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Original Research Article

Development and characterization of capsaicin creams formulated with Grewia mucilage-HPMC base

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Abstract

Purpose: Conventional topical capsaicin creams are often unavailable and unaffordable to the larger patient populations in developing countries. There is a need to formulate cost-effective alternatives using locally available polymers as cream base. This study aimed to formulate oil-in-water *Grewia mollis* mucilage-hydroxypropyl methylcellulose (GMM-HPMC) based capsaicin creams from locally available capsicum fruits and evaluation of their quality attributes.

Methods: Capsaicin was quantified from acetone extracts of *Capsicum* fruits (*C. frutescens, C. pubescens and C. chinense*) using high performance liquid chromatography (HPLC). Extract of *C. chinense* which had higher capsaicin content was used to formulate six different cream types with varied concentrations of GMM and/or HPMC as polymeric base. The creams were assessed for their organoleptic properties, pH, specific gravity, conductivity, viscosity, spreadability, oil globule size, microbial load and stability profiles using standard methods and protocols.

Results: The FTIR spectroscopic analysis confirmed the presence of capsaicin in all the *Capsicum* fruit extracts; HPLC quantification of each of the fruit extracts indicated the presence of both capsaicin; *C. chinense*: (36153 ppm) > *C. frutescens*: (7860 ppm) > *C. pubescens*: (4549 ppm) and dihydrocapsaicin; *C. chinense* (11044 ppm) > *C. frutescens* (6920 ppm) > *C. pubescens* (2828 ppm) as constituents. Formulated o/w creams were light to deep brick red in colour, with pH (6.11-6.44); specific gravity (1.00-1.03); electrical conductivity (292-1958 µS/cm); viscosity (2810-9190 mPas); spreadability (4.0-5.5 cm) and globule size (13 ± 8 µm to 91 ± 20 µm). The creams had satisfactory microbial load profiles and remained stable at 25 ± 2 °C but had varying degrees of stability at 40 ± 2 °C storage temperatures.

The optimized formulations of the creams (FB, FE and FF) contained GMM as the mono polymeric base system, while GMM_{10}:$HPMC_{10}$ and GMM_{15}:$HPMC_{10}$ were the co-polymeric base systems, respectively.

Conclusion: This study has shown the suitability of *Grewia mollis* mucilage singly used or in combination with hydroxypropyl methylcellulose as co-polymeric cream base. Formulated creams had desirable physicochemical properties and they may find better patient acceptance when compared with imported brands as a result of their potential low cost.

Keywords: Capsicum fruits, capsaicin, *Grewia mollis* mucilage-HPMC, creams

Indexing: Index Copernicus, African Index Medicus

Introduction

Neuropathic pain, especially those associated with nerve damage results from poorly controlled or long-standing diabetes, and are characterized by burning pain as well as numbness of the feet and hands [1]. Non-compliance to therapy schedules by patients suffering from neuropathic pain often affect treatment outcomes and their quality of life

[2,3]. Osteoarthritis is another common health condition amongst elderly and obese populations that presents with pain and disability, hence requires therapeutic intervention [4]. Synthetic drugs such as gabapentin are less preferred for managing neuropathic and osteoarthritic pain conditions due to the attendant side effects such as memory loss and tremors [2], which limit compliance to dosage regimen [5].

Capsaicin, 8-methyl-N-vanillyl-6-nonenamide isolated from capsicum fruit has been widely researched for its anti-inflammatory [6], anti-cancer and anti-oxidant properties [7]. However, capsaicin remains limited in its clinical application due to its poor aqueous solubility and dispersibility [8]. *In vivo* studies using rat models had revealed that capsaicin is prone to hepatic degradation and is rapidly eliminated from systemic circulation after intravenous delivery, with a half-life of about 7 min [9]. The dermal/transdermal route of drug transport is therefore an attractive alternative for the delivery of capsaicin as hepatic and gastric degradation is avoided. Also, this route may ensure a high local drug concentration at target sites [10].

The *in vitro* and *in vivo* performance of topical semi-solid dosage forms are dependent in part on their physicochemical and stability profiles, hence, formulators ensure that the active pharmaceutical ingredients used are compatible physically and chemically with the formulation excipients [11]. Capsaicin formulations are comparable to topical non–steroidal anti-inflammatory drug delivery systems used in the management of osteoarthritis [12]. These capsaicin containing topical dosage forms include hydrogels [13], patches [14], lotion [15], nanoemulsions [16] and microemulsions [17].

