Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

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Characterization and optimization of clove oil-loaded nanomicelles for the possible topical use of bacterial infection-led atopic dermatitis

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Abstract

Background Atopic dermatitis is an abnormal skin condition that impacts a significant number of people in the US, with an estimated 9.6 million children and 16.5 million adults being affected by it. The study aimed to characterize and optimize clove oil-based nanomicelles for the possible topical use of bacterial infection-led atopic dermatitis. Clove oil-loaded nanomicelles were produced and carefully analyzed for vesicle diameter, polydispersity index (PDI), zeta potential, morphological attributes, entrapment efficiency, in vitro release, stability, dermatokinetic parameters, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging behavior and antibacterial activity. Different models, such as Korsmeyer, Higuchi, first order, and zero order were employed to evaluate the in vitro release from the formulations.

Results The average size of the clove oil nanomicelles was found to be 100.15 nm with a PDI of 0.2104; they were observed to be darker against a light background. The evaluated mean zeta size was 121.3 nm, the zeta potential was − 15.31 mV. The inhibitory concentration 50 (IC_{50}) of the formulation was 61.32 ± 0.98 µg/mL; clove oil was 73.56 ± 1.63 µg/mL, against ascorbic acid was 54.51 ± 0.79 µg/mL. Among the four models tested for in vitro release kinetics, the Korsmeyer Peppas model was followed by the nanomicelles formulation. Clove oil nanomicelles generated a higher concentration of 148.68 w/v on the skin epidermis within 1.5 h, whereas the conventional formulation exhibited 55.287 w/v. Moreover, clove oil nanomicelles generated a higher concentration of 125.84 µg/mL on the skin's dermis within 2 h, whereas the conventional formulation produced 68.263 µg/mL. The nanomicelles also inhibited bacterial growth within a 24-h period.

Conclusions The study presents initial evidence regarding the potency of clove oil-based nanomicelles and their enhanced efficiency on the skin. Thus, the prepared formulation can further be studied and incorporated for the possible use against bacterial infection-led atopic dermatitis.

Keywords Clove oil, Atopic dermatitis, *Syzygium aromaticum*, Nanomicelles

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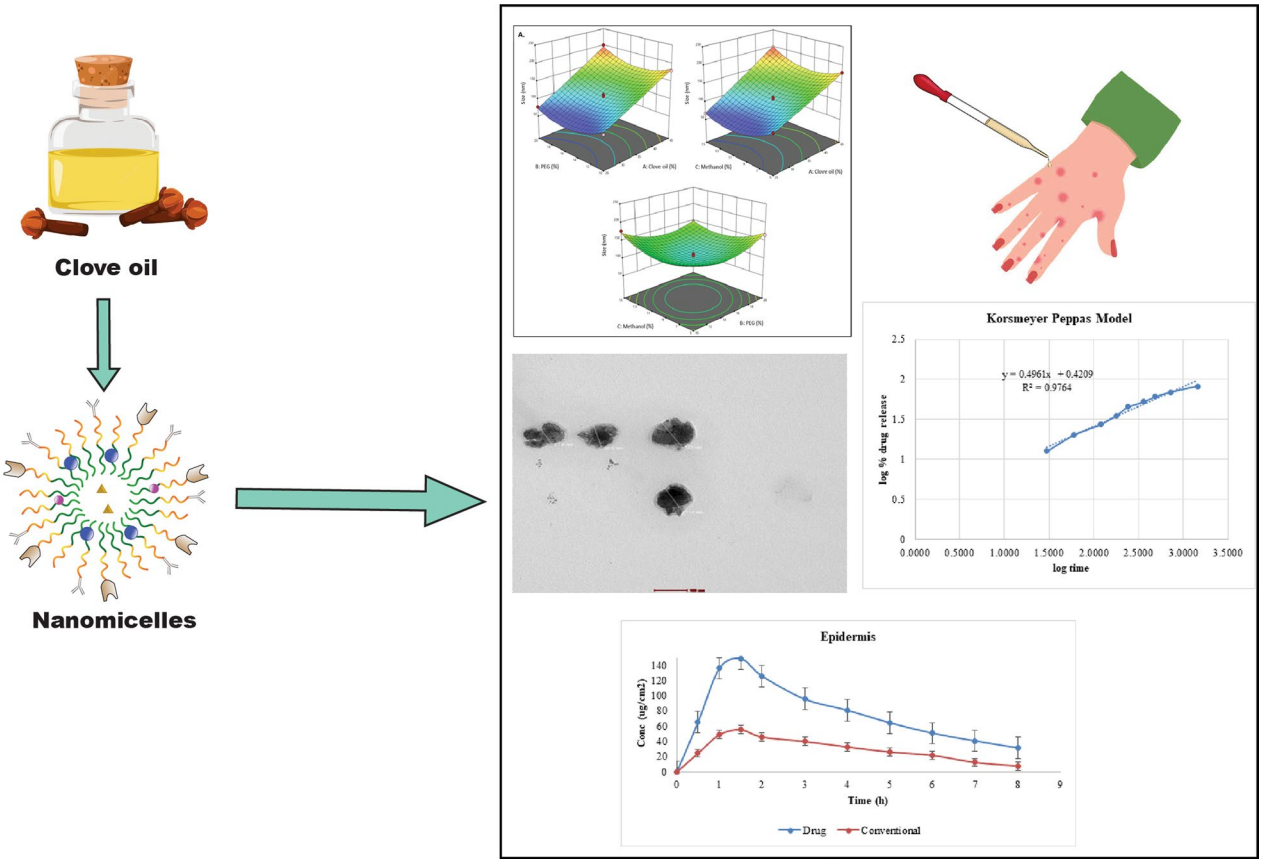
Graphical abstract



1 Background

Atopic dermatitis (AD) has been a prevalent condition of skin inflammation that affects about 9.6 million youngsters and 16.5 million adult individuals [1, 2]. It is a persistent disorder that frequently starts in the early years of life and can last throughout later life. Nowadays, the disorder has an early onset in teenagers and adults. The pathophysiology of AD is complicated and includes a dysregulated immune function with a damaged skin barrier [3]. The epidermal layer serves as a functioning protective layer, and skin abnormalities are the most common pathologic features. Critical proteins required by the epidermis are intercellular proteins, including transglutaminases, keratins, and filaggrin. Problems arising from such proteins cause the skin to allow the entry of allergens and microbes [4–6].

When exposed to UV radiation, free radicals are produced by cells, such as reactive species of oxygen and nitrogen. Enhanced stress from oxidation has been seen on the skin of those afflicted with atopic dermatitis [7, 8]. In addition, individuals impacted by AD are more susceptible to contracting viral, bacterial, and fungal infections [9, 10]. The microbial environment of atopic skin differs noticeably from that of typical skin. It has been observed that *Staphylococcus aureus* colonizes in over ninety percent of patients and is hypothesized to be the cause of the seriousness of the disease, although it is detected in fewer than ten percent of the healthy population [11, 12].

Several pathogenic processes may work together to perpetuate typical signs of illness in AD, such as remodeling of the skin surface, eczematous lesions, dryness, and pruritus owing to persistent inflammation [6]. Some present therapies for AD have been centered on skin layer repair and the administration of corticosteroids, in addition to topically and systemically administered immunosuppressants, as single or combination medications. Yet, such pharmaceutical therapies are ineffective and have significant negative consequences [13].

Syzygium aromaticum, often recognized as cloves, are small, dried flower buds of the Myrtaceae genus. The

plant has become widely spread across the islands in Indonesia and has recently expanded to many regions around the world [14, 15]. Buds and leaves of the clove plant are used for commercial purposes, and they are usually gathered by hand. The proper growth and development of buds and leaves are ensured by the use of phytohormones during the pre-budding period. Besides being used as an ingredient in spices, clove has gained a lot of attention due to its significant antioxidant and antibacterial properties [16]. The availability of many different chemicals having antioxidant properties in considerable quantities is ascribed to clove's efficient involvement with the suppression of several degenerative illnesses [17, 18]. Clove oil (CO) has long been utilized to heal wounds and burn injuries as it is non-toxic, safe, and biodegradable. For centuries, it has been utilized to address health issues of the liver, stomach, colon, and nerves, flatulence, nausea, and vomiting. Clove oil has been used in Asia to treat several diseases caused by different pathogens such as tuberculosis, cholera, scabies, and malaria. In America, it has been utilized to treat infections caused by protozoans, candida, bacteria, and viruses [19]. It has been used as a pain reliever for toothaches as well as a cure for tooth infections. Furthermore, its usage in numerous industrial settings has been reported, as it is widely employed in fragrances and cleansers [20].

