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Optimization and Characterization of New Surfactant from *Thevetia peruviana* Seed Oil via Saponification Reaction

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Abstract

In this study, a new surfactant was formed from *Thevetia peruviana* seed oil via saponification reaction. Oil extraction from the *Thevetia peruviana* seed was carried out using Soxhlet extraction method with n-hexane as the solvent. Oil optimization process was conducted using response surface methodology (RSM) in the Design Expert 13 tool. Characterization of the oil and formulated surfactant were conducted to determine the physicochemical properties. The results of the physicochemical properties of the oil showed that the saponification value was 218.79 (mg KOH/g), free fatty acid 14.03 (mg/L), acid value 28.05 (mg/g), specific gravity 0.9 (g/cm^3), iodine value 9.39 (mg/L), pH 4.04, density 0.884 (g/cm^3) and viscosity at room temperature was 31 (°C). In addition, the foam ability was obtained to be 83.5 (cm^3), flash point 4.2 (°C) and the optimal oil yield was 61.3%. Oil optimization results showed that the p-values obtained was 0.0011 which showed significant of the model, while the f-value was 3.43 and the lack of fit indicated insignificant which suggest that the experimental data fitted very well with the actual data.

Keywords: *Optimization; Characterization; Thevetia peruviana seed oil; Surfactant.*

1. Introduction

Thevetia peruviana is a plant that belongs to the family known as Apocynaceae [1]. It is commonly called yellow oleander and good for medicinal purposes [3]. *Thevetia peruviana* is grown in the Northern part of Nigeria and known in Hausa as '*Gamboje*' [11]. The leaves of the plant are green in color and have good medicinal value when used in traditional medicine [2]. *Thevetia peruviana plant* frequently grown in the tropical and shrub areas and bears yellow like flowers [4]. *Thevetia peruviana* plant can withstand most soil and drought weather [6]. *Thevetia peruviana* is a potential source of nutrition for animal feeds, food and showed anti-microbial activity, anti-inflammatory activity and contained oil from the seeds [5]. The seed of *Thevetia peruviana* is non-edible *and* contains about 64-67 percent oil and the oil could be used for bio-oil, biofuel and soap (surfactant) [10]. The application of *Thevetia peruviana* seed oil for biofuel yielded good results [9]. The biodegradability of *Thevetia peruviana* oil and production of biodiesel was observed in the work of [11]. Oil characterization and production of biodiesel from *Thevetia peruviana* seeds was investigated by [13]. Optimization and potential energy use of *Thevetia peruviana oil* for biodiesel in Nigeria was observed in the work of [12]. [16] reported that *Thevetia peruviana* could be use as feedstock for production of grease. The work of [15] reported that the oil from *Thevetia peruviana* seeds could be used for soap (surfactant). [14] observed that *Thevetia peruviana* seed oil could be used for production of liquid soap. [7] noted that the use of *Thevetia peruviana* seed oil for soap formulation was the presence of saponin [10]. The work of [6-8] reported that the formulation of surfactant (soap) from *Thevetia peruviana* seed oil will be effective for use in enhanced oil recovery application based

on its hydrophilic head group. [19-21] stated that the use of biosurfactant which are environmentally safe and cost-effective could serve as alternative to the imported synthetic surfactant. Therefore, the focus of this study is to optimize the extracted oil from *Thevetia peruviana* seed using design expert 13, formulate a surfactant via saponification reaction and determine the physicochemical properties of the oil and formed surfactant.

2. Oil optimization

Extraction of oil involves Soxhlet extraction, mechanical process, solvent extraction, enzymatic and ultra-sonic extraction methods [17]. Normal-hexane extraction method has been mostly used because of its high oil yield, easy to recover, high ability to accommodate other solvents, non-polar, good solubility of oil [16]. However, because the oil extraction process has different methods and steps, it is important to determine the optimum parameters that influences its oil recovery operation. Oil optimization considered keeping one factor constant while changing the other factor [14]. Response surface methodology (RSM), using design expert is ANOVA statistical model that describes the relationship between the response, experimental parameter and the optimum factors with the number of experiments runs [17]. This method (RSM) is used to optimize the extracted oil process. Oil optimization process can use the designs such as central composite design (CCD) and Box-Behnken design with factorial design [15].

