RESEARCH PAPER

Development and Characterization of Transfersomal Gel Containing Caesalpinia sappan L. Extract for Enhanced Topical Antibacterial Delivery

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ABSTRACT
Transfersomes are ultra-deformable lipid vesicles that have shown great potential in enhancing transdermal drug delivery. Caesalpinia sappan L. is a medicinal plant rich in brazilin and phenolic compounds with proven antibacterial activity. Despite the potential, its topical efficacy is limited by poor skin penetration. Therefore, this study aims to develop a transfersomal gel containing C. sappan extract, evaluate the effect of Tween 80 concentration on vesicle characteristics, and assess its antibacterial activity against Staphylococcus aureus. Transfersomes were prepared using the thin-film hydration method with varying Tween 80 concentrations and characterized for particle size and pH. The optimized transfersomal formulation was then incorporated into a carbopol-based gel and evaluated for physicochemical properties, stability, and antibacterial activity using the agar well diffusion method. The results showed that increasing Tween 80 concentration significantly reduced vesicle size ($p < 0.05$), with the smallest particles obtained at the highest surfactant concentration. Transfersomal gel demonstrated acceptable physicochemical characteristics and exhibited significant antibacterial activity against Staphylococcus aureus compared to the negative control ($p < 0.05$). This study indicates that transfersomal gel is a promising delivery system to enhance topical antibacterial effectiveness of C. sappan extract.
Keywords: Transfersome, Caesalpinia sappan, antibacterial gel, drug delivery system, Tween 80
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INTRODUCTION

The stratum corneum acts as the primary barrier limiting the effectiveness of topical drug delivery, particularly for hydrophilic and high-molecular-weight compounds. Consequently, conventional topical formulations often fail to deliver adequate drug concentrations into deeper skin layers, resulting in reduced therapeutic efficacy. To overcome this limitation, advanced vesicular drug delivery systems have been developed, among which transfersomes have gained significant attention.

Transfersomes are ultra-deformable lipid vesicles composed of phospholipids and edge activators that enable the components penetrate the skin by adapting their shape in response to hydration gradients. Due to their high elasticity, transfersomes can traverse narrow intercellular spaces of the stratum corneum, leading to enhanced drug permeation and sustained release profiles[1,2]. Several studies have reported the successful application of these vesicles for topical and transdermal delivery of anti-inflammatory, antimicrobial, and cosmetic agents[3,4].

Transfersomes have been widely investigated as ultra-deformable vesicular carriers capable of enhancing drug penetration through the stratum corneum. Their ability to adapt to the skin's microenvironment allows for improved drug deposition at the target site with reduced systemic exposure. Recent studies have highlighted the potential of transfersomes as a promising approach for topical drug delivery, particularly for drugs with limited skin permeability when formulated conventionally[10].

Several transferosomal gel formulations have been successfully developed for topical applications[11]. reported that a mometasone furoate-loaded transferosomal gel exhibited improved physicochemical characteristics and enhanced in vitro performance compared to non-transferosomal formulations. Similarly, demonstrated that lamivudine-loaded transferosomal gel showed suitable vesicle size, stability, and skin applicability, supporting the versatility of transfersomal systems for topical use[12]. More recently, developed an acyclovir-loaded transferosomal gel and reported enhanced topical delivery

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performance, emphasizing the importance of formulation optimization in achieving effective drug delivery[13].

According to previous studies, Caesalpinia sappan L. is a traditional medicinal plant widely used in Southeast Asia. The heartwood of this plant contains brazilin, flavonoids, and phenolic compounds that exhibit antibacterial, anti-inflammatory, and antioxidant activities[5]. Several studies have demonstrated that *C. sappan* extract is effective against Gram-positive bacteria, particularly *Staphylococcus aureus*. However, the clinical application of *C. sappan* extract in topical dosage forms remains limited due to poor skin penetration and instability of conventional formulations.

Recent advances in nanotechnology-based drug delivery systems have shown the potential of transfersomal gels as effective carriers for enhancing dermal drug penetration and improving therapeutic outcomes[6]. Despite extensive investigation on transfersomal systems, only a few studies have focused on incorporating *C. sappan* extract into a transfersomal gel and systematically evaluating the effect of surfactant concentration on vesicle characteristics and antibacterial performance. Therefore, this study aims to develop a transfersomal gel containing *C. sappan* extract, investigate the influence of Tween 80 concentration on vesicle size and physicochemical properties, and evaluate its antibacterial activity against *Staphylococcus aureus*. The results are expected to contribute to the development of effective plant-based topical drug delivery systems using transfersomal technology.

