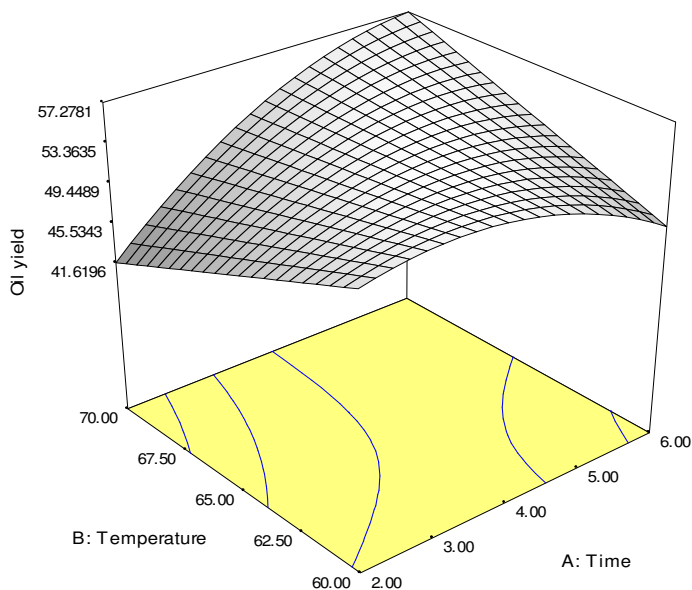


Fig. 4. 3D response surface plots showing effect of temperature, time and their interactive effect on oil yield for petroleum ether

Figs. 5 and 6 show the effect of solid/solvent ratio and time on oil yield using n-hexane and petroleum ether as solvents respectively. The effect of time is observed to be more pronounced compared to the effect of solid/solvent ratio on the oil yield in both solvents.

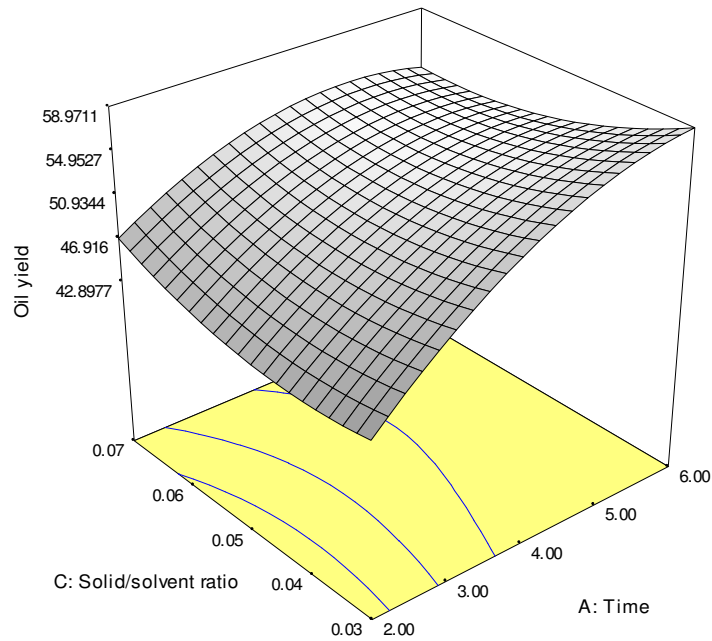


Fig. 5. 3D response surface plots showing effect of solid/solvent, time and their interactive effect on oil yield for n-hexane