3. Materials and methods

3.1. Materials

Thevetia peruviana seeds were obtained in Abubakar Tafawa Balewa University (ATBU). It was packed in bags and stored in a cool place to avoid sample contamination and decay. The shell was cracked using a mortal and hammer to break into smaller sizes and later sieved it into smaller particles of 0.9 mm while the shell was disposed. Other materials include n-hexane (solvent), heating mantle, retort stand, conical flask, thimble, condenser, weighing balance and spatula.

3.2. Methods

Thirty grams (30g) of the *Thevetia peruviana* seeds sample was weighed using a weighing balance to obtain the mass and placed into the thimble for the Soxhlet extraction process. 150 mils of normal-hexane (solvent) was poured into the conical flask containing the n-hexane and heated using a heating mantle of specification (ZNCL-TS500ML). The Soxhlet extractor has three main components: A reflux which circulates the solvent, a thimble (filter paper) which retains the solids to be laved, and a siphon process, which periodically empties the thimble (filter paper). The solvent (n-hexane) was heated to reflux, while the condenser ensures that the solvent vapor cools, and drips back down into the chamber that housed the solid materials. In addition, when the Soxhlet chamber was full, the chamber was emptied by the siphon and the solvent returned to the distillation flask. The above procedures were repeated for several times for about 2-3 hours, in order to obtain a large quantity of oil. Rotary evaporator was used to recover the solvent after the oil from the seeds was recovered. The extracted solids remained in the thimble and were discarded. However, after extraction process, the mixture of n-hexane was distilled and the oil was recover from the solvent separately. The initial mass of the sample was weighed and denoted as (M_1) before extraction. The mass of the extracted oil was weighed and denoted as (M_2). The percentage oil yield was calculated using the formula;

$$\% \text{ oil yield} = \frac{\text{Weight of oil recvoverd } (M_2)}{\text{Weight of sample seed used } (M_1)} \times 100 \qquad (1)$$

3.3. Experimental design and oil optimization

The oil optimization process was conducted using Design Expert 13. Twenty (20) experimental design was obtained using the response surface methodology (RPM). The experimental design matrix has three (3) numeric factors such as solvent to biomass ratio (mL/g), time (h),

and temperature (℃), as the factors considered at two different levels (low and high values). The oil yield is the response in %. Table 1 is the factors considered in this study. More so, the Soxhlet extraction process was conducted based on the design matrix. To obtain the optimal oil yield, the resulted experimental runs results were inputted into the design matrix of the generated design matrix with the operating conditions of the runs and the response parameters were fitted to a quadratic model to improve its optimization performance. The oil optimization was investigated using the analysis of variance (ANOVA) results.

Table 1: Factors for the experimental design

Factors	Low value	High value	Symbol
Solvent to biomass ratio (mL/g)	20	40	A
Time (h)	1	3	B
Temperature (℃)	50	70	C

4. Characterization (physicochemical) of the oil and biosurfactant

4.1. Determination of the saponification value

One and half gram (1.5 g) of the extracted oil was poured into a conical flask of 150 mils and weighed (M). Ethanol of 10 mils and potassium hydroxide of 0.5 N was added into the mixture and stirred continuously. The mixture reaction was refluxed using condenser water on a water bath for one hour. The solution result was then cooled and titrated against a 0.5 N of hydrochloric acid (HCL) solution with addition of 1mil of phenolphthalein as an indicator. The process was repeated without the sample (blank experiment). The equation 2 was used for the calculation of the saponification value.

$$S_v = \frac{T_v \times N \times 56.1}{M} \quad (2)$$

where; S_v = saponification value; M = mass of sample; T_v = titrated value; N = equivalent factor

4.2.Determination of the acid value

One and half gram (1.5 g) of the extracted oil was weighed (M) and poured into a conical flask of 150 mils. 10 mils of ethanol and ether of equal volumes were mixed thoroughly. Potassium hydroxide which had been neutralized with 0.5 N was added into the mixture and stirred continuously. The resulted mixtures were heated for 15 minutes using heating mantle until there was complete dissolution of the samples and later allowed to cool. 1mil of phenolphthalein was added into the mixtures as an indicator. 0.5 N potassium hydroxide was titrated with the mixtures. The process was repeated without the sample (blank experiment). The equation 3 was used for the calculation of the acid value.

