Original Article

Formulation and Evaluation of Neem (*Azadirachta indica* A. Juss) Seed Oil Suppositories

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Abstract

Background: Neem seed is very vital because of its rich lipid content and bitter constituents. **Aims:** This study was designed to provide a scientific rationale for the preparation and use of Neem seed oil as suppositories using dika fat (DF), and macrogol (MG), as bases. **Materials and Methods:** The suppositories which were prepared by fusion method using a pre-calibrated mould, were characterized using parameters like appearance, crushing strength, weight variation, melting point, pH, liquefaction time and in-vitro release according to standard procedures. **Results:** Results show that, the suppository strengths were in the order; bland, MG (25.00 ± 1.50N) > DF (12.90 ± 0.72 N), while those containing medicaments were NSM1 (20.00 ± 1.92) > NSD1 (12.90 ± 0.94) > NSM2 (12.70 ± 1.24 N) > NSD2 (10.00 ± 1.35 N). The pH of medicated formulations were NSM1 (6.50 ± 0.01), NSM2 (6.54 ± 0.03) > NSD1 (5.73 ± 0.04) and NSD2 (5.07 ± 0.03). Melting point values show that, macrogol base had mean values of 36.80 °C ± 0.62 and 36.40 °C ± 0.46 for NSM1 and NSM2 respectively, whereas, those with DF gave an average melting point values of 32.10 °C ± 0.87 and 30.90 °C ± 0.79 for NSD1 and NSD2 respectively. **Conclusion:** Results obtained showed that suppositories prepared with Macrogol (MG) base exhibited better physicochemical properties than Dika fat (DF) base suppositories, therefore water soluble bases may be bases of choice in the delivery of neem seed oil.

Keywords: *Anti-inflammatory, dika fat, macrogol, neem seed oil, suppository.*

Introduction

Neem tree also known as Dogonyaro, Indian lilac, wonder tree, and village dispensary, has its place in the family Meliaceae, and is a very significant tree native to India.[1] The plant matures speedily to a great height and yields fruits and flowers in season. It grows in the tropics and is very widespread with several parts of the plant (leaves, roots, fruits, seeds, and bark) having medicinal properties such as antipyretic, antiviral, and antifungal. It has great potential not just in medicine but also in the areas of pesticides and agrochemicals. Other uses include water purification, removal of dyes, timber, firewood, fertilizer, and as animal feed.[2] The plant is able to adapt well to different climatic conditions. The major phytochemical components of neem are terpenes and limonoids. Azadirachtin, meliacin, gedunin, and salannin are constituents that elicit biological activity. There are two naturally occurring species of neem, *Azadirachta indica* A. Juss. and *A. excelsa* Jack, which are native to India, Philippines, and Indonesia.[3]

Neem seed is very vital both because of its rich lipid content and the presence of an enormous number of bitter constituents (azadirachtin, azadiradione, fraxinellone, nimbin, salannin, salannol, vepinin, vilasinin, etc.) in substantial quantities. Neem kernel lipids are analogous to the standard glycerides from other oil seeds and comprise oleic acid (50%–60%), palmitic acid (13%–15%), stearic acid (14%–19%), linoleic acid (8%–16%), and arachidic acid (1%–3%).[4] It is brownish yellow, nondrying oil with an acrid taste and unpleasant odor.

Pharmacological properties of neem seed oil (NSO) are abound in literature, for example, Naik *et al.*[4,5] investigated the pharmacologic and mechanistic properties and reported a dose-dependent increase in anti-inflammatory effect of the NSO in albino rats with a proposal that NSO could act by inhibiting cyclooxygenase. Also Jagadeesh *et al.*[6] investigated the effect of NSO as an anti-inflammatory agent compared to that of indomethacin, a known anti-inflammatory drug, their results showed that NSO exhibited significant anti-inflammatory activity. In addition, NSO showed lower ulcerogenic

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property compared to indomethacin. In a similar study, Akihisa *et al*.[7] reported the isolation and anti-inflammatory properties of 17 limonoids from neem seeds. There have been NSO dermatological dosage formulations reported in literature such as creams[8] and ointments,[9] but there appear to be no rectal route dosage formulations, especially using the bases explored in this study. Therefore, as a result of the impressive anti-inflammatory actions of NSO, a rectal dosage form for the treatment of inflammatory conditions in the rectal region and anus was developed.

