Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 13, Issue 3, 2023, 273
https://doi.org/10.33263/BRIAC133.273

Xanthium strumarium L. Extract Loaded Phyto-Niosome Gel: Development and *In Vitro* Assessment for the Treatment of *Tinea corporis*

Prakash Rajak [1,*], Evana Patra [1], Arka Karmakar [1], Biman Bhuyan [1]

[1] Department of Pharmaceutical Sciences, Faculty of Science and Engineering, Dibrugarh University, Dibrugarh-786004, Assam, India
* Correspondence: prakash@dibru.ac.in (P.R.);

Received: 10.04.2022; Accepted: 21.06.2022; Published: 10.07.2022

Abstract: Herbal drugs, believed to be safer and do not exhibit such side effects, are used in traditional medicine. The drug's gross therapeutic value is improved when herbal drug delivery methods are combined with modern scientific processes. The ultimate goal of the study is to develop a phyto-niosomal-based drug delivery system that encapsulates *Xanthium strumarium* L. methanolic extracts. The niosomal dispersion has been evaluated for particle size, PDI, optical microscopy, zeta potential, entrapment efficiency, % drug loading, and *in vitro* release study, and based on the results, formulation N6 containing cholesterol: surfactant ratio of 1:3 with a span 60 was incorporated into carbopol gels (G1 to G4) having concentrations of carbopol 934 0.5%w/w, 1.0%w/w, 1.5%w/w and 2.0%w/w respectively. The phyto-niosomes, *in vitro* release profile, and *in vitro* antifungal assay were evaluated. Supported on results obtained, the niosomal dispersion was found to have an improved release profile with 52.83±0.75% release backed by the zone of inhibition data compared to rest formulations. Thus, phyto-niosomes could be a promising carrier for the methanolic extract of *Xanthium strumarium* L. Antifungal activity for treating *Tinea corporis* exclusively due to their modest production and effortless production scale-up.

Keywords: *Xanthium strumarium* L.; *Tinea corporis*; phyto-niosome; *Trichophyton rubrum*.

1. Introduction

The zeal to attain and maintain the dos of premium health has always engulfed the human mind since immemorial. Such endeavor led to the evolution of treatment and delivery systems crosswise to humankind. One panorama that all these systems feature in common is that they are almost exclusively supported by remedies constituted in nature with a deep-rooted tralatitious accent. Thence, it would not be an overstatement to express that herbal medicine has an abundant and upstanding history, which is longer and stronger than the nowadays-contemporary medicine system, whose foundation is firmly rooted in the deep ancient ages and its patrons drawn from the hoary antiquity.

Topical drug delivery across the skin can render many advantages, such as bestowing sustained drug release, minifying fluctuation in plasma drug levels, circumventing first-pass metabolism, ameliorating patient compliance, and catering to local (dermal) or systemic (transdermal) effects [1,2]. Yet, the obstruction role of the skin, exerted by the horny layer of the stratum corneum, impairs the penetration and absorption of drugs [3]. Therefore, novel

topical drug delivery systems, with the use of nanotechnology in dosage form design, have been utilized to subdue the issues pertaining to skin barriers [4].

Liposomes are well-established nano-sized lipid vesicles that render potential value in topical drug delivery. They are formed by one or manifold lipid bilayers that enclose a discrete aqueous phase. Liposomes furnish numerous advantages as drug delivery carriers- for instance, they are biodegradable, non-toxic, and competent in encapsulating water-soluble and oleophilic substances [5-9]. They are analogous to the epidermis concerning their lipid composition, which largely enables them to perforate the epidermal barrier compared to the remaining conventional dosage forms. The majority of topically applied liposomes onto the cutis will accumulate in the upper layers of the stratum corneum and serve more as a "reservoir", providing a more localized activity [10-14].

In order to boost skin permeation of encapsulated molecules, alterations in composition and structure of stereotypical liposomes were made to generate new classes of lipid vesicles with flexible and ultra-deformable properties in particular niosome transferosomes and ethosomes. Niosome is one of the nanocarriers with unique characteristics, mainly consisting of a non-ionic surfactant and sometimes cholesterol and its derivatives. These nanoparticles mostly resemble the characteristics above as that of liposomes. However, they are considered more stable [15,16]. Niosome is thought to amend the horny layer properties by reducing trans-epidermal water loss and augmenting smoothness by replenishing lost skin lipids following fusion to corneocytes [11,17]. Niosome feature the ability to modify the structure of the stratum corneum through their surfactant properties to make the layer looser [11,18].

Niosome is utilized as nano-medicines, having the sustained-release property that also alters absorption through the skin and assures drug release in precise quantity [19]. Niosome proffer advantages, as they are osmotically proactive, chemically stable, and feature a prolonged storage period compared to liposomes [20,21]. They hold eminent compatibility with biological systems and low toxicity because of their non-ionic activity. They hold advanced potentiality for entrapping oleophilic substances into vesicular bilayer membranes and hydrophilic drugs in the aqueous compartment. Phyto-niosomes technology is successfully applied to capsulize herbal extracts and phytochemicals, relinquishing significant results *in vivo* and *in vitro* pharmacokinetic studies [11,22]. Studies have shown that Phyto-niosomes are promising carriers for topical delivery of caffeine, gallic acid, and black tea extract in skin care products [23]. Thus, developing and assessing topical gel containing niosome with herbal extracts can be an enthusiastic move to address *Tinea corporis*.

Tinea corporis is a superficial dermatophyte contagion defined by inflammatory or non-inflammatory lesions on the glabrous skin (i.e., skin regions except the scalp, groin, palms, and soles) [24]. Three anamorphic asexual genera cause dermatophytoses: Trichophyton, Microsporum, and Epidermophyton. *Trichophyton rubrum* is a common dermatophyte and is resistive to destruction because of its cell wall. This defensive barrier contains mannan, which may inhibit cell-mediated immunity, hamper the proliferation of keratinocytes, and heighten the organism's resistivity to the skin's innate defenses. *Tinea corporis* is oftentimes seen in typically hot, humid climates. *T. rubrum* is the most frequent infectious agent in the world and is the source of 47% of corporis cases [23,25,26]. Various topical antifungal preparations are accessible in markets, such as ketoconazole and clotrimazole. They have broad-spectrum activeness against systemic and superficial mycosis. They are absorbed readily but incompletely and sometimes exhibit several hypersensitive reactions, moderate burning at the

application site, blisters, vexation, pain, and redness. However, herbal drugs are safer and have little potential to exhibit side effects [27].

