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Original Research Article

Design, Development and Evaluation of the Repellent Activity of *Azadirachta indica* Oil-Based Solid Lipid Microparticles against *Aedes aegypti* (Linn)

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ABSTRACT

The use of repellents has shown to be a practical and economical way of preventing mosquito-transmitted diseases. The aim of the study was to design, formulate and characterize *Azadirachta indica* (neem) oil based solid lipid microparticulate (SLMs) creams and thereafter evaluate its repellent activity against the *Aedes aegypti* mosquito. The oil was extracted from fresh leaves of *Azadirachta indica* using a soxhlet apparatus. The solid lipid microparticles (SLMs) were prepared using hot homogenization method. The stability of the formulated SLMs creams was evaluated by measuring the pH and particle size at determined time intervals. The morphology, encapsulation efficiency, loading capacity, FTIR analysis, skin irritation ability and mosquito repellence of the SLMs were also determined. The SLMs composed of both spherical and non-spherical particles and exhibited a slight decrease in the pH and an increase in particle size over time. The neem oil encapsulation efficiency of the SLMs were in the range 52 – 71% while loading capacity were between 2.6 and 10.3 g/100 g lipid. The Fourier-transform infrared (FT-IR) spectroscopy revealed no strong chemical interaction(s) between the oil and excipients. The SLMs formulations showed no sign of skin irritation and produced higher and more prolonged repellence activity than the unformulated oil but lower than the commercial mosquito repellent. The study successfully developed SLMs loaded with *Azadirachta indica* oil which significantly increased the mosquito repellent activity of the oil and thus represents a possible alternative to synthetic mosquito repellents.

Keywords: Solid lipid microparticles, Mosquito repellents, *Aedes aegypti, Azadirachta indica* A. Juss, Lipid based drug delivery systems.

Introduction

Vector-borne diseases have been reported to pose a major challenge in public health and mosquitoes are one of such vectors that transmit a large number of the pathogens responsible for these diseases.[1] The *Aedes aegypti* mosquito is one of the most clinically important vectors with the ability to transmit deadly diseases such as yellow fever, dengue, chikungunya, and the Zika virus.[1,2] The fact that vaccines have not been developed for many of the diseases transmitted by the *Aedes aegypti*, as with the dengue and zika, has made vector control a major strategy in the prevention as well as management of outbreaks.[3,4] This relies heavily on the use of insecticides either in the reduction of breeding sites or in the reduction of the density of adult mosquitoes.[3,5] The organophosphates (such as temephos) as well as insect growth regulators (such as diflubenzuron and methoprene) have generally been used in the control of mosquito larvae.[6,7] Pyrethroids, on the other hand, are usually deployed against the adult mosquito population.[6,7] The continuous use of these substances have resulted to undesirable effects on humans, non-target organisms as well as the environment.[8,9] Furthermore, the repeated use of these substances has been reported to bring about resistant populations of the *Aedes aegypti*,[10] thereby creating the need to develop safer alternative approaches for the control of the insects.

Personal protective measures such as the use of repellents have also been reported to play a vital role in reducing the transmission of diseases by preventing infected mosquitoes from biting humans.[11] N, N-diethyl-meta-toluamide, (DEET) has been used as a gold standard for repellents for many decades,[2] however, there are some challenges associated with its repeated and prolonged use. These include toxicity issues in humans, ranging from mild side effects such as skin eruptions to severe effects like seizures[12] and induction of encephalopathy in children, especially at high concentrations.[11] It has been reported to have undesirable effects on other mammals and non-target insects.[13] These challenges have inspired researchers to explore non-toxic, biodegradable as well as more eco-friendly plant sources of insect repellents in order to scale down the negative effects of the synthetic insect repellents on humans as well as the environment. Moreover, plant-derived insect repellents exhibit very low chances of development of resistance since these substances have very diverse phytochemical compositions which exert different mechanism of action, unlike the synthetic agents with insect repellent activity, which are usually composed of a single chemical active ingredient.[14] Plant-derived insect repellents have also been investigated over the years due to their cost-effectiveness. Many plants such as *Ocimum gratissimum*, *Thymus vulgaris*, *Cymbopogon nardus*, *Cymbopogon citratus* and *Azadirachta indica*, have been reported to exhibit repellent activities against many insects, mosquitoes inclusive.[1] However, one of the major drawbacks of using plant-derived repellents is the fact that these substances have short-lived actions. As