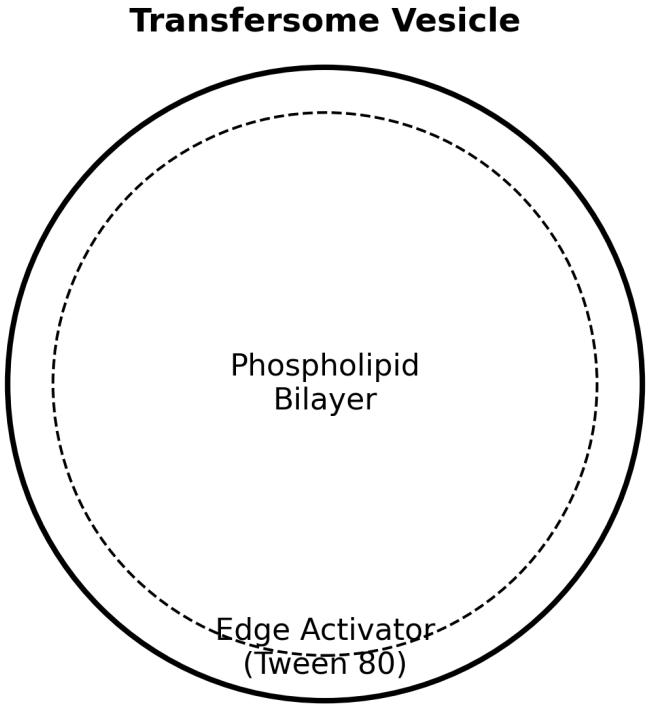

Figure 1: Schematic representation of transfersomes composed of phospholipid bilayers and edge activator (Tween 80), illustrating their ultra-deformable structure.

MATERIALS AND METHODS

Materials

C. sappan heartwood was obtained from Central Java, Indonesia. Soy lecithin, Tween 80, carbopol 940, and other analytical-grade reagents were used in this study. Meanwhile, *Staphylococcus aureus* was obtained from a certified microbiology laboratory.

Preparation of *C. sappan* Extract

The powdered heartwood was extracted by maceration using 96% ethanol, which was concentrated using a rotary evaporator and subjected to phytochemical screening.

Preparation of Transfersomes

Transfersomes were prepared by applying the thin-film hydration method. Soy lecithin and Tween 80 at different concentrations were dissolved in chloroform and evaporated to form a thin lipid film. This was hydrated with phosphate buffer saline (pH 7.4) containing *C. sappan* extract and sonicated to obtain nanosized vesicles.

Table 1: Composition of *C. sappan* Extract-Loaded Transfersomes.

Formulation	Extract (mg)	Soy Lecithin (mg)	Tween 80 (mg)	PBS pH 7.4 (mL)
F1	243	1215.0	122.7	ad 60
F2	243	1215.0	183.2	ad 60
F3	243	1215.0	276.8	ad 60

Characterization of Transfersomes

Particle size and polydispersity index (PDI) were determined using a particle size analyzer. pH of transfersomal dispersion was also measured.

Preparation of Transfersomal Gel

The optimized transfersomal formulation was incorporated into a carbopol-based gel, which was evaluated for organoleptic properties, pH, viscosity, spreadability, and adhesion.

Table 2: Composition of Transfersomal Gel

Component	Concentration (% w/w)	Function
Transfersomal suspension	25.0	Active ingredient
Carbopol 940	1.5	Gelling agent
Glycerin	7.0	Humectant
Propylene glycol	5.0	Penetration enhancer
Methyl paraben	0.15	Preservative
Purified water	ad 100	Vehicle

Antibacterial Activity

Antibacterial activity against *Staphylococcus aureus* was evaluated using the agar well diffusion method, and clindamycin gel was used as the positive control.

Statistical Analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) or non-parametric tests as appropriate, with significance set at $p < 0.05$.