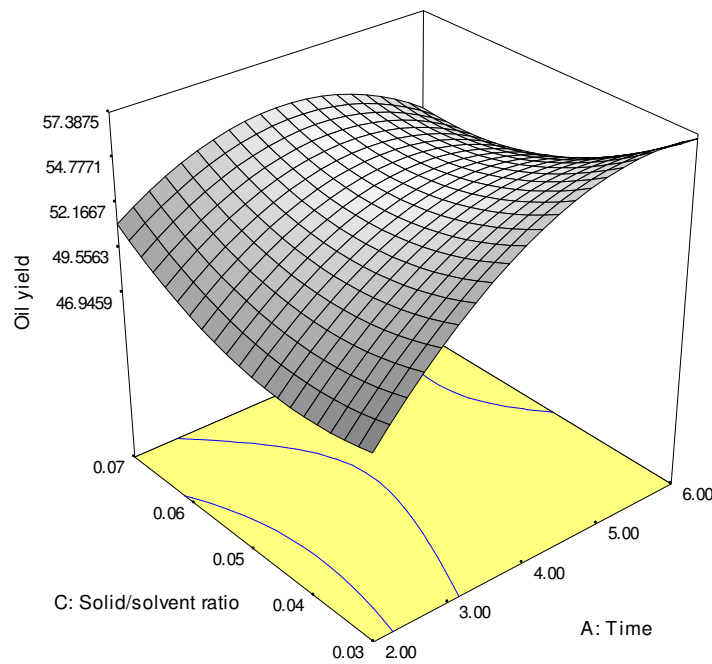


Fig. 6. 3D response surface plots showing effect of solid/solvent, time and their interactive effect on oil yield for petroleum

Using the design expert optimization tool, the oil yield (Y) response was maximized within the design space. Since no unique solution was

given, the optimal solution chosen was based on economic considerations [16] (reduced temperature and time corresponds to reduced

operating costs of the extraction process) and not necessarily the highest oil yield value. The optimal oil yield chosen for *Parinari* oil extraction and the corresponding conditions are as indicated in Table 4. Further validation experiments conducted at the predicted optimal condition was 60.4%. This value is in reasonable agreement with the predicted optimal yield. The value also compares well with literature value [20].

Table 4. The physico-chemical properties of *Parinari* oil

Properties	Values
Saponification value (mgKOHg ⁻¹)	241.9
Iodine value (I ₂ /100g oil)	170.2
Acid Value (mgKOHg ⁻¹)	3.61
Oil yield (%)	64
Viscosity (mPa s)	48.22
Refractive index (25 °C)	1.4641
Specific gravity	0.887

3.4 Physicochemical Characteristics of the Extracted Oil

The physico-chemical properties of the *Parinari* oil are as presented in Table 5. *Parinari* oil has a relatively high iodine value and the oil can be conveniently classified as a drying oil comparable with linseed oil. High iodine value and refractive index are indicative of high level of unsaturation in an oil. Drying oils are raw materials for the oleochemical industry. The results further suggest the use of the oil for as potential raw material in the paints industry based on its properties [3,5].

Table 5. Optimum values for *Parinari* oil extraction process

Process parameters	Optimum values
Time (hr) A	3.7
Temperature (°C)B	60
Solid/solvent ratio(g/ml) C	0.03
Solvent type D	n-Hexane
Oil yield (%)	61.43
Desirability	

4. CONCLUSION

Several parameters such as extraction time, extraction temperature, solid/solvent ratio and solvent type affecting solvent extraction of *Parinari polyandra* oil were investigated. Their effects on oil yield were clearly shown. This work

has demonstrated the use of CCD based response surface methodology for determining the conditions leading to the optimum yield of *Parinari* seed oil extraction. The developed second order polynomial equations was suitably employed in *Parinari* seed oil extraction process, where the analysis of the interactions of extraction time, temperature, solid/solvent ratio and solvent types (n-Hexane or Petroleum ether) were considered.

The optimal conditions were indicated as extraction time of 3.7 hours, extraction temperature of 60°C, solid/solvent ratio of 0.03g/ml using n-Hexane as the solvent. The physicochemical analysis of the extracted oil further suggests its potential application as a drying oil, a renewable raw material in the paints industry. Further work is ongoing on the effect of other extraction parameters such as particle size on the oil yield and the effect of processing parameters on the quality of *Parinari* oil. This work also indicates the suitability of the Design Expert tool for experimental design and statistical analysis of *Parinari* oil extraction considering the effects of both numerical and categorical factors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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