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Formulation of Quercetin and Azelaic Acid Based Liposome and Evaluation of Its Invitro Antibacterial Activity

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ABSTRACT:
Liposomes act as promising drug carriers for topical application because they can penetrate through statum corneum {the outer layer of the epidermis (skin)} easily and provide a moist environment favorable for woundhealing. In this study, an azelaic acid and quercetin-based liposome was formulated and characterized by FESEM. The invitro antimicrobial activity of the liposome against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli was evaluated by agar well diffusion technique. The liposome showed the greatest efficacy against Staphylococcus aureus followed by Pseudomonas aeruginosa and Escherichia coli. This study suggests that the Azelaic acid and quercetin-based liposome has good antibacterial properties and can be used to treat bacterial infections caused by Staphylococcus aureus, Escherichia coli, and Pseudomonasaeruginosa.

Keywords: Liposome, Azelaic acid, Quercetin, Antibacterial activity, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli.

I. INTRODUCTION

Liposomes are small artificial vesicles of spherical shape that are made of cholesterol and natural non-toxic phospholipids. They can be used to carry both lipophilic and hydrophilic drugs. Liposomes containing topical dosage ensure localized activity with little to no systemic activity. Liposomes are more effective and less toxic than conventional topical formulations (Khandekar et al., 2015; Heliyon 8., 2022, Int J Mol Sci., 2023). Liposomal topical formulations can as a solubilizing matrix for poorly soluble drugs, penetration enhancers of the active ingredient into the skin, local depot (microreservoires) for sustained drug release, etc. Quercetin is a flavonoid present in the human diet and is known for its antimicrobial, antiviral, and antioxidant properties. According to current research, the antibacterial mechanism of quercetin mainly includes destroying the cell wall of bacteria and changing the cell permeability, affecting protein synthesis, reducing enzyme activity, and inhibiting nucleic acid synthesis (Dengyu et al., 2020; Saleh et al.,2023). Azelaic acid is a saturated 9-carbon atom dicarboxylic acid (COOH-(CH2)7-COOH) that owes its name to the fact that it was originally obtained from the oxidation of oleic acid by nitric acid. It is naturally found in cereals like barley, wheat etc. Present evidence indicates that azelaic acid inhibits the growth of bacteria by interfering with protein synthesis. The presence of Azelaic acid reduces DNA and RNA synthesis (Holland & Bojar., 1993).

Escherichia coli is a gram-negative bacterium. Numerous strains of Escherichia coli have evolved as opportunistic and commensal pathogens (Groisman and Ochman., 1996). It has been reported that 46%, 25%, and 21% of Escherichia coli isolates that cause wound infections are resistant to ampicillin, tetracycline, and fluoroquinolones respectively (Petkovsek et al., 2009). Pseudomonas aeruginosa is a gram-negative bacterium that has shown innate resistance to many antibiotics. A part of the challenge is that excessive antibiotic use can promote the selection and multiplication of resistant isolates. P. aeruginosa is the most common cause of bacteremia in burn patients and 14-33% of burn wounds are colonized within 10 days of admission. Staphylococcus aureus is a gram-positive bacterium and is involved in the pathogenesis of respiratory and skin infections, they cause gastrointestinal, and urogenital diseases and wound contamination.

S. aureus shows resistance to virtually all the older antibiotics (Neu., 1992).In this study, the aim was to synthesize a Quercetin and Azelaic acid-based liposomal formulation and evaluate its efficacy against bacterial pathogens.

II. MATERIALS AND METHODS

Collection of Bacterial strains used

In this study, P.aeruginosa (Gram negative), S.aureus (Gram positive), E.coli (gram

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negative) Strains were used for anti-bacterial studies. All the strains were obtained from Bioline Laboratory, Coimbatore followingethical guidelines.

Formulation of liposome

Thin film hydration technique (utilizes an organic solvent to dissolve lipids) discussed by Bangham was usedto synthesize the liposome (Idoudi et al., 2023). Accurately weighed soya lecithin was dissolved in chloroform and stirred using a magnetic stirrer. Aqueous drug solution (1% Quercetin and 3% Azelaic acid) that contained sorbitol / stearic acid was then mixed, followed by the addition of cholesterol. A milky suspension formed was stirred well to mix it properly. The mixture was then sonicated for a cycle of 10 minutes using probe sonicator. Suspension was now taken in a round bottom flask and attached to rota- evaporator. The rota-evaporator water bath temperature was set at 45oC with a rotation speed of 120 rpm. After the formation of the thin film around the round bottom flask, it was hydrated with 5 ml of phosphate buffer (pH 6.8) and stirred vigorously to for small vesicles of liposome. The mixture was then subjected to sonication for 2 cycles of 10 minutes each to reduce particle size (Chai & Park.,2023; Huang et al.,2024).

Field emission scanning electron microscopy

FESEM analysis of the liposome was carried out in the Department of Nanoscience and Technology in Bharathiar University, Coimbatore. A lyophilized sample of the liposome was used for analysis.

