

Antibacterial and Biofilm Inhibitory Effects of Rutin Nanocrystals

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Abstract: Rutin is a nontoxic bioactive agent that extensively exists in fruits and vegetables with several therapeutic properties, essentially attributed to its effective anti-inflammatory and antioxidant actions. Rutin possesses poor bioavailability and low aqueous solubility, limiting its therapeutic applications. The expansion of nanoparticulate systems is one of the technical routes to enhance the solubility and bioavailability of rutin. The present study aimed to examine the antimicrobial effects of rutin nanocrystals (RNs). A minimum inhibitory concentration (MIC), biofilm, and attachment inhibitory effects of RNs were evaluated for several bacterial strains compared with bulk rutin. It was shown that the aqueous dispersion of RNs was much more effective than rutin against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus mutans*, *Klebsiella pneumonia*, and *Acinetobacter baumannii*. The results revealed that rutin's antimicrobial activity improved by reducing particle size up to the nano-sized range in the MIC test. RNs and rutin didn't show any biofilm inhibitory effect.

Keywords: rutin; nanocrystals; antimicrobial activity.

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1. Introduction

Curcumin, rutin, quercetin, and resveratrol are well-known natural compounds that are helpful in biomedicine due to their excellent safety, cost-effectiveness, and broad biological impacts [1-4]. Flavonoids' many pharmacological/biological effects, including ROS scavenging, anti-inflammatory, immunomodulatory, antibacterial, and cancer-fighting properties, have made them excellent prospective medicines [4-6]. As a lipophilic component, Rutin is soluble in organic solvents such as pyridine, methanol, and ethanol [7, 8]. In addition, rutin has limited stability and bioavailability, and these physicochemical characteristics are mostly attributable to its low water solubility [8, 9]. Rutin has significant antioxidant capabilities due to its reducing characteristics on various oxidizing species such as superoxide, peroxy, and hydroxyl radicals [10]. Besides, it has pharmacological properties such as anticancer, antibacterial, and anti-inflammatory properties [11-13]. Rutin has been demonstrated to prevent the prooxidant effects of certain flavonoids by accelerating the production of oxygen radicals [14]. Rutin could be useful in biomedical applications due to its nontoxic chemical structure [15, 16].

Many investigations have looked at flavonoids' anti-biofilm potential, including rutin's biofilm inhibitory efficacy against drug-resistant biofilm-producing bacteria [17-21].

Rutin has been shown to have potent antibacterial properties against a wide range of bacteria [22]. Rutin has been shown to have strong antibacterial, and antifungal action against pathogens such as *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Candida krusei* and have been reported [23]. According to the studies, rutin displays effective actions against gram-negative and gram-positive bacteria. The study on the antibacterial effect of flavonoids is conflicting, perhaps because of inter-and intra-assay differences in susceptibility examinations. On the other hand, several studies revealed a relation between flavonoid structure and antibacterial activity. Furthermore, several studies have found an association between flavonoids' action and the inhibition of DNA gyrase. Furthermore, it has been suggested that (–)-epigallocatechin gallate and sophoraflavone G inhibit the function of the cytoplasmic membrane and that licochalcones A and C prevent metabolism of energy [21].

Nanotechnology uses innovative techniques to modify matter at an atomic or molecular level, and it has found widespread use in all disciplines of research [24, 25]. In the field of biomedicine, developing drug delivery via nanoparticulate systems has resulted in a marked rise in technological innovation [26, 27]. Nanomedicine has opened up new possibilities for the pharmaceutical industry's future [28, 29]. Increased bioavailability of active substances is a benefit of nanotechnology-based medication nanoformulations [30].

The present study aimed at investigating the antibacterial and biofilm inhibitory effect of RNs against *S. aureus*, *P. aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus mutans*, *Klebsiella pneumonia*, *A. baumannii*. Furthermore, bacterial cell attachment was studied to evaluate the effect of RNs on the adherence of bacteria to the surfaces.

2. Materials and Methods

2.1. Preparation of rutin nanocrystals.

RNs were prepared in our previous study via the ultra-sonication method and solvent evaporation process [31] (Figure 1). The prepared RNs displayed a mean particle size of 75 ± 0.16 nm. Moreover, the results of SEM (Figure 1a) showed that the RNs had aggregated quasi-spherical, uniform small nanoparticles (Figure 1b). The RNs displayed crystalline states according to peak intensities (Figure 1c). The FTIR spectra evaluated the chemical composition of RNs, and there was no difference between the absorption bands of the bulk rutin and RNs (Figure. 1d) [31].