The suppository is a solid delivery system designed for placement into the rectum where they melt, dissolve, or disperse, and show a local or a systemic effect. Suppositories can be manufactured by using lipophilic and hydrophilic bases. The advantage of the local administration as suppository is that it can be used when oral delivery is not convenient. In addition, first pass effect is avoided.

The aim of this study was to use the oil hauled out from the neem seed as an active ingredient in an anti-inflammatory suppository formulation using macrogol (MG) and dika fat (DF) as bases.

Materials and Methods

Materials

Irvingia gabonensis seeds were purchased from Karmo market, Abuja, Nigeria and *A. indica* seeds were obtained from National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. Other materials used were *n*-hexane (Lobachemie, Mumbai, India), aluminum foil (Novena foil, China), liquid paraffin, polyethylene glycol 1000 and 4000 (Emprove Exp Merck, Darmstadt, Germany), sodium dihydrogen orthophosphate and sodium hydroxide (AnalaR grade), nutrient agar (Sigma Life Sciences, St. Louis, Missouri, USA), ferric chloride (Sigma-Aldrich, St. Louis, Missouri, USA), and distilled water (NIPRD Laboratory, Nigeria).

Methods

Extraction

The plant material, *I. gabonensis* seeds were purchased from Karmo market, Abuja, Nigeria. The material was validated at the herbarium section of NIPRD, Abuja, where a voucher sample (NIPRD/H/6983) was deposited. The seeds were milled and 380 g of the pulverized seed was weighed out using the analytical balance (Mettler Toledo, Greifensee, Switzerland) and then macerated with *n*-hexane at a ratio of 1:10. The mixture was left for approximately 72 h after which the supernatant was poured out, filtered, and concentrated in water bath (Karl Kobb, Derieich, West Germany) at approximately 100°C, the ensuing extract was weighed and kept at room temperature until further use.

Neem seeds were harvested from the medicinal garden of NIPRD and validated at the herbarium of the institute where a voucher specimen (NIPRD/H/6987) was deposited. A quantity of 200 g of pulverized neem seeds using a blender (Qlick, Japan) was macerated with *n*-hexane at a ratio of 1:10, the mixture was stirred and allowed to stay still for eight days according to the previously used method.[9] The supernatant was decanted, filtered using a Whatman no. 1 filter paper, and concentrated in a water bath (Karl Kobb) at approximately 100°C, the subsequent extract was weighed, packaged in a sterile container, and stored at room temperature.

Preparation of neem seed oil suppositories using different bases

DF from a plant source that is naturally occurring was used to represent lipophilic base, whereas MG was used as a water-soluble base. An oil-in-water emulsion of NSO using surfactants, such as Tween 80 and Span 20, was first made before incorporating into the base. Pour molding method was used for the manufacture of the suppositories in pre-calibrated mold with different bases. Calculated displacement values were used in defining the various final quantities of the bases used. The suppository mold was properly cleaned and lubricated with liquid paraffin. Appropriate quantities of bases and NSO as presented in Table 1 were weighed differently into a beaker and positioned in a water bath (Karl Kobb) at approximately 60°C to melt. In the case of MG base, an emulsion was initially formed based on the required hydrophilic and lipophilic balance of NSO experimentally determined to be 12. The mixtures were vigorously stirred together with melted bases at approximately 50°C, using magnetic stirrer (VWR, Langenfeld, Germany) to allow for homogenous mixture. This procedure was repeated for production of placebo suppositories as control formulations. The mixture was poured into the mold until it overflowed, the top was filled as the solidifying mixture was shrinking. The mold content was allowed to solidify and

Table 1: Composition of suppository formulations

Ingredients (g)	MG	NSM1	NSM2	DF	NSD1	NSD2
Neem seed oil	-	4.92	9.84	-	4.02	8.04
Tween 80	-	4.22	4.74	-	-	-
Span 20	-	0.78	0.26	-	-	-
PEG 1000 (80%) + PEG 4000 (20%) to	98.40	98.40	98.40	-	-	-
Dika fat to	-	-	-	80.3	80.3	80.3

MG = macrogol base alone, NSM1 = 5% w/w neem seed oil + macrogol base, NSM2 = 10% w/w neem seed oil + macrogol base, DF = dika fat base alone, NSD1 = 5% w/w neem seed oil + dika fat base, NSD2 = 10% w/w neem seed oil + dika fat base

the suppositories were thereafter removed and packaged in aluminum foil until further experiments were conducted.

