

CURCUMIN-LOADED SELF-EMULSIFYING SYSTEMS: A NOVEL APPROACH TO COUNTER *STAPHYLOCOCCUS AUREUS* IN DIABETIC FOOT ULCERS

Falak Niaz

⁷Department of rehabilitation and Allied Health Sciences Riphah Internation, Malakand Campus, Pakistan

falak.niaz@Riphah.edu.pk

Keywords

Diabetic foot ulcers, *S. aureus*, curcumin, self-emulsifying drug delivery systems (SED DS)

Article History

Received: 22 October, 2024
Accepted: 15 December, 2024
Published: 31 December, 2024

Copyright @Author

Corresponding Author: *
Falak Niaz

Abstract

Persistent hyperglycemia and related consequences, such diabetic foot ulcers (DFUs), are hallmarks of diabetes mellitus (DM), that dramatically raise the risk of morbidity and mortality among the diabetic individuals. Multidrug-resistant organisms such as *Staphylococcus aureus* (*S. aureus*) are commonly found in DFUs, which makes treatment more difficult with current antibiotics. A possible remedy is curcumin, a polyphenolic substance having antibacterial qualities. Its bioavailability is increased when it is administered using Self-Emulsifying Drug Delivery Systems (SED DS).

Methodology: A six-month cross-sectional experimental study was carried out to check the antibacterial activity of SED DS loaded curcumin. SED DS loaded with curcumin (cu-SED DS) were made with a variety of oils, co-surfactants, and surfactants. A Zetasizer was used to characterize the formulations for droplet size, zeta potential, and polydispersity index (PDI). Agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) techniques were used to measure antibacterial activity.

Results: The most effective cu-SED DS formulation against *S. aureus* was F1, which exhibited the lowest MIC value ($0.0507 \pm 0.02 \mu\text{g/mL}$) and a significant inhibition zone ($14 \pm 1 \text{ mm}$) among the five. Characterization showed that F1 was stable with a zeta potential of -2.34 mV , a PDI of 0.089, and a droplet size of 490.3 nm. The antibacterial efficacy of formulations varied F1 has better activity than others.

Conclusion: Curcumin-loaded SED DS formulations offer a novel and effective approach to managing DFU-associated *S. aureus* infections.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by persistent hyperglycemia due to insulin insufficiency or malfunction (1). This condition, if uncontrolled, leads to organ damage and failure, including diabetic foot ulcers (DFUs) vulnerable to Gram-positive infections. Addressing these complications is crucial, given DM's global prevalence. The projected global count of individuals with diabetes is set to reach 592 million by 2035,

comprising 10% of the worldwide prevalence. Notably, gestational diabetes affects 21 million women, with an escalating annual rate. Moreover, approximately 175 million cases of undiagnosed diabetes exist

(2). North Africa and the Middle East exhibit the highest diabetes prevalence (10.9% in adults), while the Western Pacific region displays the most significant caseload and incidence rate (37.5%). Varied diabetes classifications have

been compared based on genetics, diagnostics, criteria, and causative factors (3).

Patients with DM face various complications, notably diabetic foot ulcers (DFUs), a prevalent concern leading to heightened mortality and amputation rates. DFUs, once initiated, frequently progress, elevating amputation risks. Amputation rates are fifteen-fold higher in diabetic patients than non-diabetics (4). Global DFU prevalence is approximately 6.3%. Regionally, Asia, North America, Europe, Africa, and Oceania report rates of 5.5%, 13.0%, 6.5%, 5.1%, and 3.0%, respectively. In the United States, 15-25% of diabetes patients develop DFUs, while Pakistan's National Diabetes Survey notes a 4-10% prevalence, escalating ulcer and amputation risks (5,6). DFUs can deteriorate, affecting bones if untreated, with Wagner classification gauging severity based on ulcer depth, osteomyelitis onset, and gangrene (7).

Diabetic foot ulcers (DFUs) host a diverse microbe mix, including *Enterococcus* spp., *Staphylococcus* spp., *Acinetobacter* spp., and *Klebsiella* spp., often from patient flora. Tissue-destructive microbes like *Proteus*, *Pseudomonas*, and *Enterococcus* spp. worsen DFU conditions. *Citrobacter* spp. prevalence in DFUs is around 10%, less in non-DFUs. *Pseudomonas*, *Staphylococcus*, and *Acinetobacter* spp. are common in both DFUs and non-DFUs, contributing to chronic infections (8). *S. aureus* and *Streptococcus* species dominate diabetic foot infections (DFIs), with MRSA strains comprising 33% of isolated microbes (9,10). MDROs, associated with prolonged antibiotic use, prevail in up to 70% of cases, while multidrug-resistant *Pseudomonas aeruginosa* is linked to prolonged antimicrobial use (11,12). MDROs extend hospital stays and may elevate amputation risks (13,14). Studies underscore MDRO spread risks but further research is needed for clinical outcomes and interventions (14).

Abundant in plants, polyphenolic compounds are traditional antimicrobial agents with therapeutic potential. They include ellagic acid, chlorogenic acid, rutin, and catechin, proven effective against diseases like diabetes, cancer,

and cardiovascular issues (15). Compounds in tea, turmeric, berries, and dark chocolate offer antioxidant benefits but face limited bioavailability, spurring research into biotransformation to enhance absorption (16).

