

# Formulation, Evaluation and Anti-Hemorrhoidal Activity of Suppositories Containing *Moringa Oleifera* Lam. Seed Oil

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## ABSTRACT

The plant *Moringa oleifera* has been reported to have various ethnomedicinal uses, of particular interest is the anti-inflammatory effect of the seed oil. In this study, suppository formulations containing Moringa seed oil (MSO) were developed for the management of inflammatory conditions of the anorectal region. The suppositories were prepared using a water soluble base, macrogol (MG) and a fatty base, dika fat (DF), obtained from *Irvingia gabonensis* seeds; they were evaluated for appearance, hardness, weight variation, melting point, pH, liquefaction time and *in vitro* release according to standard pharmacopoeia procedures. Anti-hemorrhoidal activity of the formulations in laboratory rats were also evaluated. Results show that all the suppositories prepared had good physicochemical properties. *In vivo* studies revealed that the optimized preparation containing dika fat was effective in reducing hemorrhoids induced in rats. Therefore, this study demonstrates the propensity of Moringa seed oil suppositories in the treatment of anorectal inflammatory conditions.

**Keywords:** Moringa seed oil; suppositories; Dika fat; Macrogol; Anti-inflammatory.

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## INTRODUCTION

The plant *Moringa oleifera* Lam. is a species of the family, Moringaceae; it is native to South Asia (India, Pakistan, Bangladesh and Afghanistan) but has been cultivated in the Philippines and the Sudan, Latin America and Africa (Fahey, 2005). In Nigeria, *Moringa oleifera* has become naturalized and is popularly known as “Okwe-beke” by the Igbos, “Zogale” by the Hausas, and “Ewe igbale” by the Yorubas) (Evbuomwan, Dick, & Chioma, 2017).

The plant is called the “miracle tree” because of its action against a wide range of ailments. Preparations of the various parts of the plant have been reported to have antimicrobial, anti-inflammatory, anticancer, hepatoprotective, antioxidant, cardiovascular, antiepileptic, antidiabetic, diuretic, anthelmintic, antiulcer and wound healing activities (Mishral et al., 2011; Amrutia, Lala, Srinivasa, Shabaraya, & Moses, 2011; Gupta et al., 2012; Rastogi, Bhutda, Moon, & Aswar, 2009).

The seeds particularly possess constituents that make them useful in the treatment or management of anti-inflammatory diseases (Saini, Sivanesan, & Keum, 2016), traditionally, the seed oil has been used for the treatment of rheumatism, warts, arthritis, mineral and vitamin deficiency (Fahey, 2005; Mishral et al., 2011). Moringa seed oil can be obtained from the seed kernels using organic solvents like n-hexane; the extracted oil is pale yellow, sweet, non-sticky, non-drying and resistant to rancidity (Olaleye & Kukwa, 2018; Lalas & Tsaknis, 2002). This fatty oil contains palmitic, behenic, stearic and arachidic acids as major parts of its fatty acid contents in addition to small traces of cerotic, lignoceric, myristic, margaric, erucic and caprylic acids. The oil also contains oleic acid as the predominant fatty acid i.e. 73.57 % of the total fatty acids and about only 1.2 % polyunsaturated fatty acids (Ogunsina et al., 2014).

Studies have shown the anti-inflammatory properties of Moringa seed oil; Suryadevara, Doppalapudi, Sasudhar, Anne and Mudda (2018) developed a cream formulation using Moringa seed oil and found that the cream reduced carrageenan-induced paw edema by 70 %, which was similar to that reported for Ibuprofen. In a similar study by Somnath et al. (2015), microemulsion formulations of Moringa seed oil were also found to reduce carrageenan-induced paw edema for up to 3 h. Another study showed that the hydro-alcoholic extract of *M. oleifera* seeds and its chloroform fraction were able to reduce acetic-acid induced colitis in rats (Minaiyan, Asghari, Taheri, Saeidi, & Nasr-Esfahani, 2014).

Hemorrhoids are in essence a cluster of tissues and muscles that line the anal canal, but they are inappropriately used as such when these tissues and muscles become swollen or inflamed. Symptoms of “hemorrhoids” include rectal bleeding,

pain, protrusion and treatment is often initiated by insertion/application of non-steroidal anti-inflammatory drug products into the rectal region (Perry, 2019). Herbal remedies have also been exploited in the management and treatment of hemorrhoid inflammation (Eshghi et al., 2010; Hamidpour & Rashan, 2017).

Suppositories are solid bodies of various weights and shapes, adapted for introduction into the rectal, vaginal, or urethral orifice where they melt, soften, or dissolve at body temperature to release the stored drug. They are usually formulated using lipophilic or hydrophilic bases (Goodman, 2001). Incorporation of Moringa oil in this dosage form could be used in managing such diseases like “hemorrhoids”.

Therefore, the aim of this study was to formulate oil extracted from Moringa seeds into suppositories using water soluble base; macrogol (MG) and fatty base; dika fat (DF) and to investigate the anti-hemorrhoidal activity in laboratory rats.