There are a number of factors that promote drug permeation across the skin, including the concentration of the drug, formulation type, inclusion of mucoadhesive agents and viscosity enhancers [18]. For example, placebo-controlled *in vivo* studies have shown that capsaicin cream (0.075%) needed to be applied three or four times daily for 6 weeks for effectiveness [19, 20]. Nevertheless, recent studies have revealed that capsaicin formulations containing ≥ 1.0% capsaicin such as capsaicin patch (8.0%) may prolong duration of drug action, reduce dosing frequency and improve patient adherence [13]. Aqueous creams are particularly attractive to patients for topical application because they are less greasy and readily washed off skin and clothes [21]. In order to enhance drug permeation with aqueous formulations, permeation enhancers such as propylene glycol are incorporated into topical creams [22].

Commercially available capsaicin creams in Nigeria are imported, expensive and frequently associated with supply shortages caused by regulatory timelines. This situation of unavailability of capsaicin cream to patients and the high cost of importation justify the need to develop cost-effective local alternatives.

Hydroxypropyl methyl cellulose (HPMC) is a semi-synthetic polymeric excipient with viscosity enhancing and controlled release potential [23]. *Grewia mollis* gum mucilage (GMM) has been used to prepare semi-solid dosage forms and it has been investigated for its mucoadhesive and viscosity-enhancing properties [24]. Capsaicin cream formulation with the aforementioned polymeric agents (HPMC and GMM) as cream base agents could be useful to manage pain associated with osteoarthritis and neuropathy. Thus, this study sought to develop and characterize 1.0% w/w capsaicin creams containing optimized concentrations of GMM and HPMC as polymeric excipients.

Methods

Materials

Capsaicin reference standard (99% purity) and acetone (Merck, UK), ethanol (96%), hydrochloric acid solution (1.0%) and sodium hydroxide pellets (Sigma, Germany). Methanol and acetonitrile of chromatographic grade (MRS Scientific Ltd, UK), HPMC (Shijiazhuang Jianxin Cellulose Co., Ltd, China), coconut oil (KTC Edibles Ltd, UK), propylene glycol (Inner Mongolia Pulis Chemical Co., Ltd, China), methyl paraben (Xian Faithful Biotech. Co. Ltd, China), propyl paraben (Arshine Pharmaceutical Co., Ltd, China), glycerol (Xian Henrikang Biotech. Co. Ltd) and tween 80 (LinylGuoli Chemical Co., Ltd, China). Sabouraud dextrose agar, tryptone soya agar, mannitol salt agar, Pseudomonas centrimide agar, eosine methylene blue agar, Salmonella Shigella agar and MacConkey agar (Biotech Microbials B.V).
Fresh *Capsicum* fruits (*Capsicum frutescens, Capsicum chinense L.* and *Capsicum pubescens*

L.) were collected and identified by Mr. T.I. Adeleke of the Department of Pharmacognosy, University of Lagos, Nigeria. These fruits with herbarium number FHI 112648, FHI 112650 and FHI 112651, respectively, were authenticated at the Forestry Research Institute of Nigeria (FRIN) Herbarium, Ibadan. They were stored at room temperature prior to capsaicin extraction. *Grewia mollis* mucilage/gum was extracted by aqueous maceration of the inner stem bark of *Grewia mollis* Juss (Tiliaceace) and purified in the laboratory of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria.

Capsaicin extraction

Capsicum frutescens, Capsicum pubescens and *Capsicum chinense* fruits were dried and pulped in a blender. Pilot extractions were undertaken with methanol and acetone for extraction optimization. Subsequently, 100 g each of the powdered *Capsicum* fruits were separately subjected to a 2 h extraction in a soxhlet apparatus using 1.5 L of acetone repeatedly for four (4) times at room temperature. Thereafter, the supernatant layers of the acetone extracts were clarified by filtration and concentrated using a water bath (37 °C) to final volumes of 50 mL each and refrigerated.

Capsaicin identification

Using the Fourier transform infrared spectroscopy (Tensor 27 Platinum ATR-FT-IR spectrometer (Bruker, UK), the presence of capsaicin in the extracts was evaluated. Their FT-IR spectra were obtained by scanning the extract that have been blended with potassium bromide and punched into a pellet from wave number of 4000 to 650 cm^{-1}, using resolution of 4 cm^{-1} of the instrument. Data was processed based on the average of 32 scans per spectrum generated by the instrument.

Capsaicin quantification

High Performance Liquid Chromatographic (HPLC) method was used to analyze the three capsaicin extracts [25]. The HPLC instrument (Agilent Technologies 1200 series, UK) was coupled with a reverse phase C18 column, 150 mm x 4.6 mm x 5 µm (Zorbax Eclipse XDB, Agilent Technologies, Germany), quaternary pump, VWD UV detector operated at 222 nm and degasser. The system was maintained at 25 °C. The manual Rheodyne injector withdrew 20 µL samples each time for analysis; mobile phase was water and acetonitrile (50:50), which was run in an isocratic mode at a flow rate of 1.0 mL/min. Chromatographic data was analyzed using ChemStation Software Rev P.04.03. Standard curve of capsaicin was obtained by analyzing six standard solutions of known concentrations in triplicates.

