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Evaluation of Ibuprofen Emulsion Formulated Using *Moringa Oleifera* Seed Oil

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ABSTRACT

This study was carried out to evaluate the physicochemical properties of ibuprofen emulsions formulated using *Moringa oleifera* seed oil as the oil phase.

Moringa oleifera seeds were de-hulled, dried ad pulverized into powder. A 100 g quantity was extracted by Sohxlet extraction using petroleum ether. The extracted moringa seed oil was characterized based on refractive index, specific gravity, ash and moisture content. It was used to prepare ibuprofen emulsions using the wet gum method. The prepared emulsions were evaluated based on physicochemical properties such as pH, viscosity and organoleptic properties.

The extracted oil was golden yellow in colour with characteristic odour. It has refractive index, density, and specific gravity of 1.461±0.002, 0.92±0.01 g/dm, and 0.92±0.01 respectively. The ash and moisture contents were 2.86% and 6.42% respectively. The pH of the prepared ibuprofen emulsions were between 4. 9±0.00 and 5.2±0.01 even after 4 weeks of stability study. The viscosity of the emulsions ranged from 1500±0.00-2500±0.01 mPas even after 4 weeks stability study. All the emulsions were the oil-in-water type.

Stable ibuprofen emulsions were formulated using moringa seed oil as the oil phase and either acacia or Tween 80 as the emulsifying agent. The emulsions were comparable in physicochemical properties and stability to those prepared using arachis oil as the oil phase.

KEYWORDS: Ibuprofen, emulsion, moringa seed oil, acacia, Tween 80

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INTRODUCTION

An emulsion is a mixture of two basically immiscible liquids which are thermodynamically unstable. It is a close mixture of two liquids that are immiscible which shows an acceptable shelf life near room temperature. Emulsions are biphasic systems of two liquids that are immiscible, of which one of the liquids (the internal or dispersed phase) is dispersed uniformly as globules within the other phase (the external or continuous phase). Macro emulsions, or simply emulsions, are dispersions of one liquid in another, having globule sizes that range between 1 and 100 µm (which can be expanded to 0.5 µm and 500 µm in special cases). The globules within this size range are usually big enough to sediment under the influence of gravity. The two immiscible liquids are brought together by a third substance called the emulsifier or emulsifying agent. Emulsions are important dosage forms used for poorly soluble drugs. Emulsions are administered through oral, topical and parenteral route[1, 2]. Emulsion can be used to deliver low aqueous soluble drugs orally. For instance, upon oral administration of a drug that is delivered through oily phase of emulsions, the oil droplet is absorbed through normal absorption mechanism of oil, together with the active drug. Also, therapeutically active oil can be given in the emulsion form[3].

Emulsions may be oil-in-water (o/w) or water-in-oil (w/o). Oil-in-water emulsions may occasionally change to water-in-oil emulsions and vice versa and this is called inversion. Multiple emulsions can be prepared in the form of oil-in-water-in-oil (o/w/o) or as water-in-oil-water (w/o/w). These emulsions can also invert however to produce “simple” emulsions. A w/o/w emulsion will produce an o/w emulsion[4].

Ibuprofen is a propionic acid derivative non-steroidal anti-inflammatory drug (NSAID) that is widely used to relieve pain, tenderness, inflammation, and stiffness caused by gout, rheumatoid arthritis and osteoarthritis. It is utilized in the treatment of fever, muscle ache, post-surgical pain and

menstrual pain[5, 6]. Ibuprofen is a nonselective inhibitor of the two cyclooxygenase isoforms (COX-1 and COX-2). The undesirable actions of the drug on the gastrointestinal tract and the aggregation of platelets are caused by the inhibition of COX-1, while the antipyretic, analgesic and anti-inflammatory actions of NSAIDs are due to COX-2 inhibition[7]. Nevertheless, the function of each isoform of COX on antipyretic, analgesic, anti-inflammatory activity, and the severity of gastric damage of NSAIDs is unknown and various compounds can produce various degrees of physiological effects and gastric damage[5, 6]. Ibuprofen is usually; the most readily prescribed and utilized NSAID. The anti-inflammatory properties of ibuprofen may be lesser than that of some other NSAIDs, nevertheless, it's antipyretic and analgesic activities are humongous[7].

Moringa oleifera is a perennial softwood tree having low quality timber. It can be called horseradish tree, drumstick tree or Ben oil tree[8]. Moringa tree is known as Okwe Oyibo by the Igbo people, Ewe Ile by the Yoruba people and Nugyekai by the Hausa people of Nigeria[9]. *Moringa Oleifeira* Lam belongs to a monogeneric family of trees and shrubs called Moringaceae, which is made up of a single genus with known species of which *M. Oleifera* is the most popular and utilized. It is a quick growing, aesthetically pleasing small tree; it can grow up to four (4) meters and can bear fruit within the same first year[9].