$$\text{Acid value} = \frac{T_v \times N \times 56.1}{M} \quad (3)$$

4.3. Determination of the iodine value

One and half gram (1.5 g) of the extracted oil was weighed (M) and poured into a conical flask of 150 mils. 10 mils of chloroform were added to the extracted oil (sample). Wijs reagent (20 mils) was also added into the sample and mixed thoroughly. 100 mils of distilled water and 20 % potassium iodide (10 mils) was added into the mixture and stirred vigorously. The resulted mixture was titrated with 0.1 N sodium thiosulphate and a yellow colour disappeared. The process was repeated without the sample (blank experiment). The equation 4 was used for the calculation of the iodine value.

$$\text{Iodine value} = \frac{T_v \times 1.269}{M} \quad (4)$$

4.4.Determination of the free fatty acid (FFA)

The free fatty acid was determined by dividing the average of the acid value of the extracted oil. The equation 5 was used for the calculation of the free fatty acid.

$$\text{FFA} = \frac{\text{AV}}{2} \quad (5)$$

4.5.Determination of the specific gravity

The specific gravity was calculated using the formula

$$\text{Specific gravity} = \frac{W_1 - W_2}{W_3 - W_2} \quad (6)$$

where; W_1 = specific gravity of the bottle + extracted oil; W_2 = specific gravity of the bottle when empty; W_3 = specific gravity of the bottle + water.

4.6. Determination of the density

The density was determined using the equation

$$\text{Density} = 1000\ \frac{kg}{m^3} \times \text{Specific gravity} \quad (7)$$

5. Formulation of new surfactant

300 mils *of Thevetia peruviana* oil was weighed and poured into a conical flask of 150 mils. Potassium hydroxide (KOH) of 3g was poured into a conical flask containing *Thevetia peruviana* oil and mixed continuously. Potassium hydroxide was used as the base for the formulation of the surfactant. The mixture was heated for 10 minutes using a heating mantle and stirred continuously until a creamy viscous liquid as soap was observed. the reaction for the process is shown below.

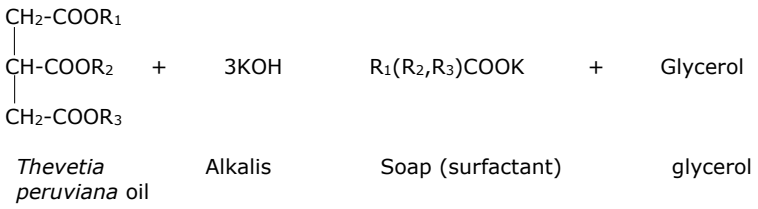

Figure 1. Saponification reaction (formulation of new surfactant).

6. Results and discussion

Table 2. Experimental design matrix.

Run	Factor 1 A: Solvent to biomass ratio (mL/g)	Factor 2 B: Time (hours)	Factor 3 C: Temperature (℃)	Response Oil yield (%)
1	40	3	70	60.0
2	20	1	70	52.0
3	30	2	60	61.3
4	20	3	70	57.0
5	30	2	50	61.0
6	40	3	60	58.2
7	20	1	50	52.0
8	30	2	60	61.2
9	40	1	70	53.0
10	30	2	50	58.0
11	30	1	60	57.2
12	30	3	60	60.0
13	30	2	60	61.1
14	20	2	60	56.0
15	40	3	60	60.8
16	20	1	50	57.0
17	30	1	60	55.0
18	30	2	70	60.0
19	30	2	60	58.0
20	40	1	50	42.0

The oil optimization matrix design consists of solvent to biomass ratio, time and temperature while the response is the oil yield which was obtained from the experimental runs (Table 2). The table showed the variations of the different factors based on the oil optimization using central composite design (CCD) method which aid to achieve the different amounts of oil yield. The minimum oil yield was obtained at solvent to biomass ratio of 40 (mL/g) having oil yield at 42% with temperature of 50°C and time of one hour. In addition, the maximum amount of oil yield was obtained at 61.3 % at solvent to biomass ratio of 30 (mL/g), and temperature of 60 °C and time of two hours. The increase in oil yield may be associated with the time, temperature, and the mass of the solvent. The contribution of the factors for increased oil yield could be as the result of the increased biomass oil due to the higher kinetic energy attained by the molecule solvent at higher temperature, which may also be responsible for breaking the bond in the biomass. Increasing the time of the oil extraction can also give room for higher oil yield as observed in the experimental runs.