2.2. The antibacterial effects of bulk rutin and RNs.

Bacterial isolates used in this study were isolated from Tabriz, Iran, during 2020. MRSA isolates were primarily screened by the resistance to cefoxitin disk (30 µg) according to Clinical & Laboratory Standards Institute (CLSI) guidelines.

The antibacterial effects of RNs and bulk rutin were assessed by determining minimum inhibitory concentration (MIC) via the broth microdilution technique in Cation-Adjusted Mueller–Hinton Broth (CAMHB). The broth microdilution method was performed according to protocols of the Clinical & Laboratory Standards Institute (CLSI) [32]. The lowest

concentration of a compound considered as MIC completely inhibited the bacterial growth, which was recognized by the unaided eye.

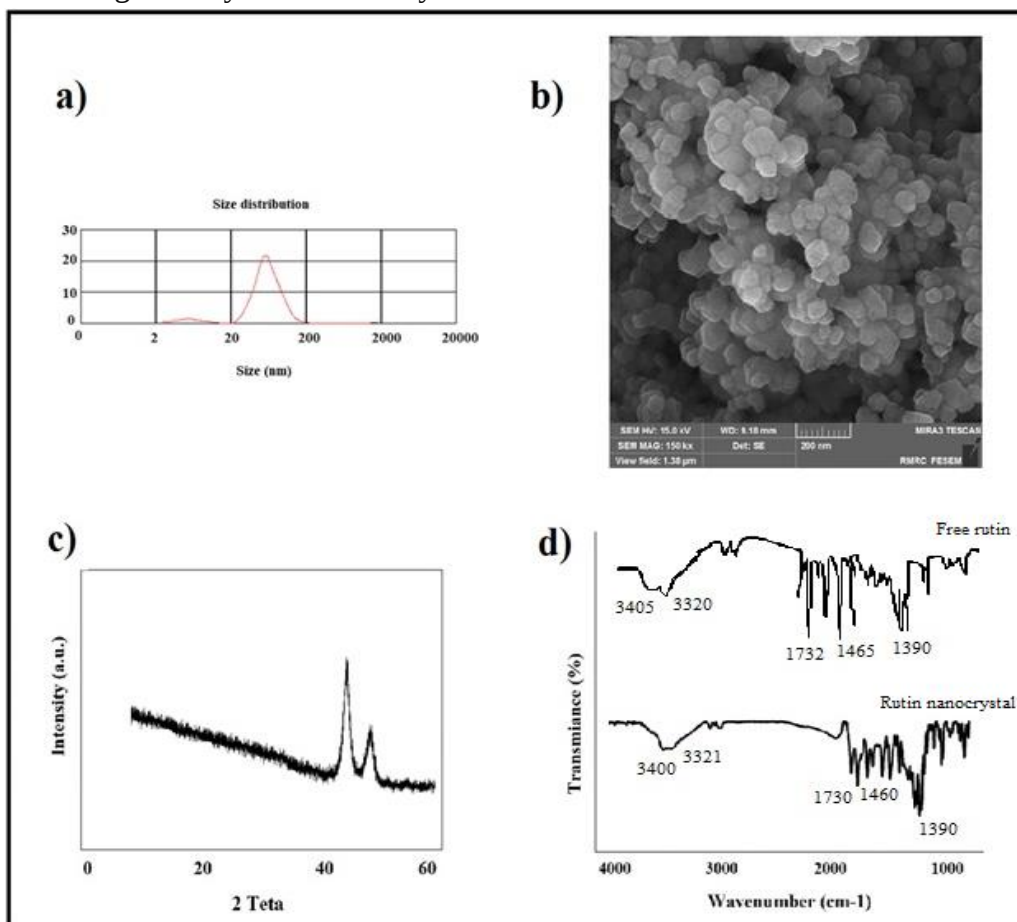


Figure 1. Physicochemical characterization of the RNs; (a) The particle size distribution, (b) Image of SEM, (c) The pattern of XRD, and (d) FTIR peak of RNs and bulk rutin. (Adapted from [31], which is under the Creative Commons Attribution License).

2.3. Biofilm Inhibitory effect of RNs and bulk rutin.

The antibiofilm effect of RNs and bulk rutin were assessed via minimum biofilm inhibitory concentration (MBIC) determination. First, 100 μ L from 0.5 McFarland bacterial suspension was added to the microplate wells containing 100 μ L of TSB. The generation of biofilm of bacteria was stimulated by the addition of 0.25% glucose to TSB. TSB. The bacterial suspension was cultured at 35°C for 24 hours in a microplate. Following this, the serial concentrations of RNs and bulk rutin were added to the wells, and it was incubated for 20 hours at 35°C. The content of the wells was removed, and wells were washed by sterile water and violently shaken. The OD at 650 nm was assessed on a microplate before and after incubation for 6 hours at 35°C. The MBIC was measured as the lowest level of a compound that led in a variation of OD 650 at or below 10% of the mean of OD of two positive control wells [33].

