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Evaluation of the Physicochemical, Antimicrobial and *in vivo* Wound Healing Properties of Castor Oil-Loaded Nanogels

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ABSTRACT

Medicinal plants are used exclusively to treat and manage different diseases, especially in poor communities. Castor oil was reported to have potent antibacterial, antifungal, and leishmanicidal activity. This study was conducted to assess the physicochemical, antimicrobial, and *in vivo* wound healing properties of castor oil-loaded nanogels. Castor oil was extracted from powdered castor beans by solvent extraction using hexane. The extracted castor oil (oil phase) was combined with Tween 80 (surfactant) and polyethylene glycol (cosurfactant) and was mixed with distilled water as the aqueous phase using the titration method to prepare castor oil-loaded nanoemulsion The optimized nanoemulsion, NM2 was used to prepare nanogels using either carbopol or sodium carboxymethylcellulose as gel base. The extracted castor oil, nanoemulsions, and nanogels were assessed for their physicochemical and antimicrobial properties *In vivo* wound healing and skin irritation studies were conducted using nanogel formulation BF5. The extracted castor oil, nanoemulsions, and nanogels showed good antimicrobial activity against the test organisms. The nanoemulsions have an average droplet size of 78.71 nm (NM1) and 72.30 nm (NM2) and polydispersity index of 0.402 (NM1) and 0.222 (NM2). The nanogels prepared with sodium carboxymethylcellulose gel base have slightly better physicochemical properties, like spreadability and extrudability than those prepared using carbopol gel base however, they were less stable after one-month storage under room temperature. The wound healing activity of the castor oil-loaded nanogel was comparable to the activity of a marketed product, gentamicin ointment but unlike the ointment it will be more acceptable to the patient due to its non-greasy nature.

***Keywords*:** Castor oil, nanoemulsion, nanogel, antimicrobial, wound healing.

Introduction

Ricinus communis is a soft wooden flowering perennial shrub in the spurge family, Euphorbiaceae, commonly known as castor. The seed is called castor bean, even though, it is not a true bean. *R. communis* is currently grown worldwide in commercial quantity for ricinoleic acid-rich castor oil (seed contains 40 % oil) production.[1-3] Castor seed oil is a colourless or faintly yellow, viscid liquid that is almost odourless. It has an initial bland taste but later becomes avid and nauseating. It is fixed and dries very slowly, having a specific gravity, of 0.958. It has a refractive index of 1.4790 to 1.4805 and solidifies at -10° C to - 18°C. Its acidity is exhibited as oleic acid which is 1.5 percent.[1] Traditionally it is utilized in the treatment of skin diseases, as purgative, to assist delivery in delayed expectant mothers, and for healing of irritated or inflamed nipples among the Ebira people of Nigeria.[1] Leaf essential oil and leaf methanol extract of castor plant were reported to have potent antibacterial, antifungal, and leishmanicidal activity.[3,4]

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The extent of suppression of certain infections by different essential oils depends on the type of essential oil, its concentration, and the implicated pathogen.[5]

Nanoemulsions are clear, thermodynamically stable, oil–water dispersions stabilized by the interfacial coating of surfactant molecules with droplets smaller than 100 nm.[6,7] They are colloidal systems that are made up of an aqueous phase and an oil phase, that are stabilized with surfactants and at times by adding auxiliary surfactants. They have dispersed phase droplets that are very small (20-500 nm), interphase surfaces that are large, as well as, low surface and interfacial tension.[8,9]As a result of these, nanoemulsion enhances the absorption rate and eradicates absorption variability, as well as, assists in solubilizing lipophilic drugs, thereby enhancing their bioavailability.[9] Direct topical application of nanoemulsion is deterred and the skin permeation profile is modified due to its low viscosity.

These problems can be solved by incorporating a nanodispersion into the polymer solution to form an *in situ* nanoemulgel. A hydrophobic drug is incorporated in the oil cores of nanoemulgel, while the hydrogel cross-linked network entraps the droplets of emulsion.[8] Nanoemulgel has enhanced the topical efficacy of many, hitherto poorly permeable drugs. It also solves the problem of hydrophilicity of hydrogels, which limits the applications for the delivery of hydrophobic drugs.[8]

Wounds are injuries inflicted on living tissue that distorts its typical anatomical structure and activity. They occur as a result of physical, chemical, thermal, microbial, or immunological injury to the tissue. Irrespective of its cause and kind, wounds can inflict harm on the tissue and distort its surrounding environment. The injury can affect the skin epithelial layer's integrity and can also extend into the subcutaneous tissue disrupting associated structures like tendons, muscles, and nerves. Failure of wounds to heal normally leads to chronic wounds.[10,

[11] Wounds are associated with severe pain, they produce physical disability like immobility and activity loss. They also cause anxiety, depression, loss of self-esteem, and also premature death.[12] Most open wounds are usually associated with various bacterial and fungal infections as the underlying tissues are exposed to the outside environment. The challenge of antimicrobial resistance in treating these infections increases the complications and burden of these wounds. The wound-healing process can be retarded or even stopped by repetitive injury, which is usually associated with diabetic foot ulcers.[12] Skin wound healing occurs as a systematic process, which naturally involves four classic phases that overlap: hemostasis, inflammation, proliferation, and maturation. Skin healing following burn injuries is affected by several factors, such as the aetiology, the degree and dimension of the burn, the patient's well-being, and the types of graft or materials used for dressing burn wounds.[8] The proper healing of wounds is important for the repair of disrupted anatomical and functional status of the skin. Wound healing is one of the major complex physiological processes that start from the response to an injury to restoring the function and integrity of damaged tissues. Wound healing entails several physiological activities such as clotting, coagulation, inflammation, and the production of fresh tissues, which may follow varied timescales from minutes to numerous months or years. Development of an incomplete healing process and wound healing failure may arise due to distortions or delays to the multistage healing process. Chronic wounds can adversely affect the quality of life and a very high quality of care is needed in their treatment.[10]