Table 3. Analysis of variance for oil optimization.

Source	Sum of squares	Df	Mean Square	F Value	P value	
Model	363.69	9	40.41	8.76	0.0011	Significant
A: Solvent to biomass ratio (mL/g)	0.0000	1	0.0000	0.0000	1.000	
B: Time (hours)	137.75	1	137.75	29.86	0.0003	
C: Temperature, °C	21.90	1	21.90	4.75	0.0544	
AB	21.78	1	21.78	4.72	0.0549	
AC	20.48	1	20.48	4.44	0.0614	
BC	10.58	1	10.58	2.29	0.1609	
A^2	18.82	1	18.82	4.08	0.0710	
B^2	17.14	1	17.14	3.72	0.0828	
C^2	10.98	1	10.98	2.38	0.1540	
Residual	46.14	10	4.61			
Lack of Fit	35.73	5	7.15	3.43	0.1010	Not significant
Pure Error	10.41	5	2.08			
Cor Total	409.83	19				
Standard Deviation	2.15		R-Squared	0.8874		
Mean	57.04		Adj. R-squared	0.7861		
C.V %	3.77		Pred. R-squared	0.0664		
			Adeq precision	11.5427		

Table 3 is the analysis of variance (ANOVA). The results of the analysis showed that the p-value is significant which suggest that the p-values is less than 0.0500. The considered factors such as solvent to biomass ratio, time and temperature are significant for the oil yield. The significant model terms are A, B, AB, AC, AC, BC, A^2, B^2, C^2. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit (f-value) of 3.43 implies not significant. Non-significant lack of fit is good. More so, the Model F-value of 8.76 implies the model is significant which suggest that there is only a 0.11% chance that an F-value could occur due to noise. Similarly, the predicted R^2 of 0.0664 implies a better predictor of the response. Sometimes, a higher order model may also predict better. The coefficient of determination (R-Squared) was obtained to be 0.8874. In addition, Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 11.543 indicates an adequate prediction factor. Equation (5) is the empirical model result obtained for the oil yield in actual terms as follows;

$$Y\ (\text{Response}) = -32.23620 + 0.234298A + 14.60537B + 2.22660C + 0.165000AB - 0.115000BC - 0.025405A^2 - 2.26749B^2 - 0.019405C^2 \quad (8)$$

The analysis of variance (ANOVA), in table 3 were fitted to a quadratic model. The reason of choosing the quadratic model was because the quadratic model gave good results compared to cubic and other models used for statistical data analysis.