It is a vital crop in countries like India, Sudan and Ethiopia, and it is also grown in other parts of the world such as West Africa, Latin America and the Pacific Islands. Different parts of the Moringa tree are eaten by humans as food or medicine. *Moringa oleifera* tree grows abundantly in some parts of Nigeria, both cultivated and in the wild[8].

Moringa oleifera is a plant which has been used extensively to treat certain infections; and as food supplement. *M. oleifera* has salient uses as medicinal agent due to its content of various important antibiotics, antioxidants and nutrients such as vitamins and minerals. Extracts that posses antimicrobial activities have been isolated from various parts of *M. oleifera* such as leaves, bark and seeds[10, 11].

The oil extracted from moringa is called Ben oil and it contains 70% of oleic acid, an 18-carbon long monounsaturated fatty acid (MUFA). Oleic acid has good oxidative stability when compared with polyunsaturated fatty acids (PUFAs). It allows for longer storage and high-temperature frying processing which makes it useful in the food industry[12].

Moringa seed is composed of 36.7% by weight of moringa seed oil. Solvent extraction, especially n-hexane gives almost complete extraction of oil from the seeds, but cold press extraction gives lower yield. Moringa seed oil is golden yellow in colour and liquid at room temperature. The density and refractive index of the oil are not affected by the method of extraction[13]. The oil is edible, and closely resembles olive oil in its fatty acids composition. It possesses significant resistance to oxidative degradation[14]. Moringa seed oil is not widely used in pharmaceutical industry.

This study was done to explore the use of moringa seed oil as pharmaceutical excipient in the formulation of ibuprofen emulsions.

MATERIALS AND METHOD

Materials

Petroleum ether (JHD, China), Ibuprofen (Kores Chemical, India), Tween 80 (Guangdong Guanghua Sci. Tech Co. Ltd., China), acacia (BDH, England), arachis oil.

Collection and Identification of plant material

Moringa oleifera seeds were purchased from Main Market, Onitsha, Nigeria ad it was authenticated by Dr. Ikpefan Emmanuel, Head of Department of Pharmacognosy and Traditional Medicine, Delta State University, Abraka, Nigeria.

Extraction of Moringa seed oil

Moringa oleifera seeds were dehusked, dried and pulverized using a manual milling machine (Corona mill, China), A 150 g quantity of powdered Moringa seeds was extracted at 60^{o}C with petroleum ether using a Soxhlet apparatus for 6 h.

Evaluation of Moringa seed oil

The extracted Moringa seed oil was evaluated based on refractive index, density, specific gravity, percentage ash content and percentage moisture content.

Refractive index: The refractive index was determined using ABBE model refractometer[15].

Density: A known volume of the moringa seed oil was transferred into a pre-weighed density bottle and the new weight was recorded. The density of the oil was calculated using equation 1:

$$Density\ of\ moringa\ oil = \frac{weight\ of\ oil}{volume\ of\ oil} \quad . \quad . \quad . \quad . \quad . \quad 1$$

Specific gravity: This was done using the specific gravity bottle. The specific gravity bottle was weighed. It was filled with water and re-weighed. The specific gravity bottle was filled with the extracted moringa seed oil and re-weighed[15]. The specific gravity of the oil was calculated using equation 2:

$$Specific\ gravity = \frac{weight\ of\ moringa\ oil}{weight\ of\ water} \quad . \quad . \quad . \quad . \quad 2$$

Total ash content: A 2 g quantity of moringa seed oil was weighed in a porcelain dish and put in a furnace set at 600^{o}C to ash. The porcelain dish containing the ash was transferred from the furnace to a desiccator to cool. It was re-weighed and the total ash was calculated using equation 3:

$$Total\ ash = \frac{W_1 - W_2}{W_1} \quad . \quad . \quad . \quad . \quad . \quad 3$$

Where W_1 is the weight of sample (moringa seed oil) and W_2 is the weight of ash

Percentage moisture content: Two gram (2 g) of moringa seed oil was weighed and transferred into a pre-weighed porcelain dish and this was dried in a hot air oven at 105ºC for 1 h. The dried oil was re-weighed. Percentage moisture content was calculated using equation 4.

$$\text{Percentage moisture content} = \frac{W_1 - W_2}{W_1} \quad . \quad . \quad . \quad . \quad . \quad 4$$

Where W1 is weight of porcelain and sample before drying; W2 is the weight of porcelain and sample after drying.

