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Extraction and Characterization of *Cola Millenii K. Schum* (Monkey Cola) Seed Oil: Optimization Using Full Factorial Design of Experiment

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ABSTRACT

Plant seed oils are vital sources of oil of nutritional, pharmaceutical and industrial importance. Presently the quest for plant seed oils has increased greatly due to the diverse application of these oils. Hence the need for low-cost oil seed crop to produce inexpensive oils to meet these needs. This work focuses on the solvent extraction of oil from *Cola millenii* seeds. The extraction process was optimized using a 2-level full factorial design of experiment in terms of oil yield to determine the optimum extraction conditions. Based on the design, 8 experiments were carried out with three varying parameters: solvent volume, extraction time and sample weight. A first order regression equation best fits the experimental data. The predicted values calculated by the regression model were in good agreement with the experimental values. From our results, the condition for obtaining the optimum yield (2.71%) of the seed oil was attained when 50.00 g of the pulverized seed sample was extracted with 200.00 cm^3 n-hexane for 60 minutes. The oil has peroxide value 35.00meqKOH/g, acid value 32.00mgKOH/g, iodine value 96.57gI_2/100g and FFA 16.09%. These values obtained were in agreement with the values reported for oils which find use in the cosmetics and paint industries.

Keywords: Fatty acids, n-hexane, Oil, Monkey cola, Factorial design.

INTRODUCTION

Various plants have been exploited for oil production. Although many different parts of plants may yield oil in commercial practice, oil is extracted primarily from seeds (endosperm) of plants which grow in many different parts of the world. The characteristics of oils from different sources depend mainly on their composition; no oil from a single source can be suitable for all purposes (Muibat *et al*., 2011). The demand on seed oils is increasing due to their diverse applications and in order to meet the demand on vegetable oils due to the growing world population. These oils are being consumed directly or indirectly as ingredients in food, or serve as components of many manufactured products (e.g. soaps, skin products, candles, perfumes and other personal care and cosmetic products). Seed oils are also used in the production of biodiesel. Several oils such as *moringa* oil, sunflower oil, rape seed oil, palm oil, soya beans oil, corn oil, baobab oil and pumpkin oil (Dorado *et al*., 2004; Alcantara *et al*., 2009; Mitra *et al*., 2009; Peter *et al*., 2013) are expensive; hence, the demand for new low-cost oil seed crops for the production of inexpensive oils suitable for food, pharmaceutical and other industrial applications. One of the possible alternative crops is *Cola millenii K. Schum*, commonly known as monkey cola (English), "atewo-edun" (Yoruba) or "achiokokoro" (Igbo). *Cola millenii* belongs to the family Sterculiaceae (Ratsch, 2005). The tree grows up to 12 meters, and occasionally to 20 meters, in height with a low crown of arching branches. The bark has been reported to produce alkaloids (Adegoke *et al*., 1968). Leaves of *Cola millenii* are reported to be used in the treatment of ring worm, scabies, gonorrhea, dysentery and ophthalmic conditions (Odugbemi, 2006). The fruit is bright red in a stellate cluster. It is covered with a felted fibrous coal and has an edible kernel (Orsakeye and Ojo, 2013). The wood is white and very resilient. It is used in Nigeria and Liberia for rat traps and bows.

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The phytochemical, proximate and mineral element compositions and antimicrobial effects of different parts of the plant have been reported (Adeniyi *et al*., 2004; Ibironke *et al*., 2013; Orisakeye and Ojo, 2013). The antioxidant property of the plant has also been reported (Orisakeye and Ojo, 2013). This research work focused on optimizing the oil extraction process and physicochemical characterization of the seed oil of this plant in order to expand the scope of knowledge on the area of its applicability.

MATERIALS AND METHODS

Sample collection

Cola millenii fruit seeds were collected from farm land areas in Agbaha-Otupka, in Obadibo Local Government Area of Benue State, Nigeria. The hard epicarp seeds were sundried and cracked manually. The fleshy parts were removed and dried at room temperature. The dried sample was then pulverized and kept for further analysis.

Experimental design for oil extraction

In order to optimize oil extraction from the oil seeds, a 2-level full factorial experimental design was employed to generate the required number of experimental runs. Selected extraction variables were sample weight (SW, g), extraction time (ET, min) and solvent volume (SV, cm^3). Table 1 shows the various variable levels that were adopted. Eight (8) experimental runs (2^3) were generated by the design expert software as shown in Table 2.