Xanthium strumarium L. is an annual plant belonging to the family Asteraceae. *X. strumarium* is available between August and September. Various parts of this plant species were found to possess useful medicinal properties such as antitrypanosomal, diuretic, hypoglycemic, anthelmintic, antifungal, antileishmanial, antiulcerogenic, and anti-inflammatory activities; it is also known to inhibit proliferation of human cancer cells *in vitro* and to exert a neuroprotective activity on the central nervous system [28-31]. More than 170 chemical constituents have been isolated and identified from *X. strumarium*, including sesquiterpenoids, phenylpropenoids, lignanoids, coumarins, steroids, glycosides, flavonoids, thiazides, anthraquinones, naphthoquinones and other compounds [11]. Furthermore, caffeic acid (Phenylpropenoids), xanthiazone (thiazides), and xanthiazone-(2-O-caffeoyl)-β-D-glucopyranoside (thiazides) were also isolated and identified from methanolic extract of *Xanthium strumarium* [20]. Rodino *et al*. investigated the *in vitro* antifungal activity of the ethanolic and aqueous extracts obtained from *Xanthium strumarium. T*heir antifungal properties were tested against the plant pathogenic fungus Alternaria alternate [29]. All extracts obtained from *Xanthium strumarium* presented antifungal potential, demonstrated by the inhibition of the mycelial growth. Generally, the ethanolic extracts showed higher antifungal activity than the aqueous extracts. Similarly, Devkota and Das performed antifungal activity of leaf extract of X. Strumarium against five phytopathogenic fungi such as *Alternaria brassicae*, *Botrytis cinerea*, *Fusarium oxysporum*, *Phytophthora capsici*, and *Sclerotium rolfsii* [32]. According to their report, both distilled water and methanol extracts had varying degrees of antifungal activity on the microorganisms tested, and the methanolic extract showed better antifungal activity than the distilled water extracts of *Xanthium strumarium*.

The objective of the planned investigation was to acquire a phyto-niosomal gel-supported drug delivery method encapsulating *Xanthium strumarium* extracts for topical utilization in the management of *Tinea corporis* infection. Supported on literature survey *Xanthium strumarium* was chosen as it is utilized traditionally for antifungal treatment. After the extraction of crude extract, preformulation studies were performed. *X. strumarium* extract was integrated into niosomal vesicles and evaluated for particle size, PDI, optical microscopy, zeta potential, entrapment efficiency, percentage drug loading, entrapment efficiency, and *in vitro* release study. The optimized niosomal vesicles were incorporated into carbopol gels of assorted concentrations and evaluated for spreadability, pH, viscosity, texture analysis, *in vitro* release profile, and *in vitro* antifungal assessment.

2. Materials and Methods

2.1. Chemicals.

The plant *Xanthium strumarium* was collected from Dibrugarh, Assam, India, having latitude-27.4728327 and longitude-94.9119621. Analytical grade chemicals and solvents were utilized throughout the experiment. Ethanol, Methanol, Formic acid, Propylene glycol, Polyethylene glycol 400, Chloroform, Cholesterol, Carbopol 934, Triethanolamine, Span 40 and 60 were procured from Research-Lab Fine Chem Industries, Mumbai, India. Acetic anhydride, Glacial acetic acid, Acetonitrile, Sabouraud Dextrose Agar modified (Dextrose Agar Base, Emmons), and *Trichophyton rubrum* (Castellani) Sabouraud (ATCC 28188) were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

2.2. Plant material.

The plant was collected from the campus of Dibrugarh University, Dibrugarh, Assam, India, having latitude-27.4728327 and longitude-94.911962, in October 2017. The plant sample was authenticated as *Xanthium strumarium* L. belonging to the family Asteraceae by Dr. A.A. Mao, Scientist F & HoO, BSI, Shillong vide letter No. BSI/ERC/Tech//Identification/2017/469, dated 14.11.2017. The voucher specimen of the plant herbarium was deposited in the departmental museum for future reference.

2.3. Preparation of Xanthium strumarium extract.

Dehydrated pulverized leaves of *Xanthium strumarium* L. were utilized as the crude drug material. Extraction was performed using the continuous hot extraction mode using methanol as solvent. The methanol dissoluble components collected in the receiver were introduced to a rotary vacuum evaporator (IKA, RV 10DS96) for the absolute remotion of residual dissolvent. The product so obtained was transferred and hermetically corked in the light-resistant container.

2.4. Phytochemical screening and TLC fingerprinting of the extract.

The methanolic extract of *Xanthium strumarium* L. was screened for the presence of different classes of phytocomponents succeeding classic validated methods of detection. Chromatographic analysis of the extract was carried out using silica gel-G with a series of mobile phases to characterize different phytocomponents [33,34].

2.5. In vitro antifungal activity evaluation of Xanthium strumarium extract.

The cup diffusion method opted for antifungal assessment of the extract against *Trichophyton rubrum* (Castellani) Sabouraud (ATCC 28188). 200µl of fungal suspension was uniformly spread over solidified SDA media with the aid of a sterile spreader. 6mm diameter wells were prefabricated in the plates with the aid of a sterile borer and filled with 200µl of the respective test extract at different concentrations. Control experiments were carried out under a similar process followed by incubation at 30ºC for 96hours. The growth inhibition zone size was measured in triplicate using an antibiotic zone scale around the disks after 4 days of incubation [29]. The diameter of the zone of inhibition (exclusive of well diameter) resulting from repeats was expressed as mean ± standard deviation (SD). The data were evaluated by one-way analysis of variance (ANOVA) by SPSS 11.5 version, and P-value < 0.05 was measured as significant.

2.6. Synthesis of phyto-niosome and preparation of phyto-niosomal gel.

Phyto-niosomes were produced using the film hydration method. A lipid assemblage of cholesterol and surfactant (Span 40, Span 60, and Tween 60) at a ratio of 1:1, 1:1.5, 1:2, 1:2.5, and 1:3 was dissolved in chloroform containing 250mg of extract. The solvent was then evaporated under reduced pressure at a temperature of 55-65°C using a rotary flash evaporator until a flimsy lipid film was fabricated. The casted film was hydrated with 20ml of Phosphate buffer saline (PBS) pH 7.4 for 1 hour while the flask was kept rotating at 55-65°C in the rotary evaporator (IKA, RV 10DS96). The hydrated niosome was sonicated to get niosomal

dispersion and was set aside at room temperature (RT) for swelling of vesicles, after which it was stored overnight at 4°C [35,36].

For preparing the niosomal gel, Carbopol 934 was dispersed in a mixture of water and glycerol (30%w/w) under magnetic stirring until homogenous dispersion was formed. The niosomal suspension containing an equivalent amount of drug was centrifuged (10000rpm at 4°C for 45mins), and the vesicles were integrated into the mixture of carbopol dispersion. The dispersion was neutralized and made viscous by the addition of triethanolamine. The prepared gel formulations (G1 to G4) having concentrations of carbopol 934 0.5% w/w, 1.0% w/w, 1.5% w/w and 2.0% w/w respectively was kept at RT for stabilization [37-39].

2.7. Entrapment efficiency and percentage drug loading.

Extract ladened niosome formulations were centrifuged at 10000rpm for 45min at 4ºC using a refrigerated centrifuge (PLM 01, Orchid Scientific, Nasik, India) and cleaned thrice with phosphate buffer pH 6.8 to isolate niosome from the non-entrapped drug. The utilized λ_{max} (UV 1800, Shimadzu) for xanthiazone assessment was 279 nm in phosphate buffer pH 6.8; this compound is accountable for the antifungal activity of *Xanthium strumarium* [28,40,41]**.** All experiments were run five times, and the calibration curve specifications were as follows,

$$y = 0.02x + 0.0145$$
$$R^2 = 0.9988, n = 9$$

The vesicles were separated from the supernatant; the quantity of the entrapped drug was assessed by lysis of the vesicles. 0.5ml chloroform was added to the centrifuge tube to disrupt the isolated vesicles. The extract was diluted with methanol, and absorbance was measured at λ_{max} 279nm against methanol as blank. All experiments were run five times, and the calibration curve specifications were as follows,

$$y = 0.0212x + 0.015$$
$$R^2 = 0.9966, n = 9$$

The entrapment efficacy and drug loading of niosome are calculated as follows,

$$\%EE = \frac{\text{amount of drug in niosomes}}{\text{amount of drug is used in the formulation}} \times 100$$
$$\%DL = \frac{\text{amount of drug in niosomes}}{\text{amount of niosomes obtained}} \times 100$$

2.8 Characterization of phyto-niosome.

The fabrication of multilamellar vesicles was inveterate by examining the niosomal suspension under a transmission electron microscope (TEM). The niosomal samples were imaged on a JEM-2100 electron microscope (JEOL Ltd, Japan) at 100KV.