Encapsulation of essential oils to achieve bioactivity in nanoparticles has the potential to greatly limit vaporization as well as the diffusion rates, under-regulated release rate to surrounding environment [21, 22]. Nanomicelles can be typically made up of amphiphilic units that convene together into a spherical shell inside an aqueous system, with hydrophilic units forming shells and hydrophobic units constituting the center [23]. Hydrophobic medications are encapsulated even more effectively in the centers of micelles copolymers. Biopolymer micelles have received a lot of attention for the nano-delivery of hydrophobic medications and therapeutic chemicals [24, 25].

Based on the well documented anti-inflammatory and antimicrobial properties of clove oil and the potential of nanomicelles as a drug delivery system, we hypothesize that the development and optimization of clove oil-loaded nanomicelles will result in a topical formulation that effectively manages the symptoms of atopic dermatitis by reducing inflammation and relieving itching. In this study, we aimed to develop and optimize clove oil-loaded nanomicelles for the topical management of atopic dermatitis. The results of this study may provide valuable insights into the development of effective and safe topical formulations for the management of atopic dermatitis.

1.1 Chemical and reagents

Clove oil was purchased from Universal Biotech, India. The chemicals, such as poloxamer 188, Pluronic F127, Polysorbate 80, poloxamer 407, polyethylene glycol and ascorbic acid, were purchased from Merck, Germany. The other chemicals such as ethanol (96%), distilled water, sodium hydroxide (NaOH), Hydrochloric acid (HCl), acetonitrile, methanol, phosphate buffer solution (PBS) and tween 80 utilized in the study were purchased from Sigma-Aldrich, Germany.

1.2 Animals

Male Wistar rats were used in this study. The study protocol was sanctioned by the Institutional Ethics Committee of the faculty of Veterinary Medicine, University of Sadat City, under the approval number VUSC-020-1-23.

1.3 Clove oil solubility

Clove oil exhibited high solubility when mixed extensively with polyethylene glycol (a surfactant), revealing a solubility ratio of 1 part oil to 9 parts surfactant (PEG). Equilibrium was attained after the mixture was repeatedly spun for twelve to fifteen minutes in a vortex mixer. The prepared mixture was kept for 3 days inside an isothermal shaker at ambient temperature. After equilibration, the mixture underwent centrifugation for 15 min at a speed of 3000 rpm. Finally, the obtained mixture was diluted in a mobile phase after being filtered through a 0.45 µm membrane filter. The Shimadzu UV-1700 Spectrophotometer, Japan was calibrated at 260 nm to further assess the content of the clove oil.

1.4 Preparation of clove oil nanomicelles

The nanomicelles of clove oil were formulated by the method of thin film hydration. The surfactant [polyethylene glycol] was used for the hydrophilic part along with Pluronic F127 was used for the hydrophobic part and the clove oil were carefully weighed and mixed with methanol in a flask. The resulting mixture was thereafter positioned in a revolving evaporator under reduced pressure for 90 min at 45 °C, forming a thin film on the flask walls. The obtained film underwent hydration using de-ionized H_2O and was sonicated for 8 min in the Powersonic 405 sonicator of Hwashin Technology, South Korea [26].

1.5 Characterization of morphological structure

Transmission Electron Microscopy (TEM) from JEM-1400 of Japan was employed to analyze the morphology of clove oil nanomicelles by placing a drop of the

nanomicelles formulation on a carbon-covered grid of copper until it dried fully at ambient temperature with an applied voltage of 80 kV. The separate levels utilized in the study were low, medium, and high for the clove oil and PEG. The variables taken as dependent were the size of particles, the polydispersity index, and entrapment efficiency.

1.6 Optimization of clove oil nanomicelles

The nanomicelles were optimized using the Design Expert software, notably the Box-Behnken design (Version 12) of Stat-Ease, USA. Three levels of high, medium, and low (Table 1) were utilized to assess the three factors size of particles, polydispersity index, and entrapment efficiency along with the in vitro release of nanomicelles thoroughly. To examine the influence of these variables, the design comprised the run of the formulation with different combinations at triple points at the center. Equations in the form of polynomials provided these models with surface plots of response to evaluate the effect of linear and quadratic factors and variables. The quadratic model had the most specific and cumulative effect on the dependent variables among other models. We used PBS as the hydration medium [27].

Figure 2A–C, accordingly, illustrates the findings from the 3D models of clove oil nanomicelles on size of particles, PDI, as well as EE%. The ratio of PEG, methanol, along with clove oil was discovered to influence the size of the particles of clove oil nanomicelles. It was determined that the composition of clove oil elevated in conjunction with a decline in PEG and methanol as well as a rise in particle size. On the contrary, the size continued unchanged when the ratio of PEG to methanol remained the same as well. This result implies that the formulation's PEG, methanol, and clove oil ratios could be optimized to regulate particulate size.

Table 1 Optimization of nanomicelles with variables utilized in central composite design (CCRD)

Factors	Levels used		
	Low	Medium	High
Independent variables			
A-Clove oil%	25	35	45
B-PEG%	10	15	20
C-Methanol%	5	10	15
Dependent variables	Measurements used		
Y1-Particle size (nm)	Minimum		
Y2-PDI	Minimum		
Y3-Entrapment efficiency%	Maximum		

The ratio of PEG, methanol, and clove oil was determined to have a minor influence upon the PDI of clove oil nanomicelles. It was discovered that when the clove oil composition as well as PEG spiked the PDI slightly elevated as well. Additionally, it was found that the PDI rose as methanol and PEG levels rose. According to this finding, the formulation's PEG, methanol, and clove oil makeup could be optimized to increase the consistency of the distribution of particle sizes.

The ratio of PEG, methanol, as well as clove oil has been identified to impact the EE% of clove oil nanomicelles. It was determined that as PEG, clove oil, and methanol levels declined so did the EE%. Through a decline in methanol and a rise in PEG, the EE% declined. This result indicates the formulation's PEG, methanol, and clove oil composition can be optimized to increase the encapsulation effectiveness of clove oil. In general, the 3D graph findings offer views regarding the optimization of clove oil nanomicelles for topical atopic dermatitis treatment.

1.7 Measurement of vesicle diameter, polydispersity index, and zeta potential

Dynamic laser light scanning was used to assess the vesicle diameter, the polydispersity index, and the potential of zeta with the Malvern Nano Zetasizer ZS of the United Kingdom at 25 °C. The formulations underwent dispersion at 1 mg/mL of Milli-Q water. The index of refraction was 1.59, and the scatter angle was 90°. The testing was conducted three times for accuracy.

1.8 Efficiency of encapsulation of clove oil nanomicelles

The drug concentration within the micelles was evaluated, and unbound molecules of clove oil were isolated from the enclosed ones using a membrane filter of 0.45 µm. Following that, different aliquots of the formulated clove oil nanomicelles were lysed using methanol and sonicated in a water bath. The content of clove oil was then estimated at a wavelength of 260 nm using the ultraviolet–visible spectrophotometry (UV–Vis) system of Shimadzu, Japan. The calibration graph showed a linearity of $y=0.4916x+0.4209$ and $R^2=0.9764$ and had a range of concentration of 35 w/v that was used to compute the content of the drug. The encapsulation efficiency (EE) of CO nanomicelles was calculated with the formula:

$$\text{EE\%} = \frac{\text{Quantity of drug encapsulated}}{\text{Total quantity of drug}} \times 100$$

1.9 Studies pertaining to in vitro release of the drug

The dialysis sack diffusion technique was employed to study the liberated clove oil from the prepared micelles. The releasing media utilized in the experiment at pH 7.4 was simulated tear liquid consisting of de-ionized

water, and $CaCl_2{\cdot}2H_2O$ and NaCl in the range of 0.1–0.2% w/v, while the concentration of NaHCO3 was around 0.3–0.4% w/v. Activation of the bags for dialysis was performed before selectively putting 1 mL of the clove oil formulation and 1 mL of the conventional formulation within the gel and immersing it in 200 mL of methanol and a buffer of phosphate having a pH of 6.7. The experiment was carried out at an ambient temperature at a 400-rpm stirring rate. The nanomicelles and conventional formulations were observed through various tools like the Korsmeyer Peppas model, the Higuchi model, the first-order release model, and the zero-order release model. The amount of in vitro release of clove oil nanomicelles through the membrane was depicted as the area of diffusion by time [28].

