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Research article

NIOSOMAL FORMULATION LOADED WITH XANTHIUM STRUMARIUM EXTRACT FOR THE TREATMENT OF TINEA CORPORIS

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Abstract

Background*:Traditional medicine relies on herbal medications, which are thought to be safer and less likely to cause negative effects. When herbal drug loaded delivery strategies are combined with current scientific technologies, the therapeutic value of the pharmaceuticals involved is enhanced.*

Objective: *It is the ultimate objective of this research to formulate and evaluate Xanthiumstrumarium L. methanolic extract-based niosomal drug delivery system.Analysis of the niosomal dispersion was conducted using particle size and PDI as well as zeta potential, entrapment efficiency, percent drug loading, and in-vitro drug release study*

Methods:*The methodology includes collection, identification and evaluation of the selected plant part, formulation and evaluation and in-vitro drug release study of all niosomal formulations.*

Results and Discussion:*Based on collected findings, the zeta potential of -49.7±6.83 mV, the optimal particle size of 352.4±19.4 nm, the entrapment efficiency of 80.13±0.12 %, and the drug loading of 34.6±0.10 % were found to be enhanced. It was determined that the niosomes had an in vitro release profile and an in vitro anti-fungal effect. According to data from the zone of inhibition, niosomal suspensions had a release profile that was 52.83±0.75 % better than other formulations.*

Conclusion:*Drug delivery through niosomal formulations is gaining success rapidly at the present time and itcould be a suitable carrier for Xanthiumstrumarium L. methanolic extract for the treatment of tinea corporis*

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Keywords: *Xanthium strumarium L.*, *Tinea corporis*, niosome, *Trichophyton rubrum. Xanthiazone*.

Introduction

Since the dawn of time, the desire to achieve and maintain high levels of health has driven the growth of treatment and delivery systems all across the world. There is a common thread that binds these systems together, that they all rely on natural remedies with a strong traditional component.There can be no doubt that herbal therapy has a rich and glorious history, one that predates and outlasts the contemporary medical system, which has its roots in prehistoric eras. In the field of drug delivery, topically applied drug administration through the skin has a number of advantages, including long-term release, reduced fluctuations in plasma drug levels, avoidance of first-pass metabolism, and improved patient compliance. However, skin barriers reflect poor drug absorption and drug penetration [1]. Over the last decade, Parenteral, oral, and topical drug delivery have all been modified as a result of nanotechnology breakthroughs. The use of nanoparticles as drug carriers has the potential to raise drug specificity, bioavailability, drug penetration, drug absorption, and therapeutic efficacy while also boosting patient compliance [2].

Noisome liposomes are a modified type of liposomes created by changes in the content and structure of prototypical liposomes. Niosomes are nano-sized lipid-vesicles that have many of the same characteristics as well-known liposomes, such as being biodegradable, non-toxic, and capable of encapsulating both water-soluble and oleophilic compounds. However, because of its unique features such as flexibility, ultra-deformability, and the ability to include non-ionic surfactant, cholesterol, and its derivatives in some cases, it is regarded a new class of lipid vesicles.Apart from niosomes, ethosomes and transfersomes belong to a new class of nano lipid vesicles that are more stable than liposomes, despite the fact that these new lipid vesicles have many of the same features as liposomes [3,4]. Both trans-epidermal water loss and smoothness of the stratum corneum are expected to be improved by the use of niosomes, which have been shown to restore lost skin lipids following fusion to corneocytes and to affect the stratum corneum's structure through their surfactant characteristics [5]. In comparison to liposomes, niosomes provide advantages such as osmotically proactive properties, chemical stability, and a longer storage term. In a vesicular bilayer membrane, they can capture oleophilic compounds, whereas in an aqueous compartment, they can trap hydrophilic drugs [6]. No toxicity is attributed to niosomes because of their non-ionic activity, which supports their sustained-release properties that also modify absorption through the skin and ensure exact drug release. Capsulizing herbal extracts and phytochemicals

using Phytoniosomes technology has yielded remarkable results in both in vivo and in vitro experiments. Phytoniosome-based carriers for caffeine, gallic acid, and black tea extract in skin care products have been proven to be promising. On this basis, topical application of herbal extract loaded niosomal formulation might be a significant step forward in treating tinea corporis [7].