RESULTS

Plant Authentication

Plant authentication of *C. sappan* was carried out at the Functional Implementation Unit (Unit Pelaksana Fungsional, UPF) Hortus Medicus, Tawangmangu, Central Java, Indonesia. The plant material was obtained from Blora Regency, Central Java. Authentication was performed to confirm the botanical identity of the sample, prevent adulteration, and ensure conformity of morphological characteristics with authoritative taxonomic references. Furthermore, the specimen was identified as *C. sappan*, which belonged to the family **Fabaceae**. The authentication certificate was provided in **Supplementary Material (Appendix 1).**

Preparation of *Caesalpinia sappan* L. Simplisia

Heartwood of *C. sappan* collected from Blora Regency was manually shaved to obtain thin wood chips. Subsequently, the material was dried under direct sunlight for 4 days to reduce moisture content and inhibit the growth of undesirable microorganisms. The dried material was ground using a blender and sieved through a **40-mesh** sieve to obtain a uniform powdered simplisia.

The loss on drying of the powdered simplisia was determined and found to be **0.667%,** which complied with the requirement stated in **Indonesian Herbal Pharmacopoeia, 2nd Edition (2017).** This specified that the acceptable moisture loss for *C. sappan* simplisia must not exceed **5%.**

Extraction of *Caesalpinia sappan* L.

A total of **500 g** powdered *C. sappan* simplisia was subjected to maceration using **96% ethanol** with a material-to-solvent ratio of **1:10** for 3 days at room temperature. Subsequently, remaceration was performed using a **1:5** ratio to maximize extraction efficiency. The combined filtrates were concentrated under reduced pressure to obtain a viscous ethanolic extract.

The extraction process yielded a percentage of **10.658%,** which was consistent with previous reports using similar extraction methods and solvents[7]. This value met the minimum requirement specified in **Indonesian Herbal Pharmacopoeia, 2nd Edition (2017),** stating that the acceptable rendement for concentrated *C. sappan* extract must not be less than **8.1%.**

Phytochemical Screening

Preliminary phytochemical screening was performed to identify the presence of secondary metabolites in the concentrated ethanolic extract of *C. sappan* using standard qualitative methods. The results were summarized in **Table 3.**

Table 3: Phytochemical screening of *C. sappan* extract

Phytochemical Group	Result	Observation
Essential oils	+	Carmine red coloration
Terpenoids	+	Purple to orange coloration
Alkaloids	+	Formation of precipitate
Flavonoids	+	Red coloration
Phenolics	+	Blue, green, purple, or reddish coloration
Saponins	–	No stable foam formation
Tannins	+	Greenish-black coloration

Phytochemical screening confirmed the presence of phenolics, flavonoids, tannins, and alkaloids, which were known to contribute to antibacterial activity. These results were consistent with previous studies reporting similar phytochemical profiles for *C. sappan* extracts and support its potential use as a natural antibacterial agent.

Characterization of *Caesalpinia sappan* Extract-Loaded Transfersomes

Transfersomes containing *C. sappan* extract were successfully prepared by applying the thin-film hydration method with varying concentrations of Tween 80 as the edge activator. In this study, 3 formulations (F1–F3) were obtained as homogeneous dispersions without visible precipitation or phase separation.