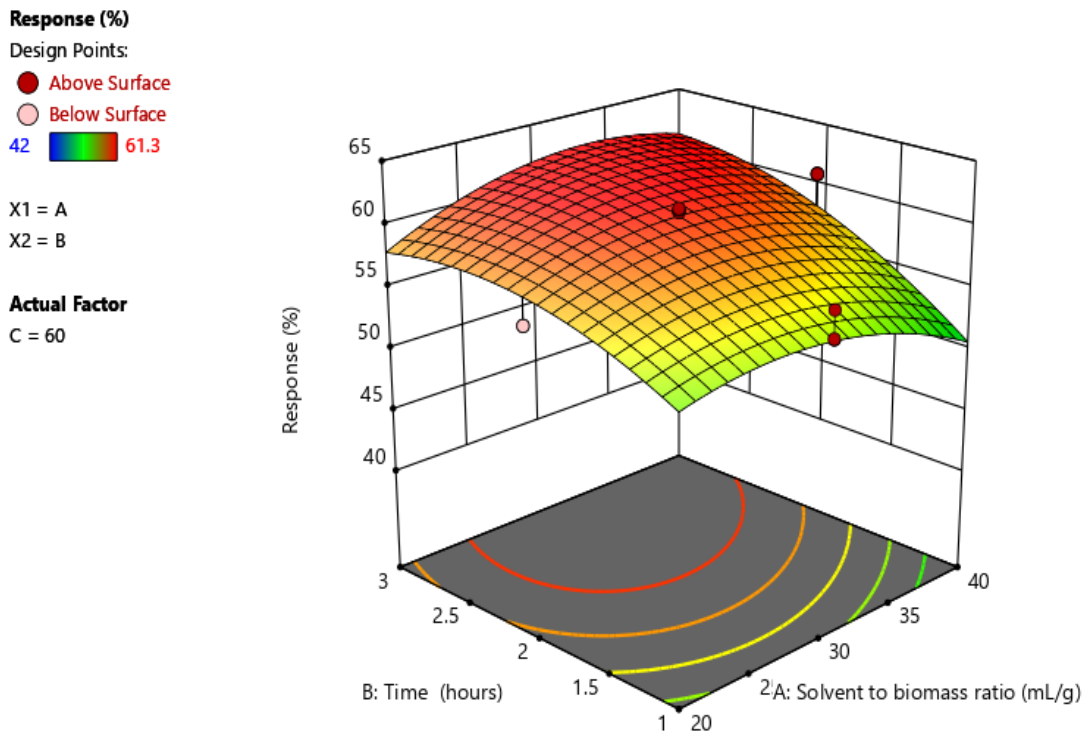

Figure 2. Effect of time and solvent to biomass ration on oil yield (response).

Figure 2 showed that increasing time have a corresponding effect on the oil yield and the solvent to biomass ratio. The optimal oil yield was obtained at 61.3% at time of 2 hours and solvent to biomass ratio of 30 (mL/g). In other hand, figure 3 showed the predicted against the actual for the oil yield. The graph suggested that the predicted value is relatively close to the actual value for the fitted data for the oil yield. This implied that the experimental data fitted the developed model very well.

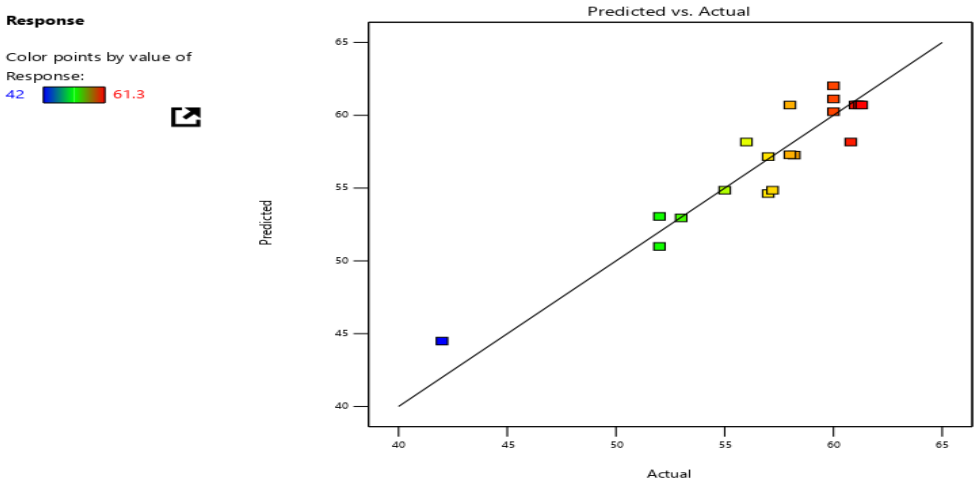

Figure 3. Predicted against actual for oil yield.

