ALTERNATIVE SOLVENT COMBINATION RATIOS FOR MORINGA OLEIFERA SEED OIL EXTRACTION

Modupe Elizabeth Ojewumi, Fidelis Olikeze, Damilola Elizabeth Babatunde

Chemical Engineering Department, Covenant University
P.M.B 1023, Ota, Ogun State, Nigeria

Moses Eterigho Emetere

Physics Department, Covenant University,
P.M.B 1023, Ota, Ogun State, Nigeria

ABSTRACT

As of recent, solvent extraction using different solvents has been found to be the most efficient method of extraction. Previously, in solvent (Soxhlet) extraction, a narrow spectrum of solvents had been put to use in extraction, with hexane being the most popular and widely used choice solvent. Hence, the use of various extraction solvents in this research work is to show that hexane could be replaced with other solvents. Additionally, Solvents were combined in different combination ratios to show that oil yield could be improved by solvent combinations as opposed to using only single solvents. From the results obtained, it was ascertained that solvent combinations actually improved oil yields since the highest oil yields were obtained from the combination of Petroleum ether (100ml), Hexane (50ml) and methanol (50ml), having 50.667% and 51.33% with the combination of Hexane (100ml) and Methanol (100ml) as opposed to when only petroleum ether was used yielding 40.667%. Therefore, the results obtained from the investigation suggests that solvent combination could prove as an adequate replacement and alternative to the use of single solvents in solvent extraction.

Keywords: Moringa Oleifera, oil yield, Solvent, Soxhlet extraction.

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1. INTRODUCTION

The *Moringaceae* is a family of oilseed trees categorised as single genus trees with about 14 reputable species. These species are not limited to *M. Oleifera*, but also *Moringa Pterygosperma, Moringa drouhardii, Moringa Stenopetala, Moringa Peregina, Moringa Cocanensis* [1]. Of the fourteen known species, *M. Oleifera* stands out as the most widely known and utilized [2]. Almost all the parts of this plant have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders [3, 4].

M. oleifera is grown world-wide in the tropics and sub-tropics of Asia and Africa [5]. Moringa is a native to India, Africa, Arabia, Southeast Asia, the Pacific and Caribbean islands, and South America [6]. Like African locust bean tree, *M. oleifera* tree is also drought tolerant plant that thrives best under the tropical climate and tolerates different soil types [7, 8]. In some parts of the world, *M. oleifera* is referred to as the 'drumstick tree' or the 'horse radish tree' [9]. In the Nile valley, the tree is known as 'Shagara al Rauwaq', which means 'tree for purifying' [10]. In Pakistan, *M. oleifera* is locally known as Sohanjna and is grown and cultivated all over the country [11]. The Hausa tribe resident in the northern part of Nigeria have been known to call it Zogali, Bagaruwa or Barambo. In the same region, Fulani inhabitants named it Garawa, or Rimimaka, while the Yoruba of the south western region call the moringa tree, Igi igbale or Igi Iyanu (Miracle Tree) and its leaves 'Ewe Igbale'. The Ibo tribe residing in the eastern coasts of Nigeria name it okwe oyibo, or odudu oyibo [1]. *Moringa* and other plants extract have been reported to possess antibacterial and antifungal properties [12 – 17]. These may account for its use in the treatment of most human ailments as the plant have been said to possess the cure to about three hundred diseases[11]. *M. Oleifera* possesses antispasmodic properties [18], antidiabetic and hepato-protective dispositions [19] and antipyretic tendencies, which make it a remedy to epileptic conditions. The leaves, roots, seeds, barks, fruits, flowers and even the immature pods operate as cardiac and circulatory stimulants, confer anti-inflammatory advantages, serve as an antiulcer [20], effective diuretic [2], effective cholesterol lowering agent [21], combatant against hypertension and various diseases of the circulatory system [22], and as a veritable antioxidant. Epidemiological studies have shown that foods rich in antioxidants provide protection against degenerative diseases including cancer, coronary heart diseases, and Alzheimer's disease [23-24]. Therefore, it is considered important to increase intake of antioxidants from dietary sources [24]. The focus of this research is to ascertain the effect of various combinations of solvents, extraction temperature and extraction time on the extractable oil yield from Moringa seeds. Solvents ranging from Hexane, methanol, petroleum ether to isopropyl alcohol were tested in different combination ratios to determine the solvent with greatest yield.
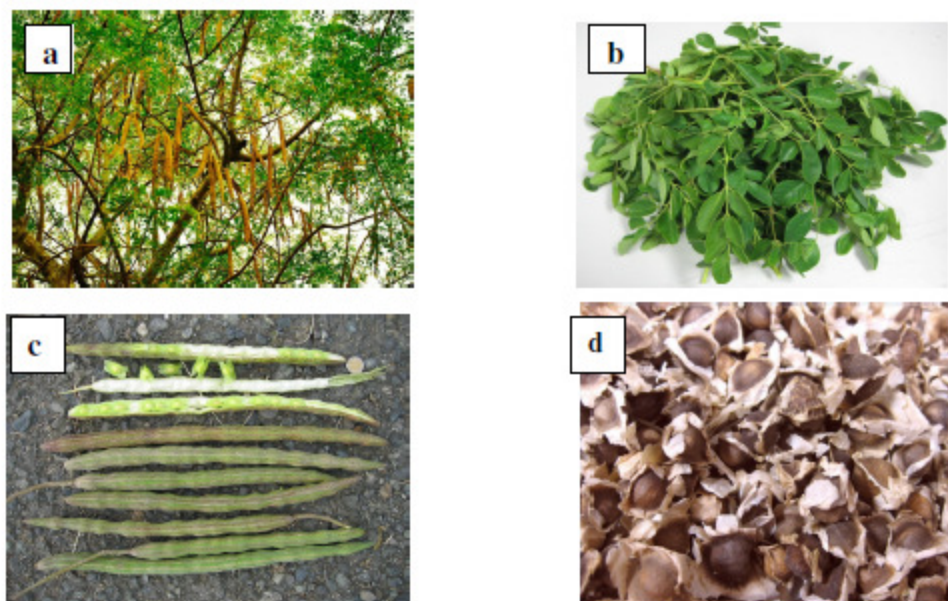
Figure 1. a, b, c, d, *Moringa oleifera* tree, Leaf, Pod and seed respectively