Like other dermatophytes, tinea corporis affects the glabrous skin, which includes the skin region except palms, soles, scalp and groin. The inflammatory or non-inflammatory lesions can be seen on the glabrous skin. Anamorphic asexual genera Trichophyton, Microsporum, and Epidermophyton all cause dermatophyte infections [8]. Trichophyton rubrum, a common dermatophyte, has a tough cell wall that makes it difficult to eradicate. Mannan, which is found in this protective barrier, may reduce cell-mediated immunity, impede keratinocyte proliferation, and improve the organism's resistance to the skin's natural defences. More often than not, Tinea corporis is found in hot, humid areas. There are approximately 47 % of all cases of corporis caused by T. *rubrum* [9]. The market offers a variety of topical antifungal medications, such as ketoconazole and clotrimazole. Mycolytics are broad-spectrum antifungal agents that are easily absorbed, but can display hypersensitivity responses, mild burning at the site of application, blisters, discomfort and redness at the site of application, and itchiness. Herbal medicines, on the other hand, are less harmful and less likely to cause negative effects.

Antitrypanosomal, hypoglycemic, antifungal, antiulcerogenic, antileishmanial, anti-inflammatory, and diuretic action are among the therapeutic qualities of *Xanthium strumarium* L. This plant, which falls under the Asteraceae family, is obtainable from August through September every year [10]. So far, 164 chemical compounds have been isolated and identified, including phenylpropenoids, lignanoids, glycosides, sesquiterpenoids, steroids, anthraquinones, and others. *Xanthium strumarium* is also known to suppress human cancer cell growth and to have neuroprotective properties in the central nervous system [11,12]. According to research, ethanolic and aqueous extracts of *Xanthium strumarium* were found to have antifungal efficacy against the phytopathogenic fungus *Alternaria alternata* when tested in vitro. All *Xanthium strumarium* extracts inhibited mycelial growth, however ethanolic extracts were more effective against fungal growth than aqueous extracts [13]. Another study investigated the antifungal efficacy of the leaf extract of *Xanthium strumarium* against five pathogenic fungi, and found that methanolic extracts were more efficacious than aqueous extracts [14].

Xanthium strumarium extracts are to be encapsulated in niosomes for topical use in the therapy of tinea corporis infection as the goal of this study. A series of preformulation investigations were carried out following the extraction of crude extracts. After that, characterization, evaluation an in-vitrorelease study of crude extract loaded niosomes were carried out.

Materials and methods

Chemicals

Acetic anhydride, Glacial acetic acid, Acetonitrile, Sabouraud Dextrose Agar, modified Dextrose Agar Base (as described by Emmons) and Trichophyton rubrum (Castellani) Sabouraud (ATCC 28188) were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Ethanol, Methanol, Formic acid, Cholesterol, Polyethylene glycol 400, Propylene glycol, Chloroform, Sorbitan monopalmitate (Span 40), Sorbitan monostearate (Span 60), Tween 60 were purchased from Research-Lab Fine Chem Industries, Mumbai, India. Throughout the experiment, chemicals and solvents with analytical grade were used.

Plant Material Collection

The plant was collected from the campus of Dibrugarh University, Dibrugarh, Assam, India in the month of October. The plant sample was authenticated as *Xanthium strumarium* L. (BSI, Shillong vide letter No. BSI/ERC/Tech//Identification/2017/469, dated 14.11.2017) belonging to the family Asteraceae.

Extraction of Xanthium strumarium

The crude drug material was prepared by dehydrating and pulverising *Xanthium strumarium* leaves.The extraction process was accomplished in a hot continuous mode utilizing methanol as the solvent. the rotary vacuum evaporator was used (IKA, RV 10DS96) for absolute remotion of leftover dissolvent after collecting the methanol dissoluble components in the receiver. The finished product was moved to a light-resistant container and hermetically sealed.

Phytochemical screening of methanolic extract of Xanthium strumarium

Classically proven techniques were used to test for the presence of several phytocomponents in the L. Xanthium extract. To identify the various phytocomponents in the extract, a chromatographic analysis on silica gel-G and a succession of mobile phases was performed [15].

In-vitro evaluation of Xanthium strumarium extract

The antifungal activity of the extract was evaluated using the Cup diffusion technique against Trichophyton rubrum (Sabouraud). Using a sterile spreader, 200 microliter of a fungus solution was evenly distributed over Sabouraud
Dextrose Agar medium that had been solidified. A sterile borer was used to form wells with a diameter of 6 mm in the plates. In order to conduct the experiment, 200 microliter of the corresponding test extract was placed into each well at variable concentrations. The same procedure was followed by 96 hours of incubation at 30^0C in the control experiments [13]. Four days after the discs were placed in the incubation chamber, we measured the size of the growth inhibition zone using antibiotic zone scale in triplicates.