Particle size and polydispersity index were ascertained by DLS technique using a computerized inspection system Zetasizer Version 7.11 connected to Zetasizer ZS Nano (Malvern Instruments Ltd, United Kingdom) at 25±1°C and at a scattering angle of 90°. The Zeta Potential of the prepared niosome was measured using a disposable folded capillary tube at 25±1°C. Particles with zeta potentials more positive than +30mV or negative than -30mV are normally considered stable [42].

2.9. In vitro release study of phyto-niosomal dispersion.

In vitro release studies were executed to similitude the release of the drug from all the niosomal suspensions. *In vitro* release from isolated niosome vesicles was carried out using a dialysis membrane with a molecular cut-off from 12 to 14000Da. 100mg niosome suspension was positioned in the dialysis bag, hermetically sealed, submerged in phosphate buffer pH 6.8, and agitated magnetically at 50rpm under a restrained temperature of 37±0.5°C. The samples were analyzed by UV spectrophotometer at a wavelength of 279nm [43]. Cumulative releases of xanthiazone from *Xanthium strumarium* extract of different niosome dispersion were investigated for a period of 12 hours [40,44]**.**

2.10. Evaluation of phyto-niosomal gel.

The spreadability of the gel formulation was ascertained by assessing the spreading diameter of 1gm of gel between two horizontal plates with 220g weight tangled on the upper plate. The consistency was measured using Brookfield Viscometer (R/S Plus Cone and Plate Rheometer, USA) connected to Rheo-3000. An adequate amount of gel was packed in the CC3-14 measuring system, and the shaft was turned at 100rpm. The viscosity data acquisition was made in triplicate.

The texture of the formulation was analyzed by the Texture Analyser (TA. XT Plus, Stable Micro Systems Ltd., UK) connected to the XT-RA dimension application. Various gel characteristics, i.e., firmness, stickiness, adhesiveness, etc., of the formulations were ascertained. During the investigation, the device was set to compression mode with a test speed of 2.0 mm/sec. The equilibrated upper cone probe was permitted to approach and pierce the sample up to a depth of 2mm above the sample holder surface. The following test conditions were delineated in the software program of the instrument: test mode-compression, pre-test speed-2mm/sec, test speed-1mm/sec, post-test speed-10mm/sec, distance-2mm, strain- 10%, trigger type- auto (force), trigger force- 5g and data acquisition rate 200pps.

2.11. In vitro drug release studies of phyto-niosomal gel.

In vitro drug release study of the niosomal gel was carried out using the same methodology followed for phyto-niosomal dispersion. However, 300 mg of niosomal gel was used for the study. Based on the characterization results, the best formulations were then selected to assess *in vitro* antifungal activity.

Dissolution data of the optimized formulations were assessed by fitting the experimental data to equations relating to different kinetic orders. The mathematical models employed to understand drug release kinetics were: $M_0 - M_t = K_0 \times t$ (zero-order kinetic equation), $\ln(M_0 - M_t) = K_1 \times t$ (first-order kinetic equation), $M_t/M_0 = K_H \times t_{1/2}$ (Higuchi kinetic equation) and $\frac{M_t}{M_0} = K_{KP} \times t_n$ (Korsmeyer-Peppas), where 'M_t/M_0' is the fraction of 5-FU released at the time 't', 'K' is the kinetic constant, and 'n' is the diffusional exponent or drug release, exponent. For moderately swelling systems of cylinder shape, n<0.5 suggests a Fickian diffusion; 0.5 < n < 1.0 supports an anomalous non-Fickian transport (both diffusional and relaxational transport); for n>1.0, the release mechanism is represented by a case-II, relaxational transport, time-independent, zero-order model [44-46]. All data are presented as the arithmetic mean values ± standard deviation (mean ± SD). Significant differences were

calculated by analysis of variance (ANOVA) by SPSS 11.5 version, and differences at $p < 0.05$ were measured as significant.

2.12. In vitro antifungal assay of phyto-niosomal gel.

The *in vitro* antifungal assay of the selected phyto-niosomal gel formulation was done in triplicate by cup diffusion method following the same process used for a methanolic extract of *Xanthium strumarium* L. using ketoconazole as the reference standard.

3. Results and Discussion

3.1. Phytochemical screening and TLC fingerprinting of the extract.

Xanthium strumarium was subjected to a hot continuous extraction process using methanol as solvent. The outcome methanolic extract (yield 22.07% w/w) was then subjected to phytochemical screening for qualitative categorization of different phytocomponents constituted in the said extract. A phytochemical test performed on the methanolic extract of *Xanthium strumarium* revealed the presence of phytoconstituents such as alkaloids, glycoside, and terpenoids. The outcomes were further quantified by means of TLC fingerprinting of the methanolic extract with specific reference to the results of the phytochemical screening. The mobile phase comprising Toluene: Ethyl acetate: Formic acid: 5:4:1 was effectively utilized in quantifying four alkaloidal components, and Toluene: Ethyl acetate: Pyridine in the ratio 8.1:0.5:0.3 was used in screening terpenoids components for the extract. With the former mobile phase, four components were elucidated, having Rf values of 0.63, 0.81, 0.93, 0.96, and the latter having Rf values of 0.67, 0.78, 0.92, respectively.

3.2. In vitro antifungal activity evaluation of Xanthium strumarium extract.

The minimum inhibitory concentration and zone of inhibition were ascertained by the Agar cup method. The antifungal activeness of methanolic extract of leaves exhibited significant against concentrations used (6000 to 15000ppm). Trials were conducted against the fungal strain *Trichophyton rubrum* (Castellani) Sabouraud (ATCC 28188) and presented the promising activity of an average of 8.34±0.52mm zone of inhibition at a minimum inhibitory concentration of 6000ppm (Figure 1).

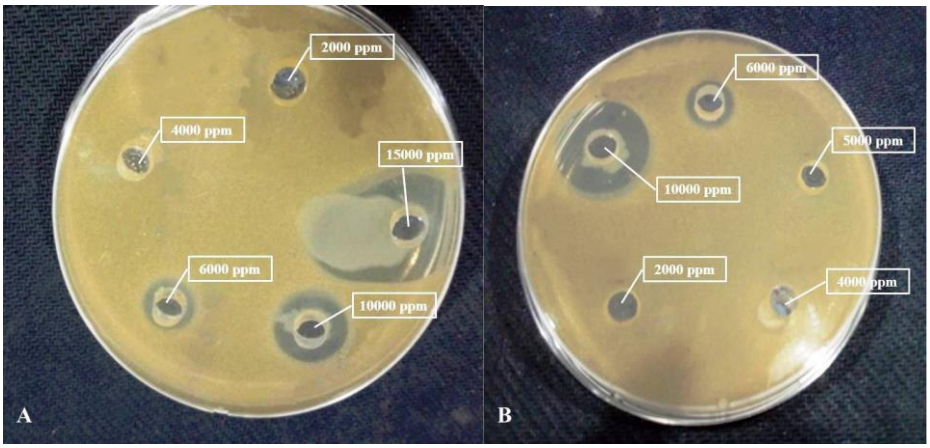

Figure 1. Images of culture plates for antifungal assay methanolic extract of *Xanthium strumarium* L. against fungal strain *Trichophyton rubrum*. In culture plate (**A**), the zone of inhibition was found at 6000ppm to 15000ppm, and in culture plate (**B**) also, the zone of inhibition was found at 6000ppm to 10000ppm.