Turmeric consists of 6.3% protein, 5.1% fats, 3.5% minerals, 69.4% carbohydrates, and 13.1% moisture. Its rhizome yields 5.8% essential oil, with curcumin at 3-4% providing its yellow color (17). Curcumin, a non-flavonoid phenol, is the active member of curcuminoids derived from *Curcuma* spp. roots. These compounds, including demethoxy-curcumin and bis-demethoxy-curcumin, have been researched for their therapeutic properties since 1991 (18). Curcumin combats hyperglycemia by reducing oxidative stress and enhancing glucose transport, insulin secretion, and pancreatic function (19, 20).

It demonstrates in-vitro antimicrobial activity against various microorganisms, affecting bacterial virulence and biofilm formation (21). Although its inhibitory activity requires higher concentrations, curcumin shows potential for synergy with antibiotics against resistant strains. Amid the rise of antibiotic resistance, curcumin analogs are being explored for enhanced activity (22). Curcumin's impact on insulin sensitivity involves multiple pathways, including glucose homeostasis and lipid metabolism (23). Lipid-based formulations used in self-emulsifying drug delivery systems (SEDDS) allow hydrophobic drugs to dissolve in the gastrointestinal tract (24). This study investigates Curcumin-loaded Self-Emulsifying Drug Delivery Systems (SEDDS) as a potential solution against Gram-positive bacteria in DFUs, contributing to enhanced therapeutic strategies for DM-related challenges.

MATERIAL AND METHODS

Study Overview

Conducted at the Microbiology Lab of the IPDM department, Khyber Medical University Peshawar, this six-month cross-sectional experimental study investigated the microbiological profile of diabetic foot ulcers. The study, initiated after ASRB approval, focused on patients with diabetic foot ulcers in

the Endocrinology department of Hayatabad Medical Complex Peshawar. Ten samples were meticulously collected from open wound lesions after surface cleaning with saline solution and swabbing. The collected samples were promptly transported in specialized media to the lab for analysis. This research aimed to contribute insights into the microbial composition of diabetic foot ulcers, utilizing a cross-sectional experimental approach in a clinical setting.

SEDDS: preparation of formulations

At first, blank SEDDS formulations were made by modifying the excipient component in their composition in context with the protocol described earlier with some interventions. To do this, various excipients (oils and surfactants and co-solvents) were measured, vortexed and homogenized, and then sonication was used for 30 min. Dynamic light scattering was utilized to evaluate the mean droplet size, polydispersity index (PDI), and zeta potential of the generated emulsions using a Zeta sizer with an E field quality of 10 v/cm and a frequency of 650 nm. For this purpose, different concentrations of excipients such as Captex 355, Captex 300, DMSO, Tween 80, Cremephor RH40, Cremephor EL, Capmul PG-8 were used to make 5 different formulations.

Characterization of curcumin loaded SEDDS:

Curcumin loaded self-emulsifying drug delivery system (cu-SEDDS) was prepared using vortex mixing and homogenization (25). Five formulations of 10mg curcumin each were created for cu- SEDDS, guided by PDI, zeta size, and zeta potential of blank formulations. Surfactant, co- surfactants, and oils were mixed to form a homogenized mixture. 1% (10mg) curcumin was added to each formulation, followed by centrifugation at 13000rpm. These formulations were then evaluated against isolated bacteria.

Agar well diffusion method

The antibacterial activity of cur-loaded SEDDS was assessed using the agar well diffusion method. The procedure involved seeding

Muller Hinton agar plates with bacterial suspensions

(0.5 McFarland) using the streaking technique. Plates were punctured to create wells (6-8mm in diameter), and 200 μ l of diluted cu-SEDDS formulation was added to each well. Distilled water (200 μ l) served as the negative control. Each plate also contained an antibiotic disk. After 24 hours of incubation at 37°C, inhibition zones were measured in millimeters around the wells to determine the antibacterial effectiveness.

Minimum inhibitory concentration (MIC)

MIC (Minimum Inhibitory Concentration) represents the lowest effective substance level for inhibiting visible organism growth. The micro broth dilution method, performed in a 96-well plate, was utilized here. Each well contained uniform broth and inoculum quantities, while the concentration of antibacterial agents varied. This technique is fundamental for testing antimicrobial susceptibility. It involves two-fold dilutions of antimicrobial solutions (e.g., 1 μ g, 2 μ g, 4 μ g, 8 μ g, 16 μ g, 32 μ g)/ μ l in broth, often in 2ml tubes or 96-well plates (25).

For clarity, 500 μ l of MHB (Mueller Hinton Broth) was added to separate tubes, and 1000 μ l stock solutions of each formulation were diluted to create concentrations following a pattern (5000, 2500, 1250, 625, 312.5, 156.25, 78.12, 39.06, 19.53, 9.76) μ g/ μ l.

Creating a standardized bacterial inoculum is essential for accurate MIC results. Isolated colonies from an overnight agar plate were dissolved in saline or broth. The suspension was standardized to 0.5 McFarland, containing 1.5×10^8 CFU/ml. A portion (0.1ml) was mixed with

9.9ml of broth to create a cell density of 1×10^6 CFU/ml. Adding this to wells with antimicrobial solution resulted in the desired 5×10^5 CFU/ml concentration.