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a result of this, various studies have been conducted to explore diverse ways of improving the duration of action and hence the efficacy of these plant-based insect repellents. For instance, fixatives have been used to improve the local delivery of essential oils for insect repellency.[11,15] The *Azadirachta indica* plant is a large tree which grows to a height of about 15 – 20 m. It is widely distributed across several countries globally and has been reported to possess extensive biological activities such as antibacterial, antifungal, anti-inflammatory, anti-malarial, antiviral, antiulcer, immunomodulatory as well as insect repellent properties.[16] Novel drug delivery systems such as nanoemulsions and microcapsules have also been successfully employed as delivery systems for plant-derived insect repellents and have demonstrated their ability to be used as a tool for mosquito control.[13,17-23] Despite the vast studies that involved the use of novel drug delivery systems for the delivery of plant-based insect repellents, there are very limited research on the use of solid lipid microparticles as delivery systems for plant-based insect repellents. The study aimed to design, formulate and characterize solid lipid microparticulate (SLMs) creams using *Azadirachta indica* oil and evaluate their repellent activity against the *Aedes egypti* mosquito.

Materials and Methods

Chemicals, reagents and solvents

The following materials were procured from their local suppliers and used without further purification: Phospholipon 90G® (Phospholipid GMbH, Köln, Germany), n-hexane (Guangdon, China), Polysorbate 80® (Sigma-Aldrich, Germany), sorbic acid and sorbitol (Merck, Germany), distilled water (The National Centre for Energy Research and Development, University of Nigeria Nsukka).

Collection and extraction of plant materials

Fresh leaves of *Azadirachta indica A. Juss* were collected in April 2019, from a local farm in Nsukka, Enugu state, Nigeria. The leaves were identified and authenticated by Mr. A. O. Ozioko of the International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State. A reference voucher (InterCEDD/966) was deposited at the herbarium of the centre. The extractions were carried out according to the method used by Egunyomi *et al*, [24] with slight modification. Briefly, the fresh leaves were milled and 50 g of the milled plant material macerated in 500 mL of n-hexane and the mixture was extracted in a Soxhlet apparatus for 8 h. The extract was then concentrated using a vacuum rotary evaporator (RE300 ROTAFLO, Fisher Scientific Ltd UK) and refrigerated at 4 ^{o}C prior to use.

Phytochemical screening

Phytochemical screening of the oil obtained was carried out employing standard procedures.[25] The presence of chemical constituents (secondary metabolites) such as alkaloids, flavonoids tannins, terpenes, saponins, steroids, phenols, and anthraquinones were investigated according to the methodology used by Adeniyi *et al*.[25]

Extraction of lipid from goat fat (Capra hircus)

Lipid was extracted from goat fat (obtained from *Capra aegagrus hircus* which was purchased in Ogige market in Nsukka, Enugu state), by wet rendering method as reported by Attama and Nkemnele[26] with slight modification. Briefly, the extraneous materials from the adipose tissue were removed. The adipose tissue (200 g) was then grated and subjected to moist heat by boiling with half its weight of water in a water bath for 45 min. The molten fat was then separated from the aqueous phase using a muslin cloth. The resulting lipid was purified by heating it with a 2% w/w suspension of a 1:9 ratio blend of activated charcoal and bentonite at 80^{o}C for 1 h. The mixture was then vacuum filtered using a Buchner funnel. The lipid was stored at room temperature prior to use.

Preparation of lipid matrix

The lipid matrix was prepared by fusion method previously reported by Attama and Nkemnele.[26] The goat fat (70 g) was melted in a beaker placed on a water bath heated to a temperature of 60^{o}C. The phospholipid, Phospholipon® 90G, (30 g) was then added to the melted goat fat and stirred until a homogenous mix was obtained. The molten lipid matrix was then allowed to cool.

Preparation of SLMs

The method of hot homogenization was employed in the preparation of different batches of the SLMs creams, using the formula in Table 1.[27] For each batch, the required quantity of neem oil was placed in a 100 mL beaker containing molten lipid matrix placed on a hot water bath heated to a temperature of 60 ^{o}C. Sorbic acid, sorbitol and polysorbate 80 were accurately measured and dissolved in water at the same temperature. The aqueous mixture was then added to the beaker containing the molten lipid matrix and neem oil, then homogenized using a digital Ultra-Turrax; homogenizer (T25 IKA, Staufen, Germany), at 10,000 rpm for 10 min. The SLMs preparations were then stored at room temperature. The procedure was repeated with various concentrations of neem oil.

Physicochemical characterization of SLM

Time dependent pH study

The pH of various cream formulations (50 mL) was determined using a validated digital pH meter (pH H12002-01 edge, Hanna Instruments, Padova, Italy) after calibrating with standard buffers. The measurement was done in triplicate and the average of the readings taken. The measurements were then repeated at room temperature on days 7, 14, 30 and 90.

Determination of particle size

Each of the SLMs formulation (20 mg) was placed on a glass slide and dispersed using distilled water. The slide was then covered with a slip and viewed with a photomicroscope (Hund®, Weltzar, Germany) fitted with an electronic image analyser (Moticam® China), at a magnification of 400×. The average particle size of the microparticles was determined (n = 50). This was repeated at room temperature on days 7, 14, 30 and 90.

