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RESEARCH ARTICLE

IN-VITRO ANTIBACTERIAL ACTIVITY OF *LACTOBACILLI* METABOLITES LOADED HYDROGEL FORMULATIONS AGAINST *PSEUDOMONAS AERUGINOSA*

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ABSTRACT
Objective: *Pseudomonas aeruginosa* is an opportunistic pathogen that may cause serious infections, ranging from localized skin infections to life-threatening systemic diseases. It is one of the resistant bacteria which lead to failure in the treatment. New treatment strategies are urgently needed for the treatment of infections caused by resistant bacteria. Researchers are interested in *Lactobacillus* species because of their antimicrobial effective metabolites. The aim of this study was comparing the antibacterial activity of lactobacilli metabolites loaded chitosan and Pluronic F127 hydrogel formulations.
Methods: Metabolites loaded hydrogel formulations were prepared by adding 20 µl of supernatant into the polymer solutions. The antimicrobial activities of two hydrogel formulations were investigated against *P. aeruginosa* using Agar Spot Method. Metabolites loaded chitosan hydrogel formulations were found to be effective against *P. aeruginosa*.
Results: No antibacterial activity was observed for metabolites loaded Pluronic F127 hydrogel formulations. When we compare these two hydrogel formulations, we observed that hydrogel formulation prepared by using chitosan released effective metabolites but Pluronic F127 hydrogel formulation couldn't release antibacterial effective metabolites.
Conclusion: The antibacterial effect of metabolites loaded chitosan hydrogel formulation suggests that this formulation could be used as an alternative treatment option in *P. aeruginosa* skin infections. However, further investigations are needed to develop a pharmaceutical product.
Keywords: Antimicrobial activity, bacterial metabolites, chitosan, *Lactobacillus sp*, Pluronic F127.

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INTRODUCTION
Antibiotic resistance is a serious public health problem that threatens the all over the world. Day by day, new multi-resistant microorganisms have increasingly been reported from different regions of the world. Failure in the treatment of these resistant bacteria leads scientists to investigate alternative treatment options[1,2,3]. *Lactobacillus* genus, which is grouped among lactic acid bacteria, comprises of Gram-positive, facultative anaerobic or microaerophilic, non-spore-forming, rod-shaped bacteria. These bacteria produce lactic acid as the major metabolic end-product of carbohydrate fermentation. Some groups of them also produce various amounts of compounds such as acetic acid, carbon dioxide, and ethyl alcohol[4,5]. Lactobacilli are the normal flora members of the human body and they commonly found in the mouth, vagina, and intestines[6]. Owing to their metabolites and antagonistic effects, they protect the human body from pathogens[7]. In a healthy premenopausal woman, the dominance of lactobacilli in the vagina is protective against the colonization of pathogenic microorganisms[8]. In recent years, the number of studies reporting the beneficial effects of flora microorganisms on human health has increased. The genus *Lactobacillus* has taken considerable attention because of their antimicrobial, immune modulatory, anti-inflammatory and anti-carcinogenic activities. These microorganisms have been the subject of many investigations to find out whether they can be used as an alternative for the treatment of diseases[3,9-12]. Chitosan, a deacetylated derivative of chitin, is a cationic polymer that consists of a long chain formed by a combination of β-1, 4-D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) by linking with β (1–4) glycosidic bonds. As a natural polymer, it demonstrates

incredible properties such as biocompatibility, biodegradability, non-toxicity, antimicrobial activity, and adsorption capability. Therefore, it has been extensively studied for its use in many fields. Chitosan is also widely used as a wound dressing due to its excellent hemostatic properties and antimicrobial activities. Its antimicrobial activity has been explained by several theories. The most accepted hypothesis is that its action on bacteria would occur by the loss of its intercellular components. Due to its positive charge, it binds to the bacterial membrane causing a change in permeability and the output of its intercellular components, thus leading to cell death. The antimicrobial activity of chitosan was observed against a wide variety of microorganisms, including fungi, algae, and bacteria, being more active against Gram-positive bacteria than Gram-negative ones[13-15].

Poloxamers, poly (ethylene oxide)–poly (propylene oxide)–poly (ethylene oxide) triblock copolymers (PEO–PPO–PEO), are synthetic polymers with thermo reversible behavior in aqueous solutions and widely used in pharmaceutical systems. They are available in different molecular weights with commercial name pluronics. Among pluronics, F127 is widely explored for drug delivery due to its thermo-reversible gelation properties, ability to solubilize hydrophobic solutes and to form micellar structure (incorporate both hydrophilic and hydrophobic drugs) and extending drug release and non-toxicity. Moreover, Pluronic F127 gels have also exhibited improvement in wound healing by stimulating expression of vascular epithelial growth factor and (VEGF) and transforming growth factor (TGF-β1) [16,17].

The aim of this study was comparing the antibacterial activity of vaginal lactobacilli metabolites loaded chitosan and Pluronic F127 hydrogel formulations against *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Lactobacillus sp. Isolates

In this study, metabolites of the seven *L. gasseri*, two *L. crispatus*, and one *L. helveticus* vaginal isolates which were previously identified in terms of species-level by analyzing the 16S rRNA gene sequence and found effective against some test bacteria were used[3].