Flow rate: A slight modification of the method of Kolo *et al*[16] was used. A 10 ml quantity of the extracted moringa seed oil was filled in a burette and the time it took the oil to flow through the orifice of the burette was noted. The flow rate was calculated using equation 5:

$$Flow\ rate = \frac{Volume\ of\ burette\ (ml)}{Flow\ time\ (s)} \quad . \quad . \quad . \quad 5$$

Preparation of ibuprofen emulsions

Ibuprofen emulsion was prepared using the wet gum method following the formula in Table 1. The oil, water and emulsifying agent (gum) ratio was 4:2:1. A 7.1 ml of Tween 80 was measured and transferred into a dry mortar and 14.3 ml of distilled water was transferred into the mortar and triturated with the Tween 80. A 0.2 g of methyl paraben was then added and dissolved in the aqueous medium as it is very soluble in water. A 1 g of ibuprofen was dissolved in 28.6 ml of Moringa seed oil and added in small quantities to the aqueous phase. The mixture was consistently triturated in a clockwise direction until the whole quantity of the oil was mixed and the primary emulsion was formed. It was then transferred into a measuring cylinder and made up to the 50 ml volume with distilled water. It was labeled and sealed. The same procedure was repeated for the arachis oil. It was also repeated using acacia as the gum and either arachis oil or moringa seed oil as the oil phase.

Table 1: Composition of ibuprofen emulsions

Ingredients	EM1	EM2	EM3	EM4
Ibuprofen (g)	1	1	1	1
Arachis oil (ml)	28.6	-	28.6	-
Moringa seed oil (ml)	-	28.6	-	28.6
Acacia (g)	-	-	7.1	7.1
Tween 80 (ml)	7.1	7.1	-	-
Methyl paraben (g)	0.2	0.2	0.2	0.2
Water (ml)	14.3	14.3	14.3	14.3
Total (ml)	50	50	50	50

Evaluation of ibuprofen emulsions

The organoleptic properties, pH, viscosity and emulsion type of the prepared emulsion were determined.

Organoleptic properties: The odour, colour and appearance of the emulsions were observed. Organoleptic properties were also observed after 4 weeks of stability studies[11, 17].

Determination of emulsion type: This was done using the drop dilution technique; 5 ml of distilled water was added to the emulsion. The emulsion was then observed for phase separation or total homogeneity of which the latter signifies it is oil in water type emulsion while the former would imply that the emulsion is water in oil type emulsion

pH: The pH meter was calibrated using standard buffer solution thereafter the pH probe was dipped into a beaker containing 50 ml of the emulsion. Upon stabilizing, the reading for pH was recorded. This was done after formulation and upon storage for four weeks[18, 19].

Viscosity: This was done using spindle 3 of a Brookfield viscometer at 28ºC. The spindle was dipped into a beaker containing 50 ml of the emulsion. After rotation, the reading was recorded. This was done after formulation and upon storage for four weeks[20 - 22].

RESULTS AND DISCUSSION

Characterization of moringa seed oil

Refractive index: The refractive index for the extracted moringa seed oil was 1.461±0.002 at 31.5ºC. This is comparable to the values obtained by Shumi and Bacha[23] (1.4625 at 40 ºC), by Babiker and Shakak[24] (1.4640) and by Premi and Sharma[25] (1.436± 0.001). The refractive index was within the acceptable limit (1.4677-1.4705)[26]. The refractive index is the ratio of the velocity of light in a vacuum to the velocity in the medium being measured[23, 24]. It is defined as how fast light propagates through a substance. Refractive index is specific for oils, within certain limits. It is related to the degree of binding / bonding saturation, but it is affected by other factors such as free fatty acids content, oxidation, and thermal treatment[15]. Refractive index affects quite a lot of things about the oil including the absorption profiles. If the absorption profile of the medium is changed, the real part of the refractive index must change as well. Since the composition of the oil changes during aging, the refractive index is also expected to increase. So refractive indices can be used to check for contaminants

Density: The extracted moringa seed oil has a density of 0.92±0.01 g/cm^3. This value was in agreement with that obtained by Babiker and Shakak[24] (0.9190 g/cm^3) and by

Premi and Sharma[25] (0.9073± 0.001 g/cm^3). Density is a measurement that compares the amount of matter an object has to its volume. The oil was found to have a density close to the density of water.

Specific gravity: The extracted moringa seed oil has a specific gravity of 0.92±0.01. The value obtained was within the acceptable limit (0.9-1.16)[26]. Specific gravity is the ratio of the density of a substance to the density of a reference substance in this case water.

