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Phytochemical Screening of Seed Oils from Some Nigerian Grown Cucurbitaceae Plant Seeds.

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Abstract

Background*: Fruits from Cucurbitaceae plant family are naturally consumed or after processed in form of juices, alcoholic beverages and jams. Seed oils have been well applicable in food as edible oil and as source of raw materials for soap and detergent production and other industrial chemical products. Consequently, a lot of attention has been devoted to this renewable bioresources from plant origin as alternative for the replacement of the existing environmentally unsafe chemical feedstock derived from fossil origin.*

Materials and Methods: *The fruit seeds (Cucumeropsis manni, Lagenaria siceraria, Cucumis melo and Citrullus colocynthi) used in this work were obtained at Sango Market, Saki, Oyo State, Nigeria. The seeds were deshelled, sundried for 2 weeks and then pulverized. All samples were stored in a screw capped container prior to analysis. Soxhlet extractor was used for extracting the oils from the seeds using n-hexane as the extractant. The oil yield were further characterized for their physicochemical parameters such as acid value, peroxide value, ester value and saponification value using method adopted by AOAC, (1990).*

Results: *The obtained oil yields ranges from 47.97 -56.30%, the least amount was identified in Cucumeropsis manni and the highest content was found in Lagenaria siceraria. The acid values of the oils were from 2.24 - 5.70mgKOH/g, where Cucumis melon had the lowest acid value and the highest amount was found in Citrullus colocynthis. The range of saponification value was from 126.22mgKOH/g in Cucumeropsis manni to 342.31mgKOH/g in Lagenaria siceraria. The smallest peroxide value was determined in Cucumis melon (0.50mgKOH/g) while the largest amount was found in Citrullus colocynthis. Ester values observed was 101.22mgKOH/g in Cucumeropsis manni and 639.54 mgKOH/g in Lagenaria siceraria.*

Conclusion: *The four seed types can be explored for their oil in commercial quantities and due to high saponification value present in Lagenaria siceraria oil; it may be used industrially in soap production and safe for human consumption.*

Keywords: *Oil extraction, Saponification value, Acid value, Ester value, POV.*

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I. Introduction

Among of the highly rich oilseeds are those of *Cucurbitaceae* family which includes bitter apple (*Citrullus colocynthis*), cucumber (*Cucumis sativus*), bitter gourd (*Momordica charantia*), watermelon (*Citrullus lanatus*), preserving melon (*Citrullus amarus*), honey melo (*Cucumis melo*) and bottle gourd (*Lagenaria siceraria*). *Cucumis melo* (*Muskmelon)* is a cucurbit species which belongs to family *Cucurbitaceae*, it is a close relative of squash, pumpkin and watermelon. It is monoecious plant that is frequently thought of as a fruit, it is an annual, trailing herb that grows well in subtropical or warm, temperate climates [1] *Lagenaria siceraria* , the bottle gourd, is a vine grown for its fruit, which can be either harvested young to be consumed as a vegetable, or harvested mature to be dried and used as a utensil [2] and watertight container. It is called Egusi Igba among Yoruba tribe in Nigeria, is a vigorous climbing plant. The *Lagenaria siceraria* seed is rich in protein (23.48%) and the minerals (73.12%), it also contains high lipid level 44.54% similar to those of the other oil seeds [3]. Several cucurbit crops such as watermelon and honey melon accumulate β-carotene or lycopene, antioxidants that are highly beneficial to human health [4]. *Citrullus colocynthis* seed is called egusi in Yoruba, Kwartowa in hausa and egwusi in igbo, it is a widely cultivated and consumed crop of the family *Cucurbitaceae* [5] having seeds of high oil contents. The fruits are bitter, antipyretic, and are useful in hyperglycemia, tumors, ulcers, asthma, bronchitis and constipation [6]. The seed of the plant fruit is used in preparation of local soup among West African. Egusi is a very good source of essential amino acids (arginine, tryptophan and methonine). Vitamins (B1, B2) and niacin as well as minerals like phosphorus, calcium, magnesium, sodium, potassium, iron and zinc [7]. *Citrullus colocynthis* fruits are traditionally used to treat constipation, oedema, bacterial infections,