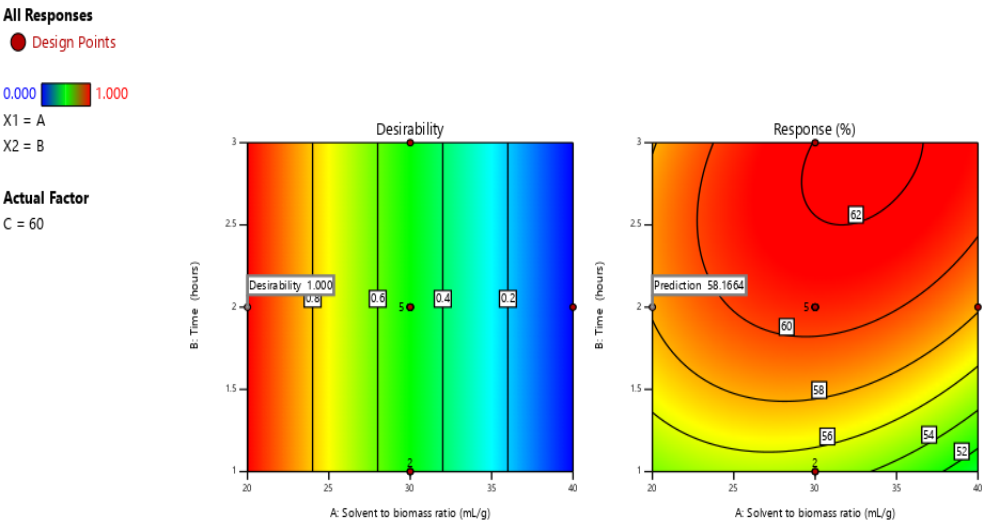

Figure 4: Effect of solvent to biomass ratio and time on the oil yield

Figure 4 showed the effect of solvent to biomass ratio for the different time intervals. The contours map within the region of (30, 2) and (25, 2.10) showed a significant response of (60, 58.16). It can also be observed that the maximum oil yield was obtained at the solvent to biomass ratio at 30 (mL/g). and time of 2 hours. Also, the contours are not very close due to the variations in the response values.

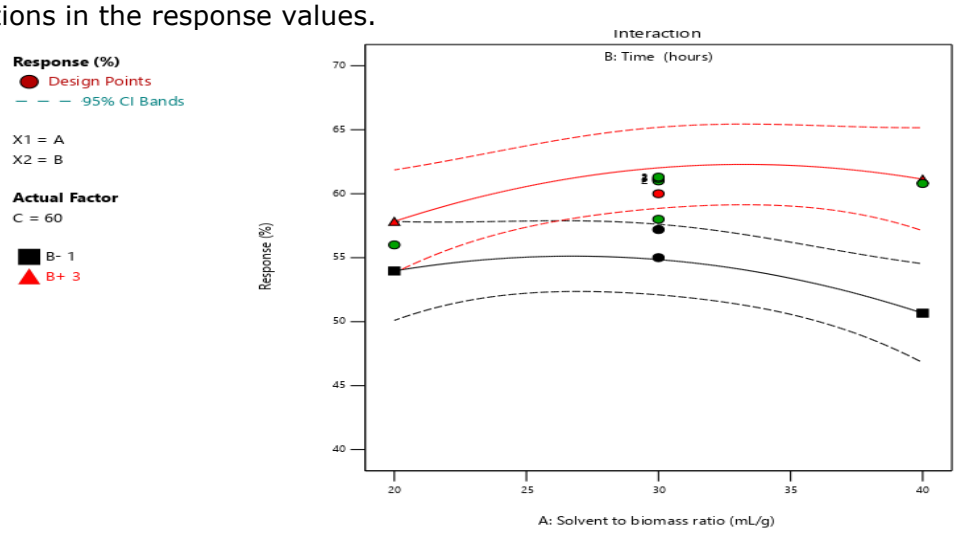

Figure 5. Interaction effect of solvent to biomass ratio.

The interaction effect of the solvent to biomass ratio and time in the response showed significant influence on the model. It can be observed from the graph the different responses in term of the solvent to biomass ration, time, and temperature on the oil yield interaction (Figure 5).