2. MATERIALS AND METHODS

2.1. Plant material

M. Oleifera seed used were purchased from a village in Kaduna State, Nigeria.

2.2. Seed preparation

De-hulling was done by pounding with a mortar and pestle. This was carried out to ensure higher extraction efficiency since the seed coats which are removed contain no oil. The chaff and foreign materials such as sand, stems, seed coats, bad seeds and dirt from the sample were handpicked to prevent the contamination of the oil. The excess moisture from the seeds was also removed by drying in order to increase extraction efficiency. The seeds were weighed before and after processing in order to ascertain the moisture expelled from the seeds. The dried seeds were ground to increase surface area and thereby facilitate higher extraction efficiency.

2.3. Essential oil extraction

Oil was extracted from 30g of dried powdered seed using Soxhlet apparatus and a heating mantle with different solvent combination ratios at different proportions with 22 runs [16]. Each run was done for four (4) hours. The Soxhlet apparatus is made up of a condenser, an extractor and a round-bottomed flask. The Samples were encapsulated in filter paper and placed in the extractor. The solvents used for extraction were placed in the flask. The solvent vaporized and rose to the condenser where it condensed. The condensed organic solvent then dripped on the sample. The sample upon contact with hot organic solvent began to secret oil which is soluble in the organic solvent. When the oil and solvent mixture had risen high enough (higher than the siphon tube), the mixture flowed through the siphon tube back into the flask. The oil was then recovered from the organic solvent by heating the mixture with a condenser coupled to the flask. The organic solvent vaporized and was then condensed back into another container.

Table 1 shows the summary of the extraction process, the solvent used, quantity, temperature of extraction and time used for the extraction process.