Table 1: Formula and composition of the SLMs creams

Batches	Plant oil (%)	Lipid matrix (%)	Polysorbate 80 (%)	Sorbitol (%)	Sorbic acid (%)	Distilled water (%)
Unloaded SLM	0	12	1.5	4	0.05	qs 100
5 A	5	12	1.5	4	0.05	qs 100
10 A	10	12	1.5	4	0.05	qs 100
15 A	15	12	1.5	4	0.05	qs 100

Key: Unloaded SLM contains 0% *Azadirachta indica* oil, 5A batch contains 5% *Azadirachta indica* oil, 10A batch contains 10% *Azadirachta indica* oil and 15A batch contains 15% *Azadirachta indica* oil

Morphology
The morphology of the various SLM batches was observed using a scanning electron microscope (Hitachi, 3400 N, Japan) with 15 kV accelerating voltage. The samples were prepared by placing 10 µg of the SLMs onto an aluminium specimen stub, dried, and sputter coated with gold prior to imaging.

Determination of entrapment efficiency and loading capacity
Entrapment efficiency (EE) and loading capacity (LC) of the formulations were determined using the method described by Chime *et al*[28] with some modifications. A Beer's calibration curve was obtained for the neem oil in Dimethyl sulfoxide (DMSO) at a concentration range of 1-5 mg% at a predetermined wavelength maximum of 275 nm. The neem oil-loaded SLMs were suspended in DMSO and separated by centrifugation at 5,000 rpm for 45 min. The supernatant was carefully separated which contained the unloaded neem oil and analysed by UV spectroscopy at predetermined wavelength maximum of 275 nm. Entrapment efficiency (% EE) was calculated using the formula:

$$\% \text{ EE} = \frac{(\text{total amount of neem oil added} - \text{the amount present in supernatant})}{\text{total amount of neem oil added}} \times 100$$

The loading capacity (LC) was calculated using the formula:

$$\text{LC} = \frac{(\text{total amount of neem oil added} - \text{the amount present in supernatant})}{\text{weight of the microparticles}} \times 100$$

FT-IR analysis
FT-IR spectra of formulations as well as that of the neem oil were obtained at a range of 4000-650 cm^{-1} according to methods by Kenechukwu *et al*.[29] Infrared spectra were obtained on infrared spectrophotometer (Agilent technologies, FTIR- Cary 630, USA) using potassium bromide of spectroscopic grade. The potassium bromate (KBr) plate was first cleaned with a trisolvent (acetone-toluene-methanol at 3:1:1 ratio) mixture for baseline scanning. The test formulation (0.1 g) was then mixed with nujol (0.1 mL) diluent. The mixture was introduced into the potassium bromate (KBr) plate and compressed into pellets, each of which was placed in the light path and the spectrum obtained.

Skin irritation test
Ethical clearance was obtained from the University of Nigeria ethical committee (DOR/UNN/19/00025) prior to the commencement of the study. The skin irritation test was carried out using the method described by James and Sunday,[30] with slight modification. Herein, five (A-E) groups each of four Wistar albino rats were used. Groups A, B, C and D were treated with 5A, 10A, 15A and unloaded SLM formulations, respectively while group E which was treated with distilled water served as the negative control.[30] The back of the animals was carefully shaved free of fur 24 h before application of the test samples. Methylated spirit was applied as an antiseptic to the shaved region with the aid of a cotton wool to prevent infections by microorganisms. An area of 40 mm x 30 mm on the shaved skin was marked. For each group, the test formulation (0.5 g), was applied topically on the shaved skin of the rats and held in contact with the skin using an adhesive plaster and a non-occlusive bandage dressing for 24 h. Thereafter, the bandage and the test materials were removed and the surface of the skin was rinsed with distilled water and 1 h later, the sites were examined for skin irritation. The skin of the rats was observed 24 h after the application of the formulations, and repeated after 48 h, 72 h, 7 days and 14 days. The animals were then euthanized using chloroform.

Evaluation of repellent activity
Preparation of mosquitoes for repellence test
Laboratory strains of mosquito larvae *of Aedes aegypti* were collected from National Arbovirus and Vector Research Centre Enugu, Nigeria and reared in the insectary, Department of Zoology, University of Nigeria Nsukka. The mosquitoes were about 6-8 days old, free from pathogens and were starved 12 h prior to the commencement of the study.

Preparation of human volunteers for mosquito repellence test
The laboratory mosquito repellence test was conducted using the standard human-bait technique according to WHO[31] and Keziah *et al*.[32] Ethical clearance was obtained from the University ethical committee (DOR/UNN/19/00025) before commencing the investigation. Also, eight human volunteers, both male and female, used in the study signed an informed consent form. An area of 4 by 4 cm (16 cm^2) was marked and cut open on plastic disposable hand gloves worn by the volunteers. The edges of the cut area were lined with masking tape.