cancer and diabetes [8]. *Cucumeropsis mannii* Naudin is popularly called "egusi- itoo" among Yoruba and agushi in Hausa, in West Africa. Melon is a cucurbit crop that belongs to the Cucurbitaceae family with fibrous and shallow root system [9]. Dehulled seeds of *Cucumeropsis manni* consist of large amounts of lipids proteins and carbohydrates. It is a rich source of minerals such as sodium, potassium, phosphorus, calcium, magnesium, manganese, iron, zinc, copper and selenium [10]. However, in order to evaluate four fruit seeds from cucurbitaceae seeds oils, we extracted their oils and further characterized for their oil properties as a potential oil source for industrial and domestic use.

II. Materials and Method

2.1 Sample Collection

Citrullus colocynthis, Cucumeropsis mannii, Cucumis melo and *Lagenaria siceraria* seeds were obtained at Sango Market, Saki, Oyo State, Nigeria. The seeds were deshelled, sundried for 2 weeks and then pulverized. All samples were stored in a screw capped container prior to analysis.

2.2 Oil Extraction

The extraction process was carried with the aid of soxhlet extractor. 40g of each sample was accurately weighed and was inserted into the extractor. 200ml of the solvent (n-Hexane) was poured into the round bottom flask. This was heated at 190^{o}C and the extraction was continuously for at least 6 hours using soxhlet apparatus. At the end of the extraction, the resulting mixture containing the oil was heated in an oven to evaporate the solvent from the oil. The extraction was done in triplicates. The percentage of the oil extracted was determined according to the method reported by [11].

2.3 Oil Yield Determination (%)

The Percentage Composition of the oil was calculated as follows:

$$\text{Oil content} = \frac{weight\ of\ oil}{weight\ of\ the\ sample\ used} 100\%$$

2.4 Oils Seed Characterization

The oils were characterized by determining their acid values, saponification values, peroxide values, ester values and iodine values in accordance to the methods used by [12].

2.4.1 Determination of Acid Value

12.5ml each of both diethyl ether and ethanol were measured into conical flask and 5g of the oil sample was weighed and added. The mixture was shaken vigorously and 0.5ml of phenolphthalein was added as an indicator and shake. The mixture was then titrated using 0.1M potassium hydroxide solution until pink colouration persisted for at least 15s was observed. Thus, the acid value is calculated using the following formula:

$$\text{a} = 5.610V_{NaOH}/w$$

Where;'' a'' is the acid value, w is the weight of the oil sample and v is the difference in the volume of potassium hydroxide used in the titration.

2.4.2 Determination of Saponification Value

2g of the oil sample was weighed into round bottom flask. 25ml of 0.5M ethanolic potassium hydroxide was added and boiled for 30 minutes in a reflux condenser. The mixture was removed from the heat source and 1ml of phenolphthalein was added changing the mixture into pink colour. The mixture was then titrated with 0.5M hydrochloric acid until the pink colour disappear (mixture changed from pink to neutral solution) [12,13]. Thus, the saponification value was calculated using the following formula:

$$S = 28.05\ v/w$$

Where, S is the saponification value, v is the difference in volume of hydrochloric acid consumed and w is the mass of the oil sample used.

