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PHYSICOCHEMICAL ANALYSIS AND PHARMACEUTICAL POTENTIAL OF MORINGA OIL EXTRACT

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Abstract: **The potential of *moringa oleifera* as a source of high quality oil that can be used for cooking, industrial and pharmaceutical application was studied. This work was intended to examine the physicochemical analysis and pharmaceutical potential of *moringa oleifera* seed oil extract. Harvested seedlings of *moringa oleifera* plant from jimeta yola in adamawa state of Nigeria produced an oil yield of 36.5%. The chemical composition indicated that *moringa* seed contained appreciable amount of oil that encouraged its extraction and characterization. Solvent extraction process was employed in its extraction. Values obtained for physical characterization of moringa were 0.9434 specific gravity, 1.4560 refractive index, 40.53 viscosity, 4.0 moisture content. Chemical characterisation of the seed oil reveal 0.08, 0.16, 184.93 mg KOH/g, 120.1 and 9 of free fatty acid, acid value, saponification, iodine and peroxide value respectively. No report has appeared on the potential of *moringa* oil for use in the pharmaceutical industry especially in the area of self emulsifying drug delivery systems (SEDDS). *moringa* oil being a very stable oil is expected to be useful as an adjunct in the formulation and clinical trials for drug formulations.**

Keywords: **extract, moringa, oil, pharmaceutical, physicochemical, SEDDS, potential.**

1. INTRODUCTION

Recently, self emulsifying oil formulations (SEOFS) have been formulated using medium chain triglyceride oil and some non ionic surfactants. Some researchers have shown that the nature of oil used in the formulation of SEOFS influenced their overall usefulness, safety and performance [24] [14]. Long chain triglyceride oils which can also be sourced from moringa oleifera oil have been used in the preparation of emulsions which can be used in drug formulations [27]. Its inclusion as an adjunct in the development of a SEOFS portends obvious advantages.

Moringa oleifera is a shrub and small deciduous tree of 2.5-10m in height. When matured the fruit becomes brown and has a 10–50 seeds inside [29]. The plant was reported to contain various amino acids, fatty acids, vitamins, and nutrients [19] and its constituents such as leaf, flower, fruit and bark and seed have been anecdotally used as herbal medicines in treatments for inflammation, cholesterol, paralysis and hypertension. Many reports described *M. oleifera* as highly potent anti-inflammatory [12], hepatoprotective [23], antihypertensive [13] and anti-tumor [18]. Also, its seed has strong coagulative and antimicrobial properties [10]. The seed oil has physical and chemical properties equivalent to that of olive oil and contains a large quantity of tocopherols [28]. *Moringa oleifera* seeds also contain between 30-35 % (w/w) of vegetable oil [25], known as“Behen” or “Ben” oil. This oil resembles olive oil in its fatty acid composition and is oleic acid-rich, which makes it suitable for edible purposes. This study tries to explore the potential of *moringa* oil for use in self emulsifying drug delivery systems based on its physicochemical properties.

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2. MATERIALS

Moringa oleifera seed was purchased at the jimeta main market in Yola Adamawa state. All other chemicals that were used were of analytical grade.

Pre-treatment of Materials:

Pods and shells of *moringa oleifera* were removed manually and the seed were grounded in a domestic blender.

Extraction of the oils:

Solvent Extraction This involves the immersion of the oil seeds in a liquid which may be a petroleum fraction or a non-flammable fluid, commonly a chlorinated hydrocarbon in which fat dissolves. The solvent is subsequently heated off and the extracted oil recovered.

The mechanical oil extraction processes are capable of removing all but 4% to 5% of the oil originally present. Solvent extraction only leaves 1% or less of the oil behind.

The crushed seed was extracted with ethanol (60-80^{o}c) in a soxhlet extractor and then the solvent was distilled off at 80^{0}c. The oil content was calculated from weight of oil and weight of seed.