It is on record that 80% of Africans rely primarily on medicinal plants for their medications. They are more affordable, easily accessible, and are linked with fewer untoward effects when compared to conventional medicines.[13] Compounds that could be used as leads in the discovery of drugs for wound healing abound in medicinal plants.[14] Medicinal plants' wound healing activities are linked to their bioactive chemical content. They include alkaloids, phenols, triterpenes, and flavonoids. These bioactive compounds have been reported to possess antioxidant and antimicrobial activities for wound healing, improve the deposition of collagen, and enhance the proliferation of both fibroblasts and keratinocytes.[15] In most instances, the process of wound healing progresses naturally. Nevertheless, the healing process can be crippled due to the presence of an infection. This may result in the extension of the inflammatory phase, interruption of the normal clotting mechanisms, disruption of leukocyte function, and angiogenesis.[16] Therefore, the vital roles played by many plants in wound healing can be attributed to their antimicrobial effects. Phenolic compounds play an important role in wound healing through their antioxidant effect against free radicals that retard the progression of wound healing.[13]

This research was conducted to assess the physicochemical, antimicrobial, and *in vivo* wound healing properties of castor oil-loaded nanogels.

Materıals Aad Methods

Materials

Glycerol, Hexane (Central Drug House (P) Ltd, New Delhi India), Polyethylene glycol 200 (LOBA Chemie Laboratory Reagents and Fine Chemicals, Mumbai India), Tween 80 (Guangdong Chemical Reagent Engineering-technological Research and Development Center, China), Carbopol 940 (Corel Pharma Chem, Gujarat, India), sodium carboxymethylcellulose (BDH Chemicals Ltd Poole England), triethanolamine (Nice Chemicals Pvt. Ltd., Mumbai, India), Chlorocresol (Lodha Chemicals India), Mueller Hinton agar (Titan Biotech Ltd, Rajasthan, India) and Sabouraud dextrose agar (Titan Biotech Ltd, Rajasthan, India).

Organisms used:

Staphylococcus aureus, Lactobacillus spp, Escherichia coli, Proteus vulgaris, and Candida albicans were obtained from the stock preparation of The Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Delta State University, Abraka.

Sample collection and identification

Castor beans were collected in the month of November 2021 from the botanical garden of the Department of Pharmacognosy and Traditional Medicines, Faculty of Pharmacy, Delta State University, Abraka, Nigeria. It was identified by Dr. Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria. It was assigned voucher number UBH-R391.

Method of extraction of castor oil

Castor beans were air-dried under shade for one week and de-shelled. The decorticated beans were dried in a tray under shade for two days to further reduce the moisture content and were milled using a manual grinder. A 550 g of the milled castor bean was macerated in 700 ml of hexane for 24 hours, and the mixture was filtered using a muslin cloth. The filtrate was centrifuged for 30 minutes at 3000 rpm using a centrifuge (Remi Elektro Technik limited, Vasai, India). The supernatant containing the oil and hexane was placed in a rotary evaporator (Rotavapor RII, Büchi Labortechnik AG, Flawil, Switzerland) at 60°C for 6 hours to separate the oil from the solvent. The oil was kept in an air-tight container

Evaluation of the extracted castor oil

The oil was evaluated considering its organoleptic properties such as colour, taste, odour, and texture. Its density, refractive index, pH, and viscosity were also determined.

Microbiological sensitivity test of castor oil

The microbial sensitivity testing of the castor oil was conducted using the Agar well diffusion method. An overnight broth was prepared using each of the test organisms, which included: *Staphylococcus aureus, Lactobacillus spp, Proteus vulgaris, Escherichia coli, and Candida albicans.* The agar plate was labeled in a clockwise direction and the surface was inoculated by spreading a small quantity of the microbial inoculum over the entire agar surface. Serial dilutions were made with the castor oil at different concentrations of 100, 50, 25, 12.5, 6.25, and 3.125. A sterile cork borer was used to punch aseptically, a 6 mm diameter hole. A small volume of the antimicrobial agent was transferred into the well using a Pasteur pipette. The agar plates (Mueller Hinton agar) containing the bacteria were incubated at a temperature of 37°C for 18-24 hours using a Uniscope SM9052 laboratory incubator, (Surgifriend Medicals, England), while the agar plates (Sabouraud dextrose agar) containing the fungi were incubated for 24 hours at room temperature. Ciprofloxacin was used as the control for the bacteria group while nystatin was used as the control for the fungus.[17]

Preparation of nanoemulsion

Castor oil-loaded nanoemulsions were prepared by titration method[18] using the extracted castor oil, Tween-80 as a surfactant, polyethylene glycol (PEG 200) as a co-surfactant, and distilled water as a continuous phase. Castor oil was mixed with the surfactant mix (2:1 of Tween 80 and PEG 200) in various ratios (1:1 to 1:30). Distilled water was added in drops into the mixture of castor and surfactant mix with continuous stirring. Several emulsions were made using different ratios of the surfactant mix and oil, however, only the 20:1 and 30:1 surfactant mix to oil ratio gave optically clear emulsions.