2.4.3 Determination of Peroxide Value

2g of the oil sample was weighed into a conical flask and 10ml of chloroform was added and the mixture was stirred. 15ml of acetic acid was added with 1ml of freshly prepared saturated potassium iodide solution and the flask was closed immediately, stirred for one minute and kept for over 5 minutes away from light at room temperature. 75ml of distilled water was then added and this was shaken vigorously. Few drops of starch solution were added as indicator to give blue-black colouration. The mixture was then titrated with 0.001N Sodium thiosulphate solution until a clear/white colour was observed [13]. The Peroxide value can be calculated by the formula:

$$\text{POV} = \frac{(V_1 - V_0) \times C \times 1000 \times T}{m}$$

Where POV = peroxide value expressed in meq/, V_1 = consumption of sodium thiosulfate solution in the main test, in mL , V_0 = consumption of sodium thiosulfate solution in the blank test, in mL, C = molar concentration (molarity) of the sodium thiosulfate solution in mol/L , T = titer of the thiosulfate solution, m = weighed portion of the oil sample in grams.

2.4.4 Determination of Ester Value

1.44g of each of the oil samples was weighed into round bottom flask and 5ml of ethanol was added. 5 drops of phenolphthalein were added to the mixture and this was titrated with 0.1M ethanolic potassium hydroxide until the mixture turns pink. 20ml of 0.5M potassium hydroxide was then added and the mixture was heated for one hour under reflux condenser. The mixture was removed from the heat source (in which the pink colour becomes light pink) and 25ml of distilled water was added along with 0.2ml of phenolphthalein solution. The mixture was then titrated to neutrality using 0.5M hydrochloric acid. Ester Value is determined by the formular: $E = 28.05\ v/w$

Where, E is the ester value, v is the difference in volume of hydrochloric acid consumed and w is the weight of the oil sample.

III. Results and Discussion

The preliminary physical parameter to be considered in judging the quality of oils is their colour, the darker the colour of the oil the worse the quality. All the oils extracted were yellow in colour; this study shows that the qualities of the oils were good and conform to a report given by Egwin *et al*., 2015. From the Table 1, the percentage oil yield for *Citrullus colocynthis, Cucumeropsis mannii, Lagenaria siceraria and Cucumis melon* seeds were 50.4%, 47.97%, 56.37%, and 51.46% respectively. However, these results were comparable with the results obtained by [14] who reported 53.20% oil yield for *Citrullus colocynthis*, [15] who reported 48.74% oil yield for *Cucumeropsis mannii* seed oil. Most of these values were within the standard range ($\geq$ **32**%) set by [16], therefore, the oil yields in this study maybe considered economically for commercial production of oil in Nigeria.

Acid Value is the amount of free fatty acid (FFA) present in oil or fat. It is used as an indicator for the edibility of oil [17], it indicates whether the oil is in good non-degradable state or not [18]. The acid value of *Citrullus colocynthis, Cucumeropsis mannii, Lagenaria siceraria* and *Cucumis melon* seed oil obtained was 5.70 mgKOH/g, 2.50 mgKOH/g, 3.927 mgKOH/g and 2.24 mgKOH/g respectively. According to [16], the maximum acceptable level of acid value in oil is 4.00 mgKOH/g, any value below this simply means the oil is good for human consumption. This study showed that the acid values obtained for *Cucumeropsis mannii, Lagenaria siceraria* and *Cucumis melon* seed oils were within the standard range (4.00 mgKOH/g) showing that the oils were in good non-degradable state which can be used for daily consumption except for *Citrullus colocynthis* seed oil which had higher acid value than the standard range, hence, the intake of *Citrullus colocynthis* seed oil should be limited until it is properly refined. It was noted that the acid value (5.70 mgKOH/g) obtained in the *Citrullus colocynthis* seed oil was not in agreement with 3.50 mgKOH/g reported by [19], and also, 2.50 mgKOH/g found in *Cucumeropsis mannii* oil was not in conformity to 1.35mgKOH/g stated by [20] and also not in agreement with study reported by [21] which was 2.90%. Each of the oil gotten from *Citrullus colocynthis, Cucumeropsis mannii, Lagenaria siceraria and Cucumis melon* seeds had saponification value of 155.68 mgKOH/g, 126.22 mgKOH/g, 342.21 mgKOH/g and 182.26 mgKOH/g, respectively.

Table 1: Phytochemical Constituents of Extracted Seed Oils.