1.10 Dermatokinetic analysis

Clove oil nanomicelles (2.5 mg/kg) were applied to the skin of rats and were analyzed using the Franz Diffusion Cell (FDC) as per the previous in vitro permeation research studies on the skin [29]. The equipment was utilized to determine the concentration and quantity of clove oil and its prepared formulation after 0, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min of skin application beginning with FDC. The residual formulation of the nanomicelles was cleared from the skin and washed with pH 7.2 saline. It was submerged for 90 s in relatively warm water at 60 °C. Clove oil was extracted using forceps to split the epidermal and dermal skin layers into tiny fragments and then soaking those pieces in 5 mL of methanol for 24 h. Upon separating these layers, the solution of methanol was filtered through a membrane, and the clove oil concentration was determined by HPLC. The analysis through HPLC was performed by preparing the stock solution of clove oil with a quantity of 1 mg/mL. It was dissolved in methanol to create multiple solutions of standard varying from 10 to 100 g/mL through dilution of stock solution. Furthermore, appropriate concentration of the extracted clove oil solution from the epidermis and dermis of the skin was taken for analysis and injected within the HPLC instrument. With the help of a 0.22 µm filter, the particulates were removed. We used a reverse-phase C18 column along with a mobile phase with ratio of methanol and water (70:30) for HPLC and set the temperature to 30 °C. The rate of flow was 1.0 mL/min to which we injected 20 µL of the prepared solutions of standard and sample within the HPLC system. The clove oil concentration for every cm^2 from the epidermal and dermal layers was measured throughout time, and the C-skin max, area under the curve 0–8 h, and T-skin max, Ke factors were calculated [30].

1.11 Confocal laser scanning microscopy

We employed confocal laser scanning microscopy (CLSM) to observe the clove oil nanomicelles along with the conventional drug. A549 cells were inoculated on 25-mm plates with glass bottom, and the dishes were then placed in the incubator for a day. Following that, cells were exposed to 2 mL of clove oil nanomicelles for treatment at timepoints of 30, 60, and 120 min at 37 °C. Cells were subsequently stained with four percent of paraformaldehyde at room temperature for 20 min after being rinsed multiple times using chilled PBS in order to eliminate liberated micelles. Finally, cold PBS was utilized to rinse the cells prior to being examined by CLSM [31].

1.12 Stability analysis

The tests for stability of the clove oil nanomicelles were evaluated for a month at ambient temperature. The stability of the formulation of clove oil nanomicelles was observed by preserving it for 90 days at a 60 ± 5% level of humidity and temperatures of 30 ± 3 °C and 40 ± 3 °C. With the help of the previously established technique, the size of particles, appearance, PDI, and EE were measured three times to establish repeatability [32].

1.13 DPPH scavenging behavior of nanomicelles

The procedure introduced by Williams et al. for 2, 2 diphenyl-1-picryl hydrazyl (DPPH) was utilized to examine the complete radical action of scavenging clove oil nanomicelles before and after encapsulation [33]. The amount of antioxidant electron donation makes the sample colorless from the color of violet at an ambient temperature. After dissolving the formulation in 3 mL of methanol, the resulting mixture was diluted in a DPPH sample consisting of a solution of methanol (0.3 mL). The process was performed in a dimly lit room for 60 min, and the mixture was stored there. The color alteration presented evidence of the antioxidant characteristics of the sample through hydrogen donation levels. With the help of a spectrophotometer at 517 nm, the mixtures were evaluated after the addition of a blank sample in methanol (3.3 mL) and the mixture (0.3 mL).

The formula used to assess the DPPH scavenging activity of clove oil nanomicelles:

$$\begin{aligned}&\text{DPPH scavenging activity (\%)}\\&= [(\text{Absorbance of ascorbic acid}\\&\quad -\text{Absorbance of clove oil nanomicelles})\\&\quad /\text{Absorbance of ascorbic acid}] \times 100\end{aligned}$$

1.14 Antibacterial activity

According to the discussed method, the analysis of antibacterial action was performed in conformity with

the Japanese Industrial Standards L 1902:2002 [34]. *Klebsiella pneumoniae* and *Staphylococcus aureus* were obtained from Merck and cultivated using peptone and other agents such as bacterial agar. Plates of Columbia agar containing five percent of sheep plasma and NaCl (0.9%) were combined, and microbes for analysis were grown for a day at ambient temperature in an aerobic environment. To evaluate the germ quantity, the samples that were incubated were removed and placed in a solution of NaCl (0.9%) with Tween 20 (0.2%). Different dilutions were put onto plates of Columbia agar and underwent incubation for a day at room temperature. Finally, an estimation of colonies was done, complete units of the colony were evaluated, and reductions in growth were observed. Antibacterial activity was observed at 4, 8, 12, 16, 20, 24, 28, and 32 h if the values were among < 0.5 (none) or 0.5 to 1 (little) or > 1 or ≤ 3 (high) from the logarithmic formula given below:

$$\begin{aligned}&\text{Log growth variation}_{(24\ \text{hours})}\\&= \text{Log colony forming units}_{(\text{negative control})}\\&\quad - \text{Log colony forming units' sample}_{(24\ \text{hours})}\end{aligned}$$

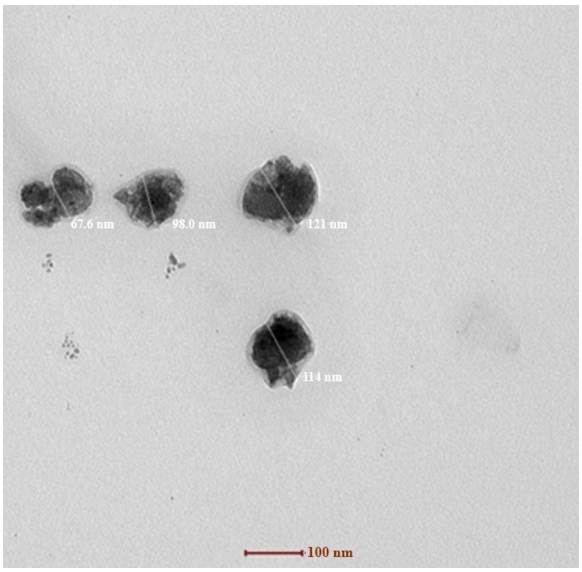


Fig. 1 Evaluation by TEM at a scale of 100 nm

1.15 Statistical analysis

The values of the findings were depicted in mean ± SD [standard deviation] after obtaining three values for each experiment. The statistical differences between the tests were evaluated by an unpaired t-test at a stable point of flux and dermatokinetic study of the formulation that was known beforehand. The level of significance utilized in this study was $p < 0.05$.

2 Results

The clove oil nanomicelles were observed to be in a darker shade against the lighter background under transmission electron microscopy. In Fig. 1, the spread

Table 2 Formulation runs for optimization of clove oil nanomicelles

Run	Factor 1 A: Clove oil (%)	Factor 2 B: PEG (%)	Factor 3 C: Methanol (%)	Response 1 Size (nm)	Response 2 PDI	Response 3 EE (%)
1	35	20	5	165.54	0.863	76.98
2	35	10	5	155.54	0.349	60.34
3	45	20	10	200.76	0.659	74.33
4	45	15	5	180.43	0.971	62.53
5	35	10	15	176.76	0.753	73.65
6	25	20	10	76.87	0.453	69.87
7	45	15	15	198.43	0.781	78.81
8	35	15	10	100.15	0.21	84.31
9	35	15	10	105.87	0.286	82.87
10	35	15	10	107.32	0.221	84.23
11	35	15	10	104.32	0.312	76.32
12	25	15	5	93.65	0.491	70.12
13	45	10	10	180.43	0.781	67.98
14	35	20	15	145.87	0.401	71.54
15	35	15	10	112.54	0.296	79.91
16	25	15	15	67.32	0.471	59.34
17	25	10	10	80.65	0.237	58.43

