Hindawi
International Journal of Polymer Science
Volume 2021, Article ID 5682182, 12 pages
https://doi.org/10.1155/2021/5682182

Research Article

Improved Antibacterial Activity of Water-Soluble Nanoformulated Kaempferol and Combretastatin Polyphenolic Compounds

Y. Santhosh Kumar,[1] Langeswaran Kulanthaivel,[2] G. S. Hikku,[1] R. Saravanan,[1] Thangavelu Lakshmi,[3] C. Kirubhanand,[4] Murugesan Karthikeyan,[5] P. Vijayalakshmi,[6] and Gowtham Kumar Subbaraj[1]

[1]*Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education (Deemed to be University), Kelambakkam, 603 103 Tamil Nadu, India*
[2]*Molecular Oncology Lab, Department of Bioinformatics, Alagappa University, Karaikudi, 630 001 Tamil Nadu, India*
[3]*Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (Deemed to be University), Chennai, India*
[4]*Department of Anatomy, All India Institute of Medical Sciences, Nagpur, Maharashtra 441108, India*
[5]*Department of Microbiology, Faculty of Medicine, Quest International University, Malaysia*
[6]*Department of Pharmacology, Asan Memorial Dental College and Hospital, Chengalpattu, Chennai, 603105 Tamil Nadu, India*

Correspondence should be addressed to Gowtham Kumar Subbaraj; gowtham_phd@yahoo.com

Received 29 June 2021; Revised 29 September 2021; Accepted 4 October 2021; Published 27 October 2021

Academic Editor: Bernabé L. Rivas

Kaempferol and combretastatin are polyphenolic compounds derived from plant sources which are known for their antibacterial activity. However, owing to their large size and water insolubility, their antibacterial activity is limited. In this context, the present study focused on the nanoformulation of kaempferol (NF-k) and combretastatin (NF-c) and their influence on water solubility and antibacterial properties. The NF-k and NF-c were prepared using the solvent evaporation method and were thoroughly characterized for evaluating the morphology, molecular vibrations, size, etc. Based on the results, it is observed that the pristine forms of kaempferol and combretastatin drugs get nanoformulated and completely soluble in water. Using particle size analyzer, the particle sizes of NF-k and NF-c were estimated as 334 nm and 260 nm, respectively, which are very fine compared to pristine kaempferol and combretastatin (5193 nm and 1217 nm, respectively). The molecular vibrations that exist in NF-k and NF-c were confirmed by the Fourier transform infrared spectra, where the nanoformulated drug showed lower intensities than the pristine form of kaempferol and combretastatin. The drug release kinetics of the nanoformulated drugs were carried out using the dialysis membrane method and were compared with their pristine forms. Owing to the size effect, the NF-k and NF-c release up to 50% of the drug in a sustained manner till 50 h showing twofold higher concentration than the control where it released 25%. The antibacterial activity was assessed by measuring the optical density at 600 nm using UV-vis spectrophotometer and displayed significant activity against gram-positive *Staphylococcus aureus* strain. The mechanisms behind the antibacterial activity of NF-k and NF-c were discussed in detail. The activation of ATP-dependent efflux pump system and the blockage of porin channels could be the cause for the bactericidal activity. Our understanding of efflux pumps and their role in antibacterial activity is still in its early stages. No studies have been performed to date using nanoformulations of kaempferol and combretastatin to investigate their roles. This complicates the determination of the exact mechanisms acting against bacterial growth when using nanoformulation drugs. Our increasing knowledge of water-soluble nanoformulation drugs and their roles in reduced bacterial activity will pave the way to developing effective treatments in the future.

1. Introduction

Bacterial diseases are an essential reason for persistent and infectious lethality. Antibiotics seem to be the preferential treatment strategy for bacterial diseases in the interest of their low-cost adequacy and incredible results. Nevertheless, a few investigations have given absolute proof that the inescapable utilization of antibiotic agents has prompted the development of multidrug-resistant bacterial strains. Previous findings have revealed that *Enterobacteriaceae* microbes express a resistance gene known as NDM-1 [1]. A significant group of antibiotics used have limited focus on translational machinery, DNA replication machinery, and cell wall synthesis. Unfortunately, microbial resistance can be created against any one of these methods of activity. The mechanism of microbial resistance comprises the expression of enzymes that change or else corrupt antibiotics, such as aminoglycosides and β-lactamase [2], alterations of cell components, like the ribosomes in tetracycline resistance, cell wall in vancomycin resistance [3], and articulation of efflux pumps, which give synchronous resistance opposing to different antibiotics [4]. To counteract multidrug resistance developed by the bacterial strains, nanoparticles are employed. However, the use of nanoparticles is inappropriate due to their method of action. The indirect contact with the bacterial cell wall facilitates antibacterial activity rather than infiltrating into the cell, which declines its commercial viability. In this context, more attention has been focused on developing novel and exhilarant nanoparticles with antibacterial activity.