Sample	**Oil yield**(%)	**Acid value**(mg/KOH/g)	**Saponification value**(mg/KOH/g)	**Peroxide value**(meg/KOH/g)	**Ester value**(mg/KOH/g)
Citrullus colocynthsis	50.40	5.70	155.68	12.20	518.15
Cucumeropsis manni	47.97	2.50	126.22	10.00	101.22
Lagenaria siceraria	56.30	3.93	342.31	8.30	639.54
Muskmelo	51.46	2.24	182.26	0.50	106.90
AOAC Standard, 1990	≥32	≤4.00	≥180	2-10	

The oil fraction with saponification value $\geq 180 mgKOH/g$ possesses low molecular weight fatty acid [16]. This study shows that the saponification value for *Lagenaria siceraria (*342.21 mgKOH/g*)* and *Cucumis melon* (182.26 mgKOH/g) seed oil has low molecular weight fatty acid which indicates their usefulness in soap making. However, the oils gotten from *Citrullus colocynthis and Cucumeropsis mannii* had saponification value

of 155.68 mgKOH/g and 126.22mgKOH/g respectively, indicating that the oils cannot be used in the production of soap due to high molecular weight fatty acid present in it. The result obtained in *Lagenaria siceraria* was in agreement with 323.87mgKOH/g reported by [22], *Cucumeropsis mannii* showed a value that is not in agreement with 220.19mgKOH/g reported by [23].

The peroxide value is the measure of oxidative rancidity of oil; high oxidative stability is possible when peroxide value is less than 10.00 mg peroxide/kg [16]. The peroxide value of *Cucumeropsis mannii, Lagenaria siceraria and* muskmelon has a low value of 10megKOH/g, 8.3megKOH/g and 0.5megKOH/g respectively. All these values fall within the