Determination of percentage mosquito repellence
The study was conducted between 7 am and 6 pm, due to the fact that the *Aedes aegypti* mosquito is a day-biter. Prior to the commencement of the study, the hand of each volunteer was washed with an unscented neutral soap, thoroughly rinsed, and allowed to dry for about 20 min. The left hand of each volunteer was used for the test formulations (unloaded SLMS, 5A, 10A and 15A) while the right hand which was untreated served as the negative control. Each test formulation was applied from the cut part of the glove exposing the skin. An attempt of the mosquito to insert its stylets was considered as a bite, recorded and then shaken off before imbibing any blood. The control hand was first exposed to mosquitoes for 1 min and the number of mosquito bites recorded before the hand treated with the test formulations. The number of bites was counted over 1 min; every 30 min and tests were discontinued after 360 min. The experiments were conducted in triplicates in separate cages. In the triplicate study, different volunteer and different formulations were used to nullify any effect of skin differences on mosquito repellence. The percentage repellence was calculated by using the following formula:

$$\text{Mosquito Repellence (\%)} = \frac{A-B}{A} \times 100$$

Where A is the mean number of mosquitoes landing on the negative control arm and B the mean number of mosquitoes landing on test arm.

Statistical analysis
The percentage protection from mosquito infestation was calculated using Microsoft Excel, 2007 and all the results subjected to SPSS analysis to calculate the arithmetic mean as well as standard deviation values. Data was analysed using one-way analysis of variance, with $p < 0.05$ considered significant statistical difference between data sets.

Results and Discussion

Phytochemical study
Plant products obtained from various plant parts such as barks, leaves, fruits, roots as well as seeds have played vital roles in phytomedicine since time immemorial. Knowledge of the chemical constituents of plants is desirable as such information provides an insight to the pharmacological effects of the plant as well as helps in the synthesis of complex chemical substances.[32] Phytochemical screening of plants is very pivotal in herbal drug formulations as it provides information on the bioactive substances present in the plant(s).[33] Table 2 shows the result of the phytochemical screening of the oil extracted and reveals that the oil is very rich in diverse phytochemicals such anthraquinones, steroids, phenols, flavonoids, alkaloids and terpenoids. The presence of terpenoids in the oil extract further supported the possibility of the plant possessing insect repellent, anti-feedant, insect growth disruption properties as well as considerable

toxicity toward insects as plant-based terpenoids are very lipophilic and tend to have strong aromas.[33]

Time dependent pH study

A time-resolved pH analysis to evaluate the stability of the various SLMs batches when stored at room temperature over a period of time is presented in Figure 1. It was observed that after 24 h, the batches had pH in the range of 5.08 ± 0.28 – 5.96 ± 0.52 and decrease after 12 weeks (4.3 ± 0.21 - 5.21 ± 0.65). The unloaded SLMs batch presented with the lowest pH throughout the study. It was also observed that there was a slight increase in pH as the concentration of the neem oil increased in the formulation.

The result of the pH study over time as seen in Figure 1 showed significant ($p < 0.05$) decline in the pH of the SLMs batches. This strongly suggests that the formulations might require a buffer in order to ensure stability over time. However, the reduction in pH was not as a result of degradation of the active ingredient, due to the fact that the unloaded SLM batch also experienced a reduction in pH over time. This reduction in pH might be as a result of the release of free fatty acid from the lipid matrix.[34] Although the formulations showed a significant change in pH over time, the pH of all the batches was somewhat within a suitable range for topical formulations as the pH of the skin ranges from 4 - 7.[35]

Particle size analysis

The results of the particle size analysis of the SLMs formulations over time are presented in Table 3. Particle size characterization for SLMs is very important in ensuring the production of a stable as well as a quality formulation due to the fact that particle size plays a vital role in the physical stability of SLMs.[36] The unloaded SLM formulation, containing no active ingredient had the lowest particle size compared to the other neem oil loaded SLMs formulations throughout the period of study. It was also observed that as the concentration of neem oil loaded in the SLMs increased, the particle size of the formulations increased with the 5A sample containing 5% neem oil having mean particle size of 16.97 ± 2.93 µm while the 10A and 15A formulations containing 10% and 15% neem oil, respectively had mean particle size of 19.44 ± 4.30 µm and 24.12 ± 3.81 µm, respectively at 24 h post production. It was also observed that although the neem oil loaded formulations experienced an increase in particle size over a period of 12 weeks, only the 5A batch produced a significant increase in particle size ($p < 0.05$). The unloaded SLMs batch on the other hand exhibited a significant decrease in particle size, over 12-weeks study period ($p < 0.05$).