Minimum bactericidal concentration (MBC)

MBC (Minimum Bactericidal Concentration) assessment follows the determination of MIC and reveals the lowest concentration of an antibacterial agent needed to completely

eliminate bacteria. Unlike MIC, which indicates growth inhibition, MBC confirms the lethal impact of the agent. In this study, the procedure involved placing a drop of culture mixed with curcumin- loaded formulation from the MIC well onto a blood agar plate. Positive and negative controls from the MIC plate were also added. After streaking and incubating at 37°C for 24 hours, the plate was examined for visible growth. Bactericidal effects were indicated by the absence of visible growth, demonstrating the effectiveness of the cur-loaded formulations.

Isolation and identification of bacterial isolates

S. aureus displayed distinct characteristics round, smooth, convex colonies on Nutrient agar, and round golden beta hemolytic colonies on Blood agar. Mannitol salt agar, where fermentation of mannitol resulted in yellow colonies surrounded by clear zones. Confirmation of bacterial identity commenced with Gram staining, revealing the retention of the purple color indicative of Gram-positive nature. Further refinement of identification utilized biochemical tests including the catalase

test, which demonstrated positivity through bubble formation upon mixing a colony with hydrogen peroxide. Additionally, the coagulase test exhibited positive outcomes with the formation of clumps when colonies interacted with plasma on a glass slide. Collectively, these techniques aided in the precise identification of *S. aureus* from pus samples extracted from diabetic foot ulcers, employing a comprehensive approach encompassing Gram stain, colony morphology, and biochemical assessments.

Preparations of curcumin loaded SEDDS

The development of curcumin-loaded Self-Emulsifying Drug Delivery Systems (SEDDs) involved the incorporation of oils, surfactants, and co-surfactants in combination with 10mg of curcumin per formulation. To achieve uniformity, each formulation was vortex mixed to attain a homogenized mixture. Multiple variations of cu-loaded SEDDs were prepared, each with specific concentrations of excipients, as outlined in the table 1 below.

Table.1 Represents different formulations of cu-loaded SEDDS and their composition
List of excipients: Polyethylene glycol (PEG), Tetra-ethylene glycol (TEG), Dimethyl sulfoxide (DMSO), Caprylic acid (CA).

Formulations %					
Excipients(%)	F1	F2	F3	F4	F5
Capmul 12	25	30	-	-	25
Creme RH40	30	-	-	35	-
Creme EL	-	25	-	-	-
CA	-	-	10	10	-
TEG	25	-	-	-	25



Castor oil	-	-	20	-	-
Black seed oil	-	-	-	-	30
PEG	20	-	25	25	-
Captex 300	-	25	-	-	-
Captex 355	-	-	35	20	-
DMSO	-	20	-	-	-
Tween 80	-	-	10	10	-
PEG400	-	-	-	-	20
Curcumin	1	1	1	1	1

Characterization of cu-loaded SEDDS

To comprehensively assess the characteristics of the developed formulations, a commercially available Zeta sizer (Malvern) was employed. This analytical tool facilitated the determination of crucial parameters including particle size, zeta potential of the droplets, and the polydispersity index (PDI). The zeta potential data were particularly instrumental

in evaluating the optimal cu- loaded formulation. The measurements were conducted in triplicate to ensure accuracy and reliability. The obtained results will play a pivotal role in guiding the selection of the most suitable formulation for further studies, based on its particle size distribution, zeta potential, and overall uniformity as reflected by the PDI value presented in Table 2.

Table.2 Table represents zeta size and zeta-potential of the cu-loaded SEDDs formulations.

Formulations	Zeta Size (nm)	Zeta-potential(mV)	Poly-disparity index
PDI			
F1	490.3	-2.34	0.089
F2	66.56	-17.3	0.265
F3	482.2	-8.87	0.495
F4	25.45	-2.25	0.122
F5	939.7	-10.3	0.704