Extraction of oil

The powdered seeds were extracted with n-hexane (b.p. 70^oC) according to the method described by Adepoju *et al*. (2013). The percentage oil yield was determined using equation one (1) below. The yield for each extraction is shown in Table 2.

Oil Yield (w/w %) = (Weight of seed oil produced)/ (Weight of oil seed powder used) x 100 …Eq. 1)

Physicochemical analysis of the seed oil

Color, refractive index, moisture content, and relative density of the crude seed oil were determined using A.O.A.C. (1990) methods, while acid value, saponification value, peroxide value, iodine value and % FFA (oleic) were determined according to the method by Kyari (2007).

Fatty acid analysis of the seed oil

Fatty acid composition of the crude oil was determined using gas chromatography. Oil sample (0.50g) was derivatized (esterified) for five minutes at 95^oC with $3.40cm^3$ of 0.5MKOH in dry methanol.

Table 1: Factors and their levels for full factorial design.

Factor	Symbol	Level	
		Low (-1)	High (+)
Extraction time (min)	ET	60.00	90.00
Sample weight (g)	SW	30.00	50.00
Solvent volume (cm^3)	SV	150.00	200.00

The mixture was neutralized using 0.7M HCl and 3.00 cm^3 of 14% boron triflouride in methanol was then added; the mixture was heated for 5 minutes at 90^oC to achieve complete methylation, before injecting it into the injection port of the GC.

RESULTS

Design expert software was used for the optimization processes. A total of eight (8) runs were conducted for the seed oil extraction using n-hexane as the extracting solvent. Table 2 below shows the actual factors considered in this study with observed experimental values. *Cola millenii* seeds gave a value of 2.70% high oil yield when 200.00$cm^3$of volume of n-hexane was used to extract 50.00g of the sample for 60 minutes. Results in Tables 3, gives the statistical analysis of the oil yield. The individual factors: sample weight (A), extraction time (B) and solvent volume (C) as well as two cross products (AC& BC), were found to be significant model terms. Figures 1, 2 and 3 show the interactive effect of these factors on the oil yield. Sample weight, extraction time and solvent volume were found to have varying effect on the oil yield. Table 4 shows the results of the physicochemical analysis of the seed oil. The saponification value, peroxide value, acid value and iodine value of the oil were high. Table 5 gives the result of the fatty acid analysis of the seed oil. From the result, the compositions of unsaturated fatty acids were higher than that of saturated fatty acids.

DISCUSSION

Optimization simply implies the use of specific methods to determine the most cost effective and efficient solution to a problem or design for a process (Okonkwo *et al.,* 2013). The need for optimum operating condition is to avoid wastages of raw materials, energy, time, e.t.c. Hence, optimization process is critical, as various parameters may potentially affect solvent extraction process. The optimum condition can be achieved by investigating multiple factors in all possible conditions.

Table 2: Experimental design matrix and response for *Cola millenii* seed oil extraction.

Std. Run	SW (g)	ET (min)	SV (cm^3)	EY (%)	PY (%)
1	30.00	60.00	150.00	1.92	1.93
2	50.00	60.00	150.00	2.60	2.60
3	30.00	90.00	150.00	1.42	1.42
4	50.00	90.00	150.00	1.90	1.90
5	30.00	60.00	200.00	2.39	2.38
6	**50.00**	**60.00**	**200.00**	**2.71**	**2.72**
7	30.00	90.00	200.00	2.40	2.41
8	50.00	90.00	200.00	2.56	2.56

SW- Sample weight, ET- Extraction time, SV- Solvent volume, EY- Experimental yield and PY-Predicted yield

Table 3: ANOVA for Factorial Model of *Cola millenii* oil extraction

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	1.39	6	0.23	1518.04	0.0196
A	0.34	1	0.34	2203.63	0.0136
B	0.23	1	0.23	1476.76	0.0166
C	0.62	1	0.62	4019.56	0.0100
AB	0.017	1	0.017	113.57	0.0596
AC	0.056	1	0.056	365.36	0.0333
BC	0.14	1	0.14	929.38	0.0209
Residual	1.531E-004	1	1.531E-004	-	-

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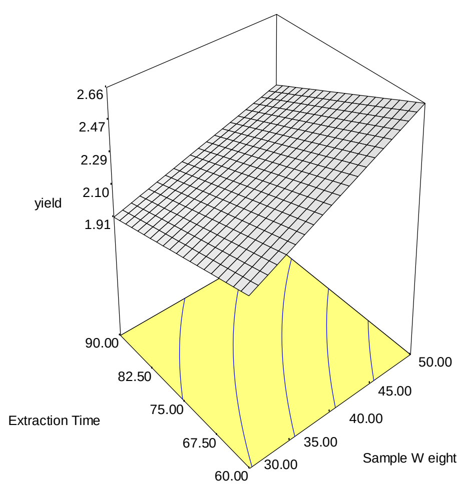