Microbial load determinations

The *Capsicum* extracts were investigated for the presence of aerobic microbes, yeast and Gram negative pathogens such as *Salmonella spps, Pseudomonas aeruginosa,* and *Escherichia coli*, in accordance to USP methods and specifications for non-sterile topical products [26] using specific media cultures. Two dilutions of the capsicum extracts were made (1 in 10 and 1 in 100). The Sabouraud Dextrose Agar (SDA) plates were incubated up-right at room temperature for 7 days (Remi Industries Ltd, Mumbai, India: Model 400053; Serial No: 111C-2368), while all other plate tests were incubated for 72 h at 37 ± 2 °C (Astell Hearson Scientific Ltd, England, Model: JBF042, Serial No: OV10045). Similar procedures were carried on *Grewia mollis* mucilage powder intended for cream preparations.

Compatibility studies

The compatibility between capsaicin in the different capsicum extracts and powdered Grewia mucilage was carried out using FTIR spectroscopy. The spectra were obtained by scanning the extract/Grewia mucilage sample from wave number of 4000 to 650 cm^{-1}, using resolution of 4 cm^{-1}. Data was processed based on the average of 32 scans per spectrum generated by the instrument.

Formulation of capsaicin cream

The *C. chinense* extract which had the highest capsaicin content as elucidated by the HPLC quantification was used to formulate six different types of aqueous creams (FA-FF) using different concentrations of the formulation ingredients as depicted in Table 1. The oily phase consisted of coconut oil, while the aqueous phase consisted of water, propylene glycol, *Grewia mollis* mucilage (GMM) and/or hydroxypropyl methyl cellulose (HPMC). Glycerol served as a solvent. Methyl and propyl parabens (preservatives) as well as Tween 80 (emulsifying agent) were

dissolved in glycerol and thoroughly stirred with GMM and/or HPMC to give a viscous homogenous aqueous phase (cream base).

Table 1: Formula used in the preparation of the capsaicin creams

Ingredients	FA	FB	FC	FD	FE	FF
Capsaicin (g)	1	1	1	1	1	1
Coconut oil (ml)	15	14	15	12	12	12
Glycerol (ml)	8	6	8	12	12	12
HPMC (g)	5	-	10	10	10	10
Grewia mollis mucilage (ml)	-	10	-	5	10	15
Propylene glycol (ml)	6	6	6	8	8	8
Tween 80 (ml)	10	10	8	10	10	10
Propyl paraben (g)	0.4	0.4	0.4	0.4	0.4	0.4
Methyl paraben (g)	0.4	0.4	0.4	0.4	0.4	0.4
Distilled water (ml)	55	53	52	42	37	32

Key: FA = GMM: HPMC (0:5); FB = GMM: HPMC (10:0); FC = GMM: HPMC (0:10); FD =GMM: HPMC (5: 10); FE = GMM: HPMC (10: 10); FF = GMM: HPMC (15:10).

The oily and aqueous phases were separately warmed over a water bath to 50 ± 2 °C. This was followed by the gradual addition of the oily phase (coconut oil) to the viscous aqueous phase (cream base) with trituration in a glass mortar to form the respective o/w emulsion. The capsaicin extracts (active pharmaceutical ingredient -API) were then thoroughly incorporated with trituration into the respective o/w viscous emulsion systems to produce homogenous cream formulations. The respective quantities of water were added to each of the cream formulations and thoroughly mixed to give the respective cream consistencies. The creams were separately packed in open-mouthed screw cap containers, appropriately labeled and stored in a cool dry place away from light until required for further characterizations and evaluations.

Evaluation of capsaicin creams

Organoleptic and physical properties

All formulations were tested for their organoleptic attributes such as colour, odour, and physical properties such as texture, homogeneity and phase separation by visual observation and by touch. Furthermore, the ease of removal of the creams applied was examined by washing the applied part with tap water. Homogeneity and texture were tested by pressing a small quantity of the formulated creams between the thumb and index finger. The consistency of the formulations and presence of coarse particles were used to evaluate the texture and homogeneity of the formulations.

Determination of microbial load of capsicum cream

The *Capsicum* creams were investigated for the presence of aerobic microbes, yeast and Gram negative pathogens such as *Salmonella spps, Pseudomonas aeruginosa,* and *Escherichia coli*, according to USP methods for non-sterile topical products [26]. The following specific media were used to incubate their corresponding organisms; Sabouraud dextrose agar (SDA) (yeast and moulds), tryptone soya agar (aerobic), mannitol salt agar (staphylococcus), Pseudomonas centrimide agar (*Pseudomonas aeruginosa*), eosine methylene blue agar (*E. coli*), Salmonella Shigella agar (Salmonella/Shigella) and MacConkey agar (bacteria coliform and bile tolerant gram negative organisms).

