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Extraction and Determination of Nutrients Composition of Moringa Oleifera Whole Seed and Oil

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Abstract: *Moringa oleifera, which is commonly referred to as "Moringa" had been widely cultivated and it is the only genus in the family Moringaceae. It is an exceptionally nutritious vegetable tree with a variety of potential uses. The Moringa oleifera seeds were gotten from a matured Moringa tree in Ile-Ife, Osun State, Nigeria. The seeds were extracted using Soxhlet apparatus method with methanol as the solvent for extraction. The proximate analysis of the whole seeds and oil was determined according to methods of A.O.A.C. (2010). Also, the physicochemical properties of the oil were carried out. All assays were carried out in triplicate, and the means and standard deviation were determined using SPSS version 20. This showed the nutrient composition presents in the Moringa oleifera whole seeds and oil. The result of the proximate analysis showed that the moisture content in the Moringa oleifera seeds (7.14%) was high compared to the moisture content in the Ben oil (2.94%). The oil content in the seeds was 39.84%. The ash content of the seeds (7.59 %) was high compared to the ash content in the ben oil (1.59 %). The crude fiber content in the Moringa oleifera seed oil (33.83 ± 1.06) was extremely high than the fiber content in the whole seeds (8.71%). The protein content of the Moringa oleifera seeds and oil was 20.51% and 19.52 %. The carbohydrate content in the whole seeds (16.21%) was extremely more than the carbohydrate content in the seed oil (2.27 ± 0.95). In addition, the result of the physicochemical properties of the Moringa oleifera oil showed that the iodine value, saponification value, Unsaponifiable matter, refractive index, acidity, and the free fatty acid of the oil were; 66.90gl/100g, 187.29 mgKOH/g, 0.91g100g^{-1}, 1.46, 0.4 %, 0.23 mgKOHg^{-1}, respectively. The PH of the oil was 6.85. Moringa oleifera seeds should be fully exploited because it has the potential to become a new source of oil. The production of useful oil from the seeds could be of economic importance of benefit to the areas where the tree is cultivated.*

Keywords*: Moringa Oleifera seeds and oil, proximate analysis, Soxhlet extraction, methanol, nutrients composition.*

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I. Introduction

In the context of global vision, the Goal 2 target of sustainable development goals (steps to zero hunger) is to "end hunger, achieve food security and improved nutrition and promote sustainable agriculture (SDG)." In contrast, target 2.2 aims to end all forms of malnutrition by 2030. Food deprivation refers to hunger, while malnutrition is a condition brought about by insufficient nutrients to meet the body's requirements for proper development and health. According to the world hunger (1) report, about 161 million people were estimated to be undernourished from 2019 to 2020, i.e., nearly one in three people in the world did not have access to adequate food in 2020 (2). Even though more than enough foods are produced to feed the global population, people who still go hungry were further increased to 811million people. In addition, the exponential population growth has been a threat to food security as time progresses (3). According to a UN's Food and Agriculture Organization report, it was stated that food security could be achieved when the agricultural sector is transformed to reach its full potential (3).

Moringa Oleifera is referred to as "Moringa", it is called by different names among different people of the world, among the Yoruba people of southwest Nigeria, it is called "Ewe Ile", among the Fulani's, "Zogale" among the Hausa's "Nugyekai" (4). Moringa Oleifera is a nutritious vegetable tree with various potential uses (5) and often grows up to 15 m in height (6,7,8). It has spreading, feathery foliage of tripinnate leaves, open crown of drooping, thick corky whitish bark, and fragile branches (9). Every part of Moringa tree can be used for food, beneficial applications (10,11), or nutritional purposes, including leaves, roots, seeds, root barks, stem bark, pods, etc. (8,12,13).

Moringa Oleifera serves as an extremely valuable food source that has been useful for human nutrition and in the development of animal nutrition to make their diet well balanced (14,15,16). Several studies have

reported that Moringa oleifera contains high digestible protein, trace metal ions, essential amino acids, antioxidants, calcium, iron, potassium, vitamins, and carotenoids which is suitable for combating malnutrition in different developing nations where malnourishment has been a major concern (17, 18, 19). Also, it contains histidine and arginine which are mostly relevant for infant nutrition (16).

According to several studies, *Moringa Oleifera* was called a miracle tree because of its various features, e.g., it contains more vitamins than carrots, more vitamin C than oranges, more calcium than milk, and more potassium than bananas (20). Also, this tree helps to increase the blood antioxidant level (21) and reduce the blood sugar level (22), as well as sustained inflammation (23). *Moringa Oleifera* has been found to be a superfood for people suffering from malnutrition and poverty because of its nutritional alternatives (3). Furthermore, Moringa seeds have a large and circular shape that grows inside the *Moringa Oleifera* tree; each Moringa pod can provide over a dozen large Moringa seeds (9). The color of the Moringa seeds is dark brown, with three papery wings extending from the seeds' main kernel. Various researchers have discovered that the seed cake contains natural coagulants that can be applied to purify and treat water with high turbidity (24, 25). The seed contains proteins, carbohydrates, vitamins A as well as vitamin B1 (26).