## **METHODOLOGY**

Plant materials used include *Moringa oleifera* and *Irvingia gabonensis* seeds. Other materials used were liquid paraffin, Polyethylene glycol 1000 & 4000 (Emprove EXP, Merck Germany), Petroleum ether (Loba chemie, India), Sodium hydroxide, Hydrochloric acid and Sodium dihydrogen orthophosphate (Analar, Germany), Nutrient Agar (Sigma Life Sciences, USA), Ferric chloride (Sigma Aldrich, USA) and Distilled and Deionised water (National Institute for Pharmaceutical Research and Development Laboratory, NIPRD, Abuja, Nigeria).

### **Animals**

Adult Wistar Albino rats (200 – 240 g) were obtained from the Animal Facility Centre (AFC) of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The animals were housed under ambient conditions of temperature  $26 \pm 1$  °C and light approximately 12/12 h light/dark cycle. They were fed on standard rodent diet with free access to clean drinking water from the Municipal water system. The experiments were carried out on animals handled according to the Institutional Animal Ethical committee guideline as reflected in the Institutional SOP No 05:003.

### **Extraction**

#### *Extraction of dika fat from Irvingia gabonensis seeds*

*Irvingia gabonensis* seeds were purchased from Karmo market, Abuja, Nigeria. They were validated at the herbarium section of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja where a voucher sample (NIPRD/H/6983) was obtained. The seeds were milled and 490 g of the pulve-

ried seed was weighed (Mettler Toledo, Switzerland), and then macerated with petroleum ether at a ratio of 1 to 5. The mixture was left for about 72 h after which the supernatant was decanted, filtered and concentrated over a water bath (Karl Kobb, Derieich West Germany) at about 100 °C. The resulting extract was weighed and kept at room temperature until further use.

#### *Extraction of oil from Moringa oleifera seeds*

*Moringa oleifera* seeds were obtained from the medicinal garden of the National Institute for Pharmaceutical Research and Development, NIPRD, Abuja. It was identified at the herbarium and a voucher number NIPRD/H/7078 was obtained. A quantity of 300 g of seed was pulverized using a blender (Qlick, Japan) and used for the continuous soxhlet extraction using petroleum ether in the ratio of 1 to 5 at 60 °C. The oil obtained was heated on a water bath (Karl Kobb, Derieich West Germany) at about 100 °C to evaporate the residual solvent. The extracted oil (MSO) was weighed, packaged in a sterile container and stored at room temperature.

#### **Preparation of MSO suppositories using different bases**

Pour moulding method was used for the manufacture of the suppositories in pre-calibrated mould with different bases. Calculated displacement values were used in defining the various final quantities of the bases used. The suppository mould was properly cleaned and lubricated with liquid paraffin. Appropriate quantities of bases and MSO as presented in Table 1 were put into a beaker and allowed to melt at 60 °C on a water bath (Karl Kobb, Derieich West Germany). In the case of macrogol base, an emulsion was initially formed based on required Hydrophilic-Lipophilic Balance (HLB) of MSO, 12. The mixtures were vigorously stirred together at about 50 °C using a magnetic stirrer (VWR Company, Germany) to allow for homogenous mixture. The mixture was poured into the mould until it overflowed; it was re-filled as the solidifying mixture was shrinking. The mould content was allowed to solidify, the suppositories were thereafter removed and packaged in aluminum foil until further evaluations were conducted. This procedure was repeated for production of placebo suppositories as control formulations.

#### **Preparation of suppositories for animal studies**

For the animal studies, 0.3 g of 5 % and 10 % MSO suppositories were prepared, this was done to adjust to the anatomic size of the rats.

**Table 1:** Composition of suppository formulations

Ingredients	MSD1(g)	MSD2(g)	MSD0(g)	MSM1(g)	MSM2(g)	MSM0(g)
Moringa seed oil	5	10	-	5	10	-
Tween 80	-	-	-	4.22	4.74	-
Span 20	-	-	-	0.78	0.26	-
PEG 1000 80 % + PEG 4000 20 %) to	-	-	-	98.4	98.4	98.4
Dika fat to	80.2	80.2	80.2	-	-	-

MSM0 = macrogol base alone, MSM1 = 5 %w/w Moringa seed oil + macrogol base, MSM2 = 10 %w/w Moringa seed oil + macrogol base, MSD0 = dika fat base alone, MSD1 = 5%w/w Moringa seed oil + dika fat base, MSD2 = 10 %w/w Moringa seed oil + dika fat base.

## Evaluation of Suppositories

### Appearance

Six suppositories were randomly selected from each group including placebo and they were observed as an intact unit and also after splitting them longitudinally. Colour, odour, shape, the absence of fissuring, pitting, exudation, sedimentation and the migration of the active ingredients were also assessed.

### Weight uniformity

The weight uniformity test was carried out as designated in the British Pharmacopoeia (BP, 2013). Twenty suppositories were randomly chosen from each batch of the formulations and weighed independently using an analytical balance (Mettler Toledo, Switzerland). The average weights and standard deviations were calculated.