Microbial load determinations were undertaken in triplicates for each cream formulation using established methods of microbial limit test [26]. The SDA plates were incubated up-right at room temperature for one week (Remi Industries Ltd, Mumbai, India: Model 400053; Serial No: 111C-2368) while all other plate tests were incubated at 37 ± 2 °C for 72 h (Astell Hearson Scientific Ltd, England, Model: JBF042, Serial No: OV10045).

pH

The pH of the formulations was determined at room temperature using a bench top pH meter (HI 2211 pH /ORP Hanna Instruments, UK). Briefly, one gram of each capsaicin cream formulation was dispersed in 25 mL of deionized water, and the pH was determined using the pH meter which was previously calibrated with standard buffer solutions (pH 4, 7, and 10) before each use. Measurements were made in triplicates.

Specific gravity

Briefly, the net weights of each of the creams were determined by deducting the weight of the empty pycnometer from that of the weights of the pycnometer containing the respective creams. The densities of the cream samples were

obtained by dividing the net weights of the samples by the volume (50 mL) of the empty pycnometer which is equivalent to the volume of the cream. The reference standard used was water with density as 1 g/mL, hence the pycnometer was filled to the 50 mL mark and its weight noted. The specific gravity for each cream was thus determined as the ratio of the density of the respective cream to that of water that filled the 50 mL pycnometer.

Conductivity

The Seven Easy Conductivity meter (Mettler Toledo, UK) was used to measure the conductivity of the six different cream formulations (sample FA-FF) at 25 °C. The electrode was calibrated using gas-free distilled water prior to sample analysis.

Viscosity

A Brookfield viscometer DV-I (Brookfield Engineering Laboratories, Middleboro, Massachusetts) was used to determine the viscosity of the different cream formulations following an already reported protocol [27]. The tests were carried out at 25 °C using spindle No. 4 which was rotated at 60 rpm. All measurements were made in triplicate to obtain statistically representative data.

Spreadability

Spreadability of the formulations was determined by measuring the spreading diameter of 1.0 g of each cream sample placed between two horizontal white tiles (5 × 10 cm). Determination of diameter was undertaken before and after 2 minutes of placing a standard weight (20 g) on the upper tile. The spreadability of the cream was evaluated in terms of the difference in the initial diameter and final diameter of the cream samples. Triplicate determinations were undertaken for each cream formulation.

Globule size

A smeared sample of the cream was made on a clean slide, stained with a drop of crystal violet and covered with a slip. The covered sample was viewed under the BINO CXI compound microscope (Micron Instruments Ltd, USA). Using the eyepiece micrometer, the globule sizes of the dispersed phase was determined by taking the average values from ten similarly sized globules.

Centrifugal analysis

The various cream formulations (5 g each) were placed in the centrifuge tubes (CENCOM II, JP Selecta, UK) and the equipment was ran at a speed of 5000 rpm for 15 min over 2 cycles. At the end of each cycle, the samples in the tubes were investigated macroscopically for the evidence of liquefaction or presence of any possible phase separation [5].

Stability

Stability studies were carried out on the creams using a previously reported method with modification [28]. A set of three creams for each of the six cream formulations were maintained at 25 ± 2 °C for 3 months while another set of three creams were subjected to a temperature of 40 ± 2 °C for 3 months. The cream samples were taken and evaluated for stability in terms of changes in colour, odour, pH, liquefaction or phase separation (on centrifugation) after 0, 15, 30, 60, and 90 days.

Statistical analysis

All experimental data were collected in triplicates and data expressed as mean ± standard deviation. A one-way ANOVA test, with Bonferroni post-hoc test was carried out using GraphPad Prism 8.4.3.686, with $p < 0.05$, depicting statistically significant differences between data sets.

Results

Properties of capsaicin extracts

The average volume obtained for the capsaicin extracts was 50 ± 3.0 mL. The extracts exhibited varied colour depending on the *Capsicum* fruit species with the extract of C. *frutescens* being light red in colour while those of C. *chinense* and C. *pubescens* were dark red.

The FT-IR spectra of the extracts from the three different *Capsicum* fruit species were similar. Their spectra confirmed the presence of both capsaicin and dihydrocapsaicin as shown in Figure 1a, with the former being the major constituent in all the fruit species. Typical absorption bands exhibited N-H stretch of amide (3287 cm^{-1}), aliphatic C-H stretch of methyl groups (2923, 2853 cm^{-1}), N-H bend of primary amines (1627 cm^{-1}) and C-N stretch of aliphatic amines (1243 cm^{-1}).