Table 1 Solvent, solvent quantity, time taken and extraction temperatures

Experiments	solvents (quantity)					
Runs	Pet ether	Hexane	Methanol	Isopropyl	Temperature[^{0}c]	Time [hrs]
1	200ML	0	0	0	80	4
2	200ML	0	0	0	60	4
3	100ML	100ML	0	0	80	4
4	100ML	100ML	0	0	70	4
5	150ML	50ML	0	0	70	4
6	150ML	50ML	0	0	60	4
7	50ML	150ML	0	0	80	4
8	50ML	150ML	0	0	70	4
9	0	100ML	100ML	0	80	4
10	0	100ML	100ML	0	100	4
11	0	150ML	50ML	0	90	4
12	0	150ML	50ML	0	100	4
13	0	50ML	150ML	0	80	4
14	0	50ML	150ML	0	100	4
15	100ML	50ML	50ML	0	70	4
16	100ML	50ML	50ML	0	60	4
17	50ML	100ML	50ML	0	70	4
18	50ML	100ML	50ML	0	80	4
19	50ML	50ML	100ML	0	80	4
20	50ML	50ML	100ML	0	100	4
21	100ML	0	0	100ML	70	4
22	100ML	0	0	100ML	80	4

3. PHYSICO-CHEMICAL ANALYSIS

3.1. Acid Value

1.0g of each oil sample was weighed into a 250ml conical flask. 95% alcohol (neutral alcohol) was prepared by diluting methanol with sodium hydroxide (5ml NaOH + 95ml ethanol = 100ml neutral alcohol). 50ml of neutral alcohol and 50ml benzene were added to the oil in the flask. The contents of the flask were shaken well to dissolve. The contents were then titrated against 0.1N potassium hydroxide solution using phenolphthalein as an indicator. The end point was marked by the appearance of a pale permanent pink colour and the titre value was recorded.

The acid value is calculated mathematically as:

$$\text{Acid Value} = \frac{X \times M_1 \times 56.1}{W_1} \tag{1}$$

Where,

X = volume of KOH required to neutralize the solution (ml)

M_1 = strength of KOH

W_1 = weight of oil used (g)

The number 56.1 is the atomic weight of potassium hydroxide (KOH)

3.2. Saponification Value

 1 gram of oil was weighed into 250ml dry round-bottomed flask. 50ml of 0.5ml alcoholic potassium hydroxide was added to the oil. The reflux condenser was set up and the contents of the round-bottomed flask was refluxed for about 1 hour after refluxing the mixture was allowed to cool and was then titrated against standard hydrochloric acid and the titre values were recorded. Similarly, 50ml of the same alcoholic KOH, blank (no oil added) was refluxed in a round bottom flask for 1hour, cooled and titrated against standard 0.5N HCl. The titre value were recorded and the titre value obtained were then used to determine the saponification value.

The saponification values were calculated mathematically as:

$$\text{Saponification Value} = \frac{Z \times M_1 \times 56.1}{W_1} \tag{2}$$

Where,

Z = volume of Hcl required to neutralize excess alkali (ml)

$Z = (X - Y)$ ml

X = titer value of HCl against oil and KOH after reflux (ml)

Y = titer value of HCl against KOH alone after reflux (ml)

M_1 = strength of HCl

W_1 = weight of oil used (g)

The number 56.1 is the atomic weight of potassium hydroxide (KOH)

3.3. Specific Gravity Test

This was done by measuring the density of Moringa seed oil in reference to the density of distilled water at 20°C.

$$Specific\ gravity = \frac{Weight\ of\ given\ volume\ of\ extracted\ oil}{Weight\ of\ equal\ volume\ of\ water} \tag{3}$$

4. RESULTS AND DISCUSSION

4.1. Determination of oil yield

22 experimental runs were conducted at varying temperatures to determine optimum yield with various solvent combination ratios. As shown in Figure 2, the highest % oil yields were runs 15, 16, 9, 10 and 2 with 50.667%, 51.33%, 45%, 46% and 40.667% respectively with run 16 being the highest.

$$Yield(\%) = \frac{Weight\ of\ Extracted\ oil\ (grams)}{Weight\ of\ Powdered\ moringa\ seeds\ used(grams)} * 100\% \tag{4}$$

The response surface method (RSM) was conducted to examine the optimization process. The experiment was conducted using twenty-two runs [25]. The RSM method was used to theoretically extend the runs from 22 to 39. The sixth order Gaussian equation was used to obtain the oil yield and percentage oil yield as shown in equation (5):

 f(x) = a1*exp(-((x-b1)/c1)^2) + a2*exp(-((x-b2)/c2)^2) + a3*exp(-((x-b3)/c3)^2) + a4*exp(-((x-b4)/c4)^2) + a5*exp(-((x-b5)/c5)^2) + a6*exp(-((x-b6)/c6)^2   (5)

Where x is the number of runs, a1, a2, a3, a4, a5, a6, b1, b2, b3, b4, b5, b6, c1, c2, c3, c4, c5 and c6 are the magnitude of coefficients for the oil yield and percentage oil yield.