### Determination of pH

The pH of each melted suppository was determined by a pH meter (Jenway, UK). All measurements were an average of three measurements and expressed as mean  $\pm$  standard deviation.

### Hardness/ Crushing Strength

The crushing strength, a measure of mechanical power or hardness of the sup-

pository was determined using the hardness tester (Erweka GmbH, Germany). Six suppositories randomly selected from each batch were used for the measurement. The weight at which each suppository cracked was documented in Kilogram force and converted to Newton.

### **Liquefaction Time**

Six suppositories were indiscriminately chosen from each lot for this test. Thereafter, 60 mL of phosphate buffer with a pH of 7.4 was heated up to  $37 \pm 1$  °C and maintained. Each suppository was dropped inside the buffer and the time taken for the suppository to completely dissolve or melt was noted as the liquefaction time.

### **Melting point Determination**

The melting point of MSO suppositories were determined according to the technique of Adebayo and Akala (2005). A suppository randomly selected from each batch was put in a beaker with a thermometer introduced. The beaker was immersed in a water bath (Karl Kobb, Derieich West Germany) at about 6 cm depth, controlled to a steady temperature rise of 1 °C/2 min. The temperature at which the suppository sample began to melt was taken as the melting point. The outcome was an average of five determinations. The melting point of the placebo was also determined in a similar fashion.

### ***In-vitro* release**

The release of MSO from suppository bases was determined using agar diffusion method (Aremu et al., 2019). A quantity of 0.25 mL of melted suppository was measured into a 25 mL volumetric flask and made up to 25 mL with phosphate buffer, then mixed thoroughly. Sterilized nutrient agar was poured into a plate and left to solidify, the surface of each plate was flooded with a dye and the extra solution was drained off. Two holes were bored in these plates using a 6 mm cork borer, and 0.5 mL of 0 %, 5 % and 10 % w/w of MG and DF formulated suppositories were respectively placed in the holes. The plates were then placed on a laboratory bench for 1 hour for diffusion to occur before being transferred to the incubator (Karl Kobb, Derieich West Germany) at 37 °C. The zones of colour change were measured for each sample at time intervals of 1 h, 2 h, 3 h and 12 h.

### **Fourier-transform infrared spectroscopy (FTIR)**

The method of Kauss et al. (2013) was adopted in preparation of the pellets. The suppositories were ground, triturated with potassium bromide and compressed into pellets. Infra-red spectra were obtained from the impact 410 Nicolet FTIR spectrometer (Thermo fisher Scientific, USA) between frequency range of 4000 and 650  $\text{cm}^{-1}$ .

## Gas chromatography-mass spectrometry (GC-MS)

The method of Okhale et al. (2018) was adopted. Each component was recognized by matching their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11).

## Animals

Adult Wistar Albino rats (200 – 240g) were obtained from the Animal Facility Centre (AFC) of the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja. The animals were housed under ambient conditions of temperature  $26 \pm 1$  °C and light approximately 12/12 h light/dark cycle. They were fed on standard rodent diet with free access to clean drinking water from the Municipal water system. Ethical permission for the study was obtained from NIPRD Animal Care and Ethics (NIPRD/05:3:05-03) in line with International Guiding Principles for Biomedical Research involving animals (CIOMS/ICLAS, 2012).

## Studies on croton oil induced haemorrhoids in Wistar Albino rats

Twenty-five overnight fasted rats were randomly placed into 5 groups of 5 animals each. Group 1 served as the sham group. Hemorrhoids were induced in animals in group 2 – 5. The hemorrhoid inducing agent was prepared using deionized water, pyridine, diethyl ether, and 6 % croton oil in diethylether in the ratio of 1:4:5:10. The inducing agent (0.16 mL) was dropped onto sterile cotton swab of 4 mm diameter and was carefully inserted through the anal opening up to a length of 20 mm. This was held in place for 10 seconds after which the cotton swab was removed. Twenty-four hours after induction, animals were treated as follows: Group 2 served as negative control and received no treatment, group 3 was treated with the suppository (0 % MSO), group 4 received suppository with 5 % Moringa seed oil, while group 5 was administered 10 % Moringa seed oil suppository. The suppositories were administered daily for 5 days. Twenty-four hours after administration of the last dose, the animals were euthanized by diethyl ether inhalation. Thereafter, the distal 2 cm of the anal region was isolated and weighed on a digital balance (Mettler Toledo – SNR 1113092341). The tissues were subsequently preserved in 10 % buffered formaldehyde solution for histological examination (Nishiki, Nishinaga, Kudoh, & Iwai, 1988; Azeemuddin et al., 2014).

Recto-anal Coefficient (RAC) was calculated using the formula:

$$\text{Recto-anal Coefficient} = \frac{\text{Weight of Recto-anal tissue (mg)}}{\text{Weight of animal (g)}}$$

## Statistical analysis

Values are presented as Mean  $\pm$  SEM and analyzed by one-way ANOVA followed by Dunnet's post Hoc test. Level of significance was set at  $p < 0.05$ .