Evaluation of suppositories

Appearance

Six suppositories were indiscriminately chosen from each group, including placebo, and they were observed as an intact unit and likewise after splitting them longitudinally. Color, odor, 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*.[10] Twenty suppositories were randomly chosen from each batch of the formulations, weighed independently using an analytical balance (Mettler Toledo), and the average weights and standard deviations were calculated.

Determination of pH

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

Hardness/crushing strength

The crushing strength, a measure of mechanical power or hardness of the suppository, was determined using the hardness tester (Erweka, Langen, Germany). Six suppositories randomly selected from each batch were used for the measurement. The weight under which each suppository cracked was documented in kilogram force and was converted to Newton.

Liquefaction time

Six suppositories were indiscriminately chosen from each lot, 60 mL of phosphate buffer with a pH of 7.4 was heated up to 37°C ± 1°C and maintained, the 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 NSO suppositories was determined according to the technique of Adebayo and Akala.[11] 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) at approximately 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 melting point. The outcome was an average of five determinations. The melting point of bland suppository bases was also determined similarly.

In vitro release

The release of NSO from suppository bases was determined using agar diffusion method. 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 and then mixed thoroughly. Sterilized nutrient agar was poured into a plate and left to solidify, the surface of each plate was flooded with ferric chloride solution (5% w/v) 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 was respectively placed in the holes. The plates were then placed on a laboratory bench for 1 h for diffusion to occur before being transferred to the incubator (Karl Kobb) at 37°C. The zones of color change were measured for each sample at time intervals of 1, 2, 3, and 12 h, respectively.

pH determination

Three suppositories per lot were used for pH determination. Each suppository was melted or dissolved in 100-mL beaker, and the electrode of the pH meter (Jenway) was dipped into the melted or dissolved suppository and the values obtained were documented.

Gas chromatography–mass spectrometry analysis of neem seed oil

The oil was analyzed by gas chromatography–mass spectrometry (GC–MS) according to the method by Okhale *et al.*,[12] using Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector, Kyoto Japan (operated in the electron ionization mode [electron energy = 70 eV], scan range of 45–400 amu, and scan rate of 3.99 scans/s), and Shimadzu GC–MS solution data system. The GC column was Optima-5 ms fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with a length of 30 m, internal diameter of 0.25 mm, and film thickness of 0.25 µm. The carrier gas was helium with a flow rate of 1.61 mL/min. The program used for GC oven temperature was 60°C–180°C at a rate of 10°C/min, then held at 180°C for 2 min, followed by 18°C–280°C at a rate of 15°C/min, then again held at 280°C for 4 min. The injection port temperature was 250°C, whereas detector temperature was 280°C. Helium was used as a carrier gas, at a flow rate of 1.61 mL/min. Diluted sample (1/100 in hexane, v/v) of 1.0 µL was injected using autosampler and in the split mode with a ratio of 10:90. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11).

Fourier transform infrared spectroscopy

The infrared (IR) spectra of different batches were run as KBr pellets on impact 410 Nicolet Fourier transfer infrared (FTIR) spectrometer in the frequency range 4000-650 cm^{-1}.

Results

Dika seeds gave a percentage yield of 49.5% w/w of the fat, which was obtained as a light yellow solid with characteristic odor. This yield can sustain commercial production.