Also, the sixth order of sum of sine function was used to obtain the temperature as shown in equation (6) below:

g(x) = a1*sin(b1*x+c1) + a2*sin (b2*x+c2) + a3*sin(b3*x+c3) +

a4*sin(b4*x+c4) + a5*sin(b5*x+c5) + a6*sin(b6*x+c6)   (6)

Where x is the number of runs, a1, a2, a3, a4, a5, a6, b1, b2, b3, b4, b5, b6, c1, c2, c3, c4, c5 and c6 are the magnitude of coefficients for the temperature. Above 39 runs, the oil yield and percentage yield is zero. The viability of extending the runs and its outcome were considered in Figures (3-9). Figure 3 show that if the number of runs increases to 39, the temperature of extraction of oil linearly increases. The sensitivity of the optimization process (which range from 0-2.5) was shown. At maximum sensitivity, the optimal conditions are at 29 runs at a temperature 100 ^{o}C. Figure 4 further affirm that the optimal yield is at the 29th runs. However, the maximum results are obtained at lower sensitivity i.e. 1.5. When the number of runs, temperature and oil yield are considered simultaneously, the oil yields decreases after sensitivity shifts above 2.5 (figure 5). The contour plot between sensitivity 1.5 and 2.5 show that the temperature affects the oil yield as confirmed in earlier discussion.

The optimal condition for the percentage oil yield was considered in Figure 6. It was observed that the percentage oil had significant effect on the temperature and oil yield. The possibility of an increased percentage yield after sensitivity of 2.5 was established.

Further examination on the influence of increasing on both the oil yield and yield percentages affirm that the system would have a maximum sensitivity of 4 (Figure 7). Two maxima at sensitivity 2.5 and 4 show that lower and higher temperature profiling may yield almost same results at certain condition that depends on the chemical properties of the oil. The oil yield at stable temperatures was considered in Figure 8. At lower sensitivity (1.05), the number of runs is directly proportional to the oil yield. At higher sensitivity (2.5), the maximum oil yield was at 16 runs.

The percentage oil yield at stable temperatures was considered (Figure 9). Like the oil yield, the maximum percentage oil yield also occurred at the 16 runs. However, at the 29th runs an heterogeneous reaction is expected.

4.2. Effect of extraction time on oil yield

Although a constant extraction time of four (4) hours was maintained throughout the experimental runs, it was observed that the oil extracted from the Moringa samples generally increased with increase in extraction time. Oil yield obtained (expressed in percentage) were relatively dependent on extraction time.

The increasing oil trend indicates that the oil yield has direct linear relationship with the extraction time. This is to say that an increase in the extraction time will bring about a corresponding increase in oil yield up to the optimum point. This was expected as prolonged intimate contact between seed samples and the respective solvent will result in more oil extraction.

4.3. Effect of temperature on oil yield

Results obtained from the various experimental runs showed that an increase in temperature would generally favour a corresponding increase in oil yield when single solvents were used, provided the boiling points of the solvents were not exceeded. This phenomenon is due to the fact that oils are generally more soluble at elevated temperatures. If the boiling points are exceeded, this will constitute a condensation problem which will lead to loss of extraction solvent by evaporation. When solvent combinations are in equal amounts, the extraction temperature is normally fixed at a temperature near the boiling points of the respective solvents but not very far from them. Exceeding the boiling point will lead to loss of solvent which in turn affects extraction efficiency. When solvent combinations were unequal, the

boiling point of the more abundant solvent was used as the extraction temperature and this gave higher yields than when lesser temperatures were used.