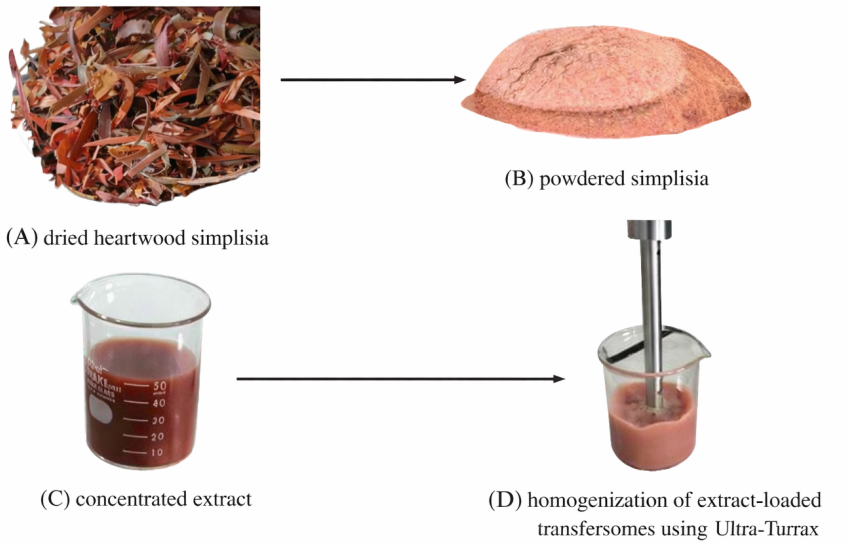

Figure 2: Visual documentation of *C. sappan* extract preparation and transfersome formation: (A) dried heartwood simplisia, (B) powdered simplisia, (C) concentrated extract, and (D) homogenization of extract-loaded transfersomes using Ultra-Turrax.

Particle size analysis showed that all formulations were in the nanometer range. As presented in **Table 4** and **Figure 4**, the mean particle size of transfersomes decreased with increasing Tween 80 concentration. F1 exhibited the largest mean particle size (202.97 ± 7.90 nm), followed by F2 (140.07 ± 4.19 nm), while the smallest particle size was observed in F3 (92.67 ± 4.58 nm). Statistical analysis using one-way ANOVA showed a significant difference in particle size among the formulations ($p < 0.05$).

Polydispersity index values for all formulations were below 0.5, indicating a relatively homogeneous particle size distribution. These results suggested that the prepared transfersomes possessed acceptable uniformity for topical delivery applications.

Table 4: Physicochemical Characteristics of Transfersomal Formulations

Formulation	Particle Size (nm)	Polydispersity Index	pH
F1	202.97 ± 7.90	< 0.5	6.4 ± 0.00
F2	140.07 ± 4.19	< 0.5	6.6 ± 0.00
F3	92.67 ± 4.58	< 0.5	6.8 ± 0.00

The effect of Tween 80 concentration on particle size was presented in Figure 2.

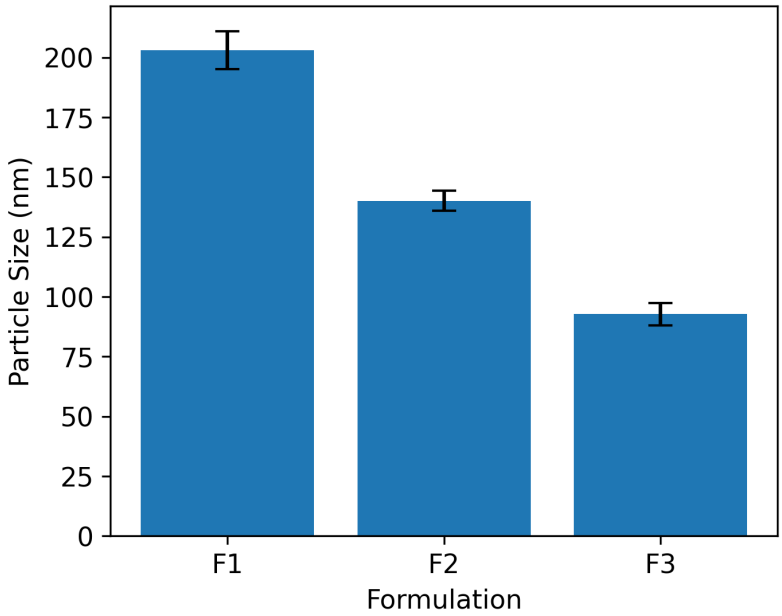

Figure 4: Effect of Tween 80 concentration on particle size of *C. sappan* extract-loaded transfersomes (mean ± SD, n = 3).

pH Evaluation of Transfersomal Formulations

pH values of transfersomal dispersions were measured to ensure compatibility with topical application. As shown in **Figure 5**, pH values of all formulations ranged from 6.4 to 6.8. An increasing trend in pH was observed with higher Tween 80 concentrations. All formulations remained in the acceptable pH range for skin application, showing their suitability for further formulation into a gel dosage form.