The neem oil loaded SLMs formulations had higher particle sizes compared to the unloaded batch and increased over the period of study (Table 3). This increase might be as a result of particle aggregation and subsequent particle growth by Oswald ripening or it might be due to crystallization of the formerly molten matrices.[36]

Morphology of various formulation batches

The micrographs showed that the formulations contained roughly spherical and non-spherical particles in the micrometer range (Figure 2). The particles in the unloaded batch appeared singly while those in 5A, 10A and 15A appeared agglomerated, with the extent of agglomeration increasing with increase in the concentration of the neem oil loaded in the lipid matrix. The shape of SLMs has been reported to be dependent on the purity of the lipids employed in the preparation of the SLMs. SLMs prepared from highly pure lipids are usually cuboidal in nature, while those obtained using chemically polydispersed lipids are typically spherical.[37] The nature of the matrices used as well as the active ingredient could also determine the particle morphology, hence the non-spherical shapes.[37] The agglomeration exhibited by the 5A, 10A and 15A batches might also be due to the presence of high concentration of the SLMs during the analysis.

Encapsulation efficiency and loading capacity

The ability of formulations to accommodate the loaded active pharmaceutical ingredient(s) is a very vital property and can be described by the encapsulation efficiency (EE) and loading capacity (LC). These parameters are affected by a range of factors such as drug concentration, nature of the lipid, method of SLMs preparation amongst others.[33] For each batch, a fixed amount of the lipid matrix was used and the concentration of the oil increased in order to evaluate the effect of increasing the concentration of the active ingredient on the EE and LC. The percentage EE and LC of the formulation batches are presented in Table 3. The result showed that the various formulations batches had very good extent of neem oil encapsulation (Table 3). The percentage EE increased with increase in the concentration of the neem oil loaded into the SLMs until 10% concentration (10A batch) after which there was a decrease. This might be due to the fact that the oil attained a saturated solubility in the lipid matrix at 10% concentration and therefore additional oil could not be solubilized within the matrix.[33] Also, the loading capacity increased with increase in concentration of the neem oil, with the 5A batch having the lowest LC (2.6 g oil/100 g lipid) and 15A the highest (10.3 g oil/100 g lipid). Overall, the result showed that the EE and LC of the SLM were dictated by the total concentration of the active ingredient (oil) in the formulation.

FT-IR analysis of the unloaded and neem oil loaded SLMs

Evaluating the compatibility of the active ingredient with the excipient(s) is a vital process in the design as well as development of novel formulations. This is primarily due to the fact that physical and chemical interactions between drugs and excipients can affect the chemical structure, stability, manufacturability, bioavailability of drugs or delivery of the drug to the patient and their therapeutic effect and safety.[37,38] The IR spectra of the neem oil, unloaded SLMs as well as loaded SLMs formulations were obtained in a bid to demonstrate the feasibility of incorporating *Azadirachta indica* oil into SLMs (Figure 3).

The FTIR spectrum of the unloaded SLM formulation as seen in Figure 3 showed a strong wide band at 3305 cm^{-1} indicatives of an –OH stretching. Prominent peaks were also observed at 2918 cm^{-1} and 2851 cm^{-1} for –CH stretching of an alkane, at 1740 cm^{-1} for –C=O stretching and at 1654 cm^{-1} for –C=C stretch. The 5A formulation containing 5% neem oil exhibited strong peaks at 3339 cm^{-1}, 2918-2815 cm^{-1}, 1720 cm^{-1} and 1640 cm^{-1} indicative of an –OH stretching, –CH stretching of an alkane, –C=O stretching and –C=C stretch, respectively. This spectrum was also similar to the 10A and 15A batches containing 10% and 15% neem oil, respectively, which displayed strong peaks indicative of an –OH stretching at 3305 cm^{-1} and 3350 cm^{-1}, respectively, and also showed prominent peaks at 2918-2815 cm^{-1} for –CH stretching of an alkane, at 1736 cm^{-1} for –C=O stretching and at 1640 cm^{-1} for –C=C stretch. The spectrum of the *Azadirachta indica* oil showed prominent peaks at 2922 $cm^{-1}$and 2856 cm^{-1} which represents the –CH stretching. Also a medium absorption peak at 1461 cm^{-1} was observed which shows the presence of C-H bend as seen in alkanes. The principal peaks of FTIR spectra of the oil and the unloaded SLMs appeared in the various formulation batches therefore, it can be deduced that there was no strong chemical interaction between the oil extracts and excipients.