Evaluation of nanoemulsion

The nanoemulsions were evaluated based on droplet size, and polydispersity index.[19]

Determination of droplet size and polydispersity index of nanoemulsion

The average droplet size and polydispersity index (PDI) of the nanoemulsions were determined by photon correlation spectroscopy using a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK).[20]

Preparation of nanogels

Eight nanogel formulations were prepared according to the formula in Table 1 by dispersing the required quantity of either sodium carboxymethylcellulose (NaCMC) or carbopol 940 in distilled water or

the nanoemulsion. Chlorocresol was added as a preservative to all the formulations except formulation F8. All the formulations contain glycerin except for formulations F2 and F6. Triethanolamine was included in formulations F5 to F8 that contained carbopol 960 to adjust the pH and thicken the gels.

Evaluation of nanogels

Physical appearance evaluation

The prepared nanogels were inspected visually for their clarity, colour, presence of particles, odour and phase separation immediately after preparation and one month after.[21, 22]

pH

The pH values of 1% aqueous solutions of the nanogels were measured using a digital pH metre.[23, 24]

Homogeneity

A little portion of the sample was felt between the thumb and the index finger to determine its homogeneity.[25]

Ease of removal

About 1 g of each nanogel formulation was spread on the skin and placed under running water to determine its ease of removal from the skin.[26]

Viscosity

The viscosity of the different nanogel formulations was determined at ambient temperature (28°C) using spindle 3 of an NDJ 5S digital viscometer (Shangai Nirun Intelligent Technology Co. Ltd. China) rotated at 6, 12, 30, and 60 rpm.[27]

Spreadability

The method used by other researchers was used.[28, 29] A 0.1 g quantity of nanogel was placed on a glass plate and diameter was recorded. It was covered with another glass and an 800 g weight was placed on it for 3 minutes. The new diameter formed by the film was recorded. The procedure was repeated three times and the average diameter was calculated.

Extrudability

The method of [30] was used. A known weight of gel was transferred into a collapsible aluminum tube and the weight was recorded. A 600 g weight was put on the tube and it was opened for 1 minute. The tube was reweighed to determine by difference the amount of gel that was extruded. The percentage extrudability was determined using equation 1:

$$\%\ Extrudability = \frac{weight\ of\ extruded\ gel}{initial\ weight\ of\ gel}\ x\ 100 \quad . \ . \ . \ . \quad 1$$

This was done in triplicate for the nanogel formulations.

Microbiological sensitivity test of the nanogel formulations

The Agar well diffusion method was used for the microbial sensitivity testing of the castor oil-loaded nanogel formulations. An overnight broth was prepared using each of the test organisms (*Staphylococcus aureus*, *Lactobacillus* spp., *Escherichia coli*, *Proteus vulgaris*, and *Candida albicans*). The agar plate was labeled in a clockwise direction and the surface was inoculated by spreading the inoculum over the entire agar surface. The nanogels were diluted with methanol to liquefy it. A sterile cork borer was used to punch aseptically, a 6 mm diameter hole. A little volume of the antimicrobial agent was transferred into the well using a Pasteur pipette. The agar plates containing the bacteria were incubated at a temperature of 37°C for 18-24 hours while the agar plates containing the fungi were incubated at room temperature for 24 hours.[17]

In vivo wound healing and skin irritation Studies

Ethical approval (REC/FBMS/DELSU/22/157) for the *in vivo* study was obtained from the ethical committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. Formulation (F5) was chosen for the *in vivo* skin irritation and wound healing activity because among all the nanogel formulations it exhibited the overall best antimicrobial properties.

Skin irritation test

This study was conducted using fifteen male Wistar rats weighing 150-200 g divided into three groups (n = 5). The Wistar rats were kept in polypropylene cages and were provided with enough standard laboratory diet and water *ad libitum*. They were housed under standard laboratory conditions (28 ± 2ºC and 55 ± 5% RH). A portion of the animal's dorsal skin was shaved. A 1 g sample of the nanogel was applied to the properly shaven skin of the rats in the test group and the animals were returned to their cages. Gentamicin ointment was applied to animals in the standard group whereas nothing was applied to those in the control group. After 24 hours of exposure, the test sites were observed for any change in colour or skin morphology (erythema or oedema).[31, 32]

Wound healing test

Nine male Wistar rats divided into three groups (n = 3) (test group, positive control group, and the negative control group) were used. Formulation F5 of the nanogel was used in the test group, Gentamicin ointment was applied to the positive control group while no gel was applied to the negative control group. The Wistar rats were anaesthetized with 50 mg/kg of Ketamine HCl intraperitoneally under aseptic conditions.[33] An injury of about 2 cm in diameter was inflicted on each of the rats followed by immediate application of the nanogel to the test group and gentamicin ointment to the second group. The diameters of the injuries were measured using a vernier calliper. A 1 g quantity of the nanogel, gentamicin ointment, or nothing was applied to animals in the respective groups daily for two weeks. The time for wound healing was determined from the day of wound creation to re-epithelization till the scars were off.[7] The wound contraction rate was determined by measurement of the diameter of the injuries every two days until complete healing of the wound and was compared with the wound-creation day, that is, day 0. The percentage of contraction was calculated using equation 2:[7, 34]

$$Contraction\ \% = \frac{[\text{Wound } area\ on\ \text{day } 0\ (cm) - \text{ Wound } area\ on\ current\ day\ (cm)}{\text{Wound } area\ on\ day\ 0\ (cm)}\ x\ 100 \ldots 2$$