3.3. Entrapment efficiency and percentage drug loading.

Xanthium strumarium extract was effectively entrapped within the lipid bilayers of the present study vesicles, with the percentage of entrapment efficiency ranging from 52.45±0.43 to 78.23±0.18 (Table 1). The type of entrapped substance, the features of the vesicle material, and the properties of surfactants utilized for preparing the phyto-niosomal dispersion show an indispensable role in determining entrapment efficiency. The percentage of drug loading fluctuated between 12.9±0.14 to 29.5±0.13 (Table 1). The experimentation's findings indicated the surfactant's impact and proportionality on entrapment efficiency and drug loading. Out of the three surfactants (Span 40, Span 60, and Tween 60) utilized in the formulations, maximal % drug loading (29.5±0.13) and % entrapment efficiency (78.23±0.18) were observed with span 60 at a cholesterol and surfactant ratio of 1:3 (N6). This might be affiliated with the amphiphile's high glass transition temperature of the amphiphile and the gel state nature of its bilayers, which fallouts in the low permeability of vesicles.

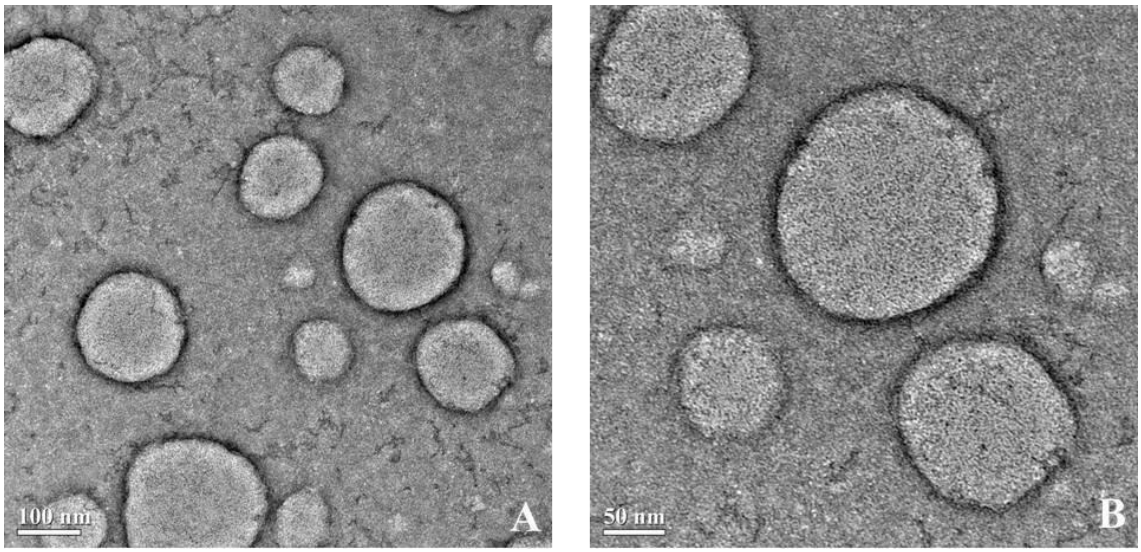

Figure 2. TEM images of methanolic extract of *Xanthium strumarium* L. containing phyto-niosome at (**A**) 100nm and (**B**) 50nm scale.

3.4. Characterization of phyto-niosome.

The bilayer vesicles were processed with the assemblage of the amphiphilic ingredients and cholesterol at varied ratios. All utilized surfactants formed niosome suspensions in the presence of cholesterol. The Transmission Electron Microscopy (TEM) micrographs at 50nm and 100nm measured in Figure 2 support the fabrication of bilayer vesicular structures from the surfactants by the film hydration method. Niosome were uniform in size and nearly spherical in appearance. The particle sizes of various formulations are presented in Table 1.

The vesicle sizes of the niosome ranged from 372.4±29.6 to 891.5±56.3nm. The mean size of the niosome is augmented with a progressive increment in the HLB value in various sorbitan ester surfactants. It has also been witnessed that increment in surfactant concentration points to gain vesicle size. The formulation N10 has a minimal ratio of surfactant (Span 60), indicating the smallest mean vesicle size of 372.4nm. It was also witnessed that the HLB value of the surfactant portrayed a key role in the vesicle sizes, as the formulation having surfactant with minimal HLB value (HLB value of span 60 is 4.7) found to have smallest vesicle sizes as compared to the formulations having surfactant with upper HLB values (HLB value of tween 60 is 14.9 and span 40 is 6.7).

Table 1. Characterization of the phyto-niosomal dispersion containing methanolic extract of *Xanthium strumarium* L.

Batch	Surfactant	Cholesterol: Surfactant	Mean size* (nm)	Zeta potential* (mV)	% Entrapment Efficiency*	% Drug Loading *
N1	Span 40	1:3	891.5±56.3	-28.9±5.0	65.08±.45	20.4±0.21
N2	Span 40	1:2.5	870.1±32.9	-27.0±4.5	60.65±0.90	18.8±0.23
N3	Span 40	1:2	817.9±43.18	-26.6±6.12	55.11±0.77	15.2±0.75
N4	Span 40	1:1.5	763.2±47.1	-22.7±7.65	53.65±0.40	13.9±0.054
N5	Span 40	1:1	729.8±49.08	-20.3±5.19	52.45±0.43	13.2±0.51
N6	Span 60	1:3	697.6±53.8	-34.8±6.54	78.23±0.18	29.5±0.13
N7	Span 60	1:2.5	662.9±44.3	-30.8±4.09	75.82±0.64	26.8±0.25
N8	Span 60	1:2	603.5±41.7	-25.6±4.26	73.16±0.82	24.3±0.17
N9	Span 60	1:1.5	571.1±37.5	-24.5±3.71	72.18±0.36	20.4±0.26
N10	Span 60	1:1	372.4±29.6	-20.3±2.03	70.13±0.31	19.8±0.75
N11	Tween 60	1:3	731.6±23.8	-47.5±7.43	72.39±0.26	18.8±0.75
N12	Tween 60	1:2.5	708.8±47.01	-32.8±4.64	70.88±0.54	16.0±0.18
N13	Tween 60	1:2	627.6±55.4	-29.7±5.15	69.8±0.21	15.4±0.59
N14	Tween 60	1:1.5	609.6±49.02	-30.6±4.85	65.72±1.49	13.8±0.16
N15	Tween 60	1:1	521.3±35.9	-26.4±4.03	60.23±0.26	12.9±0.14

3.5. In vitro release study of phyto-niosomal dispersion.

In vitro release studies were executed to study the release of the drug from all the niosomal suspensions. On the ground of cumulative amount of xanthiazone released from *Xanthium strumarium* extract of different niosome dispersion was considered for a period of 12 hours.