Calculation [5]

$$\% \text{ oil yield} = \frac{\text{weight of oil obtained}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Determination of saponification number of oils:

Exactly 2 g of oil was weighed and 250cm^3 of O.5M alcoholic potassium hydroxide was added and the flask connected to the reflux condenser. The solution was refluxed for 40 min then titrated while hot against standard 1M HCl using phenolphthalein as indicator until the pink colour of the indicator turns colorless. A blank test was also carried using only 25cm^3 alcoholic KOH against the same acid. The above procedure was done for the two oil samples.

$$\text{Saponification value :-} \quad \frac{(V_O - V_1) \times C \times 56.1}{\text{Weight of oil sample (m)}}$$

Where V_O = Titration of blank

V_1 = Titration of sample

C = Normality of the HCl or exact concentration of standard HCL

56.1 = The M.W of KOH

M = weight of oil sample

Determination of iodine value by Dam's method:

All the reagent solutions prepared were arranged on the table. 10ml of fat sample dissolved in chloroform were pipette out onto an iodination flask labeled as "TEST". 20ml of Iodine Monochloride reagent was added to the flask. The contents were mixed in the flask thoroughly. Then the flask was allowed to stand for half an hour incubation in the dark. A blank was set up in another iodination flask by adding 10ml Chloroform to the flask. To the blank, 20ml of Iodine Monochloride reagent was added and the contents mixed in the flask thoroughly. The blank was incubated in dark for 30 minutes. Mean while, the TEST from incubation was taken after 30 minutes and 10 ml of potassium iodide solution was added into the flask. **T**he stopper and the sides of the flask were rinsed using 50 ml distilled water. The "TEST" was titrated against standardized sodium thiosulphate solution until a pale straw colour was observed. About 1ml starch indicator was into the contents in the flask, a purple colour was observed. **T**he titration was continued until the color of

the solution in the flask turned colourless. The disappearance of the blue colour was recorded as the end point of the titration. Similarly, the procedure was repeated for the flask labeled 'Blank'. The end Point values of the blank were recorded. The iodine number was calculated using the equation below: [5]

Volume of Sodium thiosulphate used = [Blank- Test] m

Iodine no of oil

$$= \frac{\text{Equivalent wt of iodine} \times \text{volume of } Na_2S_2O3 \text{ used} \times \text{Normality of } Na_2S_2O_3 \times 100 \times 10^{-3}}{\text{Weight of oil sample used for analysis (g)}}$$

Equivalent Weight of Iodine = 127

Normality of sodium thiosulphate ($Na_2S_20_3$) = 0.1

Determination of Acid Value and Free Fatty Acids:

Each oil sample (1.0 g) was weighed and dissolved with 50 ml of ethanol in a conical flask. Two drops of phenolphthalein indicator were added and titrated to pink end point (which persisted for 15 mins) with 0.1 N potassium hydroxide solution (KOH). Acid value was calculated using the equation below [21]

$$\text{Acid value} = \frac{56.1 \times V}{m}$$

where 56.1 = equivalent weight of KOH

V = volume in ml of standard volumetric KOH solution used

C = exact concentration of KOH solution used (0.1N)

M = mass in grams of oil sample

Determination of moisture content in extracted oil sample:

A crucible was weighed and into it was transferred 5g of the oil sample. The sample was dried to a constant weight in the oven at 105-110^{0}c and the crucible with the content was cooled in a desicator and weighed

$$\text{Moisture content (\%)} = \frac{\text{loss of weight}}{\text{Wt of sample}} \text{ X } \frac{100}{1}$$

Determination of Peroxide Value:

1.0 g of the oil sample was weighed into a clean dry 200cm^3 conical flask. To the sample 1.0g of powdered potassium oxide was added and 20cm^3 of a mixture of glacial acetic acid and chloroform was added (2:1). The conical flask was then placed in a steam bath for 30 sec. The boiling was continued vigorously for another 30 sec. The content was quickly be poured into a flask containing 20cm of 5% potassium iodide solution. This mixture was then titrated against 0.02 sodium thiosulphate solution using starch as indicator. The same procedure was carried out with the blank solution (a solution containing all reagents except the oil sample) [22]

Calculation:

$$\text{Peroxide value} = \frac{10 \times (ST-BT) \text{ x } N}{\text{Wt of oil (m)}}$$

Where ST= sample titer value i.e volume of Na2SO3 for determination of test sample in ml

BT= blank titer value ie volume of Na2SO3 for determination of blank solution in ml

m = weight of oil sample

N = normality of sodium thiosulphate

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Determination of Density and Specific Gravity:

A cylinder (10cm^3) was washed and dried in an oven at 105-110 ^{O}C and allowed to cool. It was then weighed empty using an electric weighing balance and recorded. Exactly 5cm^3 of water was measured into the cylinder using pipette and weighed with the cylinder

Weight of empty cylinder = WI

Weight of cylinder and water = W2

Weight of water (W2-W,) = DW

Initial volume of water measure= VI

Density of water (DW) = DW/V, Cm^3

Also 1g of the oil sample was weighed and delivered directly into water in the cylinder and the displaced volume was recorded. The room temperature taken for both oil sample was at 34^{0}C

Mass of oil sample = 1 g

The displaced volume (volume of 1g of sample in water = V2)

Calculation:

$$\text{Density (Es)} = \frac{\text{mass of sample}}{\text{Density of equal volume of water}}$$

$$\text{Specific gravity S (g)} = \frac{\text{Density of sample}}{\text{Density of equal volume of water}}$$

3. RESULTS AND DISCUSSION

Physicochemical analysis of *moringa oleifera* oil extract:

Table 1: Percentage oil yield of *moringa* seed oil (MSO)

	MSO
% Oil Yield	36.5%

Results obtained from table 1 showed that the percentage oil yield of *moringa* seed is 36.5%. These high percentage of the oil made from the seed of *moringa oleifera* made the oil a distinct potential for the oil industry. The mature seed of any plant gives oil yield of 22-38% oil [17]. Jamieson (1939) reported a 40% yield by weight of the seed of *moringa oleifera*. Variation in oil yield may be due to the difference in the variety of plant, cultivation, climate, ripening stage, harvesting time of the seeds and the extraction method used.

Table 2: Physical properties of the extracted oils

Property	MSO
Specific gravity	0.9434
Viscosity	40.52
Refractive index	1.4560
PH	6.08
Colour	Amber
Moisture content	4.0

Specific gravity:

From data obtained from table 2, the specific gravity of *moringa* oil extract was found to be 0.9434. SSMO (2003) reported 0.915-0.924, 0.912-0.925, 0.918-0.926 and 0.918-0.923 specific gravity of sesame, groundnut, cotton and sun flower respectively. The higher value of *moringa* oil extract could be attributed to its source.

Viscosity:

Viscosity value of 40.53 was obtained for *moringa* oil extract. *Moringa* value was lower than values obtained by other investigators. Oils with lower values of viscosity and density are highly appreciable to consumers. In order to design an advanced technological process these properties are very important parameters. Oils are mixtures of triglycerides (TGs) and their viscosity depends on the nature of the TGs present in the oil. The viscosity changed due to the different arrangement of the fatty acids on the glycerol backbone of the triglyceride molecule. Therefore, viscosity is related to the chemical properties of the oils such as chain length and saturation/unsaturation. At room temperature of 35 °C the viscosity is high in Mustard oil as compared to Corn oil [16]. They explained that the viscosity and density decreases with an increase in unsaturation and increases with high saturation and polymerization. Viscosity also depends on sheer stress and temperature [15].

Refractive index:

The refractive index of *moringa* was found to be 1.4560.The results obtained from this study showed that the refractive index value of *moringa* lies within the range recommended by SSMO (2003). It reported that the refractive index varies with the specific gravity. Highest values were obtained with larger molecular weight oils. It was also found that increase in saturation caused an increase in refractive index value.

Moisture content:

The moisture content of *moringa* was found to be 4.0. Ahmed reported a lower value of 3% for sunflower seeds. The moisture content of *moringa* lies within the range of 3.9%-13.2% as reported by other researchers [1].

Table 3: Chemical properties of extracted oils of moringa oleifera

Property	MSO
Free fatty acid value	0.08
Acid value (mg KOH/g of oil	0.16
Saponification value	184.93
Iodine value	120.1
Peroxide value	9

Free fatty acids:

The percentage of *moringa* oil free fatty acids (FFA) as oleic acid, were calculated. The result was shown in table 3. Free fatty acid of 0.08 was found in the present study for *moringa* oil. Acid value for fresh oil extracted from stored sunflower seeds were reported to be 2.112 [2]. Acid value for virgin groundnut oil should not be more than 4mg/KOH/g oil while that of non virgin oil should not be more than 0.6mg KOH/g oil as recommended by Codex Alimentarius Commission (1993).