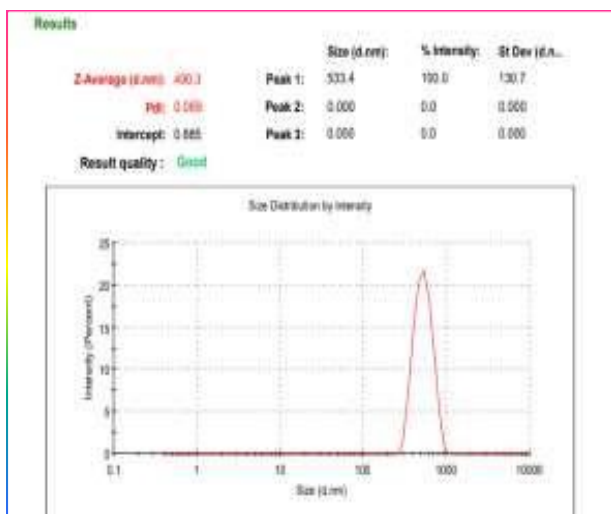


Fig.1(a) Zeta size of the formulation F1

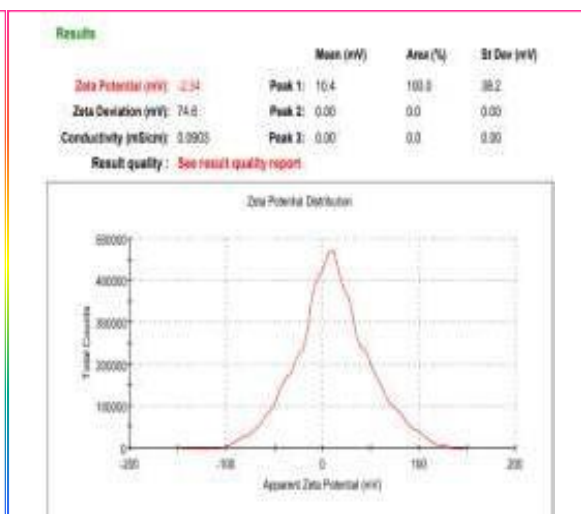
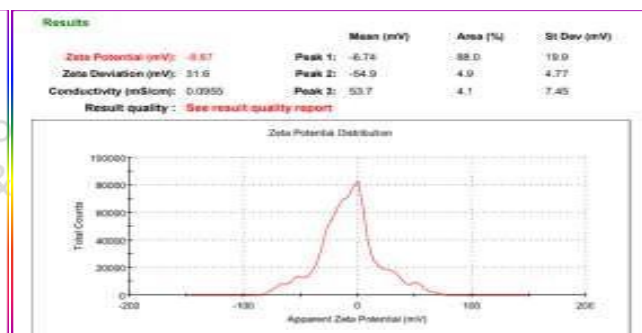