Figure 1: Response surface for two dependent factors of sample weight and extraction time

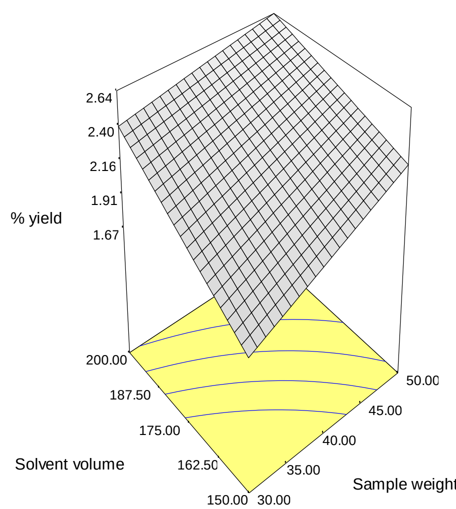

Figure 2: Response surface for two dependent factors of sample weight and solvent volume

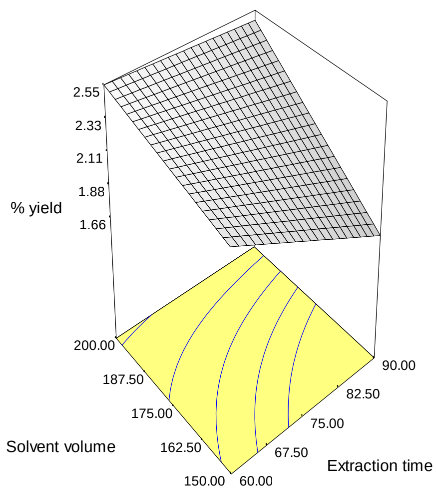

Figure 3: Response surface for two dependent factors of extraction time and solvent volume

Table 4: Physicochemical properties of the seed oil

Physicochemical properties	***Cola millenii* seed oil**
Colour	Light brown
Refractive index	1.467
Relative density	0.938
Peroxide value	35.00meqKOH/g
Acid value	32.00mgKOH/g
Saponification value	238.43mgKOH/g
%FFA (oleic acid)	16.09
Iodine value	96.57gI_2/100g

Table 5: Fatty acid composition (FAC) of the seed oil

Composition	**Form**	**Percentage**
Saturated Fatty Acids		**31.50**
Caprylic	C8:0	0.56
Myristic	C14:0	1.89
Palmitic	C16:0	19.39
Stearic	C18:0	9.66
Unsaturated Fatty Acids		**68.50**
Cis-oleic	C18:1	5.55
11-Octadecenoic	C18:1	11.80
Linolelaidic	C18:2	29.52
11-14-Eicosadienoic	C20:2	19.67
Brassidic	C22:1	1.96

Design expert 6.0.6 software was employed to analyze the result from Table 2. The analysis of variance (ANOVA) of the regression equation for oil extracted (Table 3) was done using F values at confidence level of 95% (α=0.05) to determine the effects of the main and interactive factors. From Table 3, the three linear terms (A, B & C), and two cross products (AC& BC) were significant (i.e. Prob<0.05) model terms for the oil extraction process. There is only a 1.96% chance that a "Model F-Value" this large could occur due to noise. The Model high F-value (1518.04) with low P- value (0.0196) implied a high significance for the regression model of the oil extraction (Yuan *et al.*, 2008). The predicted determination coefficient value of 0.9930 is in reasonable agreement with the adjusted determination coefficient value which was 0.9992 for the analyses. "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable; in this case the ratio of 112.461 indicated an adequate signal. Therefore, the model can be used to navigate the design space. Diagnostics case statistics study, carried out for the oil shows a close relationship between the actual value and the predicted value (Table 3) for all model terms. The model was validated; values obtained had no outlier, an indication that the precision and accuracy of the analyses were good. The Normal probability of Studentized residual plots, studentized residuals versus predicted values, and Box-Cox plot for power transforms was also good.

