FORMULATION AND EVALUATION OF TOPICAL ANTIMICROBIAL EMBELIN NIOSOMAL CREAM

Kritika R. Saboo[a], Rohit R. Ghadge[a], Dhanashree P. Sanap[a]* and Sneha A. Agrawal[b]

(Received 30 December 2023) (Accepted 01 June 2024)

ABSTRACT

Embelin, isolated from *Embelia ribes* berries is a major constituent isolated by the Soxhlet process and possesses antimicrobial activity. In this report, we develop and evaluate the topical antimicrobial embelin niosomal cream formulation. Niosomal formulation of embelin was formulated using a thin-film hydration method using Tween™ 60, chloroform and phosphate buffer with a rotary evaporator. This niosomal formulation was incorporated into the cream base, where both oil and water phase ingredients were prepared separately at 70 °C by constant stirring to form w/o (water-in-oil) cream formulation. This niosomal cream was evaluated for particle size, entrapment efficacy, spreadability, centrifugation, viscosity, dye test, homogeneity, *in vitro* antifungal and antibacterial activity against *C. albicans* and *S. aureus*, respectively. The zone of inhibition was calculated and further compared with the market formulations. The study proposed that niosomal formulations delivered sustained and prolonged delivery of drug with increase in bioavailability.

Keywords: Embelin, niosome, thin-film hydration, entrapment efficacy, antimicrobial tests

INTRODUCTION

Embelia ribes Burm F. is a medicinal plant that belongs to the Myrsinaceae family. It is commonly known as false black pepper or vidanga[1]. Embelin is an orange colored crystalline powder that is the major active component of *E. ribes,* having IUPAC name 2, 5-dihydroxy-3- undecyl-1, 4-benzoquinone, with molecular formula $C_{17}H_{26}O_4$ and molecular weight[2] 294.4 g mol^{-1}. Embelin is responsible for most of the biological activity, like antidiabetic, anti-inflammatory, anthelmintic, anti-cancer, anti-hypertension and analgesic activities[3]. Embelin has extremely high market potential, but unfortunately, it is not used to that extent for different reasons. Embelin has been isolated by Soxhlet apparatus using pet ether and methanol[4].

Niosomes are vesicles made of non-ionic surface active agents, which serve as novel drug delivery systems. Embelin is formulated as a niosomal cream-based formulation, where niosomal concept offers additional advantages of reduction in toxicity, increased penetration across *stratum corneum*, and modified pharmacokinetic and bioavailability[5]. Cholesterol is a steroidal substance that is an essential part of cell membranes and enhances the rigidity, fluidity and permeability of the bilayer[6]. Niosomes can be made using various methods like the ether injection method, reverse phase evaporation technique, multiple membrane extrusion methods, thin film hydration and emulsion method[7]. Niosome formulation primarily aims to target a specific body site for drug delivery and to modulate drug release over time.

MATERIALS AND METHODS

Materials

Embelin was isolated in the research laboratory at Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai. Crude Drug (Vidanga) was purchased from Yucca Enterprises, Wadala (E), Mumbai. Cholesterol (CHO) was purchased from Fine Chemical Industries, chloroform from Research Lab Chemicals Co., Mumbai, and Nonionic surfactants (Span™ 40, Span™ 60 and Tween™ 60) from S D Fine Chem. Ltd., Worli, Mumbai. Marketed antibacterial formulation i.e. T-bact Ointment of Glaxo SmithKline Pharmaceuticals Ltd., and antifungal formulation i.e. Clocip® cream of Cipla, were purchased from the local pharmacy.