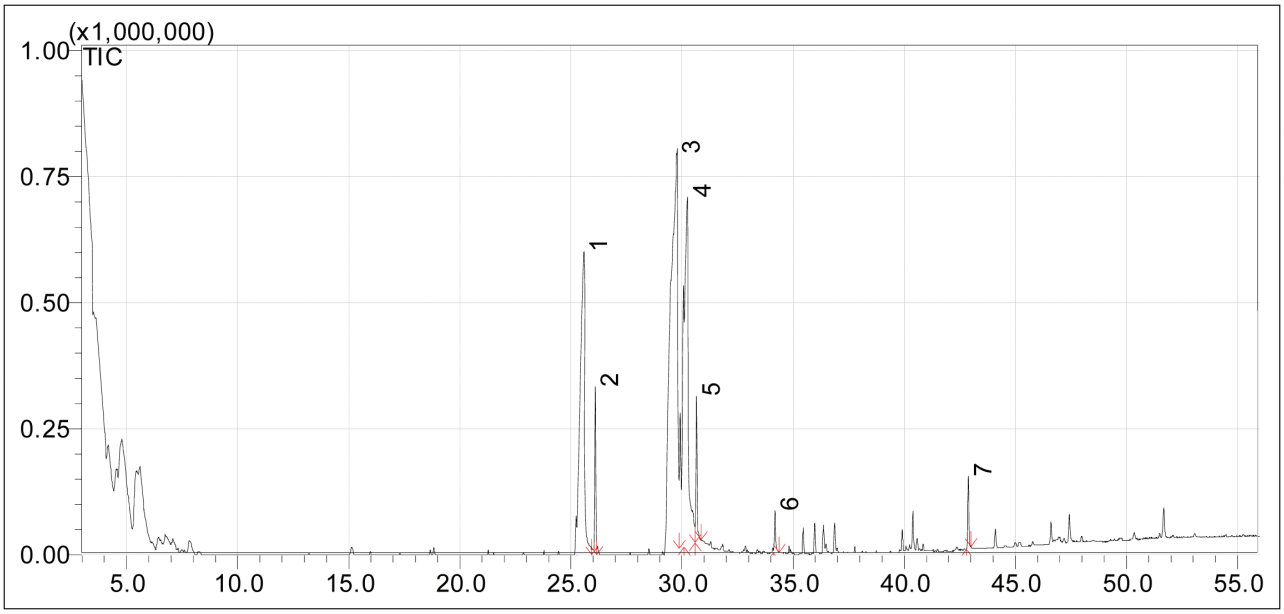


Figure 1: Gas chromatography spectrum of neem seed oil

GC of NSO revealed seven major peaks as shown in Figure 1 and which were identified from NIST library to be octadec-9-enoic acid (43.05%), palmitic acid (21.07%), stearic acid (20.22%), stearic acid ethyl ester (9.88%), palmitic acid ethyl ester (3.50%), eicosanoic acid (0.91%), and squalene (1.37%).

Figure 2 shows the FTIR spectroscopy results obtained for the different suppository formulations with or without their respective bases.

The results of the characterization of NSO suppositories including control formulations are all presented in Table 2.

Medicaments must be available for therapeutic activity from dosage forms at the site of action. For rectal administration, the drug is expected to either diffuse or partition out of the bases after melting, dispersing, or dissolving in body fluids. This has to be followed by local effect or absorption if meant for systemic effect. The release rates of the various formulations in this investigation are presented in Figure 3.