Table 1: Composition of castor oil-loaded nanogel formulations F1-F8

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8
Castor oil-loaded nanoemulsion (ml)	40.3	40.3	-	40.3	40.3	40.3	-	40.3
Distilled water (ml)	-	-	40.3	-	-	-	40.3	-
NaCMC (g)	0.5	0.5	0.5	0.5	-	-	-	-
Carbopol (g)	-	-	-	-	0.5	0.5	0.5	0.5
Chlorocresol (g)	0.05	0.05	0.05	-	0.05	0.05	0.05	-
Glycerin (ml)	2.8	-	2.8	2.8	2.8	-	2.8	2.8
Triethanolamine	-	-	-	-	q.s	q.s	q.s	q.s
Total (g)	50	50	50	50	50	50	50	50

Statistical Analysis

The data were analyzed using a 2-way analysis of variance (ANOVA) with the statistical computer software SPSS version 22.00. Mean differences were considered statistically significant at $p<0.05$.

Results and Discussion

The pictures of castor beans, extracted castor oil and the castor oil-loaded nanoemulsion are shown in Figure 1. The colour, taste, and odour of the extracted castor oil are shown in Table 2. The pH, density, viscosity, and refractive index of the extracted castor oil are shown in Table 2 and were similar to that obtained by previous researchers.[35, 36]

The results shown in Table 3 indicate that the test organisms were sensitive to different castor oil concentrations except for *S. aureus*, *Lactobacillus*, and *E. coli* which were not sensitive to 100% concentration of castor oil. This may be due to the low penetration of the agar by the 100% concentration of castor oil due to its high viscosity. Previous researchers such as Dulal *et al*[5] showed that *S. aureus* and *E. coli* were not sensitive to castor oil but Al-Mamun *et al*[3] showed that *S. aureus* and *E. coli* were sensitive to the effect of castor bean protein. The antimicrobial agent (ciprofloxacin) used as the control for bacteria showed the highest zones of inhibition in comparison with the different castor oil concentrations used. The antifungal agent (nystatin) used as the control showed a higher zone of inhibition (14.00 ± 0.05 mm) than that of the different castor oil concentrations except the 3.125% concentration of the castor oil (29.00 ± 0.13 mm).

The average droplet size and polydispersity index for nanoemulsion formulations NM1 and NM2 are shown in Figures 2 and 3 respectively. The average droplet sizes for both are in the nano range but NM2 was selected for the formulation of the nanogels because of its lower polydispersity index (PDI) value (0.222). PDI shows droplet size uniformity and acts as a crucial parameter in stabilizing nanoemulsion. It can be defined as the ratio of standard deviation to mean droplet size. The higher the value of polydispersity, the lower the uniformity of droplet size of nanoemulsion. The maximum PDI value acceptable for nanoemulsions is less than 0.30. This shows a monodispersed nanosystem which is necessary for stability.[20, 38, 39]

As shown in Figure 4 and Table 4, nanogel formulations BF1 to BF6 are opaque and cream coloured whereas BF7 and BF8 are clear and colourless. All the nanogel formulations are homogenous, have a smooth texture, and without any palpable masses. This shows proper mixing of the nanoemulsion and the gelling agent in the formulation of the nanogels.[40]

The pH of the nanogels ranged from 5.26 to 7.49. The normal skin pH range is 4.0-6.8.[41, 42] Therefore, the nanogels prepared with carbopol as the gel base have a pH that was within the normal skin pH range whereas those prepared with NaCMC gel base were slightly basic. However, there was no significant difference ($p<0.05$) between the pH of nanogel Formulations BF1-BF4 (prepared using NaCMC gel base) and Formulations BF5-BF8 (prepared using carbopol gel base). However, as shown in Figure 5, the pH of the nanogel formulations BF1-BF4 after 30 days, decreased from 7.07-7.49 to 4.9-5.7 whereas nanogel formulations BF5-BF8 decreased slightly from 5.1-6.7 to 5.0-6.4.

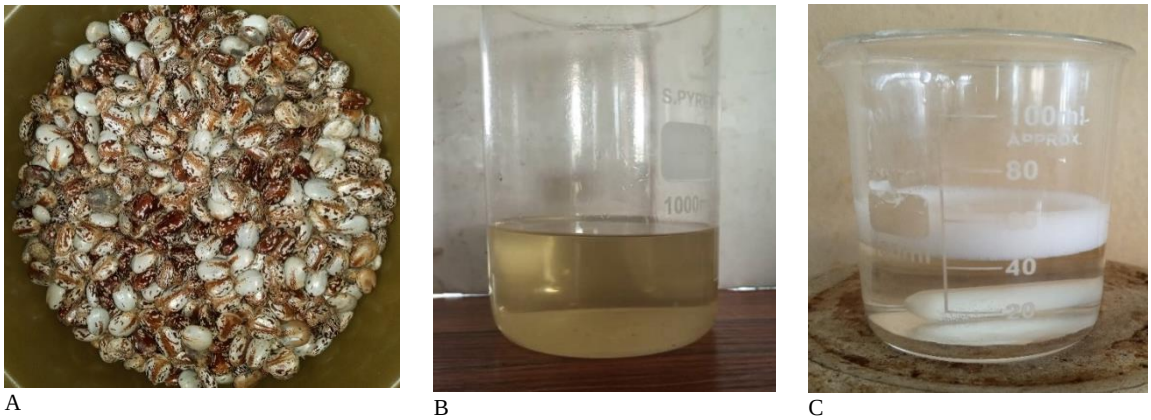
The extrudability for the nanogels as shown in Table 4 ranged from 2.23-24.44%. Formulations BF1 to BF4 showed a higher percentage of extrudability due to their low viscosity in comparison to BF5 to BF8 which showed a low percentage of extrudability due to their high viscosity. There was a significant difference ($p<0.05$) between the extrudability of nanogel Formulations BF1-BF4 (prepared using NaCMC gel base) and Formulations BF5-BF8 (prepared using carbopol gel base). This may influence the choice of container to be used. Cream jars will be preferable to collapsible tubes due to the low extrudability values for the nanogels.