Fig.1(b) Zeta potential of formulation F1



Fig.2 (a) Zeta size of formulation F2



(b) Zeta potential of formulation F2

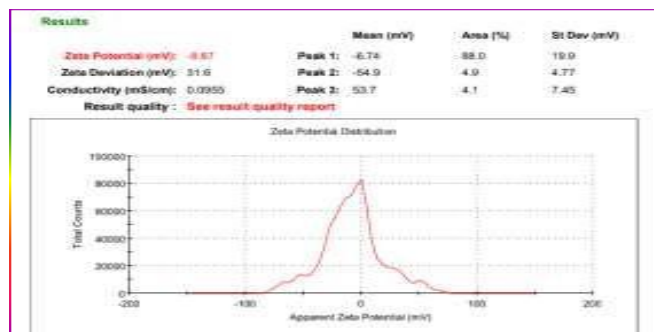


Fig.3(a) Zeta size of formulation F3

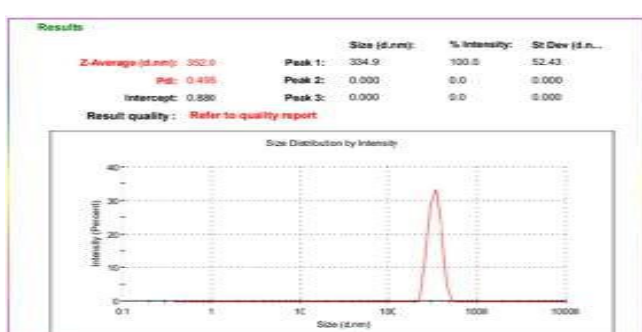


Fig.3(b) Zeta potential of formulation F3

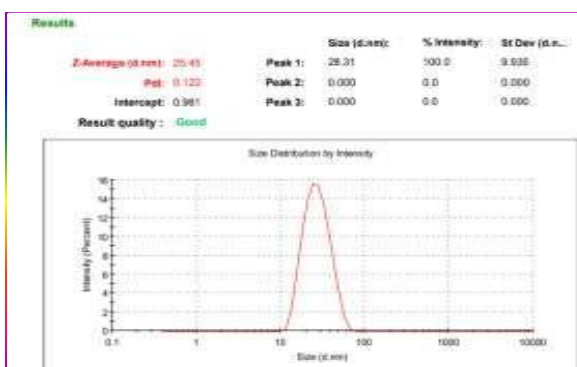


Fig.4(a) Zeta size of formulation F4

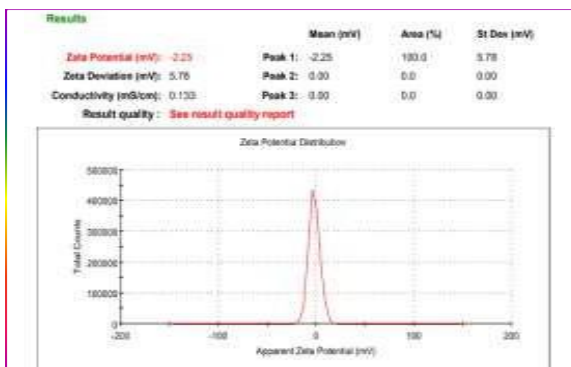


Fig.4(b) Zeta potential of formulation F4

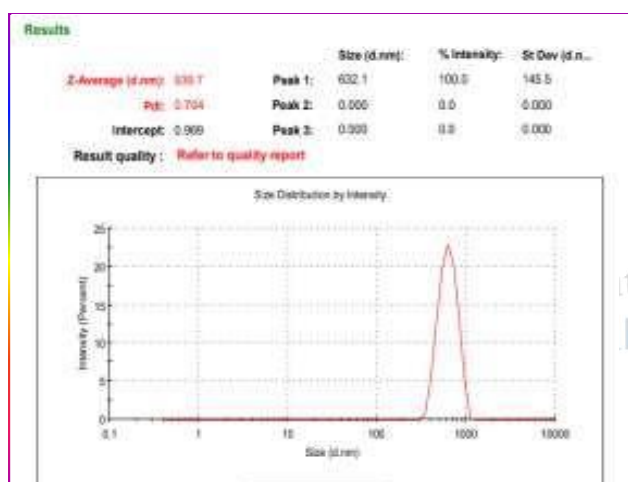


Fig.5 (a) Zeta size of formulation F5

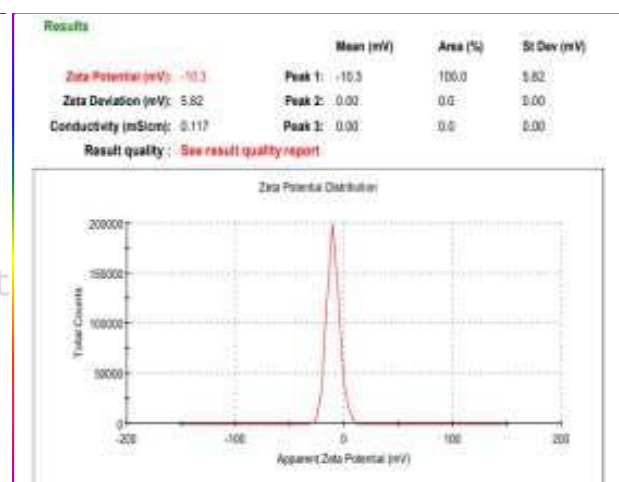


Fig.5 (b) Zeta potential if formulation

Antibacterial activity of cu-loaded SEDDs by Agar well diffusion method

In assessing the antibacterial potential of diverse curcumin-loaded Self-Emulsifying Drug Delivery Systems (SEDDS) formulations against *S. aureus*, distinct inhibitory patterns emerged. Among the formulations, F1 exhibited the highest antibacterial activity, resulting in a significant inhibition zone of 14 ± 1 . Following suit, F2 showcased a zone of 13 ± 1.5 , while F4 and F3 demonstrated zones

of 12 ± 1.5 and 11 ± 1 , respectively. Notably, formulation F5 displayed a still appreciable inhibition zone, measuring 9 ± 1.5 . All the experiments were performed in triplicate, mean and standard deviation were calculated using SPSS 22.0. The activity of different formulations of cu-loaded SEDDS was recorded against *S. aureus* as shown in the Figure 6. Data was expressed as mean and standard deviation of three independent experiment.

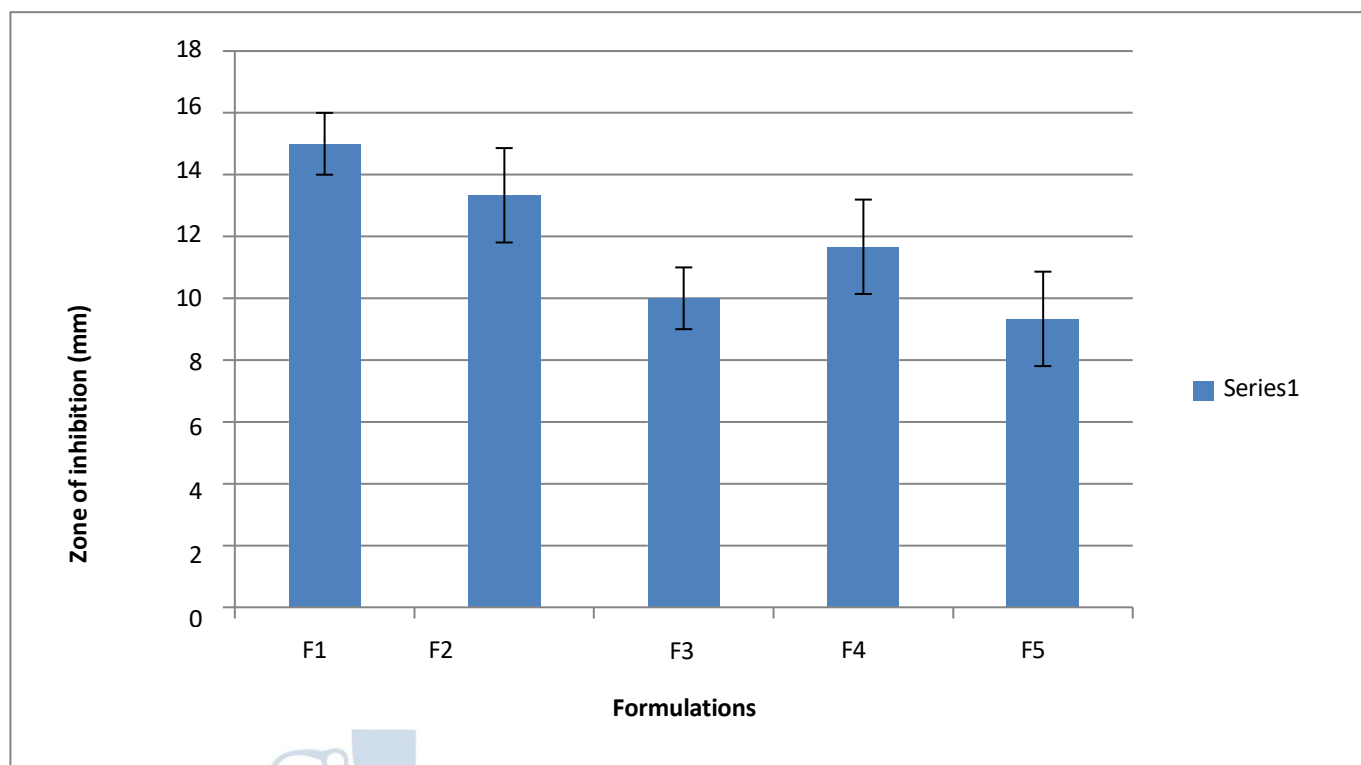


Fig 6: Cu-loaded SEDDs different formulations antibacterial activity by well diffusion method