## RESULTS AND DISCUSSION

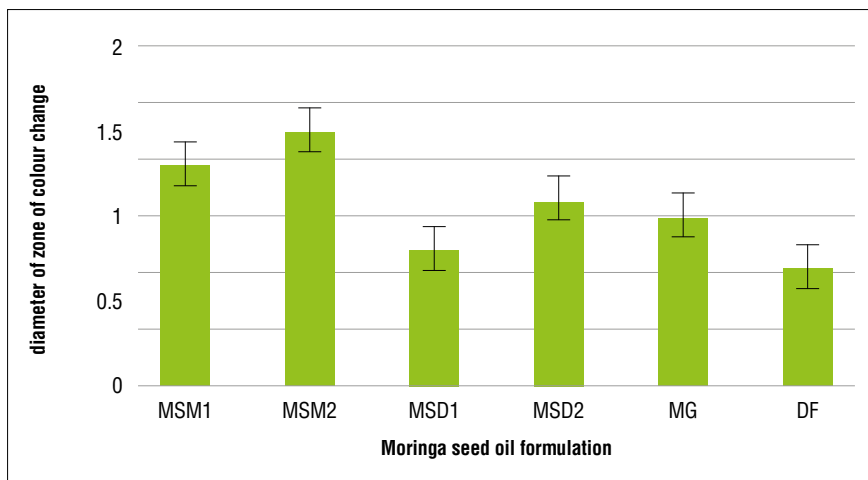
**Table 2.** Physicochemical properties of Moringa seed oil suppositories

Parameters	MSM1	MSM2	MSD1	MSD2	MSM0	MSD0
Shape	Torpedo	Torpedo	Torpedo	Torpedo	Torpedo	Torpedo
Colour	White	Off-White	Milky	Cream	White	Light-Yellow
Mean Weight (g)	2.39 $\pm$ 0.02	2.32 $\pm$ 0.02	1.97 $\pm$ 0.07	2.02 $\pm$ 0.01	2.39 $\pm$ 0.02	2.32 $\pm$ 0.01
Melting Point ( $^{\circ}$ C)	36.17 $\pm$ 0.49	36.73 $\pm$ 0.59	31.53 $\pm$ 0.15	31.80 $\pm$ 0.15	37.43 $\pm$ 0.45	32.27 $\pm$ 0.21
Hardness (N)	19.6 $\pm$ 2.0	13.7 $\pm$ 2.0	12.3 $\pm$ 0.3	10.7 $\pm$ 1.1	22.2 $\pm$ 1.1	16.0 $\pm$ 1.1
Liquefaction time (min)	27.3 $\pm$ 0.89	24.2 $\pm$ 0.55	40.5 $\pm$ 0.46	30.1 $\pm$ 0.42	32.4 $\pm$ 0.45	36.0 $\pm$ 0.42
Displacement value	0.65	-	0.74	-	-	-
Ph	6.37 $\pm$ 0.01	6.67 $\pm$ 0.02	5.12 $\pm$ 0.01	5.20 $\pm$ 0.15	5.97 $\pm$ 0.09	5.08 $\pm$ 0.03

MSM0 = macrogol base alone, MSM1 = 5 %w/w Moringa seed oil + macrogol base, MSM2 = 10 %w/w Moringa seed oil + macrogol base, MSD0 = dika fat base alone, MSD1 = 5 %w/w Moringa seed oil + dika fat base, MSD2 = 10 %w/w Moringa seed oil + dika fat base.

The rate at which the active ingredient is released from the suppository is shown in Figure 1





**Figure 1.** Average diameter of zone of colour change for prepared suppositories

**Table 3.** Effects of Moringa Oil suppository on Recto-anal Coefficient

Group	Treatment	Recto-anal coefficient
1	Sham	1.94 ± 0.15 <sup>c</sup>
2	Control	3.50 ± 0.30
3	MSD0	3.20 ± 0.17
4	MSD1	2.26 ± 0.17 <sup>b</sup>
5	MSD2	2.05 ± 0.38 <sup>b</sup>

Values are presented as Mean ± SEM (n = 5),

Significance compared to control, <sup>b</sup>p < 0.01, <sup>c</sup>0.001 groups (One-way ANOVA, Dunnet's Post Hoc)

The extraction yield of of dika fat from the seeds was 40.16 %w/w while that of Moringa oil was 24 %w/w. This value is lower than already reported (Efeovbokhan, Hymore, Raj, & Sanni, 2015; Eman & Muhamad, 2016; Siyanbola et al., 2015) and could be attributed to the difference in extraction solvents used. Dika fat (DF) was light yellow with its characteristic odour, Moringa seed oil (MSO) was pale yellow but with a characteristic peanut odour.