Formulation of noisome

The film hydration method was developed among various methods for the formulation of niosomes. The following cholesterol and surfactant ratios were chosen: 1:1, 1:1.5, 1:2, 1:2.5, and 1:3. In chloroform containing 250 mg of extract, selected ratios of cholesterol and surfactants such as tween 60, Span 60, and Span 40 were dissolved individually.A rotary flash evaporator was used to evaporate the solvent at a reduced pressure and a maintained temperature between 55-65℃ until a fragile lipid film was formed. At 55-65°C, the flask was continued rotating at 55-65°C for 1 hour, while the adhered film inside the round bottom flask was re-hydrated with 20 ml of Phosphate buffer saline (PBS) pH 7.4 for 1 hour. After collecting the hydrated noisome, it was placed into a beaker and sonicated using bath sonicator for 20 mins to get niosomal dispersion. The sonicated dispersion was kept aside without disturbing the system at room temperature (RT) for swelling of vesicles, and then it was stored for 12 hours at 2-5°C [16].

Characterization of extract loaded niosome

Malvern Zetasizer ZS Nano (Malvern Instruments Ltd, UK) was used to assess particle size and polydispersity index at 25±1℃ at a scattering angle of 90°. A disposable cuvette was used to assess particle size, and a disposable folded capillary tube was used to measure Zeta Potential of the obtained niosome. Both measurements were performed at a temperature of 25±1℃. Stable particles are considered for those formulations whose zeta potentials are more positive than +30mV or more negative than -30mV [17].
The fabrication and exterior morphology of multilamellar niosomal vesicles were studied using a Transmission Electron Microscope (TEM). Images of niosomal vesicles were captured using the JEM-2100 electron microscope (JEOL Ltd, Japan) at 100KV.

Determination of percentage drug loading and entrapment efficiency

The percentage drug loading of extract loaded niosomes were measured by centrifuging samples at 11000 rpm for 30 minutes at 2-5°C in a refrigerated centrifuge. To separate niosome from non-entrapped medicines, it was washed three times using phosphate buffer 6.8. The antifungal action of *Xanthium strumarium* is attributed to the compound xanthiazone, whose λ_{max} of 279 nm was previously identified using UV 1800, Shimadzu [18]. All of the tests were repeated five times. The equation of the calibration curve was obtained as following

$$y = 0.023x + 0.0142$$
$$R^2 = 0.998$$

To determine entrapment efficiency, the niosomal vesicles were first isolated from the supernatant. The vesicles were then lysed with 0.5 ml chloroform in the centrifuge tube. Methanol was used to dilute the extract, and the absorbance at λ_{max} 279nm was measured against methanol as a blank. All of the trials were repeated five times [17]. The equation of the calibration curve was obtained as following

$$\mathrm{y} = 0.0209\mathrm{x} + 0.018$$
$$R^2 = 0.9976$$

The following expressions are used to calculate entrapment efficacy and drug loading

$$\%EE = \frac{(\text{amount of drug present in niosomes})}{(\text{amount of drug is given in the formulation})} \times 100$$

$$\%\ Drug\ Loading = \frac{(\text{amount of drug present in niosomes})}{(\text{amount of niosomes formed})} \times 100$$

In-vitro drug release study of niosomal suspension

All the niosomal suspensions were tested for drug release in vitro to compare the results. Isolated niosome vesicles were used to perform in vitro release tests on membranes with molecular limits between 12 and 14000Da. As part of the dialysis bag procedure, 100mg Niosome suspension was placed in the dialysis bag that was hermetically sealed, immersed in buffer pH 6.8 at 37±5°C, and magnetically stirred at 60 RPM for 30 minutes at 37±5°C. Analysis of the samples was done using a UV spectrophotometer at a wavelength of 279 nanometers. For a period of 12 hours, the cumulative xanthiazone outputs from various niosome dispersion *Xanthium strumarium* extracts were examined [19,20].