Table 2: Phytochemicals present in *Azadirachta indica* extracts

Phytochemicals	Inference
Steroids	+
Phenols	+
Anthraquinones	+
Saponins	-
Alkaloids	+
Tannins	-
Flavonoids	+
Terpenoids	+

Key: - = not detected, + = detected

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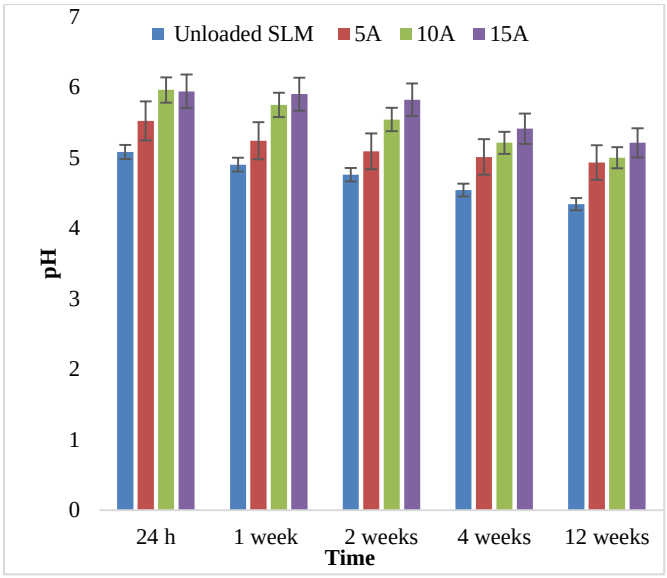

Figure 1: Mean pH (±SD) of the various formulation batches over time

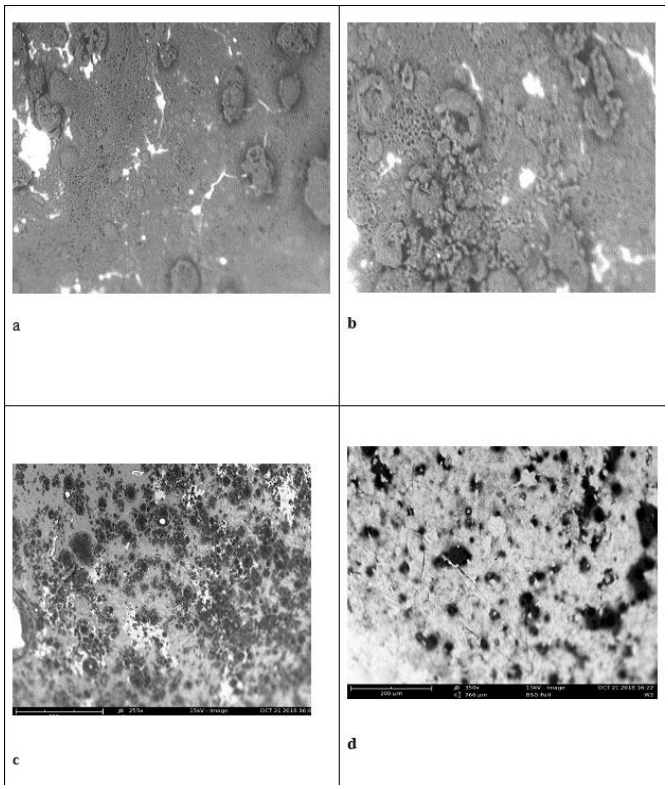

Figure 2: SEM micrographs of the SLM samples - (a) Unloaded SLM, (b) 5A, (c) 10A, (d) 15A.

Skin irritation test

Evaluation of the ability of pharmaceutical and cosmetic products containing natural products to cause irritation is a vital step in determining their biocompatibility.[39] The various formulation batches were free of any irritation on application to the skin with no signs of erythema and oedema on inspection of the application sites.

Evaluation of repellent activity

Figure 4 shows the percentage repellence of the various SLMs batches against *Aedes aegypti*. It was observed that the 5A formulation provided total protection (100% repellence) for a period of 30 min which later reduced to 78% after 2.5 h and then 42.9% after 6 h. The 10A and 15A samples containing 10% and 15% neem oil, respectively were able to provide 100% repellence for a period of 2.5 and 2 h, respectively after which their percentage mosquito repellence decreased to 60% and 43.8%, respectively by the end of the 6 h study period. The unformulated oil produced 100% repellence only at the time of application and this reduced rapidly to 98.7% after 30 min and thereafter 29.8% after 6 h. The commercial sample on the other hand, produced statistically significant higher mosquito repellence (100% protection for up to 3.5 h) than the neem oil SLMs formulations ($p < 0.05$).

The result of the mosquito repellence test (Figure 4) showed that the formulations exhibited a concentration-dependent mosquito repellent activity and as such the unloaded SLMs sample did not offer total protection at any time in the study. The 10A batch containing 10% of neem oil, which had the highest percentage EE also produced the highest repellent activity compared to the other neem oil loaded SLMs formulations. The formulations were also able to prolong the action of neem oil as they were able to provide total protection for a statistically significant longer period ($p < 0.05$) compared to the unformulated neem oil, which only provided 100% mosquito repellence on application. Although the commercial sample, showed a higher percentage repellence compared to the formulations, the use of these formulations (especially batch 10A) could serve as an alternative to the commercial sample.