When the suppositories were split longitudinally, it was observed that there was absence of fissures, the suppositories were stable and had uniform colour. There

was also absence of sedimentation and exudation indicating uniform suppository mix. All the suppositories had uniform weight; not deviating from the average by more than 5 % as specified by the British pharmacopeia (BP, 2013). This indicates that the pouring of the suppository mixture into the mould was accurately done. Uniformity of weight is of importance in the formulation of drugs as it ensures that the required osr specified amount of drug reaches the site of action.

Hardness/crushing strength is a key parameter assessed in pharmaceutical formulations as it indicates the degree to which a particular formulation resists mechanical wear and tear during handling and transportation. The results of the crushing strength of the placebo in order of their strengths were MSM0 ( $22.2 \pm 1.1$ ) > MSD0 ( $16.0 \pm 1.1$  N) while those containing medicaments were MSM1 ( $19.60 \pm 2.0$ ) > MSM2 ( $13.70 \pm 2.0$  N) > MSD1 ( $12.30 \pm 0.3$ ) > MSD2 ( $10.70 \pm 1.1$  N). Generally, hardness/crushing strength of suppositories should be at least 1.8-2 kg pressure; it was observed that suppositories without Moringa seed oil (MSO) were stronger than those containing the incorporation of the MSO. Based on this, the suppositories with the macrogol base can be said to stand a better chance of withstanding rigorous handling and other mechanical conditions.

The pH of a pharmaceutical preparation is not to be neglected as it indicates compatibility of the preparation with the site of action. The pH of the macrogol based suppositories was similar to that of the rectum which is between 6 and 8, while the dika fat based suppositories had a slightly acidic pH (5.12- 5.20), which may likely irritate the rectal mucosa.

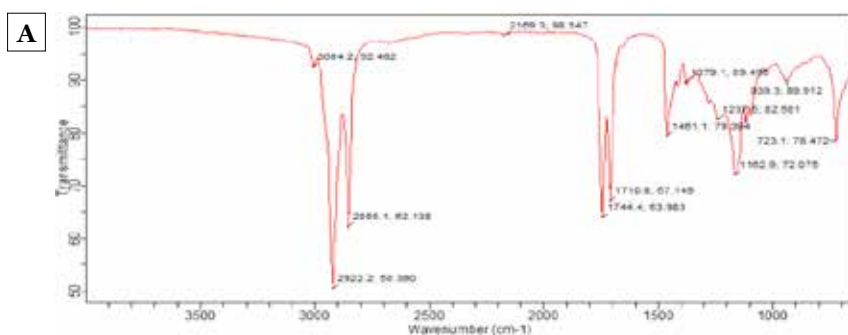
One of the most important characteristics of suppository bases is stability at room temperature, that is, it should not melt at room temperature but melt or dissolve at body temperature in order to release the active ingredient. Generally, the melting point should be less than or equal to 37 °C. It can be observed that the inclusion of the Moringa seed oil reduced the melting point of the suppositories. The melting point of the suppositories in increasing order are MSM2 ( $36.73 \pm 0.59$ ) > MSM1 ( $36.17 \pm 0.49$ ) > MSD2 ( $31.80 \pm 0.15$ ) > MSD1 ( $31.53 \pm 0.15$ ). These values were observed to be lower than the melting point of MSM0 and MSD0 (suppositories without Moringa seed oil).