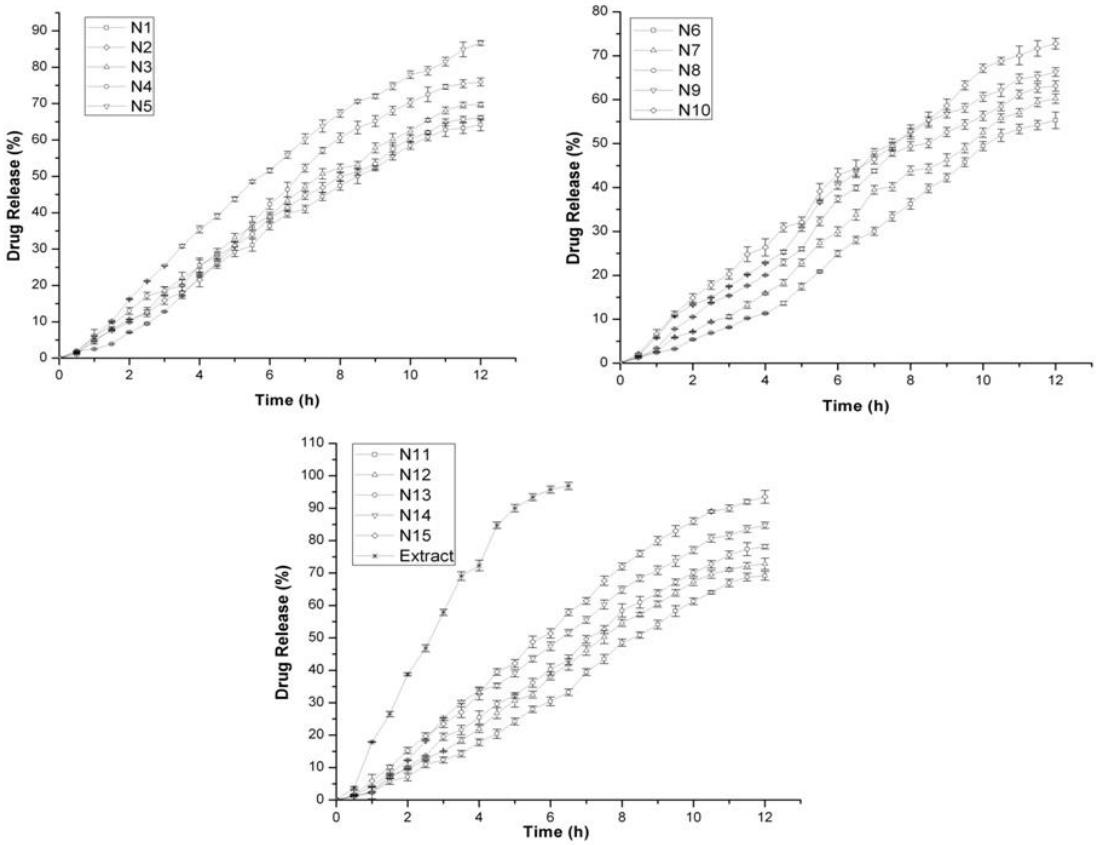

Figure 3. (**A**) Percent drug release *vs.* time profile graph for formulation N1, N2, N3, N4, N5; (**B**) Percent drug release *vs.* time profile graph for formulation N6, N7, N8, N9, N10 and (**C**) Percent drug release *vs.* time profile graph for formulation N11, N12, N13, N14, N15 and extract respectively.

Formulation N6 containing cholesterol: surfactant ratio of 1:3 with span 60 as a surfactant presented the most promising release of 55.29± 1.89 %. Tween 60, when used as a surfactant in formulation N15, demonstrated the extreme release potential of 93.52± 2.01% with cholesterol: surfactant ratio of 1:1 (Figure 3). Statistically, there were significant differences between the overall release percent of xanthiazone (*Xanthium strumarium* extract) from different niosomal formulations ($p > 0.05$). The plausive *in vitro* release profile of the formulation N6 containing span 60 as a surfactant was potentiated by optimum particle size, zeta potential, entrapment efficiency, and percent loading, thereby augmenting the selection of best formulations containing span 60 as surfactants. Hence, the formulation containing span 60 as surfactants with a cholesterol: surfactant ratio of 1:3 was emphasized during the gel preparation screening.

3.6. Evaluation of phyto-niosomal gel.

The gel formulation G1 comprising 0.5% carbopol 934 demonstrated comparatively acceptable spreadability showing an area of 10.06±0.34sq.cm/gram of the sample in contrast to the rest of the formulations (G2, G3, and G4). The data generated through the spreadability study exhibited a reciprocal relationship to the concentration of carbopol 934 utilized in the formulation (Table 2).

The viscosity of the formulations (G1 to G4) showed adequate values ranging from 3660cp to 9123cp; the proportionality of carbopol 934 used in the gel preparation has been pioneered to have a direct influence on the viscosity profile of the formulations. Nevertheless, the formulation with 0.5 % carbopol 934 (G1) presented viscosity, meeting the requirements of a gel formulation with a value of 3660cp (Table 2).

The hardness and stickiness data obtained from texture analysis are in perfect concordance with the spreadability data signifying that gel containing 0.5% carbopol-934 is much softer than the remaining formulations having the least tackiness characteristic (Table 2). Texture analysis of the gel formulation G1 constitutes a rupture force of 17.270±0.925g supported by the flimsiness, having values around 40.027±1.18mm (Table 2). The adhesiveness value indicative of the persistence characteristic of the gel on the skin surface was found to be proportionate with ratios of carbopol-934 with a peak value of 247.724 g.sec for formulation containing 2% carbopol-934 (G4). However, the gel strength of the formulations was found to be decreasing with the concentration of carbopol-934, signifying the feasibility of using a formulation containing 0.5% carbopol-934 with a maximum value of 1.982 g (Table 2).

Table 2. Evaluation of the phyto-niosomal gel containing methanolic extract of *Xanthium strumarium* L.

Parameters	Batch			
	G1	G2	G3	G4
Hardness* (g)	10.025±0.124	13.006 ±0.356	17.123±0.799	22.289±0.545
Stickiness* (g)	-7.227± 0.245	-9.160± 0.313	-11.276± 0.251	-16.859± 0.503
Gel Strength* (g)	1.982 ±0.083	1.632 ±0.064	1.415 ±0.051	1.183±0.034
Rupture Strength* (g)	17.270 ±0.925	15.154±0.75	13.921±0.48	11.721±0.633
Brittleness* (mm)	40.027 ±1.18	35. 511 ±1.09	32. 033±0.926	29.861±1.024
Adhesiveness* (g.sec)	-49.901±2.05	-92.682±5.68	-168.569±8.97	-247.724±6.93
Viscosity* (cP)	3660±59	5266±73	7960±83	9123±72
Spreadability* (g.cm/sec)	10.06±0.34	8.54±0.31	6.61±0.26	4.13±0.18

*Values are expressed as mean ± SD (n=3).