The spreadability of the nanogel formulations ranged from 2.45-6.40 cm as shown in Table 4. Formulations BF1 to BF4 prepared using NaCMC gel base showed better spreadability index in comparison to BF5 to BF8 that were prepared with carbopol gel base. This could be a result of the high viscosity of BF5 to BF8 as spreadability is indirectly proportional to viscosity. Gels produced with NaCMC are more spreadable than gels produced with Carbopol-940 due to less viscosity.[43] Gels show better spreadability when they have low viscosity.[43, 44] However, there was no significant difference ($p<0.05$) between the spreadability of nanogel formulations BF1-BF4 (prepared using NaCMC gel base) and formulations BF5-BF8 (prepared using carbopol gel base).

The viscosity of the nanogels at ambient temperature (28ºC) using spindle 3 of Brookfield rotated at 6 rpm, ranged from 3484.4 to 17310 mPas on day 0 to 2535.8 to 17210.0 mPas on day 30 (Figure 6). The viscosity curves as shown in Figure 6 indicates that the nanogels exhibit shear thinning flow behaviour both on day 0 and day 30. Figures 6a and 6b shows that the viscosity of nanogel formulations BF1-BF4 decreased markedly from 7600.4-8303.4 mPas on day 0 to 2532.9-2767.8 mPas on day 30, while the viscosity of formulations BF5-BF8 decreased slightly from 3489.4-17310 mPas on day 0 to 3484.7-17210 mPas on day 30. This may be due to a decrease in pH of the nanogel formulations containing NaCMC after 30 days as shown in Figure 5. The solution pH affects the solution viscosity of NaCMC.[45] For pH values less than 7, the solution viscosity becomes significantly lower.[45]

Table 2: Organoleptic and physicochemical properties of Castor oil

Parameter	Colour	Taste	Odour	pH	Viscosity (mPas)	Density g/ml	Refractive Index
Value	Light yellow	Bland	Odourless	5.70	3274.3	0.93	1.4754



A B C

Figure 1: (a) Castor beans (b) Castor oil (c) Castor oil-loaded nanoemulsion

Table 3: Antimicrobial sensitivity of castor oil

	ZONES OF INHIBITION[a] (mm)				
Castor oil concentration (%)	***Staphylococcus aureus***	***Lactobacillus spp***	***Escherichia coli***	***Proteus vulgaris***	***Candida albicans***
100	0	0	0	4.00 ± 0.01	3.50 ± 0.01
50	6.00 ± 0.01	11.00 ± 0.02	7.50 ± 0.05	4.00 ± 0.03	5.50 ± 0.01
25	5.00 ± 0.01	15.00 ± 0.01	10.00 ± 0.11	3.00 ± 0.01	9.50 ± 0.04
12.5	9.00 ± 0.02	17.50 ± 0.03	7.00 ± 0.03	4.00 ± 0.05	11.50 ± 0.02
6.25	8.00 ± 0.01	15.30 ± 0.02	5.50 ± 0.01	7.00 ± 0.11	12.00 ± 0.10
3.125	7.00 ± 0.01	11.50 ± 0.01	4.50 ± 0.01	0	29.00 ± 0.13
Control	16.00 ± 0.10	26.50 ± 0.02	17.00 ± 0.10	29.00 ± 0.14	14.00 ± 0.05

[a] Data was expressed as mean ± standard deviation (n = 3)

Table 4: Physicochemical properties of the nanogels

PARAMETERS	**BF1**	**BF2**	**BF3**	**BF4**	**BF5**	**BF6**	**BF7**	**BF8**
pH[a]	7.49 ± 0.00	7.07 ± 0.00	7.07 ± 0.00	7.14 ± 0.00	5.10 ± 0.00	5.26 ± 0.00	6.38 ± 0.00	6.70 ± 0.00
Clarity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Clear	Clear
Colour	Cream	Cream	Cream	Cream	Cream	Cream	Colourless	Colourless
Presence of particles	None	None	None	None	None	None	None	None
Odour	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Homogeneity	Good	Good	Good	Good	Good	Good	Good	Good
Spreadability[a] (cm)	5.20 ± 0.02	6.40 ± 0.01	5.13 ± 0.03	5.00 ± 0.01	2.43 ± 0.01	3.03 ± 0.02	2.96 ± 0.01	3.03 ± 0.01
Ease of removal	Very easy	Very easy	Very easy	Easy	Not easy	Easy	Easy	Easy
Extrudability[a] (%)	24.44 ± 0.05	20.57 ± 0.10	16.17 ± 0.06	8.38 ± 0.02	2.45 ± 0.01	2.23 ± 0.01	8.86 ± 0.04	4.77 ± 0.02
Skin Irritation	None	None	None	None	None	None	None	None

[a] Data was expressed as mean ± standard deviation (n = 3)