Moreover, it is a good source of minerals, micronutrients, and bioactive compounds such as phytates, trypsin inhibitors, saponins, flavonoids, and sterols and a source of lipids and fibers (27). Moringa Oleifera seeds contain 19–47 % oil (4,28) which is known as Ben oil and are rich in palmitic, stearic, behenic and oleic acids (28). It is non-drying oil and can be used for lighting as it burns without dense smoke (29). According to Nguyen et al., (24) and Bhutada, (30), it was reported that Ben oil is 70% high in oleic acid which makes the oil edible and can be used for food consumption. It also has a pleasant peanut smell-like fragrance, and it is more stable than all other types of oils such as canola oil, soybean oil, and palm oil when used in frying. Ben oil is considered equivalent to olive oil in terms of fatty acid composition (25) and it is also useful as vegetable cooking oil (4). Moringa seeds and oil can also be used to treat arthritis, rheumatism, and hypertension (31).

Although several studies have been carried out on the proximate analysis of the Moringa seeds (5, 13, 32) and the *Moringa Oleifera* seed oil (4, 5, 13, 33) but more emphasis have not been placed on the extraction and determination of nutrients composition of *Moringa Oleifera* whole seed and oil. Also, most of the studies have employed various solvents to extract the Ben oil from the *Moringa Oleifera* seed oil, such as petroleum ether, n-hexane, etc. This solvent takes a more extended period for extraction, about 8 hours, to get the optimum oil extracted, which results in a lower yield, while the maximum oil can be extracted in 12 hours with a higher yield of oil (34). The oil extracted from the *Moringa Oleifera* seeds using petroleum ether solvent could be directly used for biodiesel production or other industrial applications because it is not a food-grade solvent.

Also, due to the high dependency of humans on oil for domestic and industrial uses, there is a need to look for another source of oil with a better extraction method to give a higher yield. Therefore, this study not only extracts and determines the nutrient composition of *Moringa Oleifera* whole seed and oil but also used methanol as solvent for extraction of the oil from the seed as used by (35,36) and found safe for nutritional use and have high extraction yield.

II. Material And Methods

2.1 COLLECTION AND IDENTIFICATION OF MATERIALS

2.1.1 Materials: The Moringa Oleifera seeds were obtained from a matured Moringa tree in Ile-Ife, Osun State, Nigeria.

2.1.2 Apparatus/ Equipment: Analytical weighing balance, glass wool, funnel, standard flask, beaker, pipette pump/ pipette, gas, hot plate, heating mantle, fume cupboard, thermometer, wash bottles, crucible, spatula, oven, mortar and pestle hand glove, nose mask, goggles, and Soxhlet extractor.

2.13 Reagents: Hydrochloric acid (HCL), Nitric acid, methanol, deionized/ distilled water

2.2 EXPERIMENTAL METHODS

The seeds were dehulled, cleaned, sun-dried and oven-dried to a constant weight, and they were crushed using a mortar and pestle to form a paste. A 75 gram of the grounded sample was weighed and introduced into an extraction cartridge/thimble. It was placed in a 500 ml glass Soxhlet (37). The solvent container was weighed and 400 ml of methanol was added. The Soxhlet was then introduced into the water bath already set above the boiling point of methanol at 64.7°C (148.5 °F; 337.8K), which was then connected to the cryostat cooling thermostat. Four to six siphoning processes were conducted for 5 h. The solvent was evaporated in a RE 121 Rotavapor (made in Switzerland). The container with the oil was placed in on water bath for 4 h at 103°C, then in a desiccator for 30 min and weighed. The weight difference gives the percentage of the oil being extracted, i.e., the weight of the sample before extraction minus the weight of the sample after extraction, divided by the initial weight of the sample and multiplied by 100.

2.3 PROXIMATE ANALYSIS

Proximate analysis of the whole seeds and the ben oil was carried out at the Chemistry Laboratory, Redeemer's University, Ede, Osun State. Nigeria. The dried seeds were subjected to the chemical analysis (moisture content, oil content, ash, crude fiber, protein, and carbohydrate) according to the methods of A.O.A.C. (38).

2.4 DETERMINATION OF PHYSICOCHEMICAL PROPERTIES

Physicochemical properties of extracted oils from Moringa seeds oil were determined. Saponification value, Unsaponifiable matter, iodine value, refractive index, fatty acid, and acid value were determined according to (39, 40, 41).