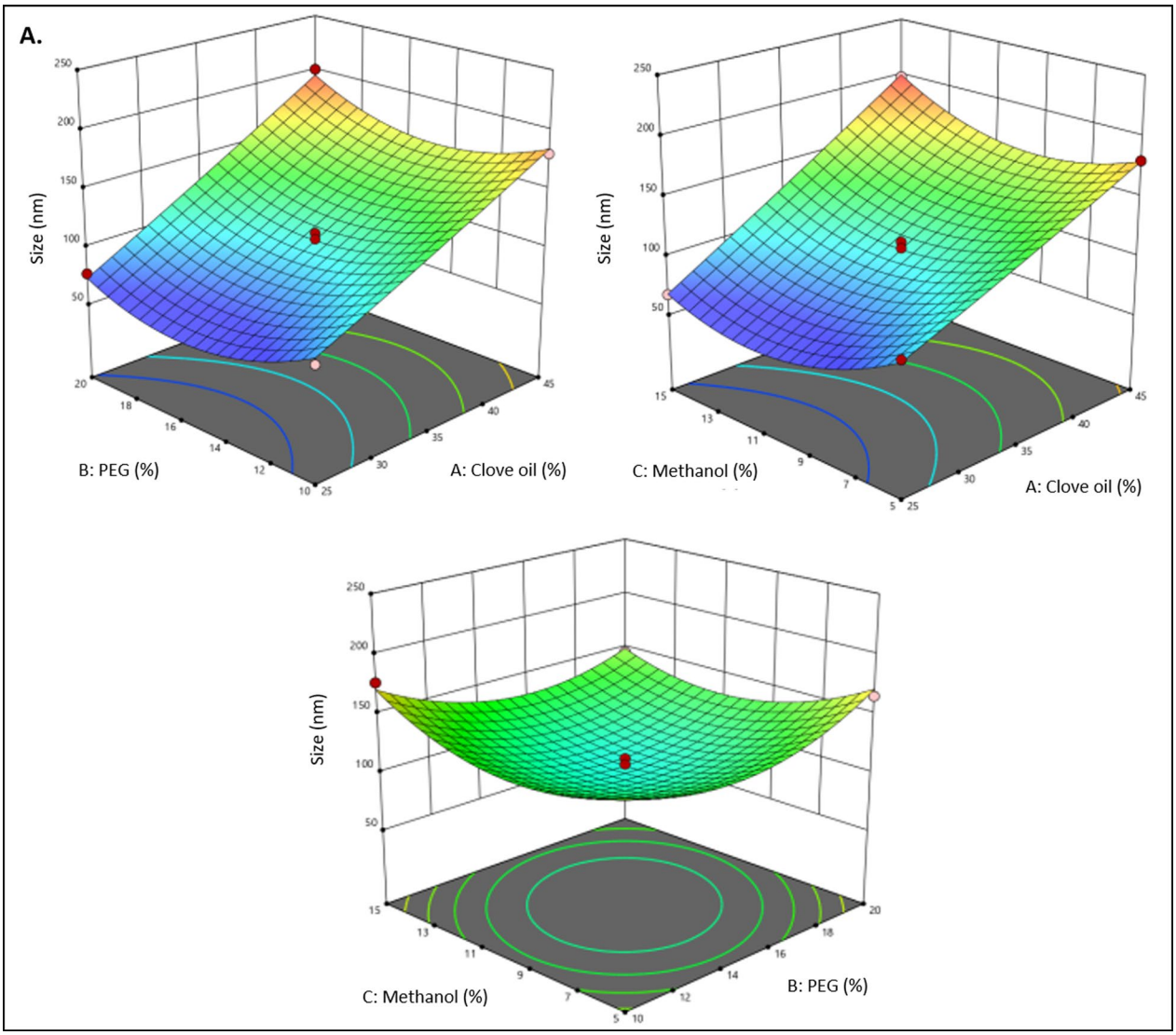


Fig. 2. 3D graphs of surface response depicting the action of clove oil nanomicelles on; **a** particle size, **b** PDI, **c** EE%

ability of the four-clove oil nanomicelles is visible, of which the sizes appear to be 67.6 nm, 98.0 nm, 121 nm, and 114 nm in width. The micelles were evaluated to be ≤ 200 nm in the nanosized range, as the mean size of the clove oil nanomicelles was determined by DLS method.

2.1 Central composite rotatable design analysis on nanomicelles

3-Dimensional surface response graphs provide a brief analysis of clove oil nanomicelles with regard to vesicle diameter (nm), polydispersity index (PDI) and encapsulation efficiency (EE%). The software performed 17 formulation runs as shown in Table 2. Figure 2A–C depicts the following graphs of the parameters:

Particle size (nm) $(Y1) = 106.04 + 55.19A - 0.5425B - 0.8475C + 6.03AB + 11.08AC - 10.22BC + 1.33A^2 + 27.30B^2 + 27.58C^2$

Polydispersity Index (PDI) $(Y2) = 0.2650 + 0.1925A + 0.0320B - 0.0335C - 0.0845AB - 0.0425AC - 0.2165BC + 0.1772A^2 + 0.0902B^2 + 0.2363C^2$

Entrapment efficiency (EE %) $(Y3) = 81.53 + 3.24A + 4.04B + 1.67C - 1.27AB + 6.77\mathrm{AC} - 4.69BC - 8.40A^2 - 5.47B^2 - 5.43C^2$

Figure 2A represents the 3D graphs of clove oil nanomicelles on particle size. The figure depicts that the size of particles increased with a decrease in PEG

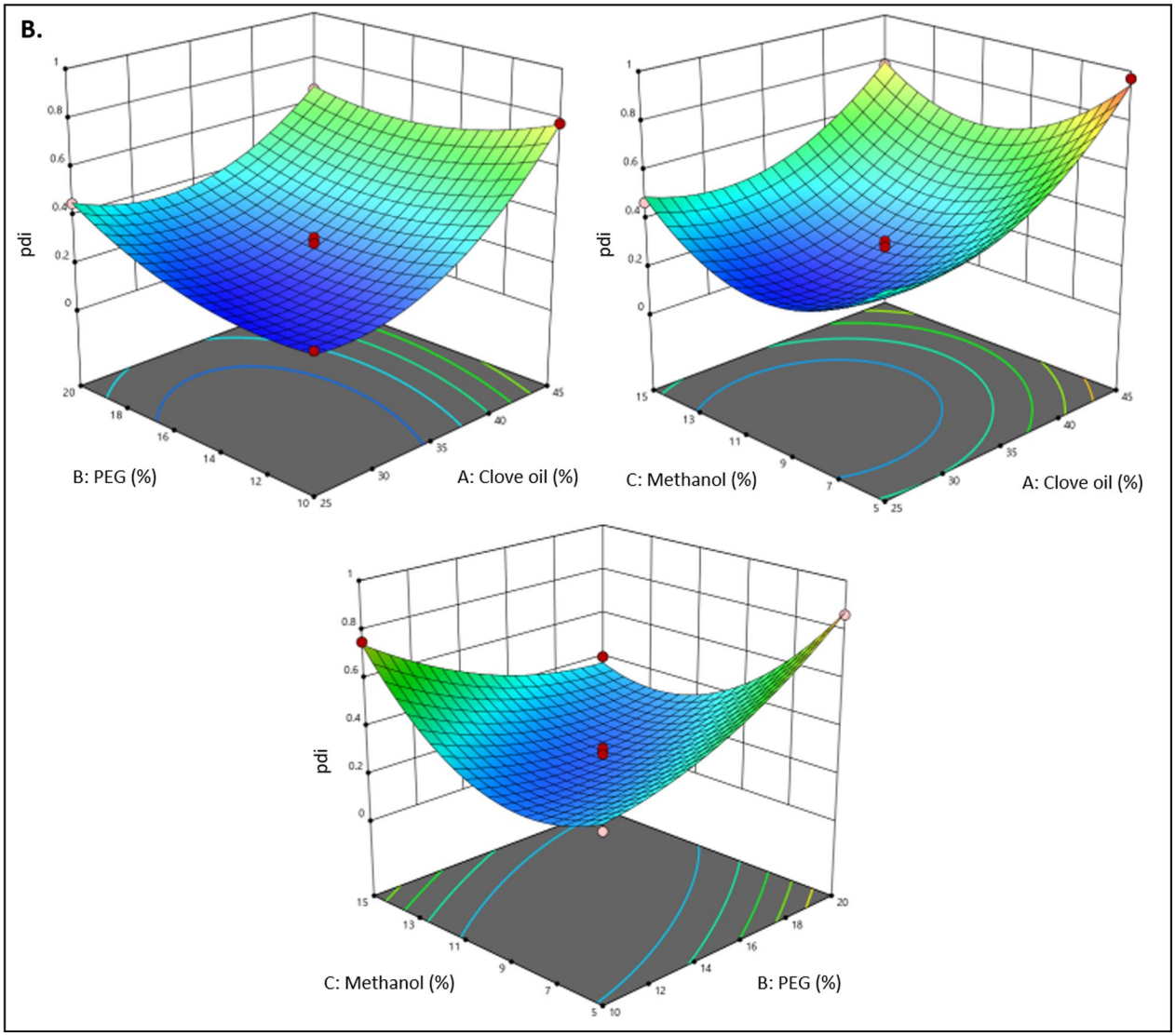


Fig. 2. continued

and methanol and an increase in clove oil composition while the size remained constant when PEG and methanol content did not change.