3.7. In vitro drug release studies of phyto-niosomal gel.

The *in vitro* release profile of the niosomal formulations in phosphate buffer pH 6.8 was found to be 52.83±0.75% for the formulation with 0.5% carbopol-934 (G1) at the 12th hour. The rest of the formulations with 1, 1.5, and 2% carbopol-934 (G2, G3, and G4, respectively) presented significantly lessened release patterns with values of 44.67±1.02%, 35.82±0.82%, and 30.42±0.59% respectively. The release profile of extract and extract gel formulated with 0.5% carbopol-934 demonstrated 96.87±1.15% release in 6.5 hours and 97.01±0.89% release, respectively. The proportion of carbopol-934 utilized in the formulations played a pivotal role in the release profile, as the other parameters viz. PEG and the number of isolated vesicles were kept unvaried (Figure 4). The justification behind extreme release from G1 may be due to the lowest carbopol proportion; the drug can be easily released through a low viscous gel. The formulations G1, G2, G3, and G4 gels contain identical niosomal suspension (formulation N6 containing span 60 as surfactants with cholesterol: surfactant ratio of 1:3); only carbopol proportion is variable, which leads to extreme drug release from G1 gel and least drug release from G4 gel. Statistically, there were significant differences between the overall release percent of xanthiazone (*Xanthium strumarium* extract) from different niosomal gel formulations ($p > 0.05$). The drug release profile was found to be better fits with a Hixson-Crowell model with an R2 value of 0.932, 0.917, 0.947and 0.984 for formulations G1, G2, G3, and G4 respectively and Peppas equation with diffusion exponent (n) of 0.443, 0.416, 0.476 and 0.441 for formulations G1, G2, G3, and G4 respectively indicating the Fickian diffusion. This finding suggests two mechanisms (i.e., erosion and diffusion) are embroiled in the release of xanthiazone (*Xanthium strumarium* extract) from different niosomal gel formulations.

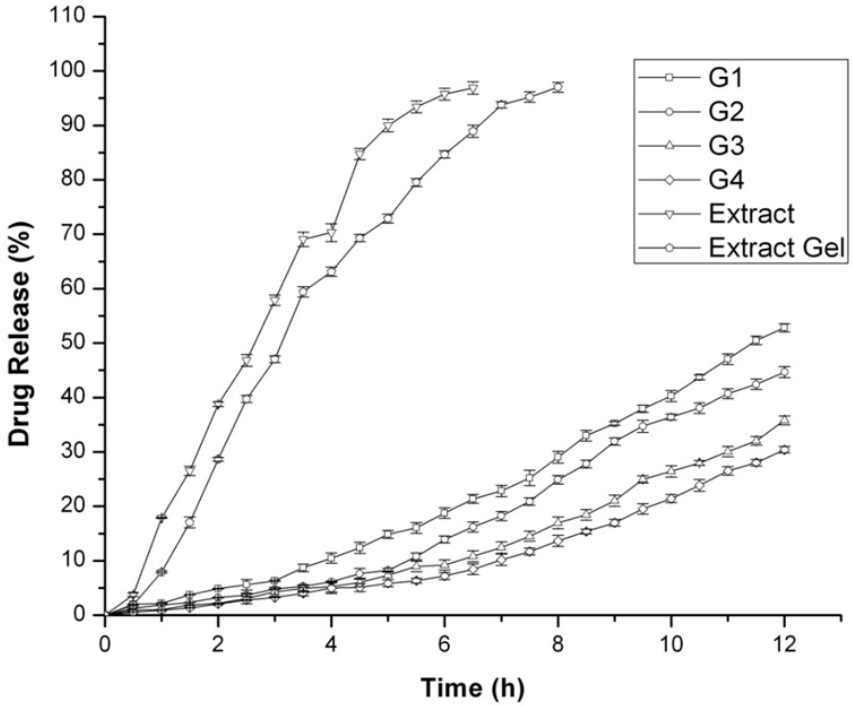

Figure 4. Cumulative drug release (%) vs. time profile graph of phyto-niosomal gels (G1 to G4) containing methanolic extract of *Xanthium strumarium* L.

3.8. In vitro antifungal assay of phyto-niosomal gel

In vitro antifungal assay of the prepared formulations was accomplished against *Trichophyton rubrum* (Castellani) Sabouraud (ATCC 28188) with ketoconazole as a reference

antifungal agent. To compare the release pattern of vesicles from gel structure formed by varied proportions of carbopol-934, gel formulations K1 to K4 containing 2% ketoconazole were prepared with 0.5, 1.0, 1.5, and 2% carbopol-934, respectively. The rest of the parameters were kept invariable to study the effect of carbopol-934 in the release pattern in terms of zone of inhibition. The highest average zone of inhibition of 17.54±2.67mm was observed in formulation G1 with 0.5% carbopol-934 after incubation of 96 hours at 30ºC, which was comparable to the zone of inhibition shown by ketoconazole with the value of 20.61±3.42mm (K_1). The *in vitro* antifungal assay of the phyto-niosomal gel indicated a clear effect of carbopol-934 on the release of the drug from the gel structure, decreasing the zone of inhibition with the increment of carbopol-934 proportion in the gel (Figure 5).

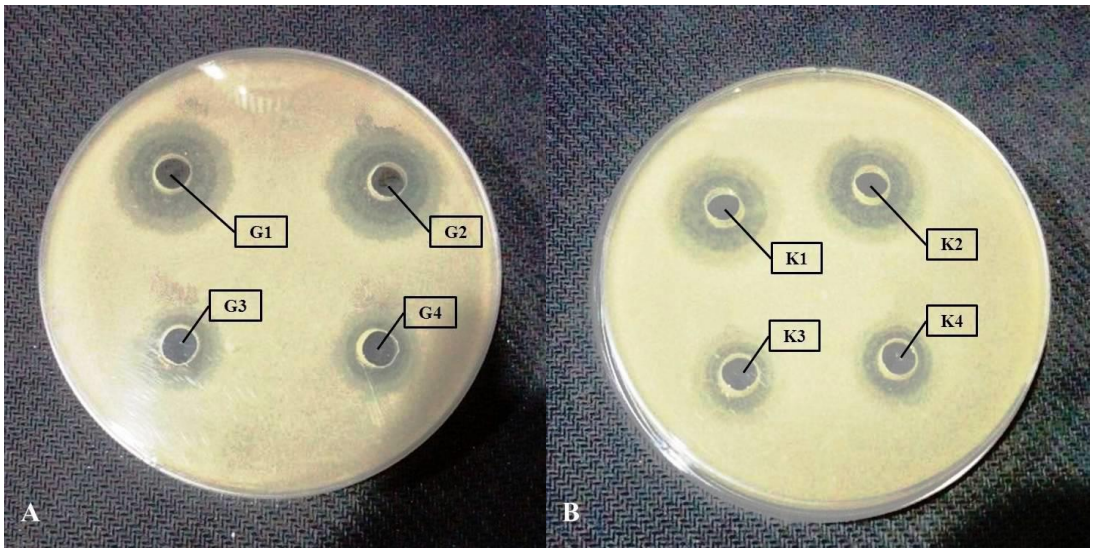

Figure 5. (**A**) Images of culture plates for niosomal gels (G1, G2, G3, and G4) containing methanolic extract of *Xanthium strumarium* L.; (**B**) Images of culture plates for ketoconazole gel (K1, K2, K3, K4).