4.4. Effect of different solvent and solvent combinations on oil yield

In this experiment, oil was extracted from Moringa seeds using four main solvents: Petroleum ether, hexane, methanol and isopropyl alcohol albeit in different combination ratios, using constant extraction times and varied extraction temperature. From the results obtained, the highest oil yields were obtained from runs 15, 16, 9, 10, 2 and 6 with yields 50.67%, 51.33%, 45%, 46%, 40.667% and 34.33% respectively. The lowest yields were from runs 21, 22, 13 and 14 with yields 20.667%, 21%, 24.667% and 24.667% respectively. Separation of the extracted oil from isopropyl alcohol resulted in the formation of a light orange jelly-like product on the surface of the oil which is believed to be responsible for the low oil yields using isopropyl alcohol. Slight charring of the oil was also noticed during solvent (isopropyl alcohol and methanol) recovery process. This may be due to the fact that isopropyl alcohol and methanol being alcohol are not inert to vegetable oils compared to the other two relatively inert solvents. At higher temperatures, by-products resulting from possible hydrolysis or trans-esterification of the oil in the presence of isopropyl alcohol or methanol may have been formed. From the physicochemical analyses carried out for the extracted oils using the four solvents in their various combination ratios, it was observed that the quality of the oils obtained was generally the same. The only obvious difference was in the colour of the oil extracted with isopropyl alcohol and methanol in the solvent mixtures which was darker than the oils extracted using just mixtures of hexane and petroleum ether. From the results of this experiment, it can be observed that combining petroleum ether, hexane and methanol in the right proportion will give higher yields than when used as a stand - alone solvents. The oil extract obtained from this experiments could be directly used for biodiesel production or for some other industrial applications but never as a food grade solvent because of its hazardous and carcinogenic potentials.
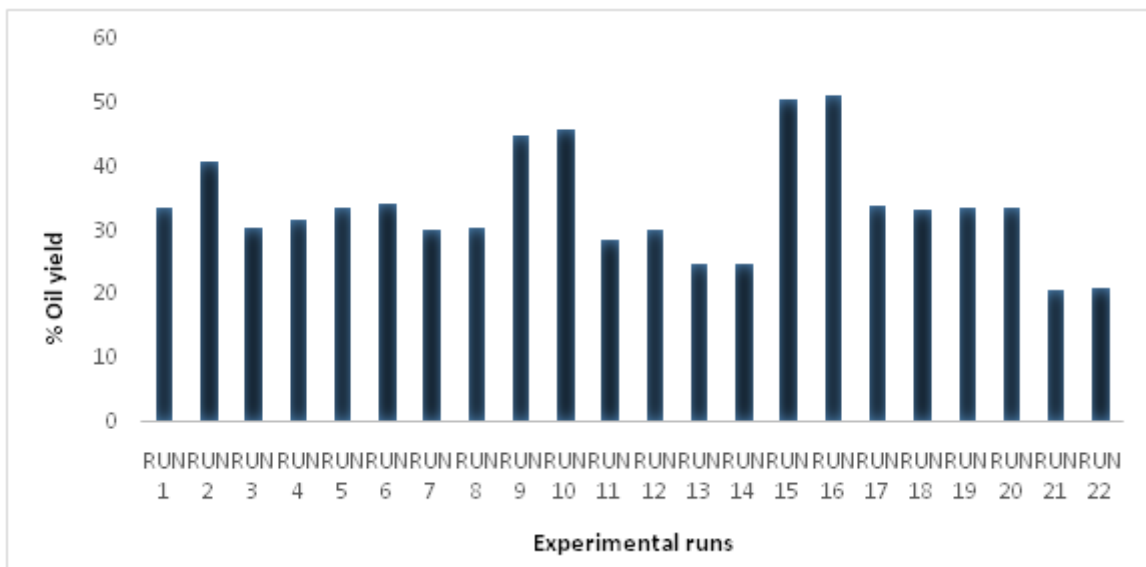

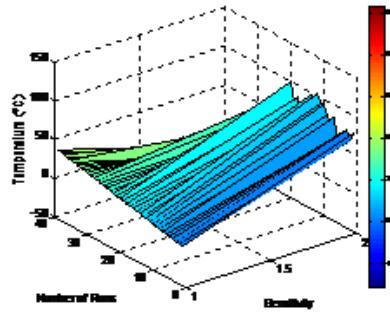**Figure 2.** Experimental runs against percentage oil yield