Figure 2B represents the 3D graphs of clove oil nanomicelles on PDI. The figure demonstrates that there was a marginal rise in the PDI in conjunction with an expansion of the clove oil composition using PEG. PDI was observed to increase with an increase in methanol and PEG content.

Figure 2C represents the 3D graphs of clove oil nanomicelles on EE%. It was shown that reducing concentrations of PEG, clove oil, and methanol led to increases in EE%. The EE% decreased with a decrease in methanol and an increase in PEG.

The size of nanomicelles had a mean peak area of 183.9 nm as depicted in Fig. 3 by the intensity with a polydispersity index (PI) of 0.2104 when observed under twenty-five-degree Celsius. The dispersant (water) was used with a refractive index value of 1.33, a viscosity value of 0.887 cP, and a dielectric constant of 78.5. The average size of zeta was 121.3 nm, while the potential of zeta and its peak were observed to be −15.31 mV. The conductivity evaluated was 0.03101 mS/cm and the zeta deviation was 6.707 mV with a quality factor of 2.432. The refractive index of the material, polystyrene latex, was 1.59 with absorption of 0.01 nm.

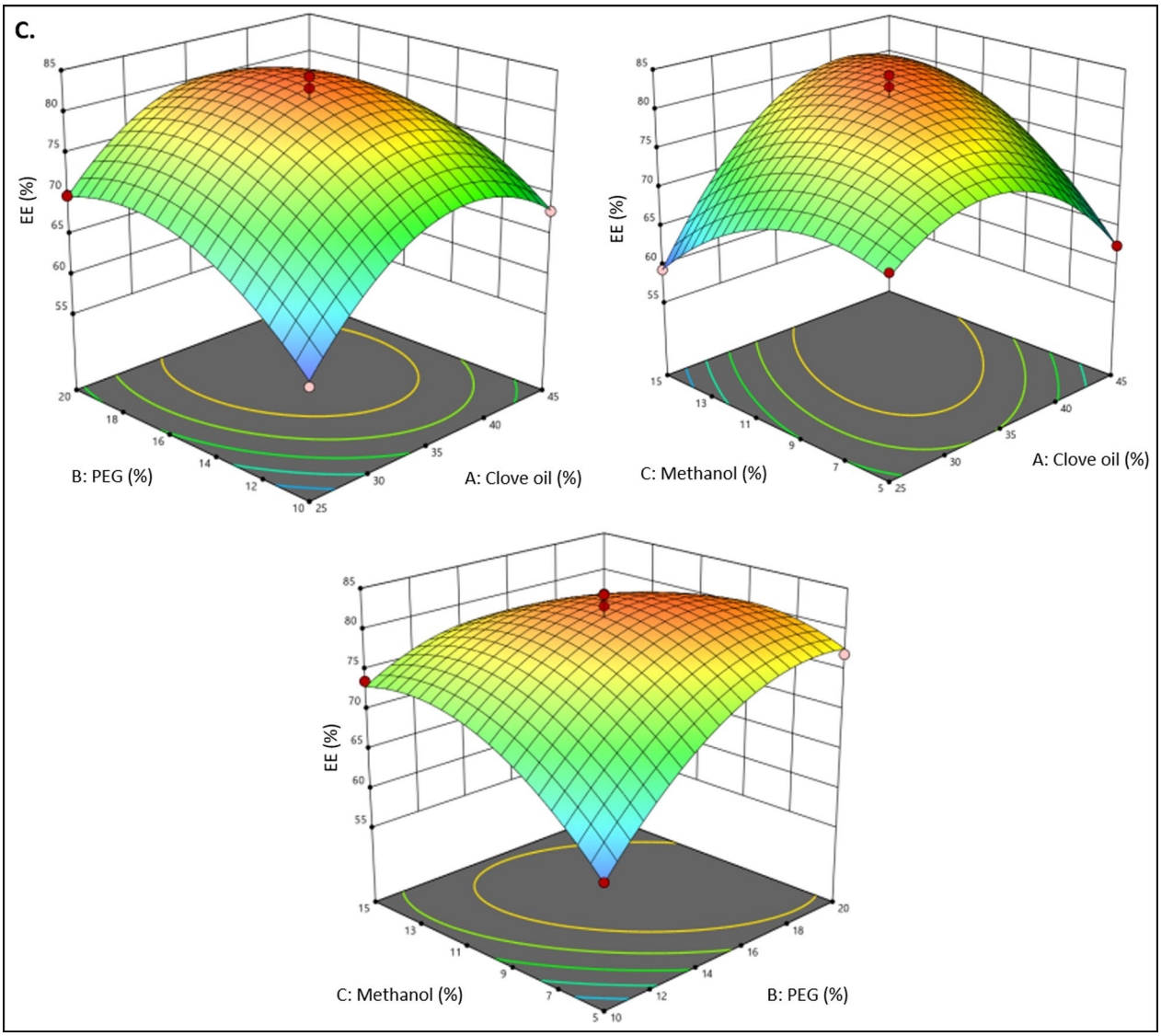


Fig. 2. continued

2.2 In vitro release analysis

The conventional formulation was compared against the clove oil nanomicelles to evaluate the release percentage of the prepared formulation. Figure 4 provides the findings of the in-vitro release analysis. The Korsmeyer Peppas model provided a linear, gradual rise as observed in Fig. 4a, with values of $y = 0.4916x + 0.4209$ and $R^2 = 0.9764$. Similarly, the analysis performed with the Higuchi model presented values of $y = 0.0221x + 0.0602$ and an R^2 value of 0.9495 as shown in Fig. 4b. The zero-order release model had values of $y = 0.0005x + 0.2636$ and an R^2 value of 0.8021 as presented in Fig. 4d. On the other hand, the first-order release model produced a decline when evaluated with values of $y = 0.0005x + 1.8889$ and $R^2 = 0.9428$ as depicted in Fig. 4c. Clove oil nanomicelles had better findings when compared with the conventional formulations, and the Korsmeyer Peppas model produced the most significant linear results. We observed 45.07% average drug release by clove oil nanomicelles while 21.16% was the average drug release by conventional drug.

2.3 Dermatokinetic analysis

The comparison between the conventional and clove oil nanomicelles formulations was evaluated by observing the amount of formulation present on the skin of rats after application. Both formulations were compared at