[a] Department of Pharmaceutics, Bharati Vidyapeeth's College of Pharmacy, University of Mumbai, Navi Mumbai - 400 614, Maharashtra, India
[b] Department of Pharmacognosy, Bharati Vidyapeeth's College of Pharmacy, University of Mumbai, Navi Mumbai - 400 614, Maharashtra, India
*For Correspondence: E-mail: dhanashree.sanap@bvcop.in

https://doi.org/10.53879/id.61.07.14496

METHODS

Embelin isolation from *E. ribes* berries

100 g of coarsely powdered *E. ribes* fruits were extracted by hot Soxhlet extraction with petroleum ether 60-80 °C. 10 cycles were run till a clear solution was obtained in the siphon. Crude extract was obtained in the round bottom flask. Residue was obtained by removal by Solvent distillation, petroleum ether used in the Soxhlet apparatus was recovered[8]. The residue was then washed with PET ether, after washing, minimum four cycles of filtration using PET ether was done. Residue obtained from this was dissolved in hot methanol and kept for crystallization for 24 h. Filtration from mother liquor gave crystals, which were washed again with hot methanol if impurities were observed. Orange-red embelin crystals were obtained (Fig. 1).

Fig. 1: Isolated embelin crystals

Embelin niosomal formulation

Preparation of niosomes

Niosomes were made by a thin film hydration method using a lipid mixture containing surfactant (Span™ 40, Span™ 60 and Tween™ 60) and cholesterol (CHO), at various ratios, as given in Table I. In a 100 mL round bottom flask, surfactant, CHO and drug were dissolved in 10 mL of chloroform and the solvent was evaporated at a temperature of 55-65 °C under low pressure. The evaporation was carried out in a rotary flash evaporator until a thin lipid film was formed[9]. The formed film was then hydrated with phosphate buffer of pH 7.4. The flask was rotated in the rotary evaporator at 55 to 65 ° for 1 h. The hydration was continued for 1 h, while the flask was kept rotating at 55-65°C in the rotary evaporator. The hydrated niosomes were sonicated in a bath sonicator for 20 min to obtain niosomal dispersion[10].

Characterization of niosomes

Morphological characterization

Niosomal solution was dropped onto a glass slide and covered with a cover slip to observe the shape of noisome vesicles. It was observed under 10X eyepiece and 45X objective lens[11] (Fig. 3).

Entrapment efficacy

The entrapment efficacy of niosomal formulations was determine using the centrifugation method. 10 mL niosomal suspension was taken in a centrifugation tube and centrifuged at 10000 rpm for 10 min at 4 °C. The clear part was used to check the free drug by using a UV/visible spectrophotometer at 294.3 nm[12].

$$\% \text{ Entrapment efficiency (\% EF)} = \frac{\text{(Amount of drug entrapped)}}{\text{(total amount of drug present)}} \times 100$$

Embelin niosomal cream formulation

Preparation of niosomal cream

All necessary materials were weighed as required for the water phase and oil phase. The oil phase consisting of Vaseline®, cetyl alcohol, stearic acid and glyceryl monostearate was melted in a water bath between

Table I: Composition of embelin niosomal formulations

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug	200	200	200	200	200	200	200	200	200
Span™ 40	200	300	400	-	-	-	-	-	-
Span™ 60	-	-	-	200	300	400	-	-	-
Tween™ 60	-	-	-	-	-	-	200	300	400
Cholesterol	40	60	80	40	60	80	40	60	80
Chloroform	20	20	20	20	20	20	20	20	20
Phosphate buffer pH 7.4	40	40	40	40	40	40	40	40	40