2.4. The effect of RNs and bulk rutin on adhesion of bacteria to surfaces.

Sub-inhibitory concentrations (0.5 MIC) of RNs and bulk rutin were applied to the wells of a 96 well microplate to assess the influence of RNs and bulk rutin on biofilm development. Fresh TSB (1:20) with and without RNs and bulk rutin were used to dilute overnight grown cultures of tested bacteria. After adding the suspension to the wells, the microplates were incubated for 24 hours at 35°C. After the content of wells was removed,

generated biofilms were rinsed with sterile water and fixed with methanol for 5 minutes. After drying the plates, it was stained for 15 minutes with a crystal violet solution. The wells were three times then the generated biofilms were solubilized via acetic acid (33%). After, the OD of wells was measured at wavelength 492 nm via the microplate readers. The wells with TSB without bacteria were considered as a negative control.

2.5. Statistical analysis.

The data were analyzed by the one-way ANOVA in the SPSS software version 20. Values of $P < 0.05$ were considered statistically significant.

3. Results and Discussion

Rutin didn't show any significant antibacterial effect on all examined bacteria in the tested concentration. The RNs inhibited all examined bacteria at different levels. The RNs showed more antibacterial effect against Gram-negative bacteria compared with Gram-positives. The MICs spectrum of RNs was 1024-4096 $\mu\text{g/mL}$ (Figure 2).

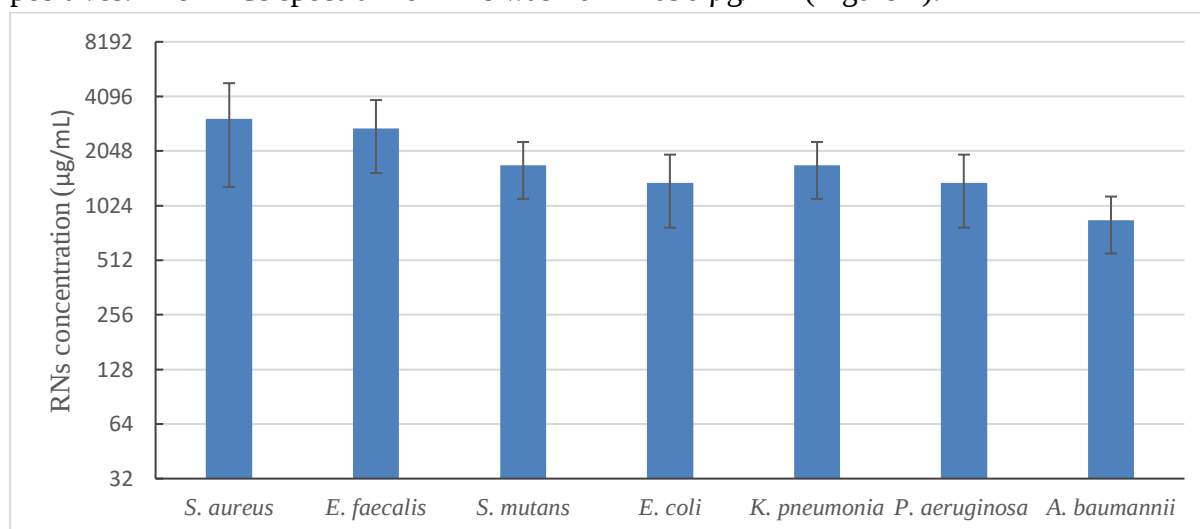


Figure 2. The inhibitory effects of RNs on bacterial isolates.

Several studies have addressed that the drug-loaded NPs may interact with microbial cells due to two probable mechanisms: (a) they may fuse with surfaces of bacterial cells and increase the permeability of cells to agents, (b) they also may be absorbed by the cell wall and act as a depot to release of antibacterial drug [34]. Bharathi *et al.* designed a rutin-containing chitosan-coated zinc oxide (CS-ZnO) nanocomposite. The antibacterial activity of the nanocomposite was evaluated against different pathogenic Gram-negative and Gram-positive bacteria, and it has been displayed that the nanocomposite was more efficient against Gram-negative bacteria [35]. When metal oxide is coated with organic polymers, the optical and structural properties of nanoparticles show remarkable changes. Bharathi and coworkers used a facile greener method to prepare chitosan (CS) coated iron oxide nanocomposite containing rutin. The prepared nanoparticles displayed antibacterial activity against Gram-negative and Gram-positive bacteria [36].