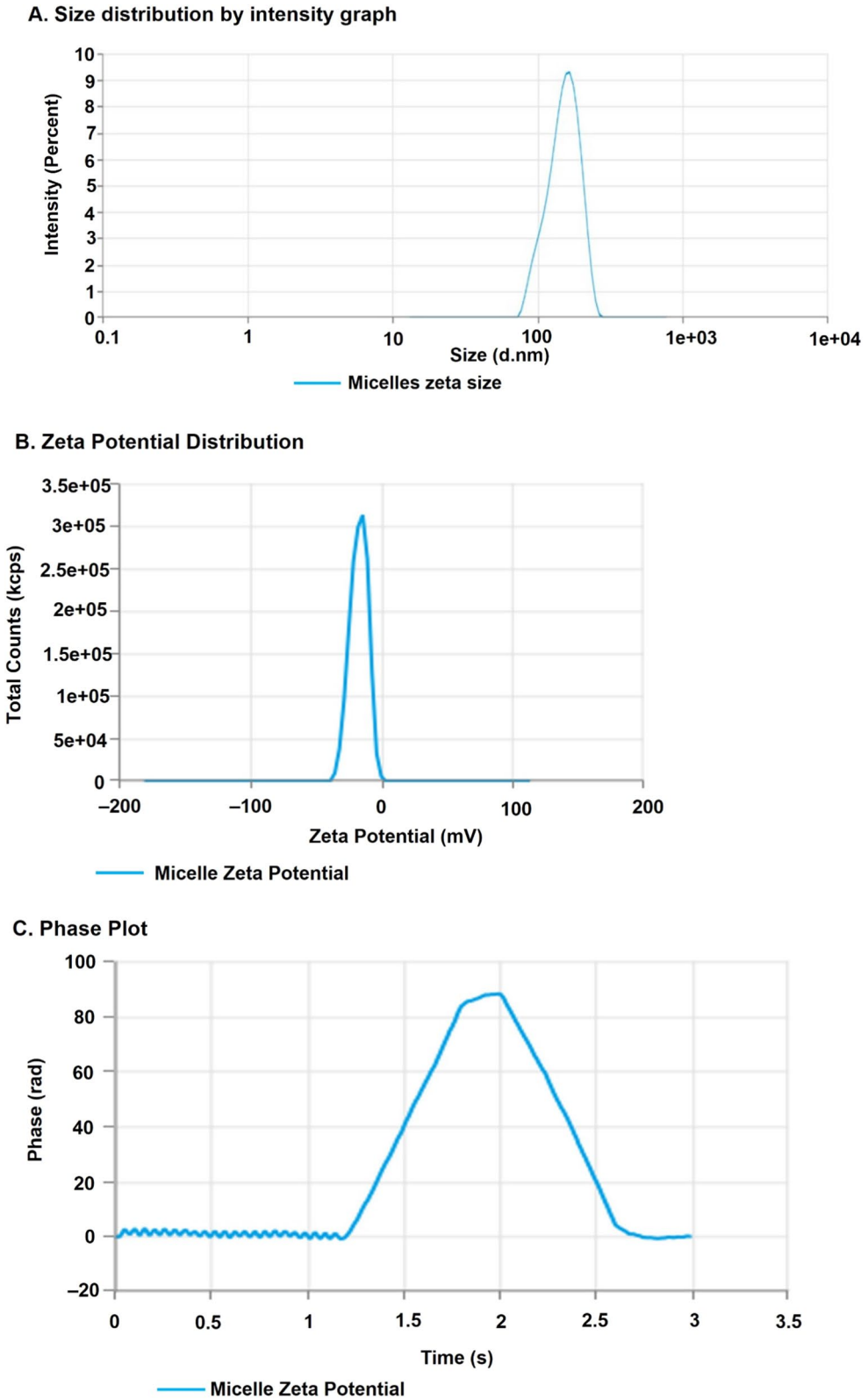


Fig. 3 Analysis of **a** size distribution by intensity graph, **b** nanomicelles zeta potential by total counts, **c** nanomicelles zeta potential by phase

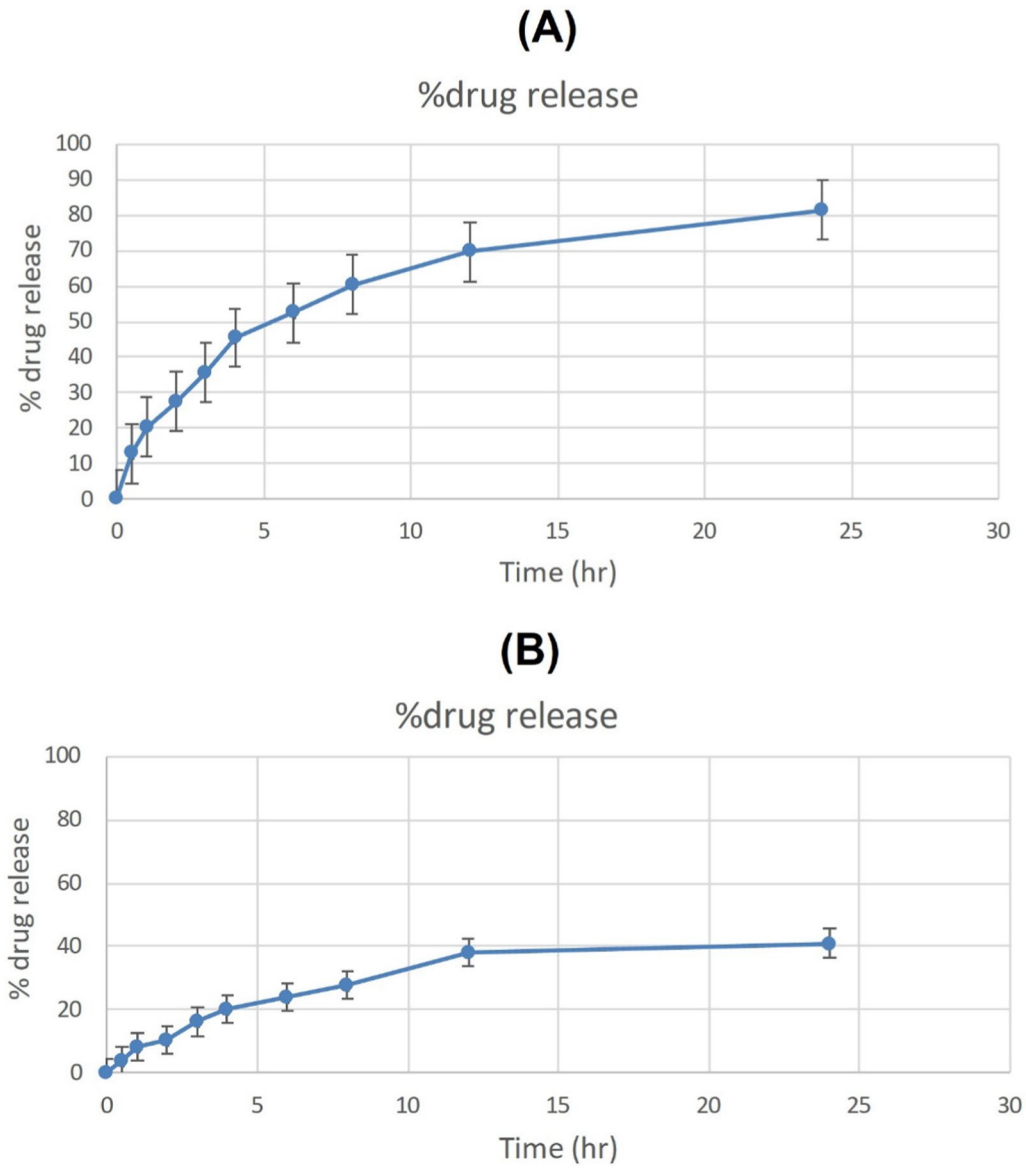


Fig. 4 In vitro release analysis **a** clove oil nanomicelles, **b** conventional formulation

various periods on the dermis and epidermis skin layers. The observed findings were estimated through analysis of variance (ANOVA). As observed in Fig. 5, clove oil nanomicelles (2.5 mg/kg) produced a higher concentration of 148.68 w/v at 1.5 h, while the conventional formulation produced 55.287 µg/mL on the skin epidermis. Similarly, clove oil nanomicelles produced a higher concentration of 125.84 µg/mL at 2 h, while the conventional formulation produced 68.263 µg/mL on the skin's dermis. The area under the curve (AUC) of clove oil nanomicelles was considerably higher in comparison with the conventional drug, while the values of Ke were lower for the nanomicelles. Table 3 presents the dermatokinetic study chart.

The confocal laser scanning microscopy (CLSM) scans can be observed in the rhodamine B-loaded formulation and rhodamine B-loaded suspension. In Fig. 6, we can observe that Fig. 6A has a lighter complex in comparison with Fig. 6B. The findings reveal enhanced penetration of the nanomicelles formulation along with its nature of elasticity when administered as a topical agent.