Result with discussion

Phytochemical screening of methanolic extract of Xanthium strumarium

Methanol was used as the solvent in a hot continuous extraction method in order to increase the extracted product yield. There were 23.01 percent extract yield after extraction, and the phytochemical screening for qualitative categorization of various plant constituents was conducted after the extraction process was completed. The methanolic extract of *Xanthium strumarium* showed the presence of phytoconstituents as alkaloids, glycosides, and terpenoids. Based on the phytochemical screening results, TLC fingerprinting of the methanolic extract was used to quantify the results. Four alkaloidal components were successfully quantified using the mobile phase composed of Toluene, Ethyl acetate, and Formic acid in the ratio 5:4:1, while terpenoidal components were detected using the mobile phase composed of Toluene, Ethyl acetate, and Pyridine in the ratio 8.2:0.5:0.3. Four components with Rf values of 0.64, 0.82, 0.94, and 0.98 were identified in the earlier mobile phase; in the latter phase, Rf values of 0.68, 0.79, and 0.93 were discovered.

Antifungal activity of methanolic extract of Xanthium strumarium

Methanolic extract of leaves showed considerable antifungal activity against doses of 6000 to 15000ppm. The Agar cup technique was used to determine the minimal inhibitory concentration and the area of inhibition. An inhibitory zone of 8.44±0.51mm with a minimum inhibitory concentration of 6000ppm has been observed in tests against the fungus Trichophyton rubrum (Castellani) Sabouraud (ATCC 28188). (Figure 1).

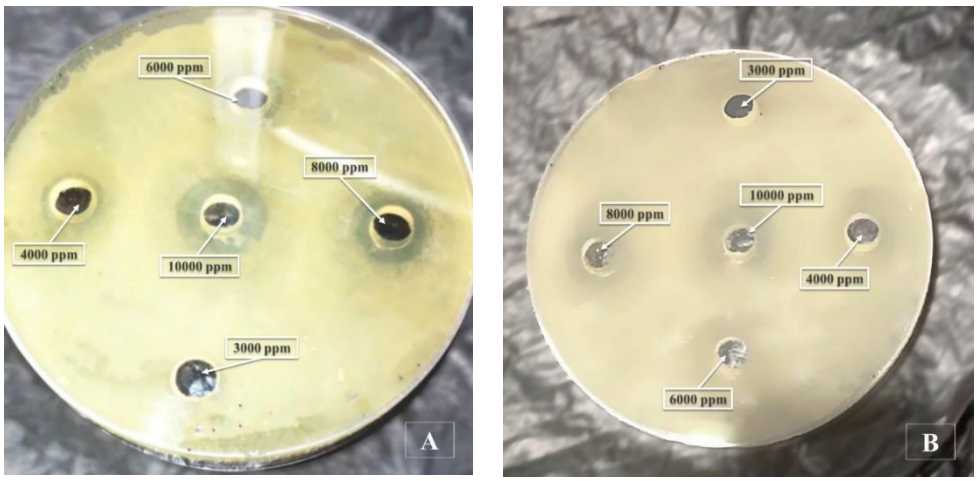

Figure 1. Antifungal assay of methanolic extract of *Xanthium strumarium*L. against fungal strain Trichophytonrubrum on culture plates A & B

Characterization of extract loaded niosome

At a scale of 50-200 nm, transmission Electron Microscopy (TEM) was employed to study the fabrication and exterior morphology of the niosomal vesicular structure. The appearance of the niosomal vesicles was found to be approximately spherical in shape and consistent in size (figure 2). Based on all formulations, it has been established in Table 1 that the particle size of the niosomal vesicles has a maximum particle size of 871.5±55.1 nm and a minimum particle size of 352.4±19.4 nm. The size of the vesicle rises in direct proportion to the surfactant concentration, which has a significant effect on it. When the HLB value of several Sorbitan ester surfactants was increased, the mean niosome size increased as well. The shortest mean vesicle size was found to be 352.4±19.4 nm in formulation NPF 2, which had the lowest surfactant ratio (Span 60). HLB value of surfactant was also discovered to influence the vesicle sizes, with a formulation including a surfactant that had a low HLB value being found to be smaller than those containing surfactants with higher HLB values (Table 1).All produced niosomes had zeta potential values ranging from -22.3±4.43 mV to --49.7±6.83 mV. The findings suggested that the formulations were stable in nature, with variations in zeta potential due to the presence of distinct functional groups (Hydroxyl, Ester, and Alkyl) in different surfactants. The composition of the surfactant and the amount of cholesterol in the sample altered the zeta potential readings.