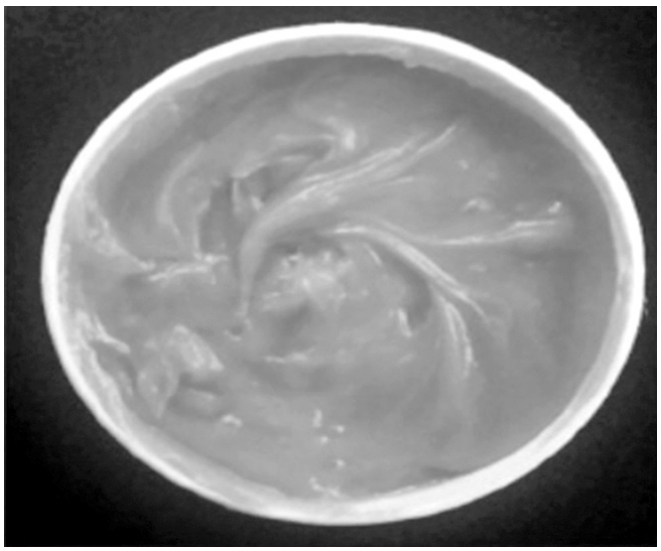

Fig. 2: Embelin niosomal cream formulations

70-75 °C. In a different container, aqueous phase containing sodium metabisulfite, propylene glycol and triethanolamine was dissolved in an embelin niosomal solution at 70-75 °C. The water phase was then slowly added to the oil phase with constant stirring until a creamy mass was obtained[13] (Fig. 2, Table II).

Characterization of niosomal incorporated cream formulations

Organoleptic characteristics

Physical appearance, color, texture, phase separation and homogeneity of the formulations were evaluated by visual observation. By squeezing a small amount of the cream between the thumb and index finger, homogeneity, consistency and texture were examined. Immediate skin feels like stiffness, greasiness and grittiness were also evaluated[14].

pH measurement

The pH was measured by a pH meter (Eutech instrument pH 510). The pH range same as skin pH was considered in the range of 4.5–6.5. The pH should neither be too acidic nor alkaline as it may cause skin irritation[15].

Centrifugation

The emulsion was heated to 50°C (122° F) and centrifuged for 30 min at 3000 rpm, and observed for any separation.

Measurement of viscosity

Brookfield viscometer was used to measure viscosity. The spindle number 3 was rotated at various rpm like 0, 1, 2, 2.5, 5, 10, 15, 20, 50, 75 and 100 rpm. All measurements were made in triplicate[16].

Table II: Composition of embelin niosomes incorporated cream

Ingredient	F1 (%w/w)	F2 (%w/w)	F3 (%w/w)	F4 (%w/w)
Vaseline®	5	7	6	8
Stearic acid	3	3	3	3
Cetyl alcohol	3	3	3	3
Propylene glycol	5	6	7	8
Glyceryl monostearate	3	3	3	3
Triethanolamine	1	1	1	1
Sodium metabisulfite	0.1	0.1	0.1	0.1
Niosomal solution	q.s. to 100g	q.s. to 100g	q.s. to 100g	q.s. to 100g

Dye test

Dye test was used to identify the type of emulsion. The emulsion was mixed with an oil soluble dye i.e. scarlet red. If the continuous phase appeared red, then it was a w/o emulsion and if the dispersed phase appeared red, then it was an o/w type of emulsion[17].

Spreadability test

The spreading diameter of 1 g of sample between two horizontal glass plates after one minute was measured to test the formulation's spreadability. The standard weight of 25 g was applied to the upper plate. Each cream formulation was tested three times[18].

$$S = M*l / t$$

where, S= spreadability, M= mass, l= length moved on glass slide, t= time

In vitro antimicrobial activity

Preparation of agar plates: Agar nutrient medium was prepared, which consisted of peptone, agar, sodium chloride, beef extract and water, and autoclaved at 121 °C at 15 psi for 15 min. The agar medium was cooled to 40-45 °C inoculum was added and then dispensed into the prepared petri dish[19].

Preparation of inoculum: *S. aureus and C. albicans* was used to check the antibacterial and antifungal activity of the topical formulations, respectively.

Preparation of agar well diffusion technique: The agar well diffusion assay was carried out using the dried inoculated agar plates that were previously produced. The wells were created by puncturing holes in the inoculated agar plates with a sterile cork borer[20]. Suitable dilutions were made for embelin, embelin plain cream (0.8, 1, 1.5 % w/w), niosomal solution and embelin niosomal incorporated cream of 1, 2, 10, 20 and 100 ppm, standard marketed antibacterial formulation T-bact and antifungal formulation Clocip®. The inoculated agar plates were incubated at 37 °C for 48 h. The zones of inhibition diameter were measured by using a ruler[21]. Minimal inhibitory