Kaempferol is a flavonoid present in many vegetables, fruits, and herbs, including grapes, tomatoes, grapes, tea, and broccoli [5]. Kaempferol shows several biological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticarcinogenic [6–10]. Combretastatin A4 is a natural stilbenoid phenol derived from South African bushwillow (*Combretum caffrum*). Combretum species have antitumor and antimicrobial activities [11, 12]. They are extensively used to treat syphilis, abdominal pains, conjunctivitis, diarrhea, toothache, peptic ulcer, dysentery, jaundice, skin, heart, and cancer diseases [13]. However, very few studies have been reported on the antibacterial activity of nanoformulated kaempferol and combretastatin. Hence, the present research is aimed at formulating the kaempferol and combretastatin in nano regime and studying their structural, drug release kinetics, and antibacterial activity against gram-positive bacteria.

2. Materials and Methods

2.1. Materials Required. Kaempferol (≥97.0% pure), combretastatin (≥97.0% pure), and sodium dodecyl sulfate were obtained from Sigma-Aldrich. Ethanol (99% pure) was purchased from Changshu Chemicals Co., Ltd., and Luria-Bertani broth was purchased from HiMedia. N-Hexane (95% pure) and dimethyl sulphoxide (DMSO, 99% pure) were purchased from SRL laboratories Pvt. Ltd. *Staphylococcus aureus* was obtained from the research collection at Chettinad Hospital and Research Institute.

2.2. Nanoformulation of Kaempferol (NF-k). The kaempferol was nanoformulated using the solvent evaporation method (Figure 1) [14]. Briefly, kaempferol was dissolved in ethanol to achieve a final concentration of 5 mg/ml. To the above solution, a known quantity of 0.2% sodium dodecyl sulfate solution (SDS) was added and stirred. Under constant stirring, hexane (antisolvent) was added to the mixture where the ratio of solvent and antisolvent was maintained at 1 : 20. Then, the mixture was continuously stirred for 3 h at 150 rpm and held at 30°C overnight. After complete evaporation of the solvent, the powder formed was crushed. It was subjected to various physiochemical characterization studies to determine the morphology, size, and molecular vibrations in NF-k.

2.3. Nanoformulation of Combretastatin (NF-c). The combretastatin was also nanoformulated using the same solvent (Figure 2) evaporation method. Briefly, in ethanol, 5 mg/ml concentration of combretastatin was dissolved, and to this solution, 0.2% SDS was added. Under constant stirring, hexane (antisolvent) was added in a dropwise manner maintaining the ratio of solvent and antisolvent as 1 : 20. The mixture was continuously stirred for 3 h at 150 rpm and kept at 30°C overnight. After evaporating the solvent, the powder formed was crushed by mortar and pestle and subjected to various physiochemical characterization processes.

2.4. Characterization Techniques. Particle size distributions of the NF-k and NF-c were determined using the dynamic light scattering (DLS) technique (Malvern Zetasizer, USA). The particle size distributions of the nanoformulated drugs were recorded by dissolving 100 μl of prepared nanoformulation into 900 μl of solvent. To elucidate the molecular vibrations that exist in the pristine and nanoformulated drugs, fourier transform infrared (FTIR) spectroscopy was used with attenuated total reflection mode. Adequate quantity of sample was placed on the crystal surface, and the spectra of NF-k and NF-c were recorded in the range 4000 cm^{-1}–500 cm^{-1} (Model BRUKER-ALPHA, Germany) using OPUS software. FTIR spectroscopy characterizes the characteristic functional groups present in the test samples originated from the different molecular bonds unique vibration and rotational energies. The morphology and size of the nanoformulated drugs were analyzed using scanning electron microscopic (SEM) images (HITACHI SU3500, Japan).

2.5. Solubility Test. The kaempferol and combretastatin both were insoluble in water in their pristine form. After nanoformulation, both drugs become water-soluble and were validated through the solubility test using Milli-Q water. Solubility test was performed by suspending 1 mg of NF-k and NF-c in 10 ml of Milli-Q water and compared with its pristine counterpart.

2.6. Drug Release Kinetic Assay. The drug release kinetic assays were carried out for NF-k and NF-c using the dialysis membrane method. Separately, the NF-k and NF-c were suspended in water. In contrast, the kaempferol and combretastatin were suspended in DMSO (as they are insoluble in water), placed in the dialysis membrane, and immersed