Conclusion

In this study, *Azadirachta indica* oil was successfully extracted using a soxhlet apparatus and n-hexane as solvent. The oil was then loaded into SLMs with lipid matrices consisting of goat fat and phospholipon 90G (70:30) using hot homogenization method. Although there was a significant decrease in the pH for the various formulations over a period of 12 weeks, the pH was in the range of 4.30 ± 0.21 - 5.96 ± 0.52 which was somewhat within the ideal range for skin preparations. The particle sizes of the neem oil loaded SLMs formulations were relatively unchanged over time. The neem oil loaded SLMs formulations showed no sign of skin irritation. The study also showed that the SLM formulations produced higher mosquito repellence than the unformulated neem oil. On the other hand, the commercial sample containing 12% DEET produced a slight statistically significant higher percentage mosquito repellence ($p < 0.05$) than the SLM formulations. However, due to the toxicity of the commercial sample as well as other synthetic repellents, the *Azadirachta indica* oil loaded SLMs represents a possible alternative to synthetic repellents.

Table 3: Encapsulation efficiency (EE), loading capacity (LC) and particle size analysis of the various SLM batches

Batch	Encapsulation efficiency (%)	Loading capacity (g extract / 100 g lipid)	Particle size (µm)				
			24 h	Week 1	Week 2	Week 4	Week 12
Unloaded SLM	-	-	15.53 ± 0.86	12.42 ± 3.91	11.55 ± 2.75	10.40 ± 3.59	8.13 ± 0.60*
5A	52	2.6	16.97 ± 2.93	15.42 ± 2.01	20.56 ± 2.39	22.29 ±1.90*	25.52 ± 2.89*
10A	71	7.1	19.44 ± 4.30	15.39 ± 2.68	19.02 ± 2.30	20.44 ± 2.24	21.80 ± 3.08
15A	68.7	10.3	24.12 ± 3.81	23.43 ± 4.27	23.95 ± 1.74	25.09 ± 3.53	26.67 ± 1.61

Key: * indicates batches with significant particle size changes

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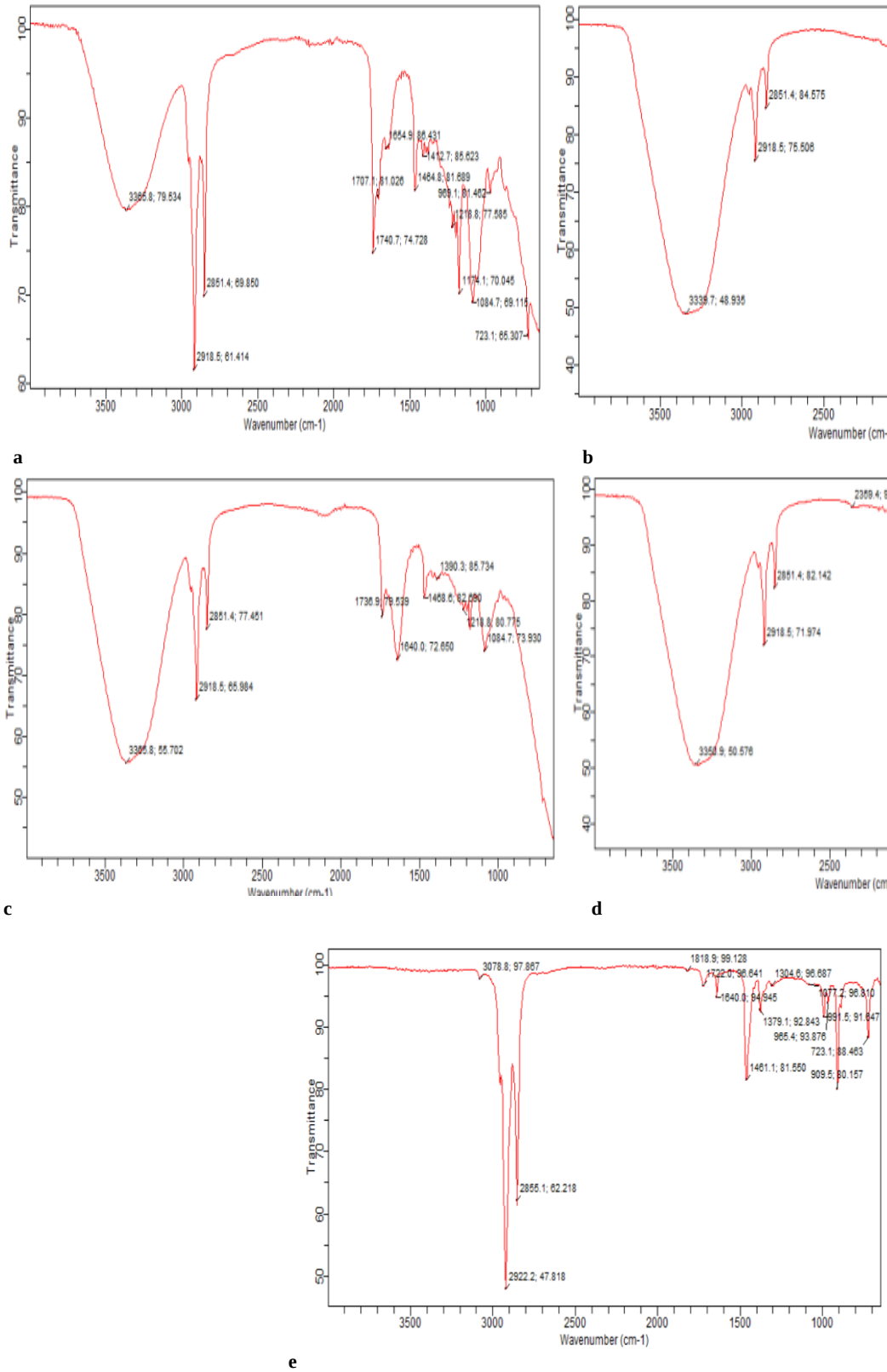