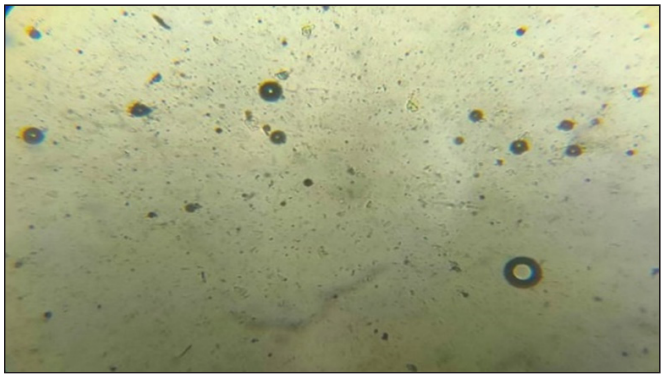

Fig. 3: Microscopic characterization of optimized niosomal vesicle [F9]

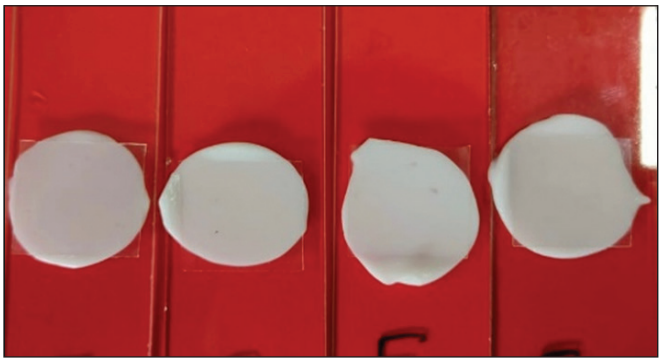

Fig. 4: Spreadability efficacy of embelin niosomes incorporated cream

Table III: Evaluation test for embelin niosomes incorporated cream

Sr. No.	Test	Results
a.	Organoleptic characterizations	
	i. Homogeneity	Homogeneous
	ii. Texture	Smooth
b.	pH	6.2
c.	Centrifugation	No phase separation
d.	Viscosity	Viscous
e.	Dye test	Confirm w/o type of emulsion
f.	Spreadability	Easily spreadable

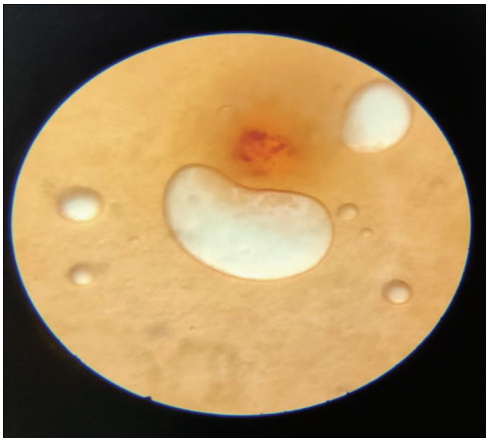

Fig. 5: Dye test exhibiting the developed optimized embelin niosomes incorporated cream as w/o emulsion type

Table IV: *In vitro* antimicrobial activity

Formulation	Zone of inhibition for *S. aureus* (mm)	Zone of inhibition for *C. albicans* (mm)
Placebo	No inhibition	No inhibition
Marketed formulation Antibacterial(T-bact):		
• 20 ppm	21.6 ± 0.11	-
• 100 ppm	24.3 ± 0.23	
Marketed formulation Antifungal (Clocip®)	-	
• 20 ppm		16.3 ± 0.05
• 100 ppm		23.3 ± 0.05
Embelin plain cream 1%		
• 10 ppm	19.3 ± 0.05	16.0 ± 0.17
• 20 ppm	20.6 ± 0.11	18.6 ±0.05
Embelin niosome incorporated cream		
• 10 ppm	20.6 ± 0.11	20.6 ± 0.11
• 20 ppm	23.6 ± 0.05	32.6 ± 0.05