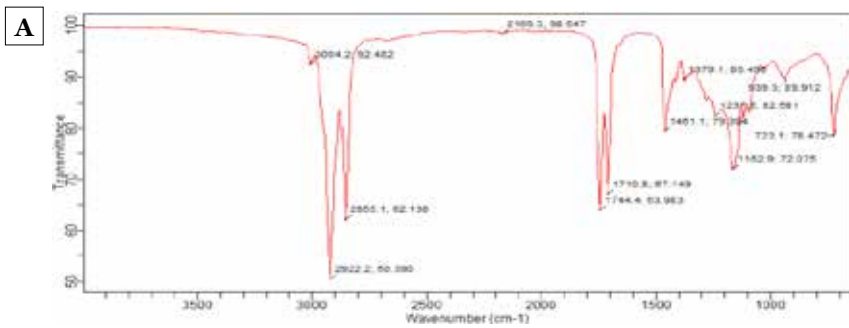
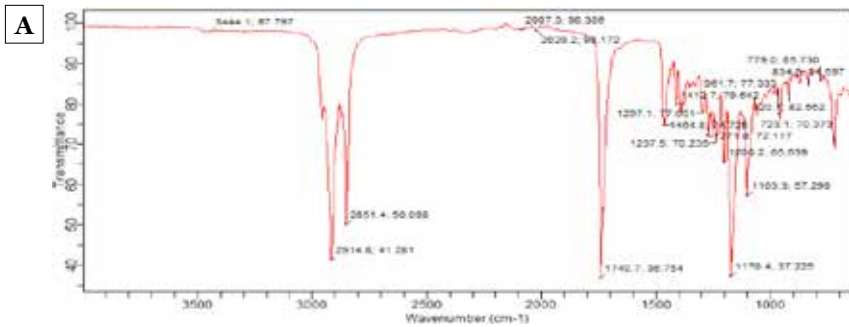
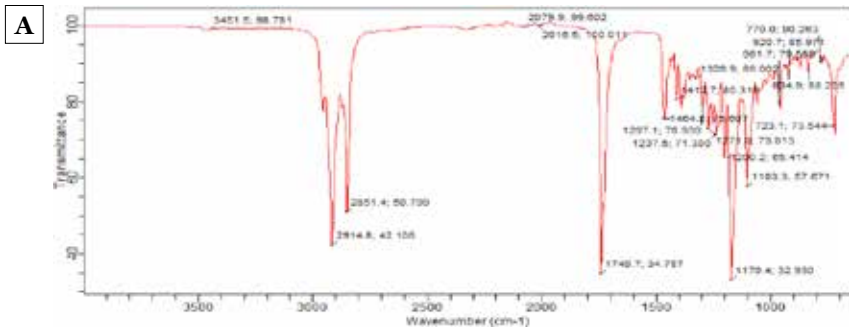
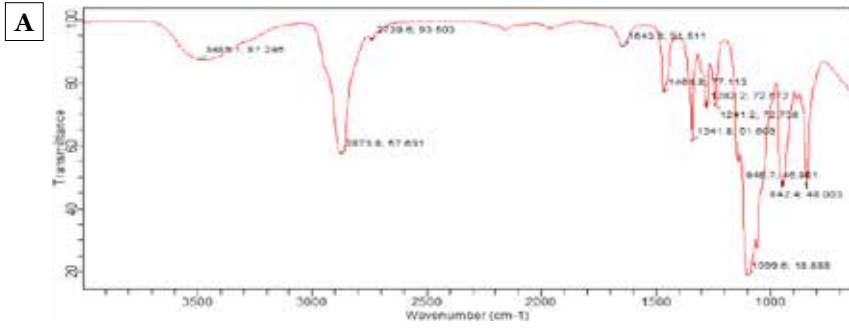
The liquefaction time is the time taken for a suppository to melt or dissolve at body temperature. The liquefaction time is a function of the melting point. It is important to note that the liquefaction time of suppositories should take no longer than 30mins (Mosbah & Mokhtar, 2016). The liquefaction time exhibited by the suppositories was satisfactory except for the MSD1 which had a liquefaction time above 30mins. A suppository which does not melt or dissolve within 30mins

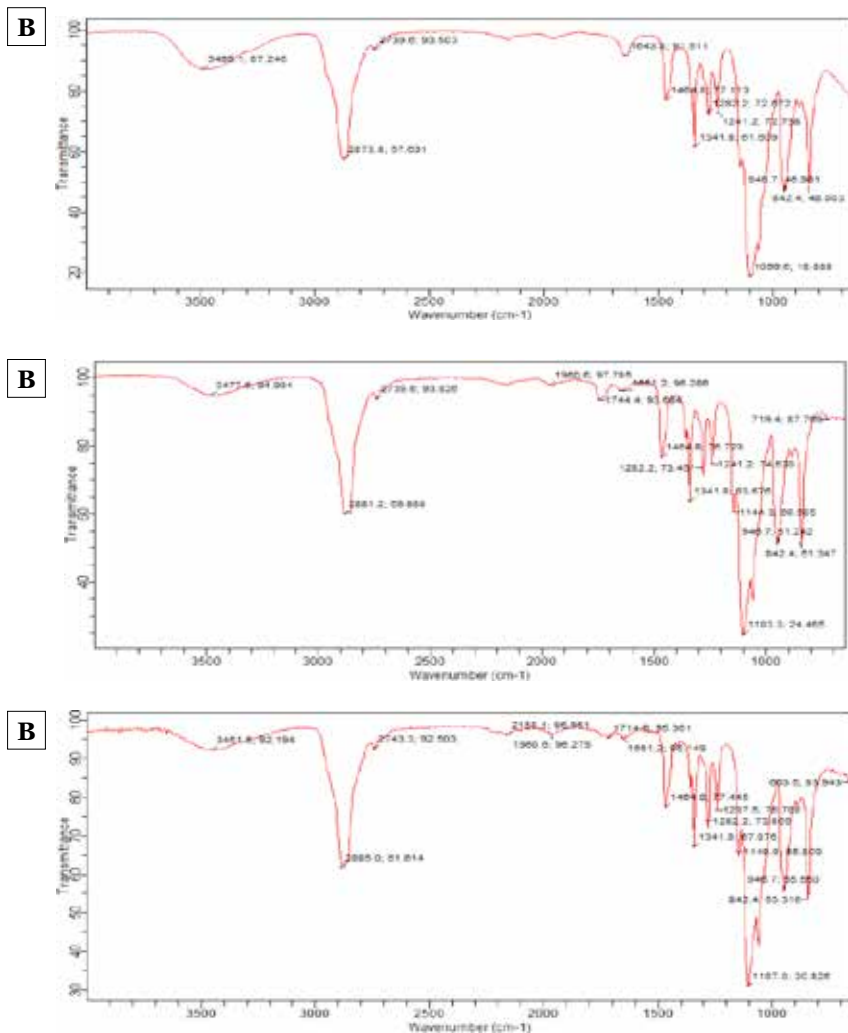
would take a longer time to elicit its action (Taha, Zaghloul, & Kassem, 2003).