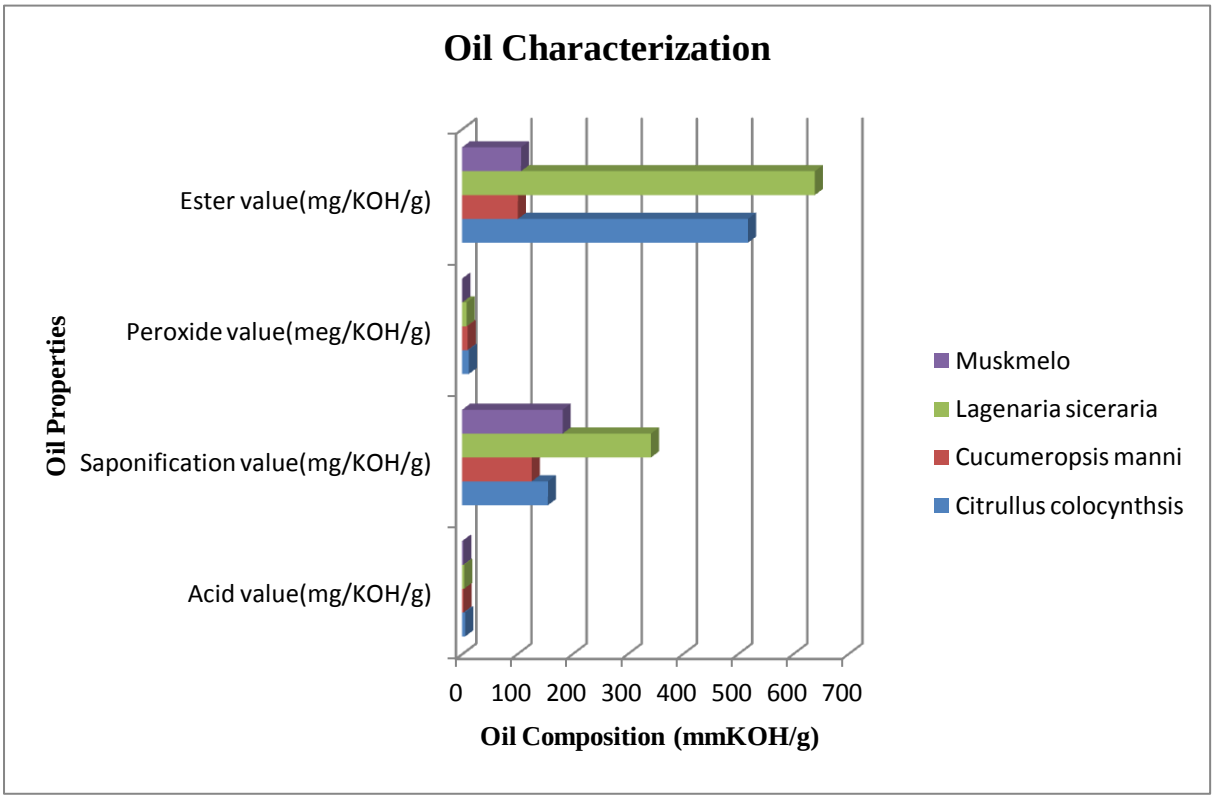

Figure 1: Oil Characterization (Chart) of the Four Seeds OIl

standard range of 10.00 mg peroxide/kg. Indicating that these oils may be stable to oxidative degradation, while *Citrullus colocynthis* oil has high peroxide value of 12.20megKOH/g, this implies that the oil is less stable to oxidative degradation i.e. it cannot be stored for longer period of time. The peroxide value obtained in this study agrees with 12.0megKOH/g reported by [24] and not in agreement with 20.00 reported by [25] for *Cucumeropsis mannii* seed oil. The ester value obtained for *Citrullus colocynthis, Cucumeropsis mannii, Lagenaria siceraria and* muskmelon were 518.15mgKOH/g, 10.122mgKOH/g, 639.54 mgKOH/g and 106.9 mgKOH/g respectively.