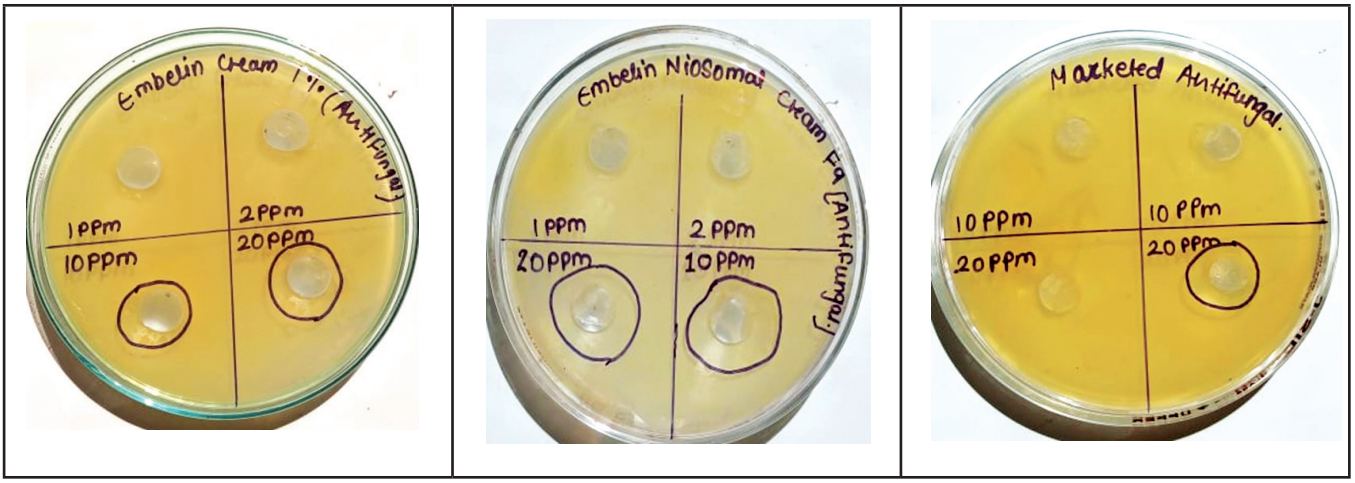

Fig. 6: Antifungal activity against *C. albicans* (A) embelin plain cream 1% w/w (B) embelin niosomes incorporated cream (F9) (C) Marketed clotrimazole antifungal cream (Clocip®)