The chromatographic quantification of the extracts revealed capsaicin elution at 6.1 min with the amounts of capsaicin in *C. pubescens* being 4549 ppm, while *C. frutescens* had 7860 ppm and *C. chinense* was 36153 ppm. Result from the compatibility study between capsaicin extract-Grewia mucilage powders is shown in Figure 1b. The spectrum obtained showed no possible interaction between the Grewia mucilage powders and capsaicin when the spectra of capsaicin in Figure 1a was compared with that of Figure 1b.

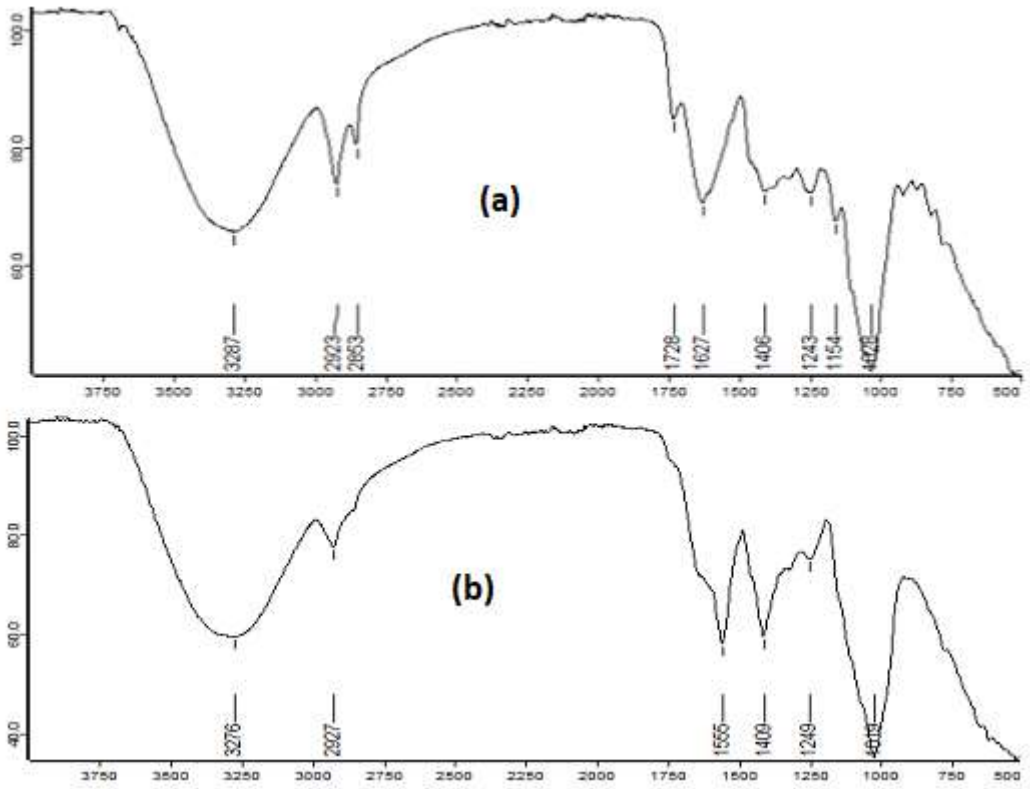

Figure 1: FTIR spectra of *Capsicum chinense* fruit extract containing capsaicin (a) and a mixture of *C. chinense* extract and *Grewia mollis* mucilage powder (b)

Table 2 shows the results from the microbial load determinations carried out on both the capsaicin extract from *C. chinense* and *Grewia mollis* mucilage powder. Their values indicated that the microbial load of both the extract and powders were within the Pharmacopoeia acceptable limits. There were no obnoxious microbes such as *Salmonella spps, Pseudomonas aeruginosa, Escherichia coli* and yeasts.

Table 2: Microbial load of *Grewia mollis* mucilage powder and capsaicin from *C. chinense*

Microbial type	Microbial load (cfu/g)		USP specification
	***Grewia mollis* mucilage powder**	**C. *chinense* extract powder**	
Total aerobic viable counts	65	42	NMT 10^5
Salmonella spps.	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent
Escherichia coli	Absent	Absent	Absent
Yeasts	Absent	Absent	NMT 10^3