Antibacterial activity of cu-loaded SEDDs by micro dilution method

In the context of combating *S. aureus*, the utilization of curcumin-loaded Self-Emulsifying Drug Delivery Systems (SEDDS) showcased noteworthy outcomes. Notably, the compound exhibited the highest activity in the form of F1, boasting a remarkable minimal inhibitory concentration (MIC) of 0.0507 ± 0.02 $\mu\text{g/ml}$. Following this, F2 and F4 emerged with considerable effectiveness, recording MIC values of 0.0583 ± 0.02 and 0.1167 ± 0.04 $\mu\text{g/ml}$ respectively. In contrast, F3 and F5 displayed relatively diminished

activity against *S. aureus*, as evidenced by their MICs of 0.3733 ± 0.16 and 0.9333 ± 0.32 $\mu\text{g/ml}$ respectively. The results illuminate the diverse antimicrobial potential of the tested compounds, firmly positioning F1 as an especially encouraging candidate for subsequent investigations. This underscores the potential impact of curcumin-loaded SEDDS in countering *S. aureus* infections. Data was expressed as mean and standard deviation of three independent experiment.

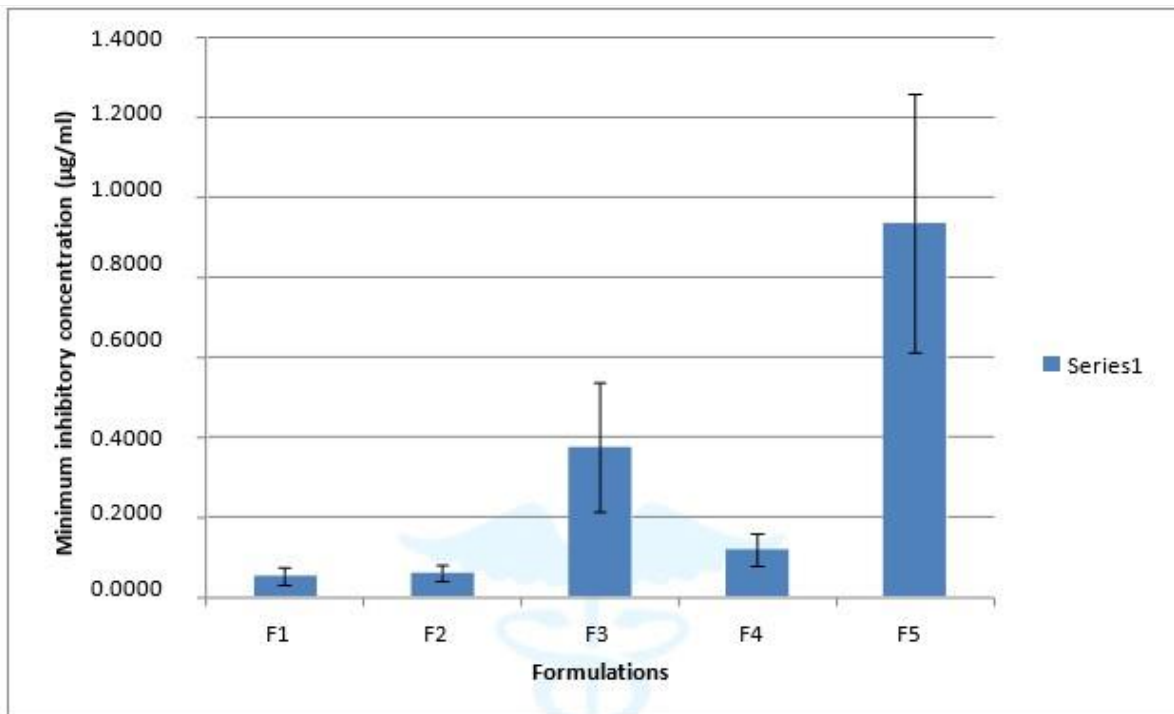


Fig 7: MIC of cu-loaded SEDDs different formulations by broth microdilution method

Minimum bactericidal concentration: Discussion

S. aureus is a noteworthy microorganism that exhibits a dual nature: as a commensal flora present in up to 30% of the population and as a causative agent for an array of human infections, ranging from soft-tissue infections to severe conditions such as toxic shock syndrome (TSS), endocarditis, and necrotizing pneumonia. In the context of diabetes-associated complications, diabetic foot ulcer (DFU) represents a significant health challenge, linked to increased mortality rates and potential implications in ischemic heart diseases (26). Our research project aligned with this context, aiming to combat DFUs by isolating *S. aureus* from clinical samples and assessing its susceptibility to curcumin, a well-known polyphenolic compound recognized for its therapeutic potential.