Obtaining metabolites

Lactobacillus isolates grown on Rogosa Agar for 24-48 hours were inoculated into tubes containing De Man-Rogosa Sharpe Broth (Merck, Germany), at 37ºC, under anaerobic conditions for 72 hours. After the incubation period, cells were removed by centrifugation (12000 g, 10 min, 4 ºC). The cell-free supernatants were filter-sterilized (0.45 µm pore size) (Minisart, Germany)[3,18].

Preparation of Chitosan and Pluronic Hydrogels

Chitosan was dissolved in 1% acetic acid solution at room temperature with continuous mechanical stirring to obtain a 1% (w/w) solution[13]. Metabolite loaded chitosan hydrogel was made by adding 20 µl of supernatant into the chitosan solution. Pluronic F127 hydrogel (20 wt%) was prepared by gradually adding under magnetic stirring at 4 ^{0}C Pluronic F127 powder to distilled water. The temperature was maintained at 4^{0}C until complete dissolution of Pluronic F127 powder[19]. Metabolite containing Pluronic F127 hydrogel was prepared by gradually adding 20 µl of supernatant into the Pluronic F127 solution under magnetic stirring at 4^{0}C.

Antimicrobial activity test

The antimicrobial activities of the metabolite loaded hydrogel formulations were investigated against *Pseudomonas aeruginosa* ATCC 27853 using Agar Spot Method[20]. Test bacteria culture grown for 18-24 hours, adjusted to McFarland 0.5, were inoculated onto Mueller-Hinton Agar plate with sterile swabs. 20 µl of each of metabolite loaded hydrogel formulations and supernatant were dropped equidistantly onto media inoculated with test bacteria. Lactobacilli supernatants as positive control were used. All the plates were incubated at 37ºC for 18-24 hours, under aerobic conditions. After incubation, the growth inhibition in Agar Spot Method was considered as a positive effect.

RESULTS AND DISCUSSION

Five *L. gasseri*, one *L. crispatus*, and *L. helveticus* metabolites loaded chitosan hydrogel formulations were found to be effective against *P. aeruginosa*. Although chitosan alone had antibacterial activity, it was found that the metabolites loaded chitosan formulations showed better antibacterial activity. Besides that, Pluronic F127 hydrogel formulation didn't provide antimicrobial activity as metabolites couldn't release from hydrogel formulations (Figure 1).

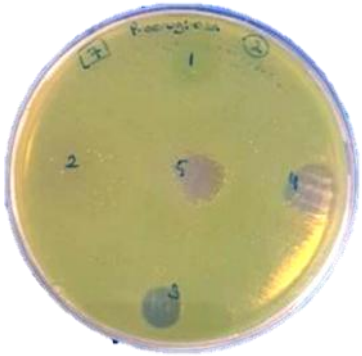

Figure 1: Antimicrobial activity of metabolite loaded hydrogel formulations against *Pseudomonas aeruginosa* ATCC 27853.

(1**:** Pluronic F127, **2:**Metabolite loadedPluronic F127 hydrogelformulation, **3:**Chitosan, **4:** Metabolite loaded chitosan hydrogel formulation, **5:**Supernatant of vaginal *L. Gasseri* isolate)

This is thought to be due to the high viscosity of the Pluronic F127 hydrogel with a concentration of 20%. When we compare these two hydrogel formulations, we observed that hydrogel formulation prepared by using chitosan released effective metabolites and therefore antimicrobial activity could be observed.Similar results were obtained in the study performed by Boonlai *et al.*,[21]. Among the hydrogels prepared with 16%, 18% and 20% (w/w) Pluronic F127, the slowest drug release was achieved with the hydrogel formulation prepared with 20% Pluronic F127. The release results werec onsistent with the microstructural features of the gel matrices. The matrix that possessed the gel barrier with high strength led to

slow drug release. In addition, the gel of 20% Pluronic possessed the smallest pore diameter and the highest pore density; therefore, the transportation rate of the drug passing through the gel network was thes lowest. In another study performed by using three P407 hydrogel formulations with concentrations at 16%, 20% and 24% (w/w) also showed that concentration of Pluronic had a significant influence on the release of drug from hydrogel formulation[22]. The concentration of Pluronic was inversely associated with ther elease of drug, i.e., the higher the Pluroniccon centration, the lower the release percentage of drug. The release rate was appreciably decreased with Pluronic concentration increasing. This indicates that P407 retarded the release of drug from formulations. This could be interpreted by viscosity of hydrogel formulations.

CONCLUSION

In conclusion, the antibacterial effect of vaginal *Lactobacillus* metabolites loaded chitosan hydrogel formulation suggests that effective metabolite loaded this formulation could be used in the treatment of *P. aeruginosa* skin infections. However, further investigations are needed to develop a pharmaceutical product.

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CONFLICT OF INTEREST

The author(s) confirm that they have no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors contributed to the study design, data collection, data analysis, data interpretation, and manuscript preparation.

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