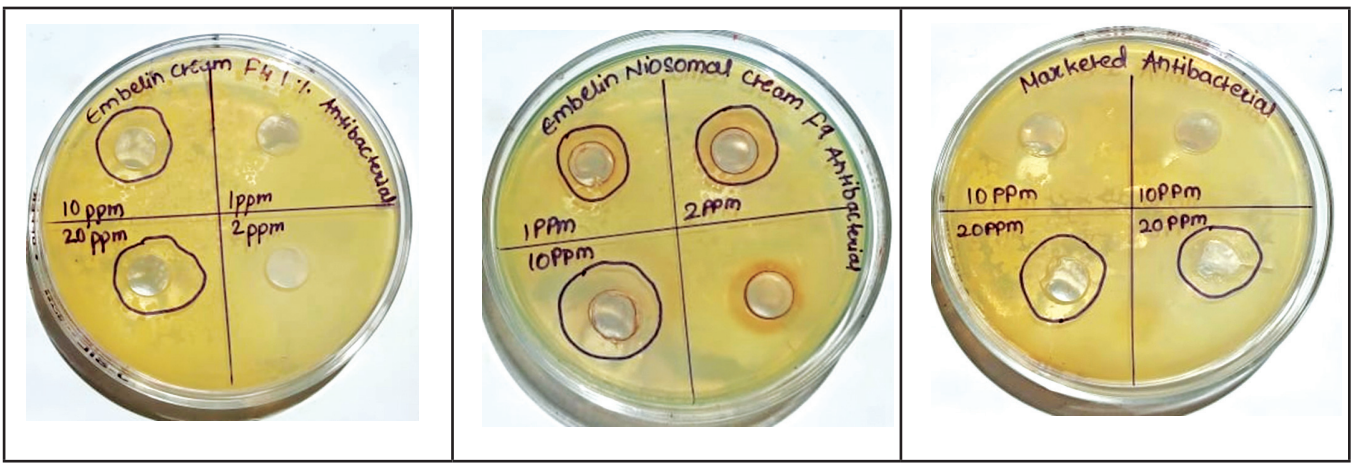

Fig. 7: Antibacterial activity against *S. aureus* (A) embelin plain cream 1% w/w (B) embelin niosomes incorporated cream (F9) (C) Marketed mupirocin antibacterial cream (T-bact, gsk)

concentration (MIC) is the maximum dilution or lowest concentration of extracts that prevent organism growth. The determination of the MIC is crucial in diagnostic labs because it assists in identifying germs that are resistant to antimicrobial agents and keeps track of the action of new antimicrobial drugs[22].

RESULTS AND DISCUSSION

Formulation and evaluation of embelin niosomes

After formulating nine batches of embelin niosomes with different ratios of surfactant, F9 was considered an optimized batch, based on the results of morphological characterization and entrapment efficacy. Morphological characterization revealed that the niosomal vesicles were spherical in shape for F7 to F9 (Fig. 7), where Tween™ 60 vesicles were used as a surfactant. Further, the entrapment efficacy test exhibited that the niosomes having the highest ratio of Tween™ 60, i.e. F9, showed maximum drug loading capacity.

Formulation and evaluation of embelin niosomes incorporated cream

Evaluation test was performed on all four batches (Table III). The main criteria to optimize the formulation was the viscosity and spreadability test (Fig. 4). On performing these tests, F4 was considered as an optimized formulation and the dye test also confirmed that it's a w/o type of emulsion cream (Fig. 5). MIC study was done to see the minimum effective concentration. The cream was made in three different concentrations i.e. 0.5% w/w, 1% w/w and 1.5% w/w. The evaluated antimicrobial test

data demonstrates that 1% w/w embelin niosomal cream is effective against both *S. aureus* and *C. albicans* and showed better zones of inhibition as compared to plain embelin cream and niosomal cream (Fig. 6). Thus we can deduce that embelin niosomes incorporated cream is more effective.

In the first step, embelin was individually measured against *S. aureus and C. albicans* to check if it has antibacterial and antifungal activity. The selected cream (F4) antibacterial activity was tested with varied concentrations of the active components (0.8, 1.0 and 1.5 %) was studied. In order to see how effective the formulation was, we compared it with the marketed formulation (Table IV). The marketed formulation was considered as the control group. No inhibition was observed in blank cream and 0.8% cream. Cream containing 1% drug had a similar antibacterial and antifungal activity to that of the control. Then we compared with zone of inhibition for niosomal cream. The niosomal cream showed higher zone of inhibition as compared to plain cream. The selected cream and niosomal formulation were compared to the marketed formulations. We observed that our niosomal formulation showed higher zone of inhibition, when compared to plain embelin cream and marketed formulation against *S. aureus* and *C. albicans.*

CONCLUSION

A thoughtfully designed *in vitro* follow-up study verified that embelin possesses both antibacterial and antifungal activity, and a more accurate comparison could be made between our formulated products and marketed products. The minimum effective concentration was found to be 1 %. We observed that 0.8 % plain cream did not show any zone of inhibition, whereas 1 % plain cream did show a zone of inhibition. The zone of inhibition of embelin niosome formulation was greater as compared to plain embelin cream and marketed formulation. So, we can conclude that embelin niosome formulation has better antibacterial and antifungal activities.