Table 4 present the physicochemical properties of the *Thevetia peruviana* seed oil. The saponification value of the oil was 218.79 (mg KOH/g). Saponification reaction is the main mechanism for soap production. However, high saponification value (218.79 mg KOH/g) indicates good soap production with high micelles formation (cloudy foam) [8]. Saponification values of 412.30 (mg KOH/g), 57.50 (mg KOH/g) and 227.21 (mg KOH/g) was reported in the work of [19,15,18]. The acid value of 28.05 mg KOH/g was obtained, the acid value was higher than 4.70 reported in the work of [19] for *Thevetia peruviana* seed oil. Also, the work [15] and [18] showed the values of 1.80 and 3.4 (mg KOH/g) lesser than the value obtained in this study. High acid values may not be good for production of soap. However, the acid value

is within the recommended range based on (ASTM). The free fatty acid was 14.03 higher than 2.40, 0.902 and 1.7 (mg/L) reported in the literature. The iodine value was obtained to be 9.39 lower than 12.60, 97.60 and 14.03 (mg/L).

Table 4. Physicochemical properties of the *Thevetia peruviana* oil.

Oil properties	This study	Ref. [21]	Ref. [17]	Ref. [20]	ASTM [16]
Saponification value (mg KOH/g)	218.79	412.30	57.5025	227.21	> 150
Acid value (mg/g)	28.05	4.70	1.80	3.4	2 -35
Iodine value (mg/L)	9.39	12.60	97.60	14.03	5-110
Free fatty acid (mg/L)	14.03	2.400	0.9024	1.7	>2.5
Specific gravity (g/cm^3)	0.90	0.91	0.89	0.86	> 0.5
Colour	Golden yellow	Golden yellow	Golden yellow	Golden yellow	-
pH	4.04	5.2	-	3.83	> 2
Viscosity at room temperature (°C) (cP)	31	30	-	28	> 20
Odour	Unpleasant	Unpleasant	Unpleasant	Unpleasant	-
Density (g/cm^3)	0.884	0.921	0.774		> 0.35
Oil yield (%)	61.3	62.7	44.04	56.50	> 30

The iodine value obtained in this work could indicate the presence of polyunsaturated fatty acid containing linoleic and oleic acids. These types of acids are used for the production of soap (surfactant). Relative gravity and density was obtained to be 0.90 and 0.884 (g/cm^3) respectively and fall within the range of the standard practices. The oil yield was high at 61.3%. High oil yield showed commercial availability of the prepared soap (surfactant). The pH of the oil was obtained as 4.04 (acidic in nature) which indicated that the seeds are really acidic.

Table 5. Physicochemical properties of the biosurfactant.

Property	This study	Ref. [20]
pH	3.85	9.75
Foam ability (cm^2)	83.5	80.0
Flash point (°C)	4.2	-
Solubility in water	Slightly soluble	Slightly soluble
Color	Creamy white	White

Table 5 showed that the pH of the prepared surfactant was 3.85 (slightly acidic). The reduction in the pH from the oil (4.04) could be the breakdown of molecules of *Thevetia peruviana* acid with the base (KOH) to obtain glycerol and soap (surfactant). The foam ability was 83.5 (cm^2) which indicate high forming capacity which could act as surface acting agent in altering the wettability of the rock properties from oil-wet to water-wet in the reservoir which will aid in the production of oil hydrocarbon via reduction of the interfacial tension of the oil**.** The flash point of the soap was 4.2 which the least temperature at which the liquid will ignite under reservoir conditions. The prepared biosurfactant was slightly soluble in water and creamy white.

7. Conclusions

The research aimed at extraction and optimization of oil from *Thevetia peruviana* seeds and its characterization. The extracted oil was used as a precursor for soap (surfactant) production for use in surfactant flooding. The physicochemical properties of the oil showed that the saponification value was 218.79 (mg KOH/g), acid value 28.05 (mg/g), free fatty acid 14.03 (mg/L), iodine value 9.39 (mg/L), specific gravity 0.9 (g/cm^3), pH 4.04, density 0.884 (g/cm^3) and viscosity at room temperature was 31°C. In addition, the foam ability was obtained to be 83.5 (cm^3) and flash point 4.2°C.The optimal oil yield was 61.3% using central composite design (CCD) of the design expert 13 tool. The oil optimization results showed that the p-values was 0.0011 which indicate the significant of the model while the f-value was 3.43 for the lack of fit and showed insignificant. This suggest that the experimental data fitted well with the actual data.

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