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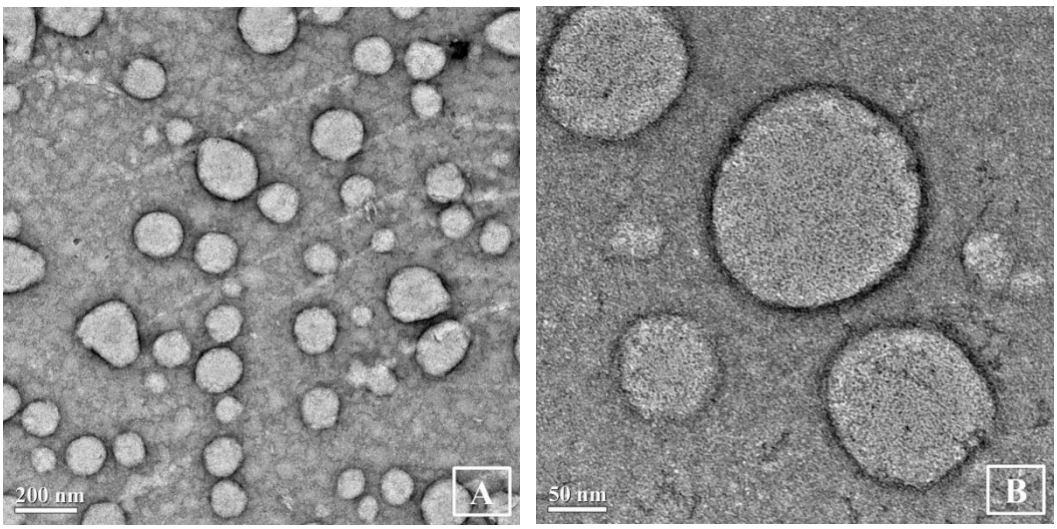

Figure 2. TEM results of niosome-containing methanolic extract of *Xanthium strumarium*L. at 200 nm (A) and 50 nm (B) scales

Determination of percentage drug loading and entrapment efficiency

There were wide variations in the percentages of entrapment efficiency in the present niosomal vesicles for *Xanthium strumarium* extract i.e., from 53.35±0.37 to 80.13±0.12 shown in the table 1. The niosomal dispersion's entrapment performance is highly dependent on the type of encapsulated component, the characteristics of the vesicle material, and the qualities of the surfactants used. The percentage of drug loading variation was found between 18.0±0.11 to 34.6±0.10 which is also shown in table 1. Research shows that surfactant and its proportionality have a significant influence over drug loading and entrapment. Only span 60 at a cholesterol and surfactant ratio of 1:3 was found to have the highest percentage of drug loading (34.6±0.10) and entrapment efficiency (80.13±0.12) among the three surfactants studied.

Table 1. Characterization and evaluation of the niosomal formulation comprising methanolic extract of Xanthium strumarium L. leaves

Batch No.	*Surfactants Used*	*Cholesterol: Surfactant*	*Zeta potential (mV)*	*Mean size (nm)*	*% Entrapment Efficiency*	*% Drug Loading*
PNF 1	Tween 60	1:1	-28.6±3.43	501.3±25.7	62.13±0.20	18.0±0.11
PNF 2	Span 60	1:1	-22.3±4.43	352.4±19.4	72.03±0.25	24.9±0.72
PNF 3	Span 40	1:1	-22.3±4.59	709.8±38.88	53.35±0.37	18.3±0.48
PNF 4	Tween 60	1:1.5	-32.6±4.25	589.6±38.82	67.62±1.43	18.9±0.13
PNF 5	Span 60	1:1.5	-26.7±3.01	551.1±27.3	73.08±0.30	25.5±0.23
PNF 6	Span 40	1:1.5	-24.9±7.05	743.2±26.9	55.55±0.34	19.0±0.024
PNF 7	Tween 60	1:2	-31.7±4.55	607.6±45.2	71.7±0.15	20.5±0.56
PNF 8	Span 60	1:2	-25.6±4.26	583.5±31.5	75.06±0.76	29.4±0.14
PNF 9	Span 40	1:2	-28.8±5.52	797.9±32.98	57.01±0.71	20.3±0.72
PNF 10	Tween 60	1:2.5	-35.0±4.04	688.8±36.81	72.78±0.48	21.1±0.15
PNF 11	Span 60	1:2.5	-32.0±3.49	642.9±34.1	77.72±0.58	31.9±0.25
PNF 12	Span 40	1:2.5	-29.2±3.9	850.1±22.7	62.45±0.84	23.9±0.20
PNF 13	Tween 60	1:3	-49.7±6.83	711.6±13.6	74.29±0.20	23.9±0.72
PNF 14	Span 60	1:3	-37.0±5.94	677.6±43.6	80.13±0.12	34.6±0.10
PNF 15	Span 40	1:3	-31.1±4.4	871.5±55.1	66.98±.39	25.5±0.18