Key: NMT = Not More than

Table 3 shows some of the physical properties of the formulated o/w creams. The pH of all the formulations were mildly acidic (6.11-6.44) and their specific gravity values ranged from 1.00 - 1.03, indicating their miscibility with water and also inferring that the creams will not cause occlusion when applied to the skin. Their electrical conductivity values ranged from 292 to 1958 µS/cm. Formulation FB that has only GMM as cream base has the lowest electrical conductivity, while Formulation FF with the highest amounts of GMM and HPMC in

combination as cream base also exhibited the highest conductivity. The viscosities of all the formulations were appreciably high and ranged from 2810- 9190 mPas. The viscosities of formulations that had co-polymeric constituents of GMM and HPMC as cream base were comparatively lower to those that contained either GMM or HPMC as base constituents. Furthermore, as shown in Table 3 the creams had appreciable spreadability which ranged from 4.0 - 5.5 cm. The globule sizes of the creams ranged from 12.97-91.0 µm. Formulation FC which has 10% HPMC as the mono polymeric constituent has the highest globule size and the smallest globular size was exhibited by FF which has GMM_{15}:$HPMC_{10}$ co-polymeric ratio concentrations. The cream formulations were all stable (no phase separation) when subjected to 2 cycles of 15 min centrifugation at 5000 rpm.

Table 3: Some physical properties of the formulated creams

Cream code	pH	Specific gravity	Electrical Conductivity (µS/cm)	Viscosity (mPas)	Spreadability (cm)	Globule Size (µm)	Stability on centrifugation
FA	6.24 ± 0.01	1.03 ± 0.02	1749 ± 16	3090 ± 11	5.5 ± 0.3	45.5 ± 4.7	Stable
FB	6.11 ± 0.03	1.02 ± 0.01	292 ± 9	9190 ± 21	0.1 ± 0.2	28.6 ± 3.6	Stable
FC	6.17 ± 0.02	1.03 ± 0.02	1127 ± 13	7590 ± 16	4.0 ± 0.1	91.0 ± 11.7	Stable
FD	6.13 ± 0.01	0.98 ± 0.01	1812 ± 18	2810 ± 8	4.9 ± 0.3	16.2 ± 4.8	Stable
FE	6.34 ± 0.01	1.00 ± 0.01	1214 ± 11	5020 ± 8	4.1 ± 0.2	39.2 ± 5.7	Stable
FF	6.44 ± 0.02	1.00 ± 0.01	1958 ± 17	3170 ± 10	4.5 ± 0.3	13.0 ± 4.5	Stable

Key: FA = GMM: HPMC (0:5); FB = GMM: HPMC (10:0); FC = GMM: HPMC (0:10); FD = GMM: HPMC (5:10); FE = GMM: HPMC (10:10); FF = GMM: HPMC (15:10)

Table 4 shows the microbial load of the various cream formulations. Results indicate acceptable microbial load, as the values of total counts of viable aerobic microbes were within the Pharmacopoeia limits. Also, there was absence of obnoxious organisms such as *Salmonella spps*, *E. coli and Pseudomonas aeruginosa* as well as yeasts and moulds.

Table 5 shows the effect of storage temperature on the pH of cream formulations at various time intervals over a period of 90 days. Results show a non-significant slight reduction in the pH of all the formulations stored at 25 ± 2 °C over the storage period of 90 days, whereas, formulations stored at 40 ± 2 °C over the same storage period showed a more pronounced reduction in the formulations pH with increasing storage period.

Table 4: Microbial load of the formulated creams

Microbial type	Microbial load (cfu/mL)						USP specification
	FA	FB	FC	FD	FE	FF	
Total viable counts	56	51	54	50	50	50	NMT 10^5
Salmonella spps.	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Escherichia coli)	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Yeasts and moulds	Absent	Absent	Absent	Absent	Absent	Absent	NMT 10^3

Key: NMT = Not More than

Table 5: Comparative pH of the formulated creams at two different storage temperature over a 90 days period

Days	pH											
	25 ± 2 °C						40 ± 2 °C					
	FA	FB	FC	FD	FE	FF	FA	FB	FC	FD	FE	FF
0	6.24 ± 0.05	6.11 ± 0.06	6.17 ± 0.06	6.13 ± 0.08	6. 34 ± 0.06	6.44 ±0.05	6.24 ± 0.06	6.11 ± 0.03	6.17 ± 0.07	6.13 ± 0.06	6. 34 ± 0.03	6.44 ± 0.05
15	6.18 ± 0.04	6.09 ± 0.05	6.10 ± 0.07	6.08 ±0.05	6.29 ± 0.05	6.40 ±0.05	6.10 ± 0.07	5.85 ± 0.06	5.96 ± 0.06	5.70 ± 0.05	6.18 ± 0.05	6.23 ± 0.06
30	6.17 ± 0.05	6.04 ± 0.06	6.05 ± 0.05	6.00 ±0.05	6.20 ± 0.04	6.35 ±0.04	6.03 ± 0.02	5.62 ± 0.05	5.85 ± 0.05	5.65 ± 0.05	6.10 ± 0.05	6.10 ± 0.05
60	6.13 ± 0.02	6.02 ± 0.03	5.95 ± 0.03	5.85 ±0.04	6.17 ± 0.03	6.23 ±0.05	5.92 ± 0.04	5.51 ± 0.05	5.72 ± 0.03	5.53 ± 0.05	6.00 ± 0.03	5.93 ± 0.05
90	6.10 ± 0.05	5.95 ± 0.07	5.85 ± 0.02	5.65 ±0.03	6.07 ± 0.05	6.20 ±0.05	5.35 ± 0.05	5.34 ± 0.08	5.69 ± 0.05	5.50 ± 0.05	5.85 ± 0.04	5.85 ± 0.06