REFERENCES

1. Lal B. and Mishra N.: Importance of *Embelia ribes*: an update, **Int. J. Pharm. Sci.**, 2013, 4(10), 3828-3838.
2. Vasu S.: Botanical pharmacognosy of the fruit of *Embelia ribes* Burm. F., **J. Pharmacogn. Nat. Prod.**, 2015, 1(1), 103.
3. Meena A. K.: Pharmacognostic and physicochemical studies of *Embelia ribes* Burm. F. fruit used in ayurvedic formulations, **Res. J. Pharm. Tech**., 2013, 6(6), 645-648.
4. Kamble V.: Phytochemical analysis, antioxidant activities and optimized extraction of embelin from different genotypes of *Embelia ribes* Burm F.: A woody medicinal climber from Western Ghats of India, **Physiol. Mol. Biol. Plants**., 2020, 26(9), 1855-1865.
5. Khoee S.: Niosomes: a novel approach in modern drug delivery systems. **Nanostructures Drug Deliv.,** 2017, 207-237.
6. Asthana G. and Sharma P.: *In vitro* and *In vivo* evaluation of niosomal formulation for controlled delivery of clarithromycin, **Scientifica,** 2016, 1-10.
7. Kazi K.: Niosome: a future of targeted drug delivery systems, **J. Adv. Pharm. Technol. Res.,** 2010, 1(4), 374 - 380.
8. Pundarikakshudu K. and Joshi H.: A simple, facile method for isolation of embelin from fruits of *Embelia ribes* Burm. F. (Vidang), **Indian Drugs**, 2016, 53, 23-27.
9. Thabet Y.: Methods for preparation of niosomes: A focus on thin-film hydration method. **Elsevier,** 2022, 199, 9-15.
10. Shirsand S. B.: Formulation and evaluation of ketoconazole niosomal gel drug delivery system, **Int. J. Pharm. Investig.**, 2012, 2(4), 201–207.
11. Awate P.S., Pimple T.P., Pananchery J.F. and Jain A.S.: Formulation and evaluation of berberine HCl as niosomal drug delivery system, **Asian J. Pharm. Res.**, 2020, 10(3), 149-159.
12. Makeshwar K.B. and Wasankar S.R.: Niosome: a novel drug delivery system, **Asian J. Pharm. Res.,** 2019, 3(1), 15-19.
13. Lendora H.: Formulation and evaluation of miconazole nitrate cream, **Asian J. Pharm. Clin. Res.,** 2018, 11(3), 319.
14. Mahendran S., Pavitra S. and Afzan M.: Formulation and evaluation of novel antiaging cream containing rambutan fruit extract, **Int. J. Pharm. Sci. Res.,** 2017, 8(3), 1056-1065.
15. Sekar M.: Formulation and evaluation of novel antibacterial and anti-inflammatory cream containing *Muntingia calabura* leaves extract, **Asian J. Pharm. Clin. Res.**, 2017, 10(12), 376-379.
16. Mohamed N.: Sorbitol based powder precursor of cubosomes as an oral delivery system for improved bioavailability of poorly water soluble drugs, **J. Drug Deliv. Sci. Technol.,** 2016, 35, 106-113.
17. Navgire T. and Pawar M.: Formulation and evaluation of cold cream, **IJCRT,** 2021, 9(9), 303-306.
18. Sabale V.: Formulation and *in vitro* evaluation of the topical antiageing preparation of the fruit of *Benincasa hispida*, **J. Ayurveda Integr. Med.,** 2011, 2(3), 124–128.
19. Mohite S.A.: Antimicrobial Activity of leaves extracts of *Jatropha curcas*, **Asian J. Pharm. Res.**, 2018, 8(1), 17-20.
20. Kumar S.: Bioinspired synthesis of silver nanoparticles using *Euphorbia hirta* leaf extracts and their antibacterial activity, **Asian J. Pharm. Res.,** 2014, 4(1), 39-43.
21. Chen M. X.: Formulation and evaluation of antibacterial creams and gels containing metal ions for topical application, **J. Pharm.**, 2016, 5754349.
22. Saiah H.: Antioxidant and antibacterial activities of six Algerian medicinal plants, **Int. J. Pharm. Pharm. Sci.,** 2016, 8(1), 367-374.