NaCMC powder exhibits high chemical and microbiological purity and stability, however, the aqueous solution is liable to degradation by microbes and preservatives should be added if prolonged storage is required.[46] Sodium carboxymethylcellulose inhibits the activity of chlorocresol considerably, thereby reducing its preservative potential.[47] The maximum viscosity and stability of carboxymethylcellulose sodium aqueous solutions is exhibited at pH 7–9, even though, they are stable across a wide range of pH (2–10). Changes in pH and ionic strength affect NaCMC because it is a polyelectrolyte.[46]

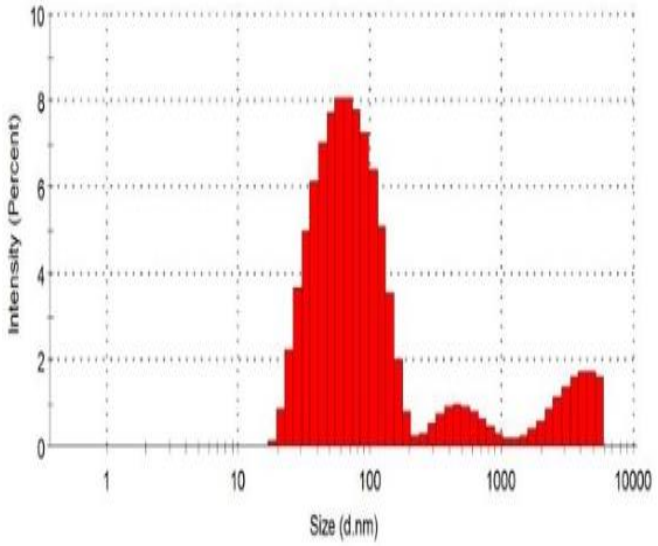


Figure 2: Size distribution by intensity of nanoemulsion formulation, NM1 (Z-Average (d.nm) = 78.71 and pdi = 0.402)

Since chlorocresol was the preservative used for the nanogel formulations, it may be likely that the interaction between it and NaCMC resulted in a change in pH and possible reduction in preservative activity that resulted in microbial degradation of NaCMC and the subsequent reduction in viscosity of the nanogels contain NaCMC gel base. Adequate information on the skin sensitization potential of individual chemicals for dermal use is very vital for hazard as well as risk assessment.[48] Gels that are too acidic irritate the skin, whereas those that are too alkaline causes scaly skin.[49]

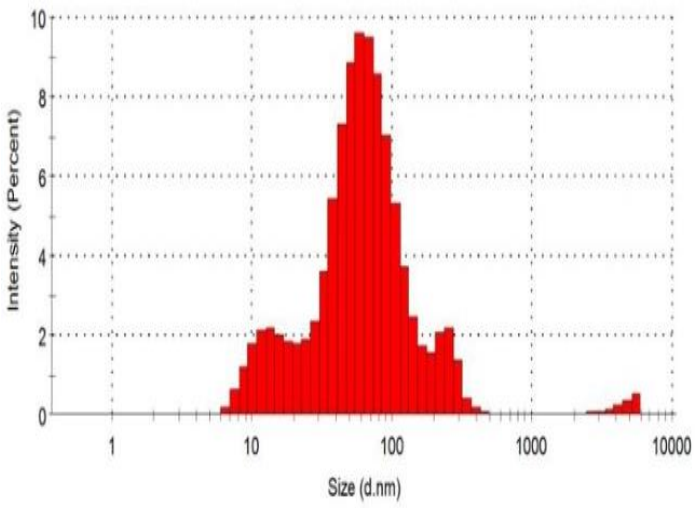


Figure 3: Size distribution by intensity of nanoemulsion formulation, NM2 (Z-Average (d.nm) = 72.30 and pdi = 0.222)

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Table 5: Antimicrobial sensitivity of the nanogels

Microorganisms	F1	F2	F3	F4	F5	F6	F7	F8
Staphylococcus aureus	9.00 ± 0.01	6.00 ± 0.01	-	-	5.00 ± 0.01	-	-	-
Lactobacillus spp.	4.00 ± 0.01	9.00 ± 0.03	-	7.00 ± .01	11.00 ± 0.05	15.00 ± 0.03	-	11.00 ± 0.02
Escherichia coli	-	16.00 ± 0.10	-	-	12.00 ± 0.04	14.00 ± 0.02	-	14.00 ± 0.05
Proteus vulgaris	-	9.00 ± 0.02	-	-	11.00 ± 0.01	14.00 ± 0.03	-	14.00 ± 0.01
Candida albicans	3.00 ± 0.01	10.00 ± 0.05	-	1.00 ± 0.01	5.00 ± 0.01	6.00 ± 0.02	-	6.00 ± 0.02

[a] Data was expressed as mean ± standard deviation (n = 3)

A B

Figure 4: Castor oil-loaded nanogels prepared with (A) Sodium Carboxymethylcellulose (B) Carbopol

No skin irritation was noticed in any of the rats used in the test and control groups. No sign of oedema or erythema was observed on the dorsal skin of the rats and these indicate that the nanogels are safe for topical application.[48] The absence of pronounced variation in normal skin surfaces and the absence of signs of inflammation connotes good skin tolerability of the nanogels.[50] Topical preparations that have pH within the normal skin pH (4.0-6.8) are considered non-irritant to the skin and this may be the reason the nanogel (pH =5.7) did not cause any skin irritation.[44]

All the test organisms were sensitive to the effect of nanogel formulations BF2 and BF5 and none of the organisms was sensitive to formulations BF3 and BF7 (Table 5). Formulations BF3 and BF7 did not contain castor oil and that may be the reason for their lack of activity. There was no significant difference ($p<0.05$) between the antimicrobial activity (inhibition zone diameter) of castor oil and that of formulation BF5. All the organisms, except *Staphylococcus aureus* were susceptible to the effects of formulations BF6 and BF8. The loss in activity against some of the test organisms by formulations BF1, BF4, BF6, and BF8 could be a result of various interactions between castor oil and some of the excipients used during the formulation process.