2.4.1 Saponification Value: The sample was melted and filtered to remove any impurities and traces of moisture. 5g of the sample was weighed into a conical flask. 50ml of alcohol KOH was added from the burette by allowing it to drain for a definite time. A blank sample was prepared using the same method. Then a reflux condenser was connected to the flask, and it was boiled gently for about 1hr. The flask and condenser were allowed to cool and rinsed with little distilled water, and the condenser was removed. 1ml of the indicator was added, and 0.5M HCl was used for titration until the pink color disappeared.

Saponification value was calculated from the equation:

$$SV = \frac{(S-B) \times M \times 56.1}{\text{Sample weight (g)}}$$

Where, S = sample titer value; B = blank titre value; M = molarity of the HCl; 56.1 = molecular weight of KOH

2.4.2 Iodine Value: 0.5g of oil was weighed into an iodine flask, and it was dissolved into 10ml of chloroform. 25ml of Wiji's iodine solution was added to it using a pipette for a certain time. The solution was mixed well and allowed to stand in a dark corner for exactly 30minutes with occasional shaking. 50ml of 15% aqueous potassium iodide was added and shacked thoroughly. 100ml of freshly boiled and cooled water was used to wash down free iodine on a stopper. It was titrated with 0.1 M sodium trioxothiosulphate solution until the yellow solution turned almost colorless. 1% starch was added as an indicator and titrated until blue coloration completely disappeared after vigorous shaking. The same procedure was used for blank tests and other samples. The iodine value (I.V) is given by the expression:

$$IV = \frac{12.69C\ (V\ 1\ -V\ 2)}{M}$$

Where, C = Concentration of sodium; V1 = volume of sodium trioxothiosulphate used for blank; V2 = volume of sodium trioxothiosulphate used for determination and M = mass of the sample

2.4.3 Free Fatty Acid: 5g of oil was dissolved in 50ml of the neutral solvent in 250ml conical flask. 3 – 4 drops of phenolphthalein indicator were added. The sample was titrated against 0.1KOH and shaken constantly until it changed to pink color.

2.4.4 Acid value: A volume of 100 ml of neutral ethyl alcohol was heated with 10 g of oil or fat sample in a 250 cm3 beaker until the mixture began to boil. The heat was removed and titrated with N/10 KOH solution, using two drops of phenolphthalein as an indicator with consistent shaking. A permanent pink color was obtained at the endpoint.

The Acid value was calculated using the expression; AV = 0.56 x No. of ml. N/10 KOH used

2.4.5 Refractive index: The Abb Refractometer was used to determine the refractive index. The programmable refractometer was first standardized using pure distilled water, whose refractive index at 20°C is 1.3330. The surface of the prisms was cleaned up with ether. Then two drops of the oil were applied at the lower prism, and the prism was closed and held in place firmly. Water was passed through the jacket at 45°C. The jacket was then adjusted, and with the help of the light source, the readings were taken. The temperature of the prism was also read and taken.

2.5 PH DETERMINATION

The PH was determined using a PH meter. It was ensured that the instrument was put on for at least 15mins to stabilize, then it was calibrated, and the reading of the sample was taken.

2.6 STATISTICAL ANALYSIS

All assays were carried out in triplicate, and the means and standard deviation were determined using SPSS version 20.

III. Result

3.1 Proximate Chemical Composition of *Moringa Oleifera* Seeds and oil

Table no 1 shows the proximate chemical composition of *Moringa oleifera* seeds and oil. It was observed that the moisture content in the *Moringa oleifera* seeds (7.14±0.04) was higher compare to the moisture content in the Ben oil (2.94±0.07). The oil content in the seeds was 39.84±0.45. The ash content of the seeds (7.59 ± 0.54) was more compared to the content in the ben oil (1.59 ± 0.35). The crude fiber content in the *Moringa oleifera* seed oil (33.83 ± 1.06) was extremely high than the fiber content in the seed (8.71 ± 0.03). Furthermore, it was discovered that both the *Moringa oleifera* seeds (20.51 ± 0.12) and the seed oil (19.52 ± 0.57) are a good source of crude protein. Also, the carbohydrate content in the whole seeds (16.21 ± 0.51) was extremely more than the carbohydrate content in the seed oil (2.27 ± 0.95).