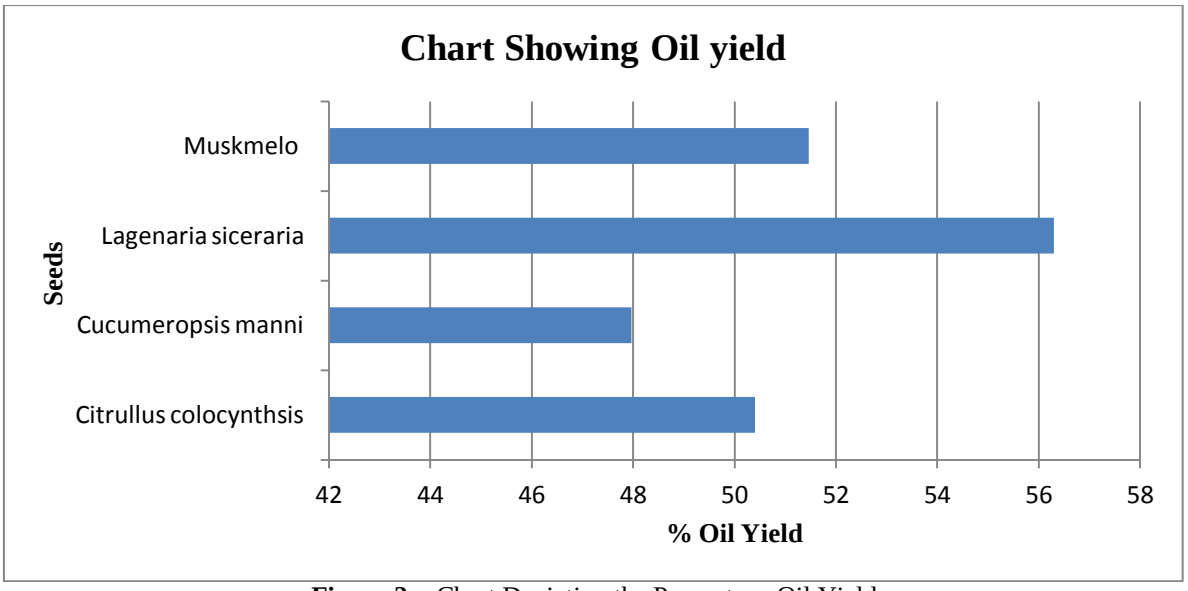

Figure 2a: Chart Depicting the Percentage Oil Yield

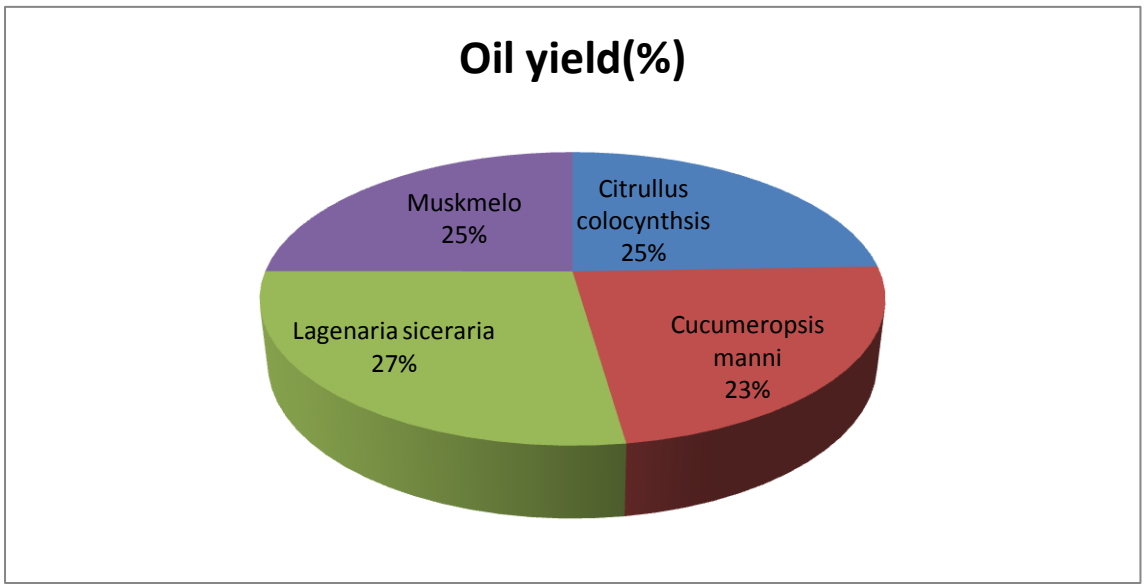

Figure 2b: Chart Depicting the Percentage Oil Yield

From figure 1, we could observe higher ester value from Lagenaria siceraria, followed by Citrullus colocynthis and similar result from other seeds oil. Saponification value of Lagenaria siceraria oil is more pronounced follow by musk melon seed oil with the least was observed from Cucumeropsis mannii. Both the peroxide and acid value of the four seed oils are very low with similar result as it was shown from the figure.
The percentage of the oil yield from the four seeds was represented in both figure 2a and 2b, the percentage oil yield from the two graphs was in the order Lagenaria siceraria > Musk melon > Citrullus colocynthis > Cucumeropsis mannii. We can then conclude that oil yield from Lagenaria siceraria and Musk melon are good source of oil for commercial and industrial purpose.

IV. Conclusion

The results obtained for the oil yields in this study were in agreement with standard value stated by [16], this implies that the studied oil seeds can be exploited for commercial purpose. The acid value and peroxide value obtained for *Cucumeropsis mannii, Lagenaria siceraria and* muskmelon seed oil revealed that the seed samples were good for domestic consumption and can be stored for long time, except for *Citrullus colocynthis* oil that has higher acid and peroxide value than the standard range by AOAC, (1990). The saponification value for each of *Lagenaria siceraria and* muskmelon seed oil was within the standard range which means the oils can be used in the production of soap.

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