4. Conclusions

The research field of pharmaceutical technology is facing more challenges in developing a novel formulation of the existing molecules rather than discovering new molecules that demand high financial involvement to increase their therapeutic effectiveness in lesser dose sizes with minimum or no side effects. The objective of the proposed investigation was to acquire a phyto-niosomal gel-supported drug delivery system encapsulating *Xanthium strumarium* L. extracts for topical utilization in treating *Tinea corporis*. Evaluations of niosomal dispersion have been executed and grounded on particle size, PDI, optical microscopy, zeta potential, entrapment efficiency, percentage drug loading, and *in vitro* release study data. The obtained information provides direct evidence that formulation N6 containing span 60 as surfactants with cholesterol: surfactant ratio of 1:3 was the most promising formulation and thus selected for incorporation into carbopol gels of different proportions (0.5% w/w, 1.0% w/w, 1.5% w/w, and 2.0% w/w). The phyto-niosomal gels (G1 to G4) were evaluated in terms of spreadability, viscosity, texture analysis, *in vitro* release profile, and *in vitro* antifungal assay. Based on results obtained, G1 gel with 0.5 % carbopol-934 encapsulating 2% phyto-niosomes was found to have an improved release profile along with a significant antifungal activity with values of 52.83±0.75% drug release at the 12th hour and 17.54±2.67mm zone of inhibition. Thus phyto-niosomes may be a promising carrier for

the methanolic extract of *Xanthium strumarium* L. Antifungal activity for treating *Tinea corporis* exclusively due to their modest production and effortless scale up.

Funding

Nil.

Acknowledgments

Nil.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. El Maghraby, G.M.; Barry, B.W.; Williams, A.C. Liposomes and skin: from drug delivery to model membranes. *Eur. J. Pharm. Sci.* **2008**, *34*, 203-222, https://doi.org/10.1016/j.ejps.2008.05.002.
2. Schäfer-Korting, M.; Mehnert, W.; Korting, H.-C. Lipid nanoparticles for improved topical application of drugs for skin diseases. *Adv. Drug Deliv. Rev.* **2007**, *59*, 427-443, https://doi.org/10.1016/j.addr.2007.04.006.
3. Bouwstra, J.A.; Ponec, M. The skin barrier in healthy and diseased state. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2006**, *1758*, 2080-2095, https://doi.org/10.1016/j.bbamem.2006.06.021.
4. Bouwstra, J.A.; Honeywell-Nguyen, P.L.; Gooris, G.S.; Ponec, M. Structure of the skin barrier and its modulation by vesicular formulations. *Prog. Lipid Res.* **2003**, *42*, 1-36, https://doi.org/10.1016/s0163-7827(02)00028-0.
5. Bozzuto, G.; Molinari, A. Liposomes as nanomedical devices. *Int. J. Nanomedicine* **2015**, *10*, 975-999, https://doi.org/10.2147/IJN.S6886.
6. Hua, S. Comparison of *in vitro* dialysis release methods of loperamide-encapsulated liposomal gel for topical drug delivery. *Int. J. Nanomedicine* **2014**, *9*, 735-744, https://doi.org/10.2147/IJN.S55805.
7. Hua, S.; Wu, S.Y. The use of lipid-based nanocarriers for targeted pain therapies. *Front. Pharmacol.* **2013**, *4*, 143, https://doi.org/10.3389/fphar.2013.00143.
8. Gugleva, V.; Michailova, V.; Mihaylova, R.; Momekov, G.; Zaharieva, M.M.; Najdenski, H.; Petrov, P.; Rangelov, S.; Forys, A.; Trzebicka, B. Formulation and Evaluation of Hybrid Niosomal *In situ* Gel for Intravesical Co-Delivery of Curcumin and Gentamicin Sulfate. *Pharmaceutics* **2022**, *14*, 747, https://doi.org/10.3390/pharmaceutics14040747.
9. Barot, T.; Rawtani, D.; Kulkarni, P. Development, characterization and *in vitro–in vivo* evaluation of Farnesol loaded niosomal gel for applications in oral candidiasis treatment. *Heliyon* **2021**, *7*, e07968, https://doi.org/10.1016/j.heliyon.2021.e07968.
10. Cosco, D.; Celia, C.; Cilurzo, F.; Trapasso, E.; Paolino, D. Colloidal carriers for the enhanced delivery through the skin. *Expert Opin. Drug Deliv.* **2008**, *5*, 737-755, https://doi.org/10.1517/17425247.5.7.737.
11. Geusens, B.; Strobbe, T.; Bracke, S.; Dynoodt, P.; Sanders, N.; Van Gele, M.; Lambert, J. Lipid-mediated gene delivery to the skin. *Eur. J. Pharm. Sci.* **2011**, *43*, 199-211, https://doi.org/10.1016/j.ejps.2011.04.003.
12. Honeywell-Nguyen, P.L.; Bouwstra, J.A. Vesicles as a tool for transdermal and dermal delivery. *Drug Discov. Today Technol.* **2005**, *2*, 67-74, https://doi.org/10.1016/j.ddtec.2005.05.003.
13. Kirjavainen, M.; Urtti, A.; Valjakka-Koskela, R.; Kiesvaara, J.; Mönkkönen, J. Liposome–skin interactions and their effects on the skin permeation of drugs. *Eur. J. Pharm. Sci.* **1999**, *7*, 279-286, https://doi.org/10.1016/s0928-0987(98)00037-2.
14. Gupta, P.; Yadav, K.S. Formulation and evaluation of brinzolamide encapsulated niosomal in-situ gel for sustained reduction of IOP in rabbits. *J. Drug Deliv. Sci. Technol.* **2022**, *67*, 103004, https://doi.org/10.1016/j.jddst.2021.103004.
15. Vora, B.; Khopade, A.J.; Jain, N. Proniosome based transdermal delivery of levonorgestrel for effective contraception. *J. Control Release.* **1998**, *54*, 149-165, https://doi.org/10.1016/s0168-3659(97)00100-4.
16. Elsewedy, H.S.; Younis, N.S.; Shehata, T.M.; Mohamed, M.E.; Soliman, W.E. Enhancement of Anti-Inflammatory Activity of Optimized Niosomal Colchicine Loaded into Jojoba Oil-Based Emulgel Using Response Surface Methodology. *Gels* **2021**, *8*, 16, https://doi.org/10.3390/gels8010016.
17. Hofland, H.; Bouwstra, J.; Bodde, H.; Spies, F.; Junginger, H. Interactions between liposomes and human stratum corneum *in vitro*: freeze fracture electron microscopical visualization and small angle X-ray scattering studies. *Br. J. Dermatol.* **1995**, *132*, 853-866, https://doi.org/10.1111/j.1365-2133.1995.tb16940.x.