Curcumin, with its diverse therapeutic properties, including broad-spectrum antibacterial activity (27), has garnered substantial attention in wound healing studies. Its ability to enhance wound contraction and accelerate healing has been noted

(28). Our study contributes to this growing body of knowledge by examining curcumin's efficacy against *S. aureus*. Confirming earlier reports, curcumin demonstrated antibacterial effects against *S. aureus*, with varying levels of potency among different formulations (29).

The formulation of curcumin-loaded Self-Emulsifying Drug Delivery Systems (SEDDS) holds substantial promise in overcoming challenges related to curcumin's low bioavailability and solubility. SEDDS provides a platform for enhancing drug absorption and systemic availability, contributing to improved therapeutic outcomes (30). Our study employed a careful selection of excipients, adhering to FDA-approved options to ensure safety (31). The incorporation of triglycerides, surfactants, and co-surfactants in specific proportions contributed to solubilizing curcumin and enhancing its bioavailability (32, 33).

Notably, our formulations demonstrated diverse oil, surfactant, and co-surfactant compositions to optimize performance. The strategic selection of surfactants such as Tween 80 and Span, along

with co-surfactants like propylene glycol (PG), facilitated the formation of stable emulsions and microemulsions (34, 35). Propylene glycol, despite its less hydrophilic nature, proved effective in enhancing microemulsion stability (35). DMSO also contributed to formulation efficiency by widening the emulsion area and improving bioavailability (36). This novel approach to curcumin delivery could potentially revolutionize wound healing strategies.

The current study's MIC values against *S. aureus* validate curcumin's bactericidal potential. The formulation's superior efficacy, reflected in the increased zone of inhibition, underscores its significance as an antibacterial agent. The standardized MIC values of curcumin against *S. aureus* in the study align with previous research, affirming its potency against this pathogen (38).

Conclusion

In conclusion, this research underscores the therapeutic potential of curcumin-loaded SEDDS formulations against *S. aureus* infections. The study not only adds to the existing knowledge of curcumin's antibacterial effects but also introduces an innovative approach to enhance its bioavailability for wound healing applications. By addressing the challenges of drug solubility and absorption, the study bridges the gap between research and practical medical interventions. However, further investigations and clinical trials are necessary to fully establish the efficacy and safety of curcumin-loaded SEDDS in managing diabetic foot ulcers and associated infections.

REFERENCES

- Mellitus DJDc. Diagnosis and classification of diabetes mellitus. 2005;28(S37):S5- S10.
- Aguirre F, Brown A, Cho NH, Dahlquist G, Dodd S, Dunning T, et al. IDF diabetes atlas. 2013.
- Kharroubi AT, Darwish HMJWjod. Diabetes mellitus: The epidemic of the century. 2015;6 6:850-67.
- Yazdanpanah L, Nasiri M, Adarvishi S. Literature review on the management of diabetic foot ulcer. World J Diabetes. 2015;6(1):37-53.
- Khan A, Junaid NJJ. Prevalence of diabetic foot syndrome amongst population with type 2 diabetes in Pakistan in primary care settings. 2017;67(1818).
- Riaz M, Miyan Z, Waris N, Zaidi SI, Tahir B, Fawwad A, et al. Impact of multidisciplinary foot care team on outcome of diabetic foot ulcer in term of lower extremity amputation at a tertiary care unit in Karachi, Pakistan. 2019;16(3):768-72.
- Mehraj MJJoOS. A review of Wagner classification and current concepts in management of diabetic foot. 2018;4:933-5.
- Sadeghpour Heravi F, Zakrzewski M, Vickery K, D GA, Hu H. Bacterial Diversity of Diabetic Foot Ulcers: Current Status and Future Prospectives. Journal of clinical medicine. 2019;8(11)
- AMINI M, DAVATI A, PIRI MJJoP. DETERMINATION OF THE RESISTANCE PATTERN OF PREVALENT AEROBIC BACTERIAL INFECTIONS OF DIABETIC FOOT ULCER. 2013;8(1):-.
- Viswanathan V, Pendsey S, Radhakrishnan C, Rege TD, Ahdal J, Jain RJTijolew. Methicillin-resistant *Staphylococcus aureus* in diabetic foot infection in India: a growing menace. 2019;18(3):236-46.
- Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini A, Chaudhry RJDc. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. 2006;29(8):1727-32.
- Gristina AG, Costerton JWJOCO NA. Bacterial adherence and the glycocalyx and their role in musculoskeletal infection. 1984;15(3):517-35.
- Richard J-L, Sotto A, Jourdan N, Combescure C, Vannereau D, Rodier M, et al. Risk factors and healing impact of multidrug-resistant bacteria in diabetic foot ulcers. 2008;34(4):363-9.
- Zhang J, Chu Y, Wang P, Ji X, Li X, Wang C, et al. Clinical outcomes of multidrug resistant *Pseudomonas aeruginosa* infection and the relationship with type III secretion system in patients with diabetic foot. 2014;13(3):205-10.