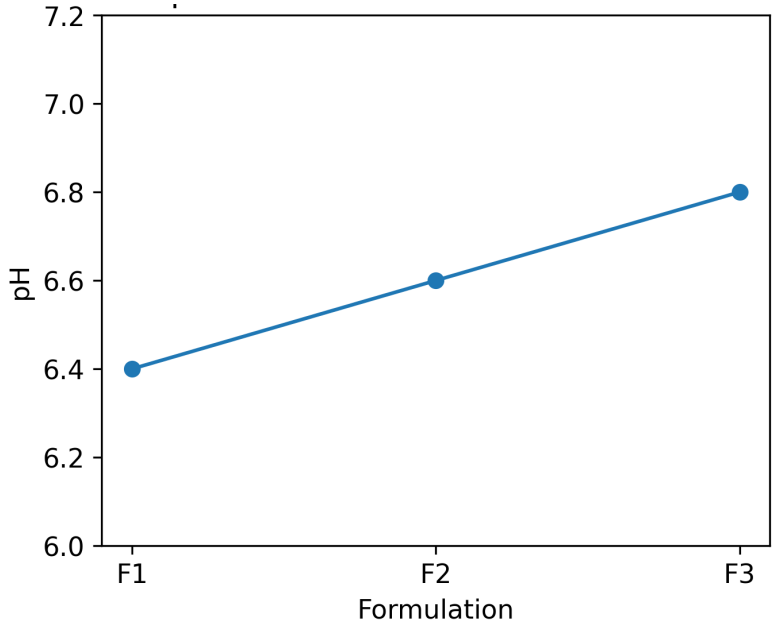

Figure 5: pH values of transfersomal formulations with different Tween 80 concentrations.

Physicochemical Properties of Transfersomal Gel

The optimized transfersomal formulation was incorporated into a carbopol-based gel system. Furthermore, the resulting gel exhibited a smooth texture, uniform appearance, and characteristic color of *C. sappan* extract. No coarse particles or phase separation were observed during visual inspection.

Physicochemical evaluation demonstrated that transfersomal gel possessed acceptable properties for topical use. pH of the gel was in the skin-compatible range, while viscosity, spreadability, and adhesion time met the standard requirements for topical gel formulations. These results indicated that transfersomal suspension could be successfully transformed into a stable gel dosage form.

Stability Study of Transfersomal Gel

Physical stability of transfersomal gel was evaluated using the freeze–thaw cycling method. After 6 cycles, changes in physicochemical parameters were recorded. As shown in **Figure 6**, slight variations were observed in pH, viscosity, spreadability, and adhesion time following the stability test.

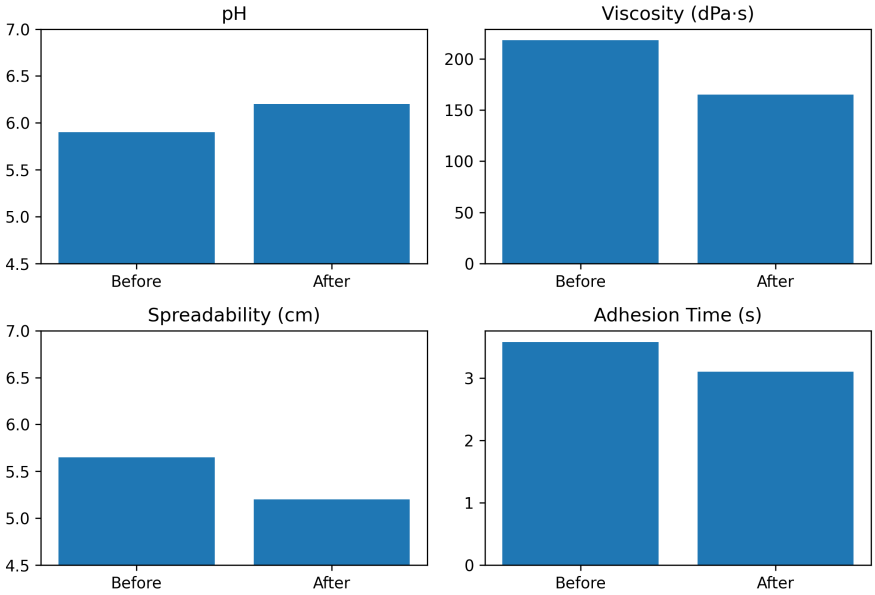

Figure 6: Physicochemical stability of transfersomal gel before and after freeze–thaw cycling (six cycles), evaluated in terms of pH, viscosity, spreadability, and adhesion time.