The wound healing activity of nanogel formulation BF5 as shown in Figure 7, was comparable to that of the positive control (gentamicin ointment) and higher than that of the negative control. There was complete wound healing on day 6 for the nanogels and the positive control, but for the negative control it was on day 8. There was no significant difference ($p<0.05$) between the wound contraction produced by formulation BF5 and the positive control, however, they were significantly higher ($p<0.05$) than the wound contraction produced by the negative control. Wound healing is one of the major complex physiological processes that start from the response to an injury to restoring the function and integrity of damaged tissues.[10] Figure 8A shows a fresh open wound inflicted on the rat on day 0 while Figure 8B shows where the wound has healed on day 8. Between day 0 and day 8, there was contraction of wound size until complete healing and eventual growing back of hairs on healed wound site. The *in vivo* wound healing test was not performed on the nanogels containing NaCMC gel polymer because the nanogels lost their viscosity within three to four weeks of storage which showed that they were unstable. NaCMC gel becomes unstable and self-decomposes when dissolved in a solution and its viscosity reduces accordingly.[45] Other influencing factors are temperature and solution pH. The viscosity of the NaCMC gel decreases on subsequent increases in temperature, it is indirectly proportional. The solution pH affects solution viscosity. For pH values less than 7, the solution viscosity becomes significantly lower. The instability of the NaCMC indicates that it cannot be used for preparations intended for long-term use and storage especially if chlorocresol is used as a preservative.[45]

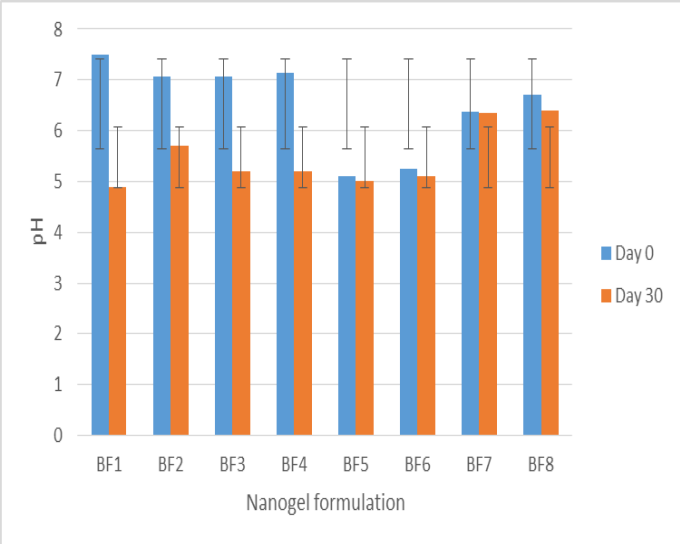

Figure 5: pH of castor oil-loaded nanogel formulations BF1-BF8 on day 0 and day 30.