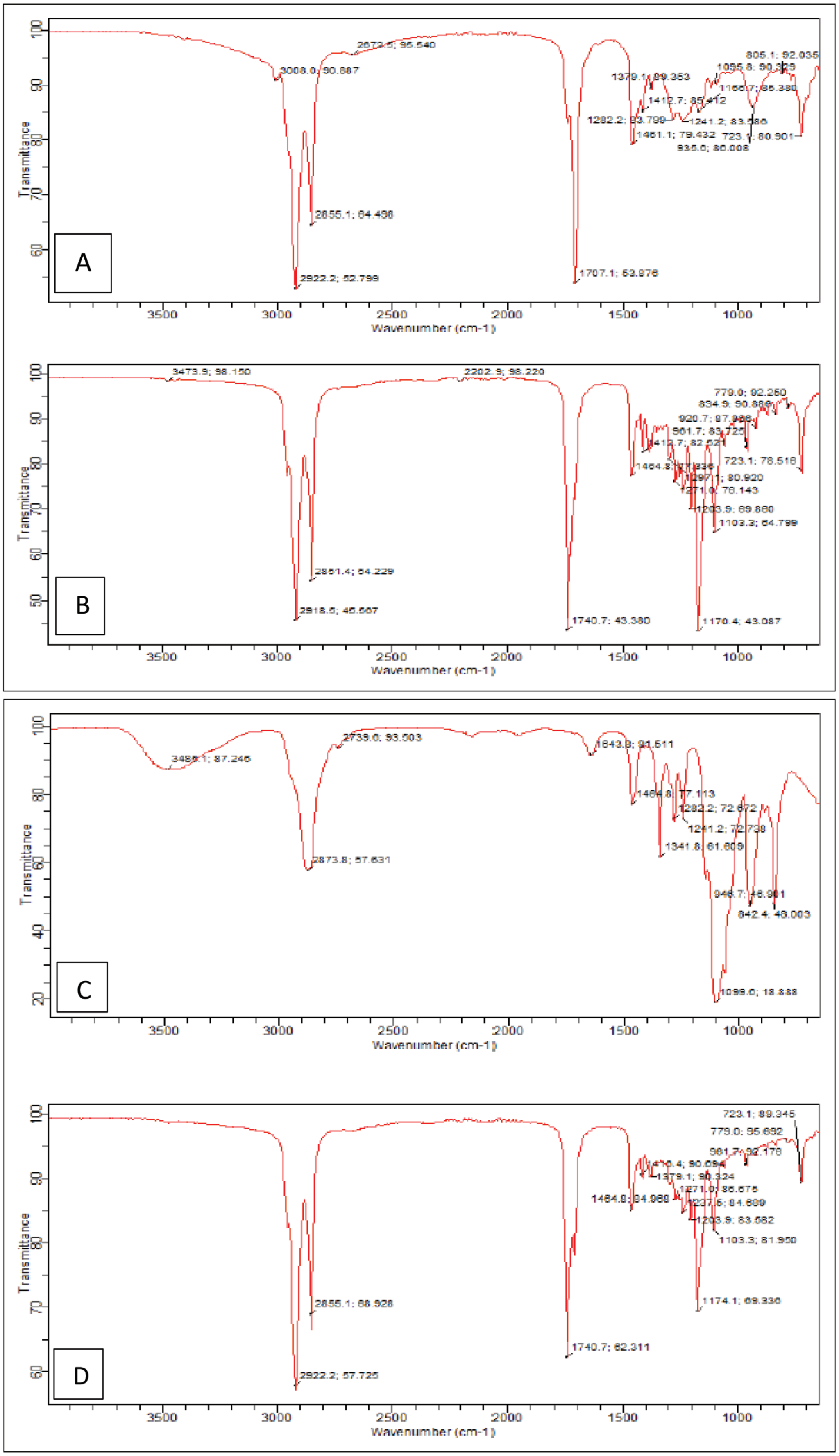
Discussion

DF yields 49.5% w/w. The fat obtained was a light yellow–colored solid with its own characteristic odor. The yield can sustain commercial production because of the availability of the seeds all year round. Neem seed kernel yields an acrid greenish yellow to brown fixed oil with garlic odor. The calculated yield was 24.5% w/w, this agrees with earlier work.[9] The availability of neem tree virtually everywhere makes it imperative for commercial exploitation. We opined that the oil yield can continuously support industrial production of various medicinal products such as suppositories, insect repellant, and dermatologicals.

Fatty acids are broadly occurring in natural fats and dietary oils and they play an imperative role as nutritious substances and metabolites in living organisms. Many fatty acids are well-known to have antimicrobial and anti-inflammatory properties.[13]

Squalene, one of the compounds identified in the GC–MS analysis of NSO, is known to play a part in immunomodulation and wound healing. This is as a result of rise in the generation of anti-inflammatory cytokines, such as interleukin (IL)-10, IL-13, and IL-14, and a consequent decrease in pro-inflammatory cytokines such as tumor necrosis factor-α.[14] Palmitic acid ethyl, which was one of the fatty acid esters identified during the analysis, also possesses anti-inflammatory activity. Stearic acid (20.22%) was one of the known compounds existing in substantial amounts. It has the ability to diminish cholestasis-induced liver injury, which causes inflammation in the liver. The various anti-inflammatory components of NSO justified the formulation as a rectal dosage form.