Eq. 2 defines the relationship between the oil yield (Y) and the coded values of sample weight (A), extraction time (B) and solvent volume (C) for *Cola millenii* oil extraction (Adepoju *et al.*, 2013). It shows that sample weight and solvent volume positively contributed to the quantity of oil extracted from the seeds; the interaction between extraction time and solvent volume also improved the oil yield.

$Y=2.24 + 0.21A - 0.17 B + 0.28C - 0.047A*B - 0.084A*C + 0.13B*C$………………………..(Eq. 2)

The 3-D dimensional surface plot representing the effect of extraction time and sample weight and their reciprocal interaction on *Cola millenii* oil yield while keeping solvent volume constant at zero level (Figure 1) shows that *Cola millenii* oil yield is favoured at higher sample weight with lower extraction time. The interaction between solvent volume and sample weight (Figure 2) shows that *Cola millenii* oil yield is favored when solvent volume increases with increased sample weight and *vice-versa*. Figure 3 shows the interaction between solvent volume and extraction time. *Cola millenii* oil yield is enhanced at high solvent volume and low extraction time. Although the 2.71% oil yield from *Cola millenii* was relatively low, it was still higher than what was reported for ginger (Shah and Garg, 2014).

Cola millenii seeds were found to contain oil, which was brown in colour. The refractive index of oils is a measure of how much a light ray is bent when it passes from air into the oil and it usually depends on the density of the oil. The refractive index and relative density of *Cola millenii* oil were found to be 1.467 and 0.938 respectively. The refractive index and relative density values of oils are, generally, physical measures for checking the adulteration of oils since different oils have characteristic density and refractive index.

The peroxide value is the predominant test for oxidative rancidity in oils. It is used to measure the extent to which rancidity has occurred during storage of oils; thus high peroxide value of oil indicates a poor resistance of the oil to peroxidation during storage (Mohammed and Hamza, 2008). The seed oil gave a peroxide value of 35.00meqKOH/g, which is comparable with the range of 0.93-56.00meqKOH/g for some for seed oils e.g. (Aremu *et al.*, 2015). The value obtained for our oil indicates that the oil is stable and may not be susceptible to oxidative rancidity if produced from fresh seeds and stored properly. The iodine value measures the degree of unsaturation in oil. Studies have shown that as the degree of unsaturation increases, the iodine value increases and the liability of the oil to become rancid by oxidation also increases. The iodine value of the seed oil is 96.57. Oils with iodine values less than 130 are regarded as non – drying oils and are suitable for paint making (Aremu *et al.*, 2015).

The saponification value of the oil was found to be 238.43. A saponification value this large indicates a high proportion of fatty acids of low molecular weight. This shows that the oil may have potentials for use in soap making and cosmetics industries and for the thermal stabilization of polyvinyl chloride (PVC) and could also be a source of essential fatty acids required in the body (Akanni *et al.*, 2005). In addition, this saponification value is within the range for edible oils as reported by Eromosele *et al.* (1994).

The acid value of oil corresponds to the amount of carboxylic acid groups in its constituent fatty acids and is given in mgKOH/g sample. The older an oil is, the higher the acid value as triglycerides are converted into fatty acids and glycerol upon aging. The acid value of *Cola millenii* oil was found to be 32.00mgKOH/g. This value is far above the range (0.00-3.00mgKOH/g) recommended for cooking oils(Oderinde *et al.*, 2009) and also higher than the values of 0.6-4.0mgKOH/g established by the Ministry of Public Health (Notification No. 205/B.E, 2543) for various types of edible fats and oils, and their mixtures. Hence this oil may not be suitable as edible oil even though the iodine and saponification values were within the limits for edible oils. Also, the %FFA value (16.09%) of the oil sample was found to be higher than the maximum limit of 5% for high grade palm oil in Nigeria (NIFOR, 1989).

The fatty acid composition of the oil is shown in Table 6. Caprylic acid is used commercially in the production of esters that are important components of perfumes and dyes. The presence of caprylic acids in the oil is another indication that the oil may find use in the cosmetic industry.

CONCLUSION

In the present research, oil was successfully extracted from *Cola millenii* seeds. The process of extraction was optimized to improve the oil yield. The various factors investigated had various degrees of effect on this yield. The sample weight and solvent volume positively contributed to the quantity of oil extracted from the seeds. The interaction of time and sample weight also improved the oil yield. Data reported here were discussed without comparing them to any standard value since no previous study had been reported on the extraction of oil from *Cola millenii* seeds. Hence, this work will serve as a reference for future studies on *Cola millenii* seed oil. Physicochemical properties of the oil show that it can find use in soap making, paint and cosmetic industries.

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