- Mumtaz R, Zubair M, Khan MA, Muzammil S, Siddique MH. Extracts of *Eucalyptus alba* Promote Diabetic Wound Healing by Inhibiting α -Glucosidase and Stimulating Cell Proliferation. Evidence-Based Complementary and Alternative Medicine. 2022;2022:4953105.
- Yang CS, Sang S, Lambert JD, Lee MJMn, research f. Bioavailability issues in studying the health effects of plant polyphenolic compounds. 2008;52(S1):S139-S51.
- Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RKJCs. Turmeric and curcumin: Biological actions and medicinal applications. 2004;44-53.
- Sharma R, Gescher A, Steward WJ. Curcumin: the story so far. 2005;41(13):1955-68.
- Aggarwal BB, Surh Y-J, Shishodia S. The molecular targets and therapeutic uses of curcumin in health and disease: Springer Science & Business Media; 2007.
- Ghorbani Z, Hekmatdoost A, Mirmiran P. Anti-hyperglycemic and insulin sensitizer effects of turmeric and its principle constituent curcumin. International journal of endocrinology and metabolism. 2014;12(4):e18081.
- Gunes H, Gulen D, Mutlu R, Gumus A, Tas T, Topkaya AE. Antibacterial effects of curcumin: An in vitro minimum inhibitory concentration study. Toxicol Ind Health. 2016;32(2):246-50.
- Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A review on antibacterial, antiviral, and antifungal activity of curcumin. Biomed Res Int. 2014;2014:186864.
- Oglah MK, Mustafa YF, Bashir MK, Jasim MH, Mustafa YFJSRP. Curcumin and its derivatives: A review of their biological activities. 2020;11(3):472-81.
- Tang B, Cheng G, Gu J-C, Xu C-HJDDt. Development of solid self-emulsifying drug delivery systems: preparation techniques and dosage forms. 2008;13(13-14):606-12.
- Tarpay MM, Welch DF, Marks MIJAa, chemotherapy. Antimicrobial susceptibility testing of *Streptococcus pneumoniae* by micro-broth dilution. 1980;18(4):579-81.
- Vu BG, Stach CS, Salgado-Pabón W, Diekema DJ, Gardner SE, Schlievert PMJTJoid. Superantigens of *Staphylococcus aureus* from patients with diabetic foot ulcers. 2014;210(12):1920-7.
- Teow S-Y, Ali S. Synergistic antibacterial activity of Curcumin with antibiotics against *Staphylococcus aureus*. Pak J Pharm Sci. 2015;28:2109-14.
- Kumari A, Raina N, Wahi A, Goh KW, Sharma P, Nagpal R, et al. Wound-Healing Effects of Curcumin and Its Nanoformulations: A Comprehensive Review. 2022;14(11):2288.
- Moghaddam KM, Iranshahi M, Yazdi MC, Shahverdi ARJIJoGP. The combination effect of curcumin with different antibiotics against *Staphylococcus aureus*. 2009;3(2).
- Ramshankar YV, Suresh S, Devi K. Novel Self-emulsifying Formulation of Curcumin with Improved Dissolution, Antiangiogenic and Anti-inflammatory Activity. Clinical Research and Regulatory Affairs. 2008;25(4):213-34.
- Chatterjee B, Hamed Almurisi S, Ahmed Mahdi Dukhan A, Mandal UK, Sengupta P. Controversies with self-emulsifying drug delivery system from pharmacokinetic point of view. Drug Deliv. 2016;23(9):3639-52.
- Maji I, Mahajan S, Sriram A, Medtiya P, Vasave R, Khatri DK, et al. Solid self emulsifying drug delivery system: Superior mode for oral delivery of hydrophobic cargos. J Control Release. 2021;337:646-60.
- Pouton CW, Porter CJ. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Advanced drug delivery reviews. 2008;60(6):625-37.
- Saxena S, Singh HN, Agrawal VK, Chaturvedi SJRgAoahwrnp. Lipid excipients in self emulsifying drug delivery systems. 2013;58424092.



Abd-Allah FI, Dawaba HM, Ahmed AM. Development of a microemulsion-based formulation to improve the availability of poorly water-soluble drug. *Drug discoveries & therapeutics*. 2010;4(4):257-66.

Jorgensen AM, Friedl JD, Wibel R, Chamieh J, Cottet H, Bernkop-Schnurch A. Cosolvents in Self-Emulsifying Drug Delivery Systems (SEDDS): Do They Really Solve Our Solubility Problems? *Mol Pharm*. 2020;17(9):3236-45.

Khan M, Ali M, Shah W, Shah A, Yasinza MM. Curcumin-loaded self-emulsifying drug delivery system (cu-SEDDS): a promising approach for the control of primary pathogen and secondary bacterial infections in cutaneous leishmaniasis. *Appl Microbiol Biotechnol*. 2019;103(18):7481-90.

Khan M, Ali M, Shah W, Shah A, Yasinza MM. Curcumin-loaded self-emulsifying drug delivery system (cu-SEDDS): a promising approach for the control of primary pathogen and secondary bacterial infections in cutaneous leishmaniasis. *Appl Microbiol Biotechnol*. 2019; 103(18):7481-90.

Ilyas M, Niaz F, Ishaq R, R. Ullah, A. Khanum. Detection of KatG Mutation in MDR *Mycobacterium tuberculosis* iso- lates by PCR-RFLP and DNA Sequencing. *Bangladesh J Med Sci* 2023; 22: 804-808.