Table no1: Proximate chemical composition of *Moringa oleifera* seeds and oil (Mean±Standard Deviation)

Proximate	Seed (Mean ± SD)	Oil (Mean ± SD)
Moisture Content	7.14 ± 0.04	2.94 ± 0.07
Oil Content	39.84 ± 0.45	39.84 ± 0.45
Ash Content	7.59 ± 0.54	1.59 ± 0.35
Crude Fiber	8.71 ± 0.03	33.83 ± 1.06
Protein	20.51 ± 0.12	19.52 ± 0.57
Carbohydrate	16.21 ± 0.51	2.27 ± 0.95

3.2 Physicochemical Properties of Moringa Oleifera Oil

The *Moringa oleifera* seed oil was observed to be brownish yellow in color and it was further subjected to analysis after the extraction of the oil. Table no2 shows the physicochemical properties of the Moringa oleifera seed oil. The iodine value, saponification value, Unsaponifiable matter, refractive index, and acidity and of the oil were; 66.90 ± 0.91, 187.29 ± 0.33, 0.91 ± 0.03, 1.46 ± 0.00, 0.41 ± 0.02. The PH of the oil was 6.85 and the free fatty acid was 0.23 $mgKOHg^{-1}$.

Table no 2: Physicochemical properties of *Moringa oleifera* edible oil extracted from Moringa seeds (Mean ± Standard Deviation)

Properties	Mean ± SD
Iodine Value (gl/100g)	66.90 ± 0.91
Saponification Value (mg KOH/g)	187.29 ± 0.33
Unsaponifiable Matter (g 100 g^{-1})	0.91 ± 0.03
Refractive index	1.46 ± 0.00
Acidity (%)	0.41 ± 0.02
Free Fatty Acid (%)	0.23±0.03

IV. Discussion

The proximate analysis of the result of Moringa Oleifera seeds and oil showed that the seeds were high in protein (20.51 ± 0.12) and the oil was high in crude fiber (33.83 ± 1.06) while the seed had low moisture content (7.14 ± 0.04) and the oil had low ash content (1.59 ± 0.35). The result on the moisture content of the Moringa oleifera seeds (7.14 %) showed that the seeds had low moisture content, which indicates that the activity of the microorganisms would be reduced, thereby increasing the shelf life of the Moringa seed. This result contradicted the report gotten by Mgbemena & Obodo (42), where the moisture content of the seeds was reported to be 9.56%. Moisture content indicates the amount of water present in the seed, and it is crucial as it serves as a benchmark to evaluate the seed's quality and stability (Shell-life). The moisture content of the seed oil (2.94 ± 0.07) also contradicted the report of Abdulkarim et al. (43), where the moisture of the oil was (7.9 ± 1.00).

The oil content of the seeds was 39.84 ± 0.45, which showed that extraction of oil from the seeds using methanol solvent yielded high oil content, which contradicted the result reported by Adegbe et al. (4), where the oil content was 32.5±7.78 with the use of n-hexane solvent. The fiber content of the whole seed was 8.71 ± 0.03, which was slightly similar to the report of Madubuike, et al. (33), who reported that the fiber content in the Moringa oleifera seed was 9.94. Also, the result showed that the oil was very high in fiber. Crude fiber content helps in bowel movement. Adequate dietary fiber intake can lower cholesterol levels and risk of hypertension (44) and prevent diverticulosis inflammation.

Furthermore, the protein content in the seeds and the oil had similar results; 20.51 ± 0.12 and 19.52 ± 0.57, respectively. As reported by Pearson (45), plant food that provides more than 12% of its calorific value from protein is considered a good source of protein. Therefore, Moringa Oleifera seeds and oil are good sources of protein. The Carbohydrate content of the oil is 2.27% which is lower than the value of 7.44% reported by Adegbe et al. (4), while the carbohydrate content of the seeds (16.21%) was very high. Iodine value is the measure of the degree of the unsaturation of the oil. A higher Iodine value indicates higher unsaturation of fats and oils. The iodine value of the oil Moringa Oleifera was 66.90. This agrees with the FAO/WHO (46) standard for edible oil, which means most of our fatty acids are saturated. The Saponification value of the oil was 187.29, which was slightly consistent with FAO/WHO (46) standard.

In addition, the value of the Unsaponifiable matter of the oil, which was 0.91, was slightly higher than the value reported by Abdulkarim et al. (47), which was 0.74. The refractive index of the oil was 1.46; this value shows consistency with FAO/WHO (46) standard. The free fatty acid of Moringa Oleifera seed oil was 0.23 mgKOHg-1 which is within the range of the FAO/WHO (46) standard. High free fatty acid causes soap formation during the alcoholysis process from its by-products.

V. Conclusion

Moringa oleifera seeds should be fully exploited because it has the potential to become a new source of oil. It contains high unsaturated fatty acids due to its high iodine and saponification values and might be an acceptable substitute for high saturated oil. The production of useful oil from the seeds could be of economic importance of benefit to the areas where the tree is cultivated.

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