In the release study carried out, it was observed that the zone of colour change increased with time. The suppositories with macrogol base had a wider zone of colour change compared to suppositories with dika fat. Generally, lipophilic drugs formulated with hydrophilic bases tend to release faster than those formulated with lipophilic bases, which is due to less affinity for the base as seen in this study.

FTIR spectrum of Moringa seed oil (Figure 2) shows prominent peaks at 2922 and 2855  $\text{cm}^{-1}$  which correspond to the asymmetric and symmetric C-H bond in the  $\text{CH}_2$  functional group. These sharp peaks could also be attributed to high lipid contents of the seed. The broadband at around 3004.2  $\text{cm}^{-1}$  can be due to the O-H stretching, in addition, the presence of N-H due to amides as a result of high protein content of Moringa seed could also be responsible for the peak observed. Peaks observed at 1744.4 and 1710.8  $\text{cm}^{-1}$  could be attributed to the carbonyl group (C=O) which is due the lipid portion of the seed. The spectrum for macrogol shows characteristic peaks at 3485.1, 2873.8, 1464.8, 1341.8, 1099.6  $\text{cm}^{-1}$  indicating the presence of O-H and C-O functional groups. Dika fat spectrum shows major peaks at 3485.1, 2873.8, 1341.8, 1099.6  $\text{cm}^{-1}$ ; the broad peak at 3485.1  $\text{cm}^{-1}$  connotes the presence of O-H stretch while the others connote the presence of C-H group as is consistent with materials containing high fatty acids content. Incorporation of macrogol into Moringa seed oil revealed loss of the seed oil characteristic sharp peaks at 2922 and 2855  $\text{cm}^{-1}$  which could be an indication of interaction. On the other hand, the major peaks present in Moringa seed oil were observed to be retained when dika fat was incorporated into the seed oil implying the absence of interaction. This justifies the use of dika fat in optimized suppository formulations of Moringa seed oil.

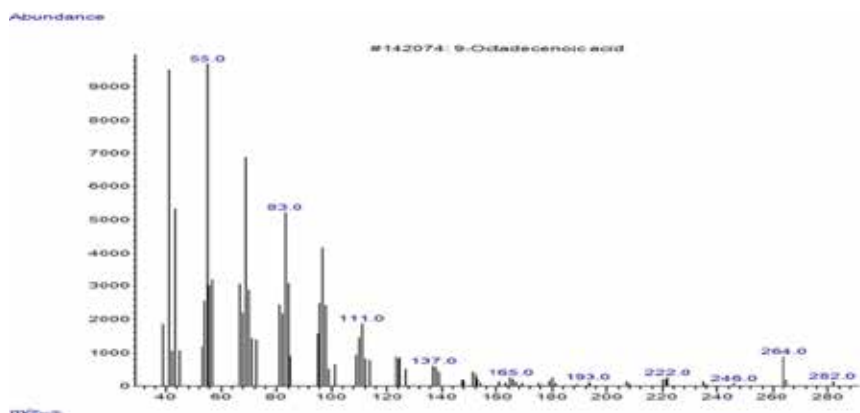






**Figure 2.** FTIR spectrum of moringa seed oil (A1), dika fat (A2), 5 %w/w moringa seed oil+dika fat (A3), 10 %w/w moringa seed oil+dika fat (A4), macrogol (B2), 5 %w/w moringa seed oil+macrogol (B3), 10 %w/w moringa seed oil+macrogol (B4).

GC-MS analysis of Moringa seed oil showed the presence of various compounds with 9-Octadecenoic acid (56.98 %) being more abundant compared to other compounds. 9-Octadecenoic acid has been found to inhibit production of inflammatory agents in RAW 264.7 cells. The compound had an inhibitory effect on nitric oxide and other inflammatory cytokines such as TNF- $\alpha$ , IL-6 (Kang et al., 2018). Another fatty acid found in the GC-MS analysis of seed oil is n-Hexadecanoic acid which through enzyme kinetics study is known to inhibit Phospholipase A(2) which is involved in initiating inflammation (Aparna et al., 2012).