Antibacterial agents are commonly efficient over planktonic bacteria may not be effective against biofilm form [37]. The application of an appropriate delivery system for antibacterial agents can be beneficial in treating infections caused by biofilm form [38, 39].

The generated biofilm is difficult to eliminate due to its resistance to most antibacterial drugs. In some cases, removal of infected tissues or apparatuses is essential for eradicating biofilm-related infections that are uncomfortable and, in some cases, impossible [40]. As a result, eliminating biofilms may need the use of new formulations or compounds that increase biofilm sensitivity to antimicrobial drugs [41]. In the present study, RNs and rutin didn't show any inhibitory effect on the preformed biofilm. To further investigate the effect of rutin nanoparticles on biofilm, its effect can be examined in combination with other antibiotics. Deepika *et al.* showed the enhanced biofilm inhibitory effect of rutin (200 µg/mL) in combination with gentamicin (2.5 µg/mL) [18].

Bacterial cell attachment was studied to evaluate the effect of RNs at sub-MICs levels (0.5 MIC) on the adherence of bacteria to the surfaces. It was observed that bacterial attachment was significantly decreased after 12 h of incubation in the presence of sub-MICs levels of RNs than rutin and controls (well-containing bacteria grown in the absence of RNs) (Figure 3).

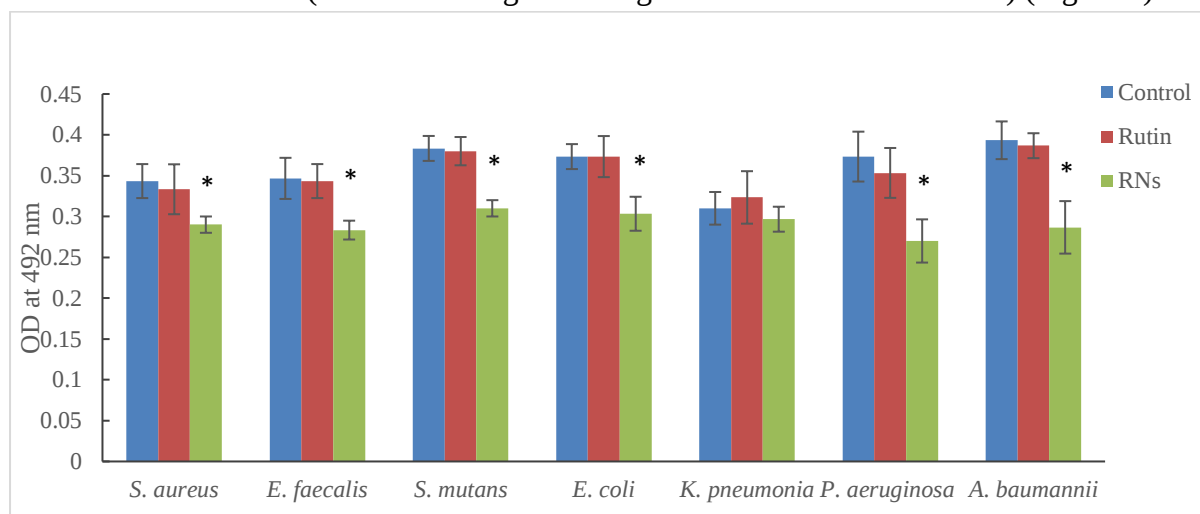


Figure 3. Effect of sub-inhibitory concentrations (0.5 MIC) of rutin and RNs on the attachment of bacteria to plastic surfaces. Bacteria were grown in the absence of nanoparticles were considered as control. * shows a statistically significant difference ($p < 0.05$).

Adherence is critical in bacterial colonization on different surfaces [42, 43]. The present study results showed that RNs reduced bacterial adherence at sub-MIC levels significantly in all bacteria except *K. pneumonia* ($P < 0.05$).

4. Conclusions

Generally, nanoparticles can be an applicable drug delivery approach with favorable characters in treating infections. The results of our study indicate that RNs have more antibacterial activity compared with rutin. Rutin and RNs didn't show any biofilm inhibitory effect. RNs decreased adherence of bacteria at sub-MIC levels.

These findings may be beneficial for applying RNs against Gram-negative and Gram-positive bacteria. However, more studies are needed to evaluate the antibacterial and biofilm inhibitory effects of rutin nanoparticles combined with other antibiotics. The *in vivo* studies on the biological activities and side effects of RNs are necessary for this application.

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Ethical Approval

The Ethics Committee of the Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1397.510) approved this study.

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Conflicts of Interest

The authors did not disclose any conflict of interest.

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