Saponification value:

Saponification value (SV) is an index of average molecular mass of fatty acid in the oil sample. The SV value obtained for the oil sample in Table 3 showed 184.93 mg KOH/g. The values are below the expected range of 195–205 mg KOH/g of oil for edible palm oils as specified by SON (2000) and NIS (1992). The lower value of saponification values suggests that the mean molecular weight of fatty acids is lower or that the number of ester bonds is less. This might imply that the fat molecules did not interact with each other [9]. Higher saponification values indicate shorter chain of fatty acids.

Iodine value:

Iodine value (IV) measures the degree of unsaturation in a fat or vegetable oil. It determines the stability of oils to oxidation, and allows the overall unsaturation of the fat to be determined qualitatively [3][4]. The oxidative and chemical changes in oils during storage are characterized by an increase in free fatty acid contents and a decrease in the total unsaturation of oils. The iodine value of *moringa* oil as iodine/100g oil was found to be 120.1.

Peroxide value:

Peroxide value (PV) is used as a measure of the extent to which rancidity reactions have occurred during storage. It could be used as an indication of the quality and stability of fats and oils [11]. The peroxide value was also found to increase with the storage time, temperature and contact with air of the oil samples. The PV values tabulated in Table 3 for *moringa* oil gave 9 meq/kg. The peroxide value determines the extent to which the oil has undergone rancidity. Peroxide value ranges are closely related to the standard value of 10 meq/kg specified by Standard Organization of Nigeria [26] and Nigerian Industrial Standard [20]. The peroxide value of *moringa* oil was found to be 9. The peroxide value may be taken as an indicator of the extent of primary oxidation products in the oil [8].

Pharmaceutical potential of *Moringa Oleifera* oil extract:

In recent years, the formulation of poorly soluble compounds presented interesting challenges for formulation scientists in the pharmaceutical industries. Up to 40% of new chemical entities discovered by the pharmaceutical industries are poorly soluble or lipophilic compounds which lead to poor oral bioavailability, high intra and inter subject variability and lack of dose proportionality [6].

In the formulation of such compounds, much attention has been focused on lipid-based formulations to improve the bioavailability of poorly water soluble drugs. Among many such delivery options like incorporation of drugs in oils [7] is employed in one of the most popular approaches of self-emulsifying drug delivery systems (SEDDS). SEDDS are mixtures of oils and surfactants, ideally isotropic and sometimes containing co-solvents, which emulsifies spontaneously to produce fine oil in water emulsions when introduced into an aqueous phase under gentle agitation [14]. Self emulsifying formulations spread readily in the gastrointestinal (GI) tract and the digestive motility of the stomach and the intestine provide the agitation necessary for self emulsification. Oils are the most important excipients. Oils help in solubilizing the lipophilic drug in a high amount. It also facilitates self emulsification and increases the fraction of lipophilic drug transported. Oils also increase absorption from the gastrointestinal tract. Both long chain triglycerides and medium chain oils with different degrees of saturation eg corn oil, olive oil, sesame oil, peanut oil, hydrogenated soyabean oil and hydrogenated vegetable oil have been used in the formulation of SEDDS.

Moringa oleifera oil having both long and medium chain trigycerides can be used in the formulation of SEDDS. From the results obtained above, it shows that *moringa* oil extract is a very stable oil less susceptible to oxidation, rancidity and decomposition and can be used in the formulation of a very stable self emulsifying drug delivery formulations. *Moringa oleifera* being very medicinal in the treatment of various ailments, its oil extract can also be an adjunct in combination with some synthetic drugs in the treatment of some health conditions. Its inclusion can help reduce the quantity of some of these synthetic drugs thereby drastically reducing unwanted side effects.

4. CONCLUSION AND RECOMMENDATION

From this study, *moringa oleifera* oil was extracted using standard procedure. The oil was subjected to physicochemical evaluation following standard methods. The results obtained demonstrated its notable good safety and stable profile. Giving the finding in this study, the oil extract from the seed of *moringa oleifera* presents useful application as nutritional supplement and also as possible pharmaceutical adjunct in self emulsifying oil formulations..

Therefore it is recommended that the oil of *moringa oleifera* be employed in the study of formulation of self emulsifying drug delivery system of specific drug to further ascertain its stability, emulsification ability and rate of release of any embedded drug from the formulation.

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