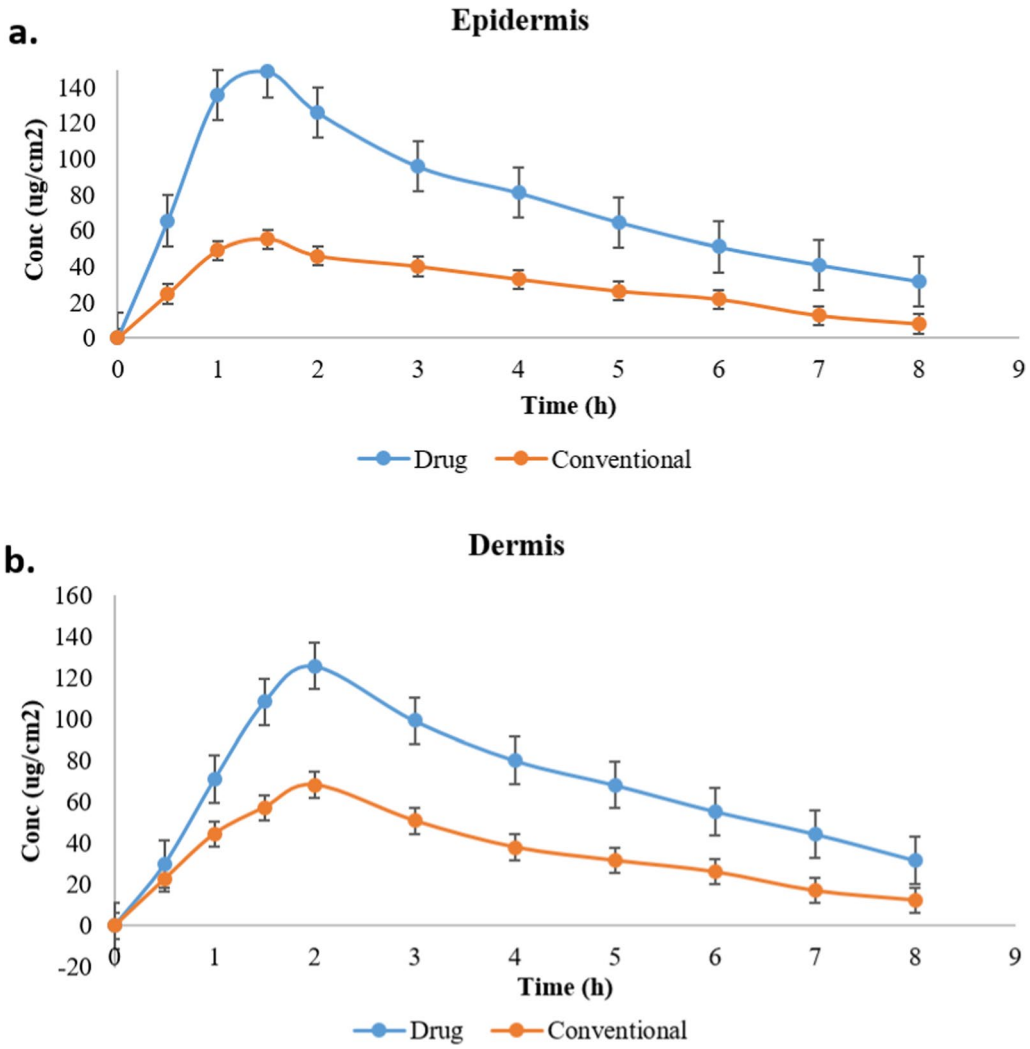


Fig. 5 Dermatokinetic analysis of formulations on; **a** epidermis, **b** dermis

Table 3 Dermatokinetic study chart

Dermatokinetic study	Tmax (h)	Cmax (mg/mL)	AUC (mg/mL)	Ke
Formulation on epidermis				
Clove oil nanomicelles	1.5	148.68	618.37	0.169
Conventional drug	1.5	55.287	236.27	0.1983
Formulation of dermis				
Clove oil nanomicelles	1.5	127.3	418.26	0.142
Conventional drug	1.5	69.2	263.5	0.171

2.4 DPPH scavenging behavior and antibacterial activity of clove oil nanomicelles

DPPH reduction characteristics through antioxidants were evaluated when the absorbing wavelength of 517 nm was lowered and an alteration from a violet color to colorless nature was found. The antioxidant effect of clove oil nanomicelles was somewhat decreased in comparison with conventional ascorbic acid. The clove oil nanomicelles' effects as antioxidants were thus proven by these findings since they did not alter the formulation due to the entrapment of the drug.

Formulation $IC_{50} = 61.32 \pm 0.98$

Clove oil $IC_{50} = 73.56 \pm 1.63$

Ascorbic acid $IC_{50} = 54.51 \pm 0.79$

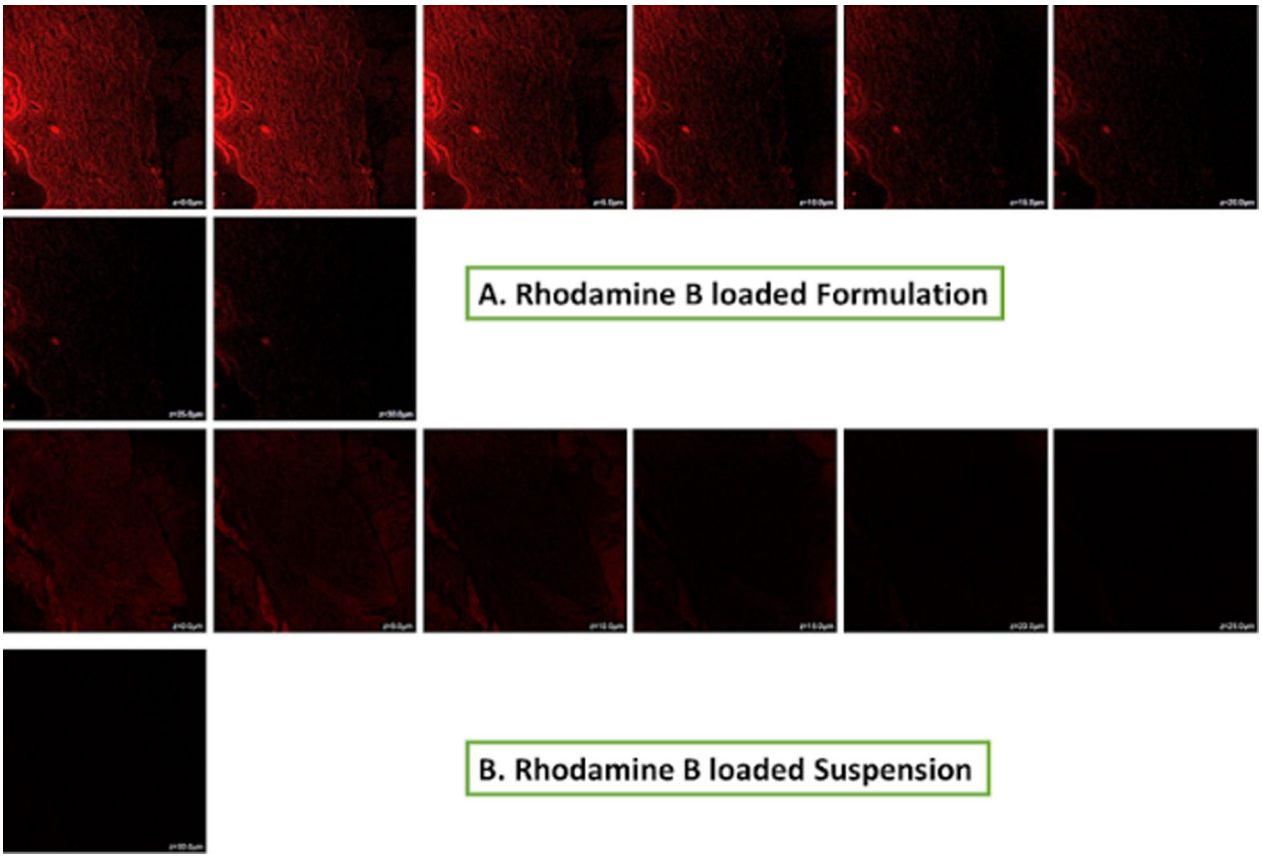


Fig. 6 CLSM scans from a perpendicular cross section of rats' optimum skin surface

On the other hand, high activity against both bacteria were found, as clove oil nanomicelles produced log variations > 3, while the conventional drug remained < 1. The maximum inhibition of bacterial growth was observed after a day of incubation time. Figure 7a depicts the inhibition observed in the bacterial culture of *Staphylococcus aureus* while Fig. 7b depicts the inhibition of *Klebsiella pneumoniae*.

3 Discussion

The present study focused on the characterization and optimization of clove oil-loaded nanomicelles for the topical management of atopic dermatitis. Since atopic dermatitis is a chronic inflammatory skin condition, it often requires effective and targeted drug delivery systems to alleviate its symptoms. Nanomicelles have gained attention as potential carriers for topical drug delivery due to their small size, improved bioactivity, sustained release capabilities, and enhanced penetration and retention at administration sites. Nanoparticles aid in the improvement of medications at the pharmacokinetic and pharmacodynamic levels [33, 34].