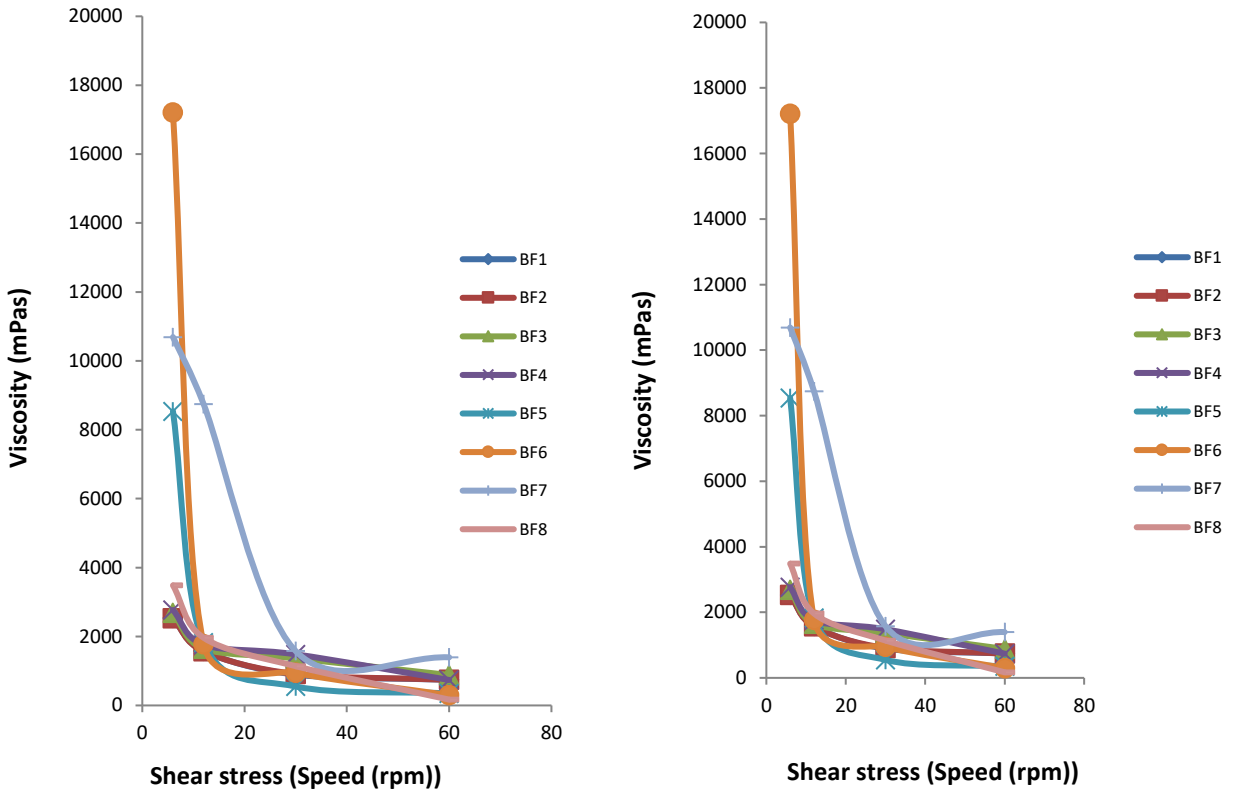

Figure 6: Viscosity curves of nanogel formulations BF1-BF8 at ambient temperature (28ºC) using spindle 3 (a) On day 0 and (b) On day 30

Conclusion

The study revealed that the extracted castor oil, its nanoemulsions, and nanogels possess antimicrobial properties against *Staphylococcus aureus*, *Lactobacillus spp*, *Escherichia coli*, *Proteus vulgaris*, *and Candida albicans*. The *in vivo* wound healing study showed that the castor oil-loaded nanogel has a comparable wound healing effect to a marketed product, gentamicin ointment.

The nanogels prepared using NaCMC gel base have slightly better physicochemical properties such as spreadability, washability, and extrudability when compared to the nanogels prepared using carbopol gel base but they were unstable after a few days of storage. Therefore, castor oil-loaded nanogels should be prepared using a carbopol gel base, however, if NaCMC gel base should be used, other compatible preservatives should be used in place of chlorocresol.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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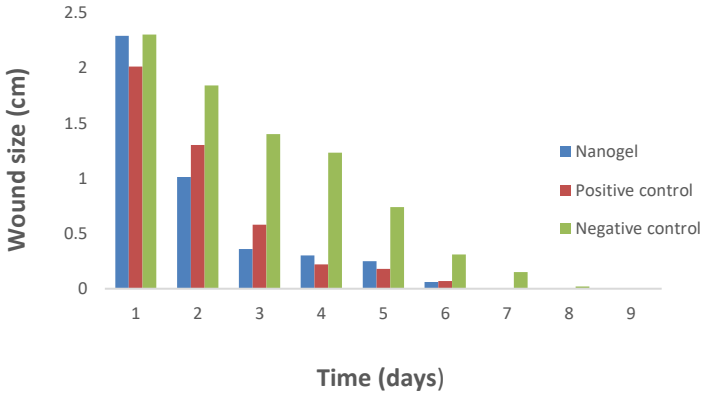
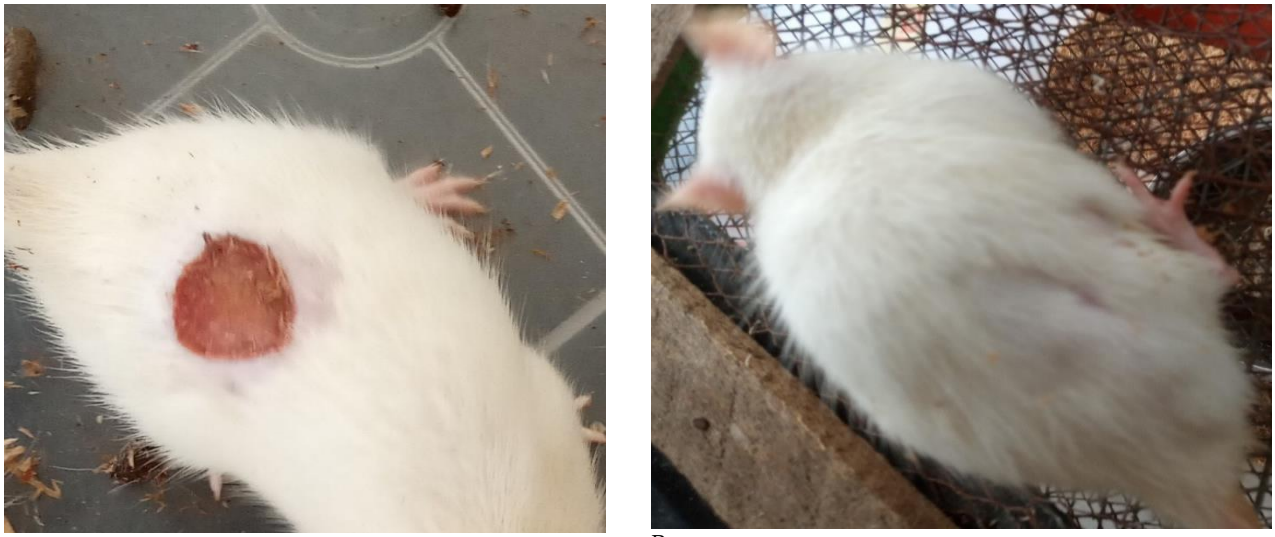


Figure 7: Wound healing activity of nanogel, positive and negative controls



A B

Figure 8: Wistar rat after application of nanogel formulation BF5 (A) day 0 (open wound), (B) day 8 (healed wound)

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