Stability (Centrifugation test)

Table 6 presents the effect of storage conditions on the stability of cream formulations at various time intervals over a period of 90 days. It was observed that formulation, kept at 25 ± 2 °C, did not show any phase separation at the completion of study. At higher temperatures of 40 ± 2 °C and 75% RH, slight instability (phase separation) was observed with most of the formulations from the 15th day of storage except FB. There was a progressive increase in phase separation as storage time increased up to the 90th day (Figure 2). Also, the colours of the formulations stored at 25 ± 2 °C did not significantly change, whereas, there were observed slight colour changes of bright red to dark red colorations for those stored at 40 ± 2 °C and 75% RH.

Table 6: Phase separation of the formulated creams at two different storage temperatures over a 90 days period

Days	Storage temperatures											
	25 ± 2 °C						40 ± 2 °C					
	FA	FB	FC	FD	FE	FF	FA	FB	FC	FD	FE	FF
0	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	- +	-	- +	- +	- +	- +
30	-	-	-	-	-	-	- +	- +	- +	- +	- +	- +
60	-	-	-	-	-	-	- +	- +	- +	- +	- +	- +
90	-	-	-	-	-	-	- +	- +	- +	- +	- +	- +

- = absence of phase separation at both cycles of 15 min centrifugation
- + = presence of phase separation at second cycle of 15 min centrifugation

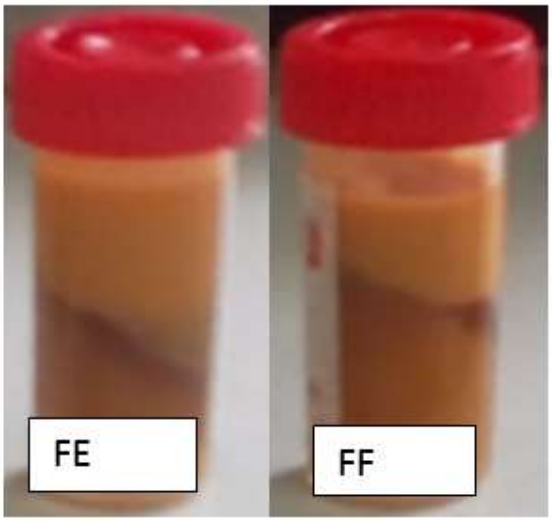

Figure 2: Cream samples (FE and FF) showing various degree of phase separation on subjection to 2 cycles of centrifugation at 5000 rpm for 15 min per cycle after 90 days storage period.

Discussion

The attributes of two polymeric substances have been explored to formulate pharmaceutically acceptable capsaicin creams. This entailed using optimized concentrations of either GMM or HPMC (as single polymeric cream base) or optimized ratio concentrations of GMM and HPMC as co- polymeric cream base/carrier.

The FT-IR absorption peaks gotten from the capsaicin extract indicated the characteristic bonds, stretches of the functional groups present in capsaicin. These bands are in agreement with the FT-IR bands previously reported [29]. Capsaicin contains phenol, ether, amide and alkene. It thus suggests that the capsaicin sourced from *C. chinense* would be suitable as an active pharmaceutical ingredient for topical formulations such as creams. The FTIR spectra of the mixture of GMM and capsicum extracts showed that the absorption peaks did not significantly change and suggest the compatibility of these two substances when used as co-constituents in pharmaceutical formulations.

The presence of both capsaicin and dihydrocapsaicin in the all the capsicum fruit species were confirmed with HPLC though their concentrations were dependent on both the fruit species and the extraction solvent used. The capsaicin content in *C. chinense* was found to be comparatively higher than other fruit species. The higher concentration of capsaicin in *C. chinense* indicated the cheaper economic utility of this fruit specie as a source of capsaicin and infers its comparatively higher potency when used in the cream formulations. However, the low microbial load of GMM and the capsaicin extract from *C. chinense* suggests a microbial profile within Pharmacopoeia specifications hence their microbiological safety when used in pharmaceutical formulations. Similar acceptable microbial load have been reported by some workers with species of *Grewia* [30, 31].