Figure 3. Optimal conditions as the number of runs influences the temperature.
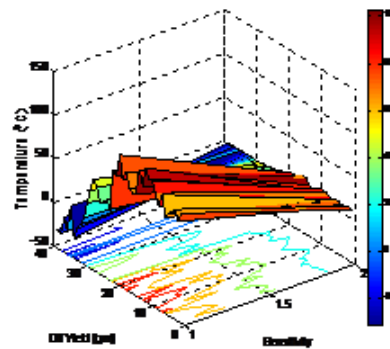

Figure 4. Optimal conditions as temperature influences the oil yield.
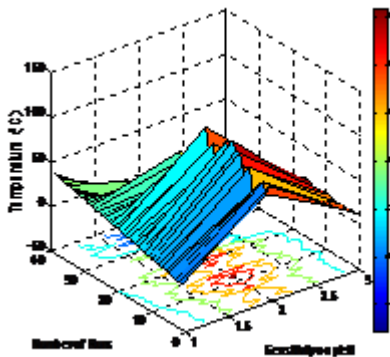

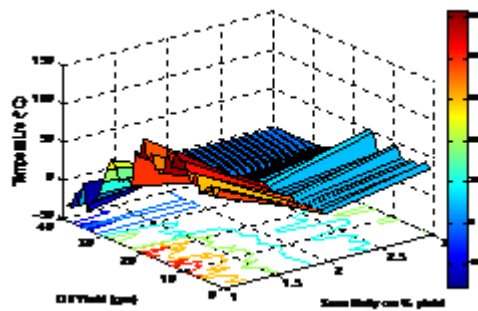**Figure 5.** Monitoring active zones against number of runs, temperature and oil yield.

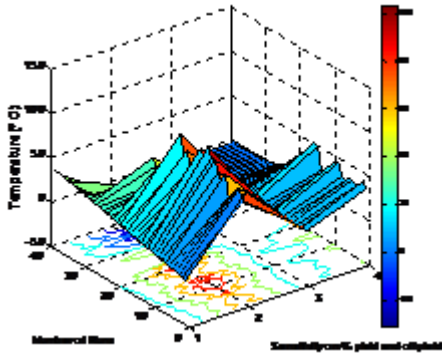**Figure 6.** Optimal % yield against temperature and oil yield

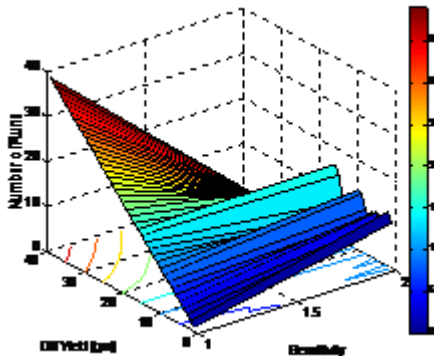**Figure 7.** Chemical properties dependence on oil yield

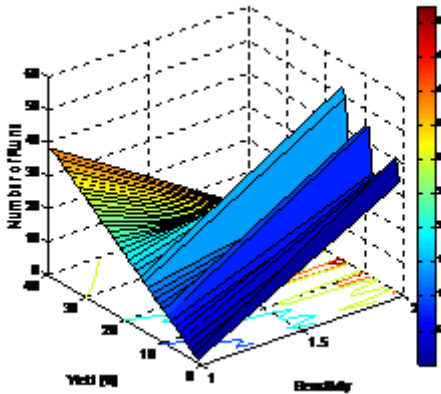**Figure 8.** Oil yield at stable temperatures.

Figure 9. Percentage oil yield at stable temperature

4.5. Physico-chemical Properties

Table 2 shows the summary of the result obtained from the analysis of the physico-chemical properties of the extracted oil.