The surfactant polyethylene glycol and Pluronic F127 was utilized for testing the solubility of clove oil during the initial stages of the study. It also helped in determining the how feasible the clove oil nanomicelles would be during the preparation. Furthermore, the method of thin film hydration was performed and the nanomicelles were developed with a consistent size along with its structure and provided a suitable formulation for topical application. The TEM images revealed that the nanomicelles possessed a uniform spherical shape with a small size range, indicating their potential for efficient drug delivery to the skin. The homogeneity of size distribution, as indicated by the polydispersity index, further supported the suitability of the nanomicelles as drug carriers [35].

Physical and covalent bonds among the polymers and the medications could help enclose the molecules of the drug within the matrices of the polymer. Encapsulating these medications for delivery can be incorporated up to the desired capacity. The entrapment efficiency of the clove oil within the nanomicelles was a critical parameter assessed in the study. High entrapment efficiency suggests that a significant amount of clove oil was successfully encapsulated within the nanomicelles, ensuring its

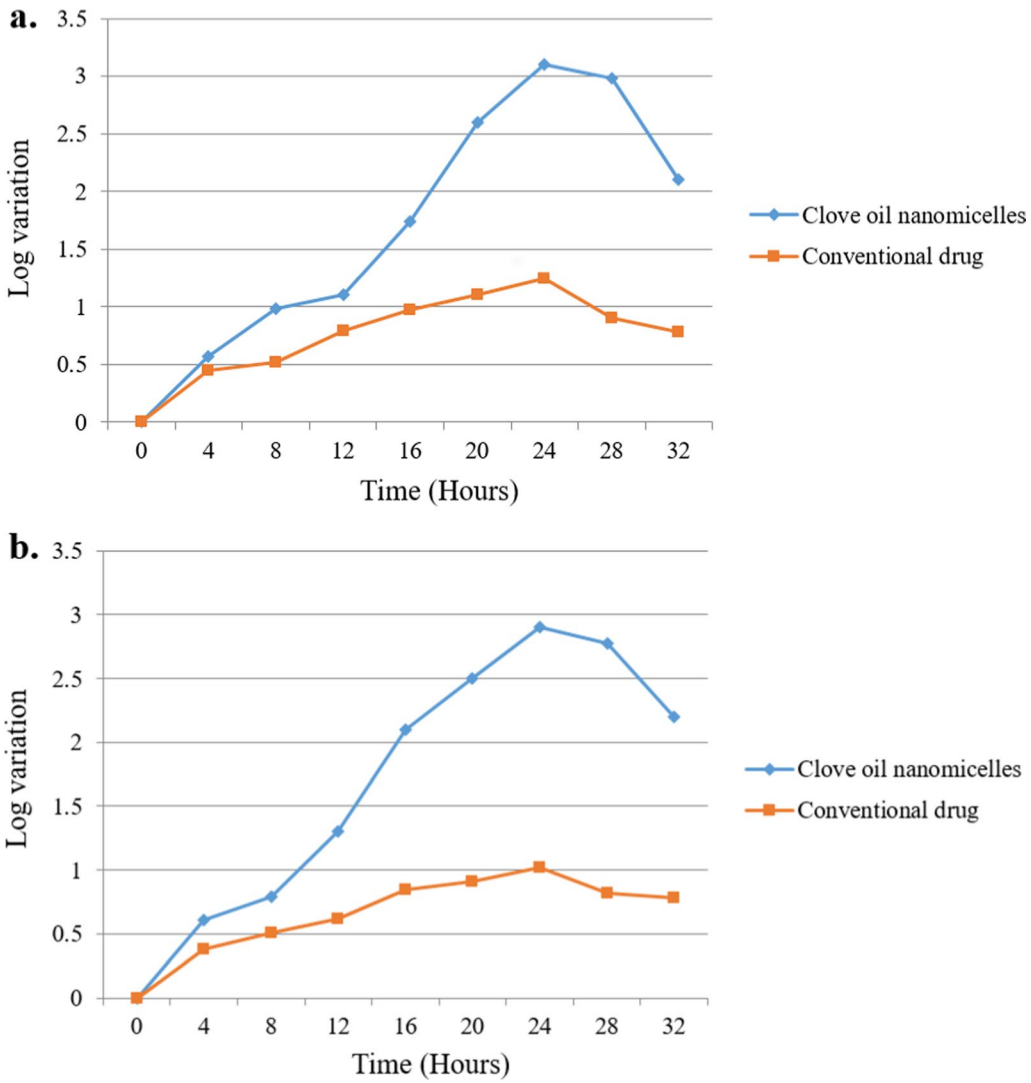


Fig. 7 Bacterial growth inhibition; **a** *Staphylococcus aureus,* **b** *Klebsiella pneumoniae*

protection and sustained release at the application site. This is a crucial factor in achieving a prolonged and controlled drug release, which is essential for managing the symptoms of atopic dermatitis effectively [36]. The benefits of polymeric nanomicelles in comparison with other nanocarriers are improved bioactivity, sustained release of drugs, lower toxicity, and enhanced penetration and retention of drugs at administration sites because of their tiny range of sizes [37]. Clove oil has strong antioxidant and antibacterial properties, although it is tough to maintain bacterial inhibition for a longer duration since the oil tends to be unstable or volatile. However, by encapsulating them into nanomicelles, their stability and performance can be enhanced. The stabilization of hydrophobic compounds in nanomicelles is due to the reduction in their surface area exposed to the aqueous environment, which minimizes their interactions with water molecules and prevents their aggregation. In addition, the surfactant molecules in nanomicelles can form a protective layer around the encapsulated compounds, shielding them from degradation by enzymes or other reactive molecules [38, 39]. As observed in the present study, clove oil nanomicelles may provide a greater advantage in the treatment of atopic dermatitis, as the dermatokinetic studies indicated their higher performance in comparison with the standard formulations utilized for treatment, and also, with their surprising benefits and characteristics, they can improve the symptoms caused by bacterial growth and provide considerable antioxidant activity.

The strength of this study includes the novelty as clove oil nanomicelles were not tested for atopic dermatitis

previously along with comprehensive characterization and optimization for topical application the potential for reduced systemic side effects compared to conventional oral or parenteral routes. While the study provides valuable insights into the formulation's physical properties, the limitations are that it does not account for the complex biological environment, cytotoxicity and potential interactions that may occur in vivo.

4 Conclusions

Atopic dermatitis has become a common disorder observed in many individuals. Nanocarriers are novel pharmaceutical components utilized in the newer era of medicines. The topical nanomicelles increase the influence of drugs and their penetration within the epidermal layer of the skin. Clove oil nanomicelles have produced efficient results in dermatokinetic evaluation, antioxidant, antibacterial, and in vitro release studies in comparison with the conventional drug for treating such disorders and can be studied further for significant incorporation as a pharmaceutical medicine. Future targeted and personalized treatments for atopic dermatitis may be made possible by nanomicelles-based delivery systems that incorporate various therapeutic approaches, including anti-inflammatory drugs, immunomodulators, and gene delivery, together into single formulation with increased effectiveness and fewer side effects.

Abbreviations

AD	Atopic dermatitis
TEM	Transmission electron microscopy
CCRD	Central composite design
UV–Vis	Ultraviolet–visible spectrophotometry
EE	Encapsulation efficiency
HPLC	High-performance liquid chromatography
FDC	Franz diffusion cell
DPPH	α, α-Diphenyl-β-picrylhydrazyl
SD	Standard deviation
PDI	Polydispersity index
ANOVA	Analysis of variance
AUC	Area under the curve
CLSM	Confocal laser scanning microscopy

Acknowledgments
The authors would like to thank the Deanship of Scientific Research at Shaqra University for supporting this work.

Author contributions
Conceptualization was contributed by GM, FA, NSA, and ASB; methodology was contributed by Munira MA, RAA, and Mohammed MM; software was contributed by RMA, and GM; validation was contributed by AFEK and FA; formal analysis was contributed by F.R.K., and ASB; resources were contributed by GM, and MSA; data curation was contributed by AFEK; writing—original draft preparation, was contributed by Munira MA, and RMA; writing—review and editing was contributed by GM, and MSA; supervision was contributed by GM, NSA, and MSA; project administration was contributed by MSA. All authors have read and agreed to the published version of the manuscript.

Funding
The authors did not receive any funding.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Institutional Ethics Committee of the faculty of Veterinary Medicine, University of Sadat City, under the approval number VUSC-020-1-23.

Consent for publication
Not applicable.

Competing interests
All of the authors have declared that they do not have any competing interests.

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Received: 9 August 2023 Accepted: 8 October 2023
Published online: 16 October 2023

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