Antimicrobial activity assay

The antimicrobial activity of the liposomal formulation was evaluated by agar well diffusion method against the gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli) and the gram-positive bacteria (Staphylococcus aureus). Petri plates containing nutrient agar were inoculated with bacterial suspension and incubated for 48 hours at 37℃. After incubation wells were bored using a sterile borer and the plant sample was added at different concentrations and incubated for 24 hours. The zone of inhibition was observed and measured.

III. RESULTS

FESEM ANALYSIS

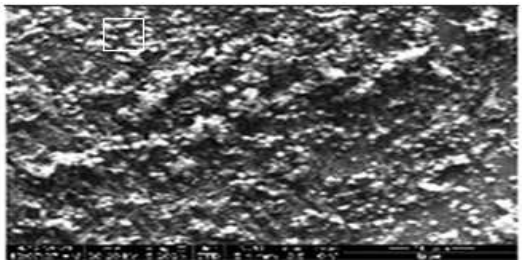

Fig 2 FESEM analysis of the liposome

The surface morphology of the liposome was analyzed at 200nm by FESEM. The liposomal vesicles were in the range of 4µm to 30µm in diameter. It was homogenously and thickly dispersed. It indicates that it is evident that liposomal formulation has greater efficacy against bacterial pathogens.

ANTIBACTERIAL ACTIVITY OF THE LIPOSOME

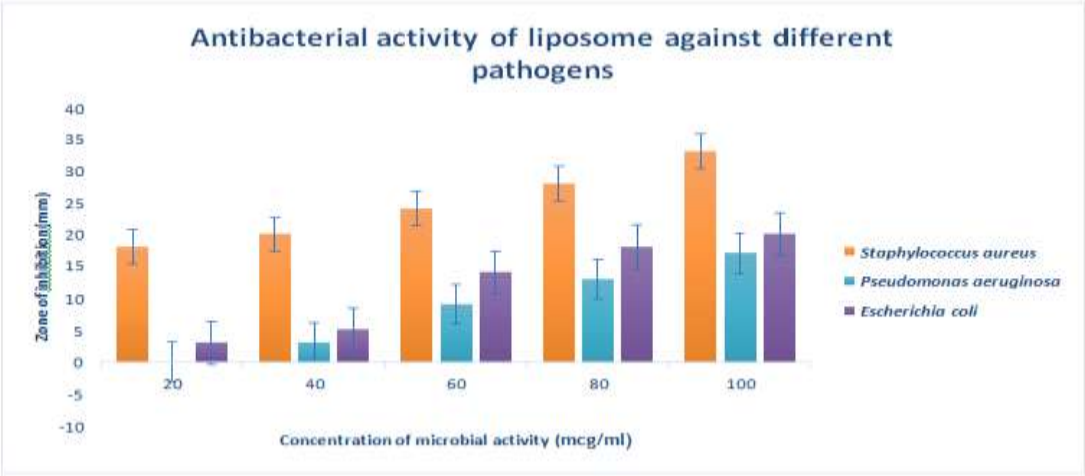

Fig 3 Antimicrobial activity of liposomal gel against bacterial species

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IV. DISCUSSION

The liposome showed antibacterial activity against all three pathogens. It showed greatest efficacy against S.aureus followed by E.coli and P.aeruginosa. The surface morphology of the liposome was studied by FESEM analysis. The liposomal vesicles were within the range of 4 to 30µm in diameter and it was thickly and homogenously dispersed. Liposomes have generated a great deal of interest. These tiny lipid vesicles can bind to, fuse with or be taken up by cells thus serving as a sort of microscopic Trojan horse, carrying drugs to places within the body, where they would not otherwise reach. In recent years, liposomes have become increasingly important as vehicles for delivery of active compounds which are used for topical management. Literature reveals that topically applied liposomal products, in comparison to existing products, enhance local effect, reduce systemic effects, optimize dosage, provide prolonged release action and be cosmetically more acceptable.

The current study was focused on developing a antibacterial liposomal gel consisting of active compounds namely quercetin and Azaelic acid which is commonly found in Centella Asiatica and Barley.The antibacterial activity of ethanolic extract of Centella Asiatica and Barley was initially studied through agar well diffusion and the results obtained showed that both Centella Asiatica and Barley had good antibacterial activity against S.aureus, P. aeruginosa and E.coli. On further analysis of the literature, it was evident that certain active compounds were responsible for the antibacterial activity, Therefore the active compounds namely quercetin and Azaelic acid was chosen and a liposome was prepared using thin film hydration method. The antibacterial activity of the Liposome against S.aureus, P.aeruginosa and E.coli was evaluated by agar well diffusion and the results showed promising effect of the liposome against the pathogens. Therefore, the liposome that was formulated showed potential to act as a promising antibacterial agent.

V. CONCLUSION

In this study a liposomal gel was formulated using Quercetin (1%) and Azelaic acid (3%). The liposome showed promising antibacterial activity against S.aureus, E.coli and P.aeruginosa. Hence further investigations such as cell line study needs to be carried out to study the toxicity of the liposomal formulation after which this liposomal formulation can be used for topical application to treat bacterial infections caused by the above- mentioned pathogens.

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