18. Kumar, P.; Verma, N. An overview on niosomes: As an auspesious drug delivery system on the bases of application. *Res. J. Pharm. Technol.* **2021**, *14*, 2896-2902, https://doi.org/10.52711/0974-360X.2021.00508.
19. Patra, E.; Rajak, P.; Bhuyan, B.; Sultana, B.; Baruah, A.; Paul, D. Applications of Niosome and Targeting Strategies in the Field of Phyto-Pharmaceuticals : A Review. *Saudi J. Med. Pharm. Sci.* **2018**, *4*, 455-467.
20. Kaur, H.; Dhiman, S.; Arora, S. Niosomes: A novel drug delivery system. *Int. J. Pharm. Sci. Rev. Res.* **2012**, *15*, 113-120.
21. Akbarzadeh, I.; Sedaghatnia, K.; Bourbour, M.; Moghaddam, Z.; Moghtaderi, M.; Samimi-Sohrforozani, E.; Quazi, S.; Far, B. Niosomes: A novel targeted drug delivery system. **2021**, https://doi.org/10.20944/preprints202112.0315.v1.
22. Barani, M.; Sangiovanni, E.; Angarano, M.; Rajizadeh, M.A.; Mehrabani, M.; Piazza, S.; Gangadharappa, H.V.; Pardakhty, A.; Mehrbani, M.; Dell'Agli, M. Phytosomes as innovative delivery systems for phytochemicals: a comprehensive review of literature. *Int. J. Nanomedicine* **2021**, *16*, 6983-7022, https://doi.org/10.2147/IJN.S318416.
23. Carod, J.-F.; Ratsitorahina, M.; Raherimandimby, H.; Vitrat, V.H.; Andrianaja, V.R.; Contet-Audonneau, N. Outbreak of Tinea capitis and corporis in a primary school in Antananarivo, Madagascar. *J. Infect. Dev. Ctries.* **2011**, *5*, 732-736, https://doi.org/10.3855/jidc.1944.
24. Lim, S.S.; Shin, K.; Mun, J.H. Dermoscopy for cutaneous fungal infections: A brief review. *Health Sci. Rep.* **2022**, *5*, e464, https://doi.org/10.1002/hsr2.464.
25. Foster, K.W.; Ghannoum, M.A.; Elewski, B.E. Epidemiologic surveillance of cutaneous fungal infection in the United States from 1999 to 2002. *J. Am. Acad. Dermatol.* **2004**, *50*, 748-752, https://doi.org/10.1016/s0190-9622(03)02117-0.
26. Williams, L. Microbial Infections: The Good, the Bad and the Ugly. In *Reference Module in Biomedical Sciences*; **2021**. DOI: 10.1016/B978-0-12-818731-9.00100-2.
27. Jartarkar, S.R.; Patil, A.; Goldust, Y.; Cockerell, C.J.; Schwartz, R.A.; Grabbe, S.; Goldust, M. Pathogenesis, Immunology and Management of Dermatophytosis. *J. Fungi* **2021**, *8*, 39, https://doi.org/10.3390/jof8010039.
28. Kamboj, A.; Saluja, A.K. Phytopharmacological review of *Xanthium strumarium* L.(Cocklebur). *Int. J. Green Pharm.* **2010**, *4* doi:10.4103/0973-8258.69154.
29. Rodino, S.; Butu, M.; Petrache, P.; Butu, A.; Cornea, C.P. Antifungal activity of four plants against Alternaria alternata. *Sci. Bull.* **2014**, *18*, 60-65.
30. Parveen, Z.; Mazhar, S.; Siddique, S.; Manzoor, A.; Ali, Z. Chemical composition and antifungal activity of essential oil from *Xanthium strumarium* L. leaves. *Indian J. Pharm. Sci.* **2017**, *79*, 316-321, https://doi.org/10.4172/pharmaceutical-sciences.1000232.
31. Sharma, K.; Kotoky, J.; Kalita, J.; Sarma, G. Traditional use of medicinal plants for anti-ringworm therapy in some parts of Kamrup District of Assam, a North Eastern State of India. *Asian Pac. J. Trop. Dis.* **2012**, *2*, S316-S319, https://doi.org/10.1016/S2222-1808(12)60173-X.
32. Devkota, A.; Das, R.K. Antifungal activities and phytochemical screening of *Xanthium strumarium*. *Bio. Bull.* **2016**, *2*, 121-127.
33. Sonam, M.; Singh, R.P.; Pooja, S. Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. *Int. J. Pharmacogn. Phytochem. Res* **2017**, *9*, 523-527, https://doi.org/10.25258/phyto.v9i2.8125.
34. Ly, H.T.; Truong, T.M.; Nguyen, T.T.H.; Nguyen, H.D.; Zhao, Y.; Le, V.M. Phytochemical screening and anticancer activity of the aerial parts extract of *Xanthium strumarium* L. on HepG2 cancer cell line. *Clin. Phytoscience* **2021**, *7*, 14, https://doi.org/10.1186/s40816-021-00252-w.
35. Shilakari Asthana, G.; Asthana, A.; Singh, D.; Sharma, P.K. Etodolac containing topical niosomal gel: formulation development and evaluation. *J. Drug Deliv.* **2016**, *2016*, 9324567, https://doi.org/10.1155/2016/9324567.
36. Maurya, K.; Singh, A.; Pandey, S.; Kumar, N.; Siddhiqui, M.A. Niosomes: Classification, preparation and application. *Int. J. Indig. Herbs Drugs* **2021**, *6*, 29-32.
37. Goyal, G.; Garg, T.; Malik, B.; Chauhan, G.; Rath, G.; Goyal, A.K. Development and characterization of niosomal gel for topical delivery of benzoyl peroxide. *Drug Deliv.* **2015**, *22*, 1027-1042, https://doi.org/10.3109/10717544.2013.855277.
38. Rao, A.A.; Arvapalli, S.; Rao, G.K.; Malothu, N.; Bandaru, N.R. Design and evaluation of acyclovir niosomes. *Res. J. Pharm. Technol.* **2021**, *14*, 4185-4188, https://doi.org/10.52711/0974-360X.2021.00724.
39. Nikam, V.; Maniyar, S. Formulation Development and Evaluation of Niosomal Gel of Collective Antifungal Agents. *Asian J. Pharm. Clin. Res.* **2021**, *15*, 64-74, https://doi.org/10.22159/ajpcr.2022.v15i2.43484.
40. Yin, R.-H.; Bai, X.; Feng, T.; Dong, Z.-J.; Li, Z.-H.; Liu, J.-K. Two new compounds from *Xanthium strumarium*. *J. Asian Nat. Prod. Res.* **2016**, *18*, 354-359, https://doi.org/10.1080/10286020.2015.1099525.
41. Aparajay, P.; Dev, A. Development and Evaluation of Eberconazole-Loaded Niosomes. *Chemistry Proceedings* **2021**, *8*, 28, https://doi.org/10.3390/ecsoc-25-11664.
42. Coviello, T.; Trotta, A.; Marianecci, C.; Carafa, M.; Di Marzio, L.; Rinaldi, F.; Di Meo, C.; Alhaique, F.; Matricardi, P. Gel-embedded niosomes: preparation, characterization and release studies of a new system for topical drug delivery. *Colloids Surf. B.* **2015**, *125*, 291-299, https://doi.org/10.1016/j.colsurfb.2014.10.060.

43. Pandey, D.; Rather, M. Isolation and Identification of Phytochemicals from *Xanthium strumarium*. *Int. J. Chemtech Res.* **2012**, *4*, 266-271.
44. Costa, P.; Lobo, J.M.S. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* **2001**, *13*, 123-133, https://doi.org/10.1016/s0928-0987(01)00095-1.
45. Schwartz, J.B.; Simonelli, A.P.; Higuchi, W.I. Drug release from wax matrices I. Analysis of data with first-order kinetics and with the diffusion-controlled model. *J. Pharm. Sci.* **1968**, *57*, 274-277, https://doi.org/10.1002/jps.2600570206.
46. Ritger, P.L.; Peppas, N.A. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *J. Control Release.* **1987**, *5*, 37-42, https://doi.org/10.1016/0168-3659(87)90035-6.