In-vitro drug release study of niosomal suspension

Over the course of 12 hours, in vitro xanthiazone release studies were conducted to determine the amount of xanthiazone was released from *Xanthium strumarium* extract of different niosomal suspension. According to the obtained data, an in-vitro release of 55.29± 1.89 % was seen in span 60 containing formulation PNF 14; this was found to be the most promising. At 1:1 cholesterol: surfactant ratio (PNF 1),

tween 60 was shown to have an extraordinary release potential of 93.52± 2.01% (figure 3). For the formulation PNF 14, which contains span 60 as a surfactant, the feasible in vitro release profile has been enhanced by an optimal particle size, zeta potential, entrapment efficiency and percent loading. This helps to increase the selection of the finest formulations including span 60 as surfactants.

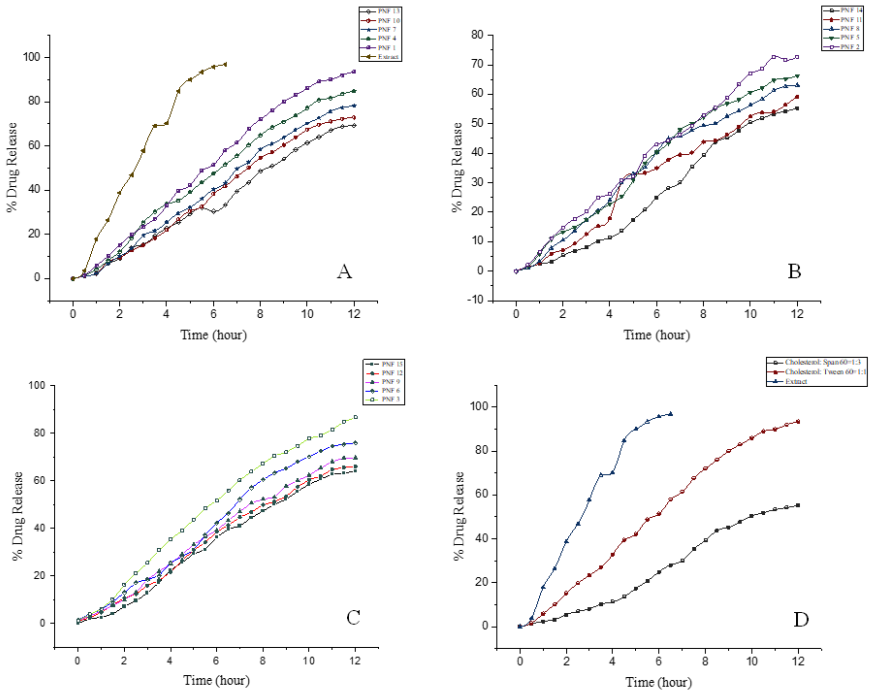

Figure 3. % Drug release vs time plot for in-vitro drug release study. In this diagram, diagram A shows the % drug release for the formulations in which Tween 60 was used at different ratio; diagram B shows the % drug release for the formulations in which Span 60 was used at different ratio; diagram C shows the % drug release for the formulations in which Span 40 was used at different ratio; diagram D shows the % drug release for the extract & formulations (PNF 1 & PNF 14) with better drug release.

Conclusion

Even in the twenty-first century, the pharmaceutical research community encounters several challenges in developing new drug delivery technologies and formulations. The current strive of the pharma world is to boost the therapeutic efficacy in lower doses with minimal adverse effect. This can be overcome by developing a modified or novel formulation of an already existing therapeutic molecule rather than finding an entirely new therapeutic molecule due to involvement of huge financial load. Several niosomal formulations with different surfactants for topical administration

in the treatment of tinea corporis were developed using *Xanthium strumarium* L. extracts in this recommended study. Particle size, PDI, surface morphology, zeta potential, % entrapment efficiency, % drug loading, and in vitro release research data have been used to evaluate niosomal formulation. It is clear from the data that the most promising formulation was PNF 14, which contained span 60 as surfactant at ratio of 3:1 with cholesterol. An enhanced release profile and considerable antifungal efficacy were observed in the niosomal formulation-14, which had values of 55.29±1.89 % drug release at 12 hours and a 19.76±2.82 mm zone of inhibition. Hence, niosomes could be a suitable carrier for *Xanthium strumarium* L. methanolic extract exhibiting antifungal activity for the management of tinea corporis only because of its modest manufacturing and uncomplicated scaling up.

Declaration of interest statement

The authors have no conflict of interest of any nature to disclose.

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