All the formulated creams had good aesthetics appearance, were smooth to feel, homogenous and showed no signs of phase separation. These qualities could be attributed to the inclusion of GMM as a polymeric carrier/base known for its viscosity enhancement, gelation and stabilization properties [32]. The use of GMM as a cream base/carrier system is novel, its material attributes could explain the better formulation outcomes of creams it contained comparative to those formulated with HPMC alone [30,33].

Another quality of the formulated creams were their acceptable pH values, they are hence expected to be non-irritant to the skin when applied topically [34]. Also, the pH profiles of topical formulations affects the chemical stability and effectiveness of such formulations [35]. This may explain the observed stabilities of these creams with inference to their effectiveness. The comparable specific gravity values with that of water exhibited by the creams suggest that they will remain as single-phase homogenous systems under low stress conditions.

The electrical conductivity of the formulations was dependent on HPMC concentration as their electrical conductivity increased with increasing HPMC concentration. Formulation FF exhibited the greatest conductivity values, inferring good emulsion stability. This finding was consistent with that of Zhang *et al.,*[2011] where they reported that some emulsifying conditions such as oil volume fraction and emulsifier composition resulted in decreased conductivity and stability [35]. Formulation FB containing GMM had comparatively lower electrical conductivity. This is an indication of the low sensitivity index of GMM, confirming the fact that natural polymers are inert and have low sensitivity.

There was a corresponding increase in the viscosity of the formulations with increase in concentrations of GMM component in the co-polymeric cream base from 5-10% and thereafter, a decrease in the viscosity at 15.0%. This implies a critical determinant role the GMM plays in the co-polymeric cream base carrier system. Though an optimum viscosity was obtained in formulation FE, containing equal concentrations of the composite polymers. The appreciably high viscosities of these formulated creams may point to the sustained release potential of these polymeric cream base/carrier systems.

Globule size of the formulations was found to be dependent on the composite polymeric constituents. Formulation FB that contained GMM as the mono polymeric base carrier system had lower globule sizes comparable to FB and FC which contained 5 and 10% HPMC, respectively. Also, formulations that contained co-polymeric base carrier system of GMM: HPMC had low globule sizes which were dependent on the concentration of GMM in the polymeric ratio blends. The effect of GMM on globule size reduction may be due to increased viscosity of the continuous phase, resulting in less frequent collision between the oil droplets of the dispersed phase, thus facilitating a reduction in their globule size [36].

Results from the stability studies of the formulated creams revealed little or no change in colour, pH and phase separation at storage temperature of 25 ± 2 °C C over a period of 90 days. This could be attributable to the high viscosities of the creams as a result of the polymeric base cream systems. This is an indication that the optimum storage condition for the creams would be at room temperature. However, creams stored at 40 ± 2 °C exhibited reduced pH, changes in colouration and different degrees of phase separations over the storage period of 90 days depending on the constituent polymeric cream base. The creams containing the co-polymeric cream base of GMM: HPMC

were more stable than the mono polymeric cream base system of either GMM or HPMC. This finding may be due to the synergistic effects of the composite polymeric systems that result in a rigid mesh-like hydrogel structures such that these formulations are able to withstand packaging, storage and environmental processes/stress variables such as temperatures and packaging systems.

The formulation (FB) that contained GMM alone as cream base was comparatively more stable than the HPMC alone formulation (FC), as it remained stable up to 15 days of storage. Formulation FE that had equal concentrations of GMM and HPMC as well as formulation FF that had higher concentration of GMM in the GMM: HPMC blend exhibited less phase separation when compared with formulations FC and FD. This infers more stability of the former formulations and is suggestive of the potential excipient usefulness of this natural polymer in pharmaceutical formulations. These observed levels of stability of the creams may be attributable to the superior viscosity enhancement attribute of GMM relative to HPMC in the formulation systems as GMM has been indicated as a viscosity enhancer [31] and HPMC as a former of strong viscous gel system [36].

Conclusion

Capsicum fruit species have been elucidated as potential local sources of capsaicin. This study has further shown the suitability of *Grewia mollis* mucilage singly used or in combination with hydroxypropyl methylcellulose as polymeric cream bases. Formulated creams (especially FB, FE and FF) had desirable physicochemical properties. These creams may find better patient acceptance when compared with imported brands because of their potential low cost.

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Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Boladale O. Silva and Modupe O. Ologunagba conceived and designed the study and supervised research work. Modupe O. Ologunagba, Asenath N. Echerenwa and Oluwadamilola M. Kolawole carried out the experiments, collected and analyzed the data, as well as prepared the preliminary manuscript. Oluwadamilola M. Kolawole revised the manuscript and carried out statistical analysis of data. All authors have read and approved the manuscript for publication.

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