Although statistically significant differences were detected between pre- and post-stability measurements ($p < 0.05$), all evaluated parameters remained in acceptable limits for topical gel formulations. No visible phase separation or crystallization was observed, showing that the gel maintained its physical integrity during the stability study.

Antibacterial Activity of Transfersomal Gel

Antibacterial activity of transfersomal gel against *Staphylococcus aureus* was evaluated using the agar well diffusion method. The results were summarized in **Table 5, Figure 7,** and **Figure 8**. Transfersomal gel produced a clear inhibition zone with a mean diameter of 11.33 ± 0.96 mm.

The positive control (clindamycin gel) showed a larger inhibition zone (18.96 ± 2.03 mm), while the negative control (gel base without transfersomes) did not produce any inhibition zone. Statistical analysis using the Mann–Whitney test revealed a significant difference between antibacterial activity of transfersomal gel and the positive control ($p < 0.05$)

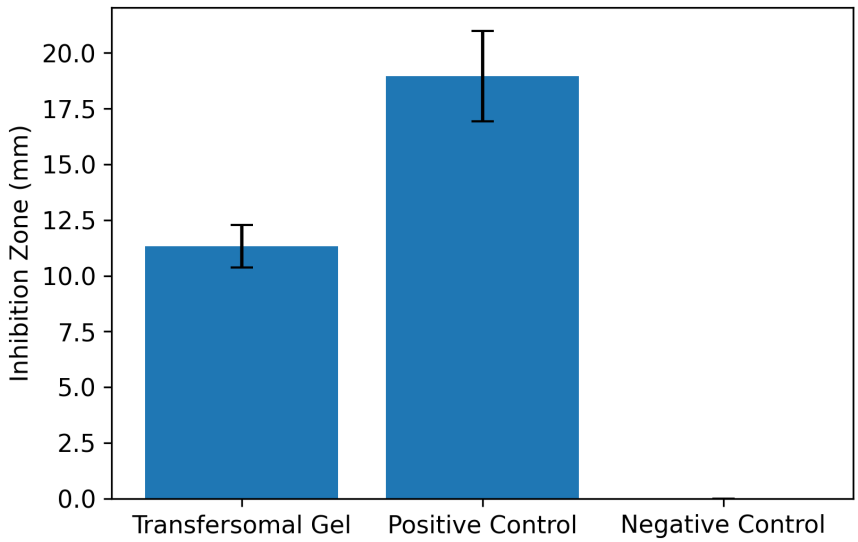

Figure 7: Antibacterial activity of transfersomal gel against *Staphylococcus aureus* expressed as inhibition zone diameter (mean ± SD).

Table 5: Antibacterial Activity Against *Staphylococcus aureus*

Sample	Inhibition Zone (mm)
Transfersomal gel	11.33 ± 0.96
Positive control (Clindamycin gel)	18.96 ± 2.03
Negative control (gel base)	0.00 ± 0.00

Figure 8. Antibacterial activity of transfersomal gel containing *C. sappan* extract against *Staphylococcus aureus* using the agar well diffusion method. TF: transfersomal gel; K+: positive control (clindamycin gel); K−: negative control (gel base).

DISCUSSION

These results demonstrated that Tween 80 concentration played an essential role in determining the physicochemical characteristics of *C. sappan* extract-loaded transfersomes. The significant reduction in particle size with increasing surfactant concentration was consistent with previous reports indicating that higher levels of edge activators enhanced vesicle deformability and reduced interfacial tension, leading to the formation of smaller and more flexible vesicles[3,4]. Nanosized transfersomes (<200 nm), as achieved in the optimized formulation, were particularly advantageous for topical delivery, as penetration was facilitated through the stratum corneum by exploiting transdermal hydration gradient[8]. The homogeneous particle size distribution observed in this study further supported the suitability of the formulation for topical application, in agreement with results reported in recent transfersomal studies focusing on dermal drug delivery systems[9].