The FTIR spectrum of the NSO shows sharp peaks between 2850–2930, 1707, and 1451 cm^{-1}, and weak but sharp peaks between 935–1380 and 3008 cm^{-1}. These peaks obtained indicate that –OH, –C=O, –CH2, and –CH3 functional groups are present. All the characteristic peaks observed in NSO spectrum were also present when NSO was incorporated into DF, implying excellent compatibility; in fact, the spectra of NSO and DF are superimposable. It is therefore opined that NSO does not pose any chemical compatibility issues with DF. On the contrary, NSO lost its characteristic peaks when incorporated into MG. Although MG retained its characteristic peak, it slightly reduced from 2873 to 2862 cm^{-1} in the presence of NSO, while losing completely the peaks attributable to NSO. On the basis of the loss of characteristic peaks alone by NSO in MG, MG does not appear as a suitable base for carrying NSO, although the release studies indicating faster release of NSO from MG base does not corroborate this observation, more so the loss of peaks may be due to molecular dispersion. The


A
B
C
D
Transmittance
Wavenumber (cm-1)
2922.2; 52.799
2855.1; 64.496
1707.1; 53.876
2918.5; 45.567
2851.4; 54.229
1740.7; 43.380
1170.4; 43.087
3488.1; 87.246
2873.8; 57.631
1099.6; 18.888
2922.2; 57.725
2855.1; 68.928
1740.7; 62.311
1174.1; 69.336