Table 2 Physico-chemical properties of extracted oil

Experiments	Solvents used	Solvent quantity (ml)	Acid value (mg KOH g^{-1})	Saponification value (KOHg^{-1})	Specific gravity
Run 1	Pet Ether	200	8.75	230.01	0.9
Run 2	Hexane	200	8.53	228.05	0.9
Run 3	Pet Ether +Hexane	100 ;100	8.69	221.06	0.9
Run 4	Pet Ether +Hexane	100; 100	8.81	221.03	0.9
Run 5	Pet Ether +Hexane	150; 50	8.58	227.21	0.91
Run 6	Pet Ether +Hexane	150;50	8.53	224.40	0.91
Run 7	Pet Ether +Hexane	50;150	8.86	218.79	0.9
Run 8	Pet Ether +Hexane	50;150	8.96	217.67	0.9
Run 9	Methanol +Hexane	100; 100	8.92	221.59	0.9
Run 10	Methanol +Hexane	100;100	8.75	221.03	0.9
Run 11	Methanol +Hexane	150;50	8.13	219.35	0.9
Run 12	Methanol +Hexane	150;50	7.97	219.91	0.9
Run 13	Methanol +Hexane	50;150	8.86	218.51	0.91
Run 14	Methanol +Hexane	50;150	8.86	218.79	0.91
Run 15	Pet Ether+Hex+Methanol	100;50;50	8.47	223.84	0.91
Run 16	Pet Ether+Hex+Methanol	100;50;50	8.53	223.84	0.91
Run 17	Pet Ether+Hex+Methanol	50;100;50	8.81	219.07	0.91
Run 18	Pet Ether+Hex+Methanol	50;100;50	8.75	218.99	0.91
Run 19	Pet Ether+Hex+Methanol	50;50;100	8.19	219.07	0.91
Run 20	Pet Ether+Hex+Methanol	50;50;100	8.25	218.79	0.91
Run 21	Pet Ether +Isopropyl	100;100	7.52	255.82	0.92
Run 22	Pet Ether +Isopropyl	100;100	7.41	256.38	0.92

Note: Pet Ether – Petroleum Ether

Hex - Hexane

4.6. Acid Value

The acid values obtained from the extracted oils were relatively higher than those quoted in literature, for example, 3.8-5.0 mg KOH g^{-1} [26, 27], but closer to 5.78-7.28 [28]. The acid value for the different runs had a range from 7.4052 (Run 22) to 8.976 (Run 8). Higher acid values generally indicate that the seed sample used for the various experimental runs were old to some extent and the conditions of the storage of the extracted oil may have initiated the breakdown of the unsaturated fatty acids by hydrolysis or oxidation. Also, the high acid values may have been necessitated by the excessive drying of the seeds at high temperatures. Higher temperatures, therefore, tend to break and destroy the cell during size reduction which tends to increase the acid formation and in turn increase acid value. The acid value range for all the experimental runs was 7.4052-8.976 which were all higher than the literature values possibly caused by aging of Moringa seeds or inadequate storage conditions.

4.7. Saponification Value

The saponification value of Moringa seed oil is the number of milligrams of KOH or NaOH required to saponify 1g of Moringa seed oil. From literature, the accepted saponification value of Moringa seed oil had a range of 181.1-252.34 [29, 30, 31]. From the results obtained from the experiments, it was observed that all the values obtained fell within this range except for the runs done with Isopropyl alcohol which had a value of 255.816 KOHg^{-1} and 256.377

KOHg^{-1}. The saponification values for all the experimental runs was from 218.790-256.377 which all fell within the acceptable values from literature except runs done with isopropyl alcohol which fell outside the range but were very close.

4.8. Specific Gravity

The literature value for specific gravity of *Moringa* seed oil was 0.89737-0.9066. The experimental values obtained from the different runs (0.90-0.915) were noticed to have been slightly higher than the stipulated acceptable limits. However, they fell within the specification range of 0.9-1.16 [27, 32].

5. CONCLUSIONS

The findings from the extraction of oil from *M. Oleifera* seeds using various solvent mixtures concluded that oil yield was dependent on time, solvent mixture, solvent mixture combination ratios and extraction temperature. The solvent mixture combination ratios proved to be the most determinant factor in oil yield. Although time was kept constant during this experiment, there was no noticeable extraction beyond 3hours for all solvent mixtures except mixtures containing methanol, which was still showing visible extraction as at 4hours.

The highest oil yields obtained at runs 15, 16, 9, 10 and 2 with 50.667%, 51.33%, 45%, 46% and 40.667% respectively with run 16 being the highest with the following solvent combinations Petroleum ether (100ml), Hexane (50ml) and methanol (50ml). Oil yield was observed to be linearly dependent ON increasing extraction temperature, provided the boiling points of the solvents were not exceeded by large values.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENTS

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