Figure 3: FTIR spectra of the of the SLM samples - (**a**) Unloaded SLM, (**b**) 5A, (**c**) 10A, (**d**) 15A, and (**e**) neem oil

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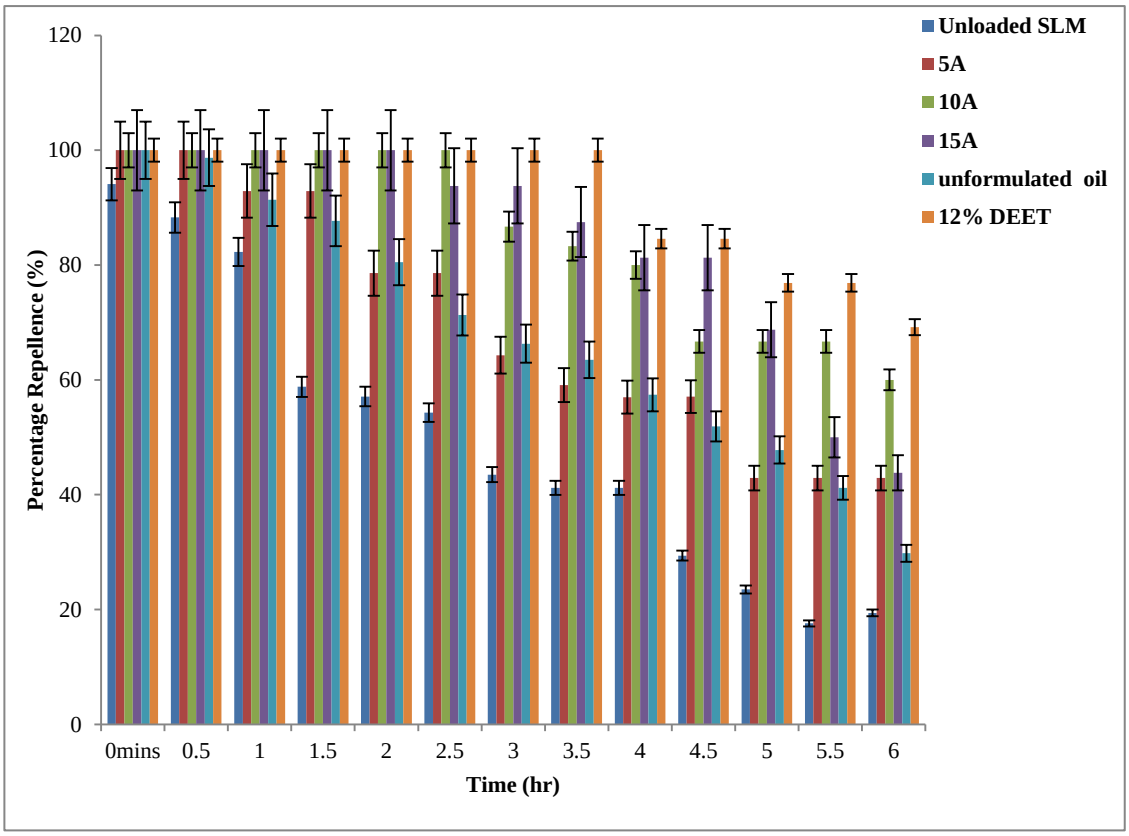

Figure 4: Mean percentage repellence of the various SLM batches (±SD)

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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