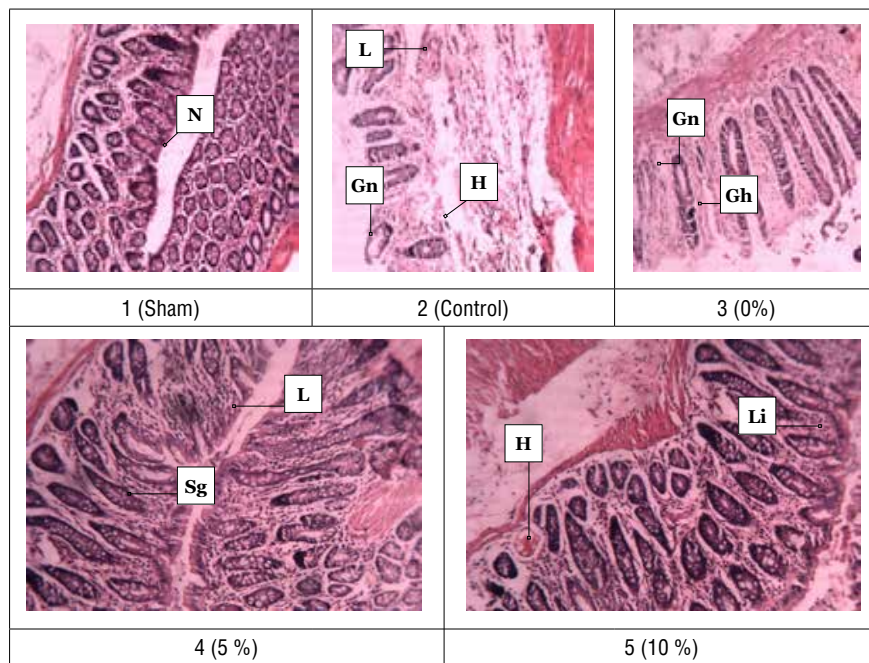


**Figure 3.** Gas chromatography spectrum of Moringa seed oil

Application of the inducing agent to the recto-anal region of rats caused significant increase in the recto-anal coefficient when compared to the sham group. Administration of suppositories prepared with dika fat (MSD1 and MSD2) caused a significant ( $p < 0.05$ ), dose dependent reduction of the RAC at  $2.26 \pm 0.17$  and  $2.05 \pm 0.38$  when compared with control of  $3.50 \pm 0.30$ . (Table 3). Symptomatic hemorrhoids occur when the integrity of the supporting tissues of the recto-anal region deteriorates; the condition is characterized by vasodilatation, inflammation reaction, haemorrhage, thrombosis and necrosis of vascular tissues in the recto-anal region (Sun & Migaly, 2016; Faujdar, Sati, Sharma, Pathak, & Paliwal, 2019). Croton-oil is widely used to induce experimental hemorrhoids in laboratory animals. Treatment with suppositories prepared with Moringa seed oil caused a reduction of the RAC which is an indication of the reduction of inflammation. In other studies, Moringa seed extract has also been shown to demonstrate anti-inflammatory activity in gastric and other tissues (Suryadevara et al., 2018; Minaiyan et al., 2014).

Histological examination showed the tissues of the recto-anal region in control rats presented with haemorrhage, infiltration of inflammatory cells, glandular hardening and necrosis. However, treatment with the suppositories caused an amelioration of tissue injury caused by croton oil. This is observed as reduction of the severity of damage caused to the tissues. Tissues of the recto-anal region in the sham group (group 1) showed normal features, whereas the control (group 2) presented with moderate haemorrhage with infiltration of inflammatory cells and glandular necrosis. Slight glandular hardening necrosis was observed with MSD0 containing no Moringa seed oil (group 3), while the groups treated with MSD1 showed infiltration of inflammatory cells with secretory glands hypertrophy (group 4) and those treated with MSD2 (group 5) presented slight hae-

morrhage and infiltration of inflammatory cells (Figure 4). The biological activity of plant products may be attributed to the component phytochemical compounds as reported by Azeemuddin et al. (2014) and Shivani, Vjayabhaskar, Rao, Kumar, & Yadav (2019) who recorded reduction of RAC and repair of gastric tissues on administration of plant products on croton oil induced hemorrhoids.



**Figure 4.** Effects of Moringa oil suppository on croton oil induced haemorrhoids in Wistar rats.

Li - lymphocyte infiltration, N - normal, H - hemorrhage, SG - normal gland, Gn - Glandular necrosis, GH - glandular hardening

In this study, Moringa seed oil suppositories formulated with macrogol and dika fat exhibited good physicochemical characteristics. *In vivo* anti-inflammatory activity of the optimized formulation show the potential of dika fat as a suppository base for the delivery of Moringa seed oil in the treatment/management of anorectal conditions like hemorrhoids.

#### AUTHOR CONTRIBUTIONS

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### **CONFLICT OF INTEREST**

Authors declare that there is no actual or potential conflict of interest with respect to this article.

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### **ABBREVIATIONS USED**

MSO-Moringa seed oil

DF-dika fat

MG-macrogol

FTIR-Fourier transform infrared spectroscopy

GC-MS-Gas chromatography-mass spectrometry

NIPRD-National Institute for Pharmaceutical Research and Development

HLB-Hydrophilic-Lipophilic Balance

MSM0-macrogol base alone

MSM1-5 %w/w Moringa seed oil + macrogol base

MSM2-10 %w/w Moringa seed oil + macrogol base

MSD0- dika fat base alone

MSD1-5%w/w Moringa seed oil + dika fat base

MSD2-10 %w/w Moringa seed oil + dika fat base



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