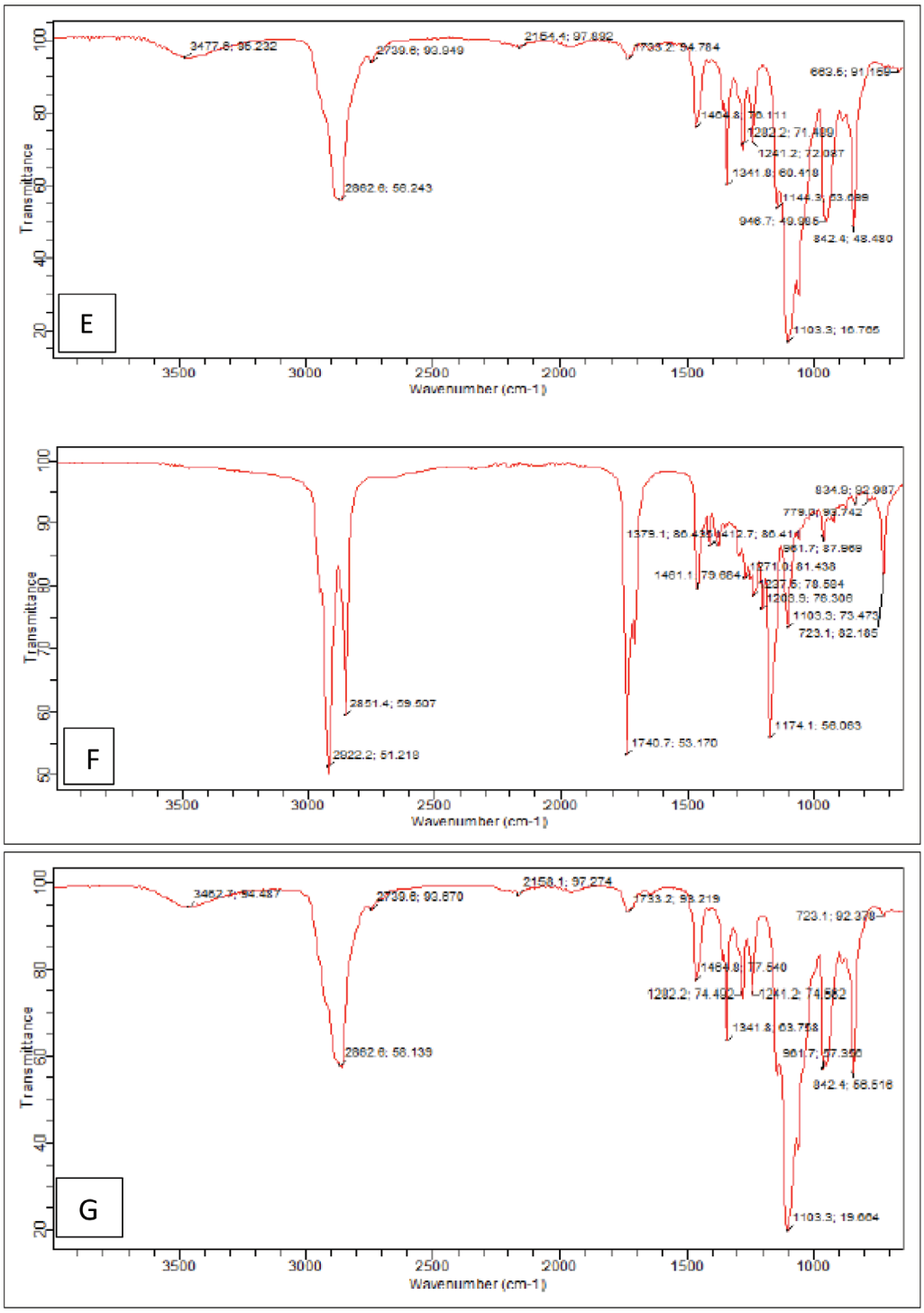


Figure 2: Fourier transform infrared spectrum of neem seed oil (A), dika fat (B), macrogol (C), 5% neem seed oil + dika fat (D), 5% neem seed oil + macrogol (E), 10% neem seed oil + dika fat (F), and 10% neem seed oil + macrogol (G)

absence of peaks related to lipids in MG formulation may be because of covering by MG.

The dissected suppositories were uniform in appearance and contained no air bubbles. There were equally no holes or brittle fracture, and thus the suppositories were not fragile and could withstand transportation and other mechanical exposures. The outcome from the weight variation experiment for all formulated suppositories was found to be within the acceptable range of the standard by the *British Pharmacopeia*.[10] No suppository deviated from the average by up to 5% as presented in Table 2. The relative standard deviations of the average weight of the suppositories were less than 3.5%, indicating accurate standardization of the mold.[15] It is important that there is constancy in the weights or measurements of dosage

Table 2: Physicochemical and release properties of neem seed oil suppositories

Physical and release parameters	NSM1	NSM2	NSD1	NSD2	MG	DF
Shape	Torpedo	Torpedo	Torpedo	Torpedo	Torpedo	Torpedo
Color	Off-white	Cream	Cream	Cream	White	Yellow
Mean weight (g)	2.38 ± 0.01	2.35 ± 0.04	1.96 ± 0.05	1.96 ± 0.03	2.45 ± 0.04	1.96 ± 0.02
Melting point (°C)	36.80 ± 0.62	36.40 ± 0.46	32.10 ± 0.87	30.90 ± 0.79	37.20 ± 0.67	32.40 ± 0.38
Hardness (N)	20.00 ± 1.92	12.70 ± 3.48	12.90 ± 0.94	10.00 ± 1.33	25.00 ± 1.50	12.90 ± 0.72
Liquefaction time (min)	30.60 ± 0.89	22.40 ± 0.55	40.00 ± 0.46	30.00 ± 0.42	30.20 ± 0.45	36.00 ± 0.42
Displacement value	0.61	-	-	0.73	-	-
pH	6.50 ± 0.01	6.54 ± 0.03	5.73 ± 0.04	5.07 ± 0.03	6.08 ± 0.03	4.57 ± 0.02

MG = macrogol base alone, NSM1 = 5% w/w neem seed oil + macrogol base, NSM2 = 10% w/w neem seed oil + macrogol base, DF = dika fat base alone, NSD1 = 5% w/w neem seed oil + dika fat base, NSD2 = 10% w/w neem seed oil + dika fat base

forms so as to ensure accurate dose delivery at the site of action.

The suppositories were assessed for hardness or crushing strength as a degree of their mechanical strength to withstand handling and transportation. The results of the crushing strength of different bases without medicament in Table 2 showed significant difference ($P < 0.05$). The order of the strengths was MG (25.00 ± 1.50 N) > DF (12.90 ± 0.72 N), whereas that of those containing medicaments was NSM1 (20.00 ± 1.92 N) > NSD1 (12.90 ± 0.94 N) > NSM2 (12.70 ± 1.24 N) > NSD2 (10.00 ± 1.35 N). The inclusion of NSO generally contributed to the lowering of the crushing strength as compared with blank bases. In addition increased plasticity (lowered crushing strength) was observed mostly with suppositories prepared using macrogol base. The higher the plasticity of the materials, the less the stress they can withstand. This notwithstanding, formulations with MG base will be capable of withstanding the rigors of handling and transportation.