The incorporation of the optimized transfersomal formulation into a carbopol-based gel resulted in a dosage form with acceptable physicochemical properties and maintained stability under freeze–thaw conditions. Although statistically significant changes were observed in certain parameters after stability testing, all values remained in acceptable limits for topical gels, indicating adequate formulation robustness. Antibacterial activity exhibited by transfersomal gel against *Staphylococcus aureus* could be attributed to both the intrinsic antibacterial properties of *C. sappan* bioactive compounds and the enhanced delivery capability of transfersomal system. Brazilin and phenolic compounds present in *C. sappan* were reported to disrupt bacterial cell membranes and inhibit nucleic acid synthesis, leading to bacterial growth inhibition[5]. The improved antibacterial performance observed in this study supported previous evidence that transfersomal carriers enhanced local drug concentration at the site of infection, thereby improving therapeutic efficacy compared with conventional topical formulations[6]. Similar outcomes were also reported in transferosomal gel formulations published in IJDDT, further confirming the relevance of the present results in the context of topical drug delivery technology.

CONCLUSION

In conclusion, the concentration of Tween 80 significantly influences the size of *C. sappan* extract-loaded transfersomes. The optimized transfersomal formulation is successfully incorporated into a gel system with acceptable physicochemical characteristics. Transfersomal gel exhibits significant antibacterial activity against *Staphylococcus aureus*, demonstrating its potential as an effective topical drug delivery system for plant-based antibacterial agents.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

This study did not involve human or animal subjects.

REFERENCE

1. Matharoo N T. Transfersomes as a transdermal drug delivery system: Permeation behaviour and future perspectives. WIREs Nanomedicine and Nanobiotechnology. 2024;16(2):e1918. DOI: 10.1002/wnan.1918
2. Chaurasiya P, Ganju E, Upmanyu N, Ray SK, Jain P. Transfersomes: A novel technique for transdermal drug delivery. Journal of Drug Delivery and Therapeutics. 2019;9(1):279–285. DOI: 10.22270/jddt.v9i1.2198

3. Vieira J, Silva AM, Souto EB. Mixed edge activators in ibuprofen-loaded transfersomes: Optimization and physicochemical characterization. Pharmaceutics. 2023;15(4):1209. DOI: 10.3390/pharmaceutics15041209
4. Khan MI, Madni A, Peltonen L, Rehman M. Lecithin-based transfersomal vesicles for enhanced dermal delivery: Formulation and evaluation. BioMed Research International. 2022;2022:8170318. DOI: 10.1155/2022/8170318
5. Insuan W, Narkrugsa W, Rachtanapun P. Brazilin content and biological activities of Caesalpinia sappan heartwood extract. SN Applied Sciences. 2024;6(1):122. DOI: 10.1007/s42452-024-06222-4
6. Souto EB, Souto SB, Zielińska A, Durazzo A. Nanotechnology-based drug delivery systems for skin applications. Pharmaceutics. 2021;13(4):593. DOI: 10.3390/pharmaceutics13040593
7. Listiana, L., Wahlanto, P., Ramadhani, S. S., & Ismail, R. Penetapan Kadar Tanin Dalam Daun Mangkokan (Nothopanax scutellarium Merr) Perasan Dan Rebusan Dengan Spektrofotometer UV-Vis. *Pharmacy Genius*, 2022. *1*(1), 62-73.
8. Matharoo, N., Mohd, H., & Michniak-Kohn. Transferosomes as a transdermal drug delivery system: Dermal kinetics and recent developments. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *16*(1), 2024. e1918.
9. Abdelwahd A, Hassan DH, El-Nabarawi MA. Optimization and evaluation of transfersomes for enhanced transdermal drug delivery: In vitro and ex vivo studies. Journal of Drug Delivery Science and Technology. 2022;68:103098. DOI: 10.1016/j.jddst.2022.103098
10. Patel R, Patel N. Transfersomes: A novel topical drug delivery approach. Journal of Drug Delivery and Therapeutics. 2023;13(2):1–8.
11. Sharma S, Kaur A. Formulation and in vitro evaluation of transferosomal gel of mometasone furoate. Journal of Drug Delivery and Therapeutics. 2023;13(3):45–52.
12. Singh R, Kumar S, Sharma V. Formulation and evaluation of lamivudine transferosomal gel for topical application. Journal of Drug Delivery and Therapeutics. 2022;12(6):112–119.
13. Verma A, Mishra R, Pandey S. Development and characterization of acyclovir-loaded transferosomal gel for enhanced topical delivery. Journal of Drug Delivery and Therapeutics. 2024;14(1):30–38.