The specific gravity of oil rises as the aromatic compounds in it rises but it reduces due to increase in the saturated compounds.

Flow rate: The extracted moringa seed oil has a flow rate of 2.2 ml/s. Flow rate is the volume of fluid which passes per unit time.

% Ash content: Ash content is defined as the inorganic residue that remains after complete combustion of any substance. The total ash value for the extracted moringa seed oil was 2.86%. This value was not in agreement with the value reported by Orhevba *et al*[14] (1.50±0.01). However, the value was below the values reported by Adegbe *et al*[9] (5.00±0.00) and by Leone *et al*[13] (6.2±0.9). The difference in value may be attributed to the different methods of extraction used or the difference in geographical locations where the plants were sourced.

% Moisture content: Moisture content or water content is the quantity of water contained in a substance. The % Moisture content of the extracted moringa seed oil was 6.42%. This is higher than the values obtained by Orhevba *et al*[14] (0.60± 0.07) but lower than that reported by Adegbe *et al*[9] (10.50±0.71) and Leone *et al*[13] (7.0±1.2).

Physicochemical properties of Emulsions

Organoleptic properties: The odour, colour and physical appearance of the emulsions are shown in Table 2. The odours of the different emulsions were characteristic of the oils used in their formulation. Emulsions prepared with tween 80 (EM1 and EM2) were white colour in colour. Formulations prepared with acacia were either light brown (EM) or ash in colour (EM4). The formulations were stable except for formulation EM4 that creamed. Creaming may be useful in special cases where concentration of emulsion is required even though generally, it has deleterious effects during storage and handling. The probability that coalescence will take place is enhanced by close proximity of the globules in a creamed emulsion and the tendency that the emulsion will invert also increases. Creaming is not a serious problem, because it can be overcome by simply shaking the emulsion but it is undesirable because it might cause the patient to take incorrect dose if not properly shaken.

Table 2: Organoleptic properties of ibuprofen emulsion formulations

Formulations	WEEK 0			WEEK 4		
	Odor	**Color**	**Appearance**	**Odor**	**Color**	**Appearance**
EM1	Distinctive smell of arachis oil	White	Good	Distinctive, smell of arachis oil	White	Good
EM2	Distinctive smell of Moringa seed oil	White	Good	Distinctive smell of Moringa seed oil	White	Good
EM3	Distinctive smell of arachis oil	Light brown	Good	Distinctive smell of arachis oil	Light brown	Good
EM4	Distinctive smell of Moringa seed oil	Ash	Good	Distinctive smell of Moringa seed oil	Ash	Good

pH: As shown in Table 3, the pH of the emulsion ranged from 4.9±0.00 to 5.2±0.01 for the freshly prepared emulsion and after 4 weeks. Ibuprofen has an acidic pH of 4.43 so from the pH it can be seen that the drug had direct impact on the acidity and acidic emulsions generally produces O/W emulsions. As a result they might be phase inversion of the emulsion. Acidic drugs are best absorbed in the alkaline medium of small intestine with higher pH.

The pH remained the same after storage for four (4) weeks indicating the stability of the preparation. The measurements of the pH values of freshly prepared formulations presented showed insignificant change after one month of observation at room temperature. This perhaps indicates that there was very minimal or no interaction among various ingredients used in the formulation and also no microbial degradation. Insignificant changes in pH of pharmaceutical products indicate chemical stability[27].

Viscosity: The viscosity of emulsions was between 1500±0.00 and 2500±0.01 mPas (Table 3). All emulsions maintained the same viscosity after storage for four weeks. This indicated good stability.

Emulsion type: All the prepared emulsions were of the oil in water type (Table 3).

Table 3: Physicochemical properties of Ibuprofen emulsion formulations (n = 3)

Formulations	pH	pH (after 4 weeks)	Viscosity (mPas)	Viscosity (after 4 weeks) (mPas)	Emulsion Type
EM1	5.2±0.00	5.2±0.00	1500±0.01	1500±0.01	O/W
EM2	5.1±0.02	5.1±0.01	2000±0.00	2000±0.02	O/W
EM3	5.2±0.01	5.2±0.00	1500±0.02	1500±0.00	O/W
EM4	4.9±0.00	4.9±0.00	2500±0.01	2500±0.00	O/W

CONCLUSION

This research work showed that moringa seed oil was successfully used as the oil phase in the formulation of stable ibuprofen emulsion using either acacia or Tween 80 as the emulsifying agent. Ibuprofen emulsions prepared using moringa seed oil was comparable in stability to those prepared using arachis oil as the oil phase.

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