The pH values of medicated formulations were NSM1 (6.50 ± 0.01), NSM2 (6.54 ± 0.03) > NSD1 (5.73 ± 0.04) and NSD2 (5.07 ± 0.03) as shown in Table 2. The pH of NSO alone was found to be 6.46–7.07.[8] The pH levels of NSM1 and NSM2 appear compatible with the pH of rectum (6–8) unlike those of NSD1 and NSD2 that tend toward acidic. Products with low pH if introduced into the rectum have the tendency of irritating the patient and this may lead to loss of compliance.

It is important for bases to have their softening or melting point above the average room temperature in the tropics to prevent untimely and undesirable melting of suppositories before their use but low enough to melt at the body temperature. Formulations with MG base had mean values of 36.80°C ± 0.62°C and 36.40°C ± 0.46°C for NSM1 and NSM2, respectively, but those with DF gave an average melting point of 32.10°C ± 0.87°C and 30.90°C ± 0.79°C for NSD1 and NSD2, respectively. The lower melting point can be attributed to the inclusion of NSO in the base resulting into weakening of the intermolecular forces of the various fatty acids in the network of both the oil and the fatty base. For the formulations with MG base, this weakening effect appears absent, thereby making it a better base as far as the melting point is concerned. The melting point can withstand storage temperature and at the same time, it will be able to dissolve at body temperature.

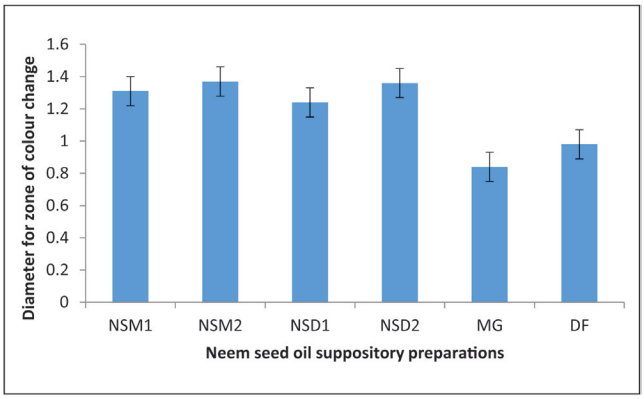


Figure 3: Average diameter for zone of color change for prepared suppositories

The liquefaction time was also evaluated to ensure that the prepared suppositories would dissolve or melt within the body temperature of 37°C so as to enable the release of the active pharmaceutical ingredient to elicit desired pharmacological action. Generally, liquefaction time should not take longer than 30 min.[16] This is important to enhance patient acceptability and prompt release of drug.[17] Formulations with MG base, NSM1 and NSM2, had mean values of 30.60 ± 0.89 and 22.40 ± 0.55 min as shown in Table 2. The introduction of NSO lowered the melting point of the prepared suppositories, thereby lowering the liquefaction time. A suppository that does not liquefy easily may exert irritant action on the rectal mucosa, and release of the drug from the base may take longer time.

The zone of color change of agar increased with time as shown in Figure 3 for all the samples except control formulations (without NSO). All formulations containing NSO showed release with MG base showing higher zone of color change. Lipophilic drug substances are known to have less affinity for hydrophilic vehicle bases and tend to escape out faster at the site of action. Also, the rate of release of phytochemicals from the bases was found to be time dependent. The nature of the base and temperature were found to have effect on the rate of diffusion of active ingredients from different vehicles.[9]

Conclusion

In this study, NSO suppositories prepared with both MG and DF showed fair physicochemical characteristics, although

there was a reaction between MG and NSO according to FTIR result, further studies are necessary to rule out possible molecular dispersion of NSO into the base. Therefore, either DF or MG after further investigation may be used as base in the formulation of NSO as an anti-inflammatory product for alleviation of painful hemorrhoids. The anti-inflammatory effect of the suppositories is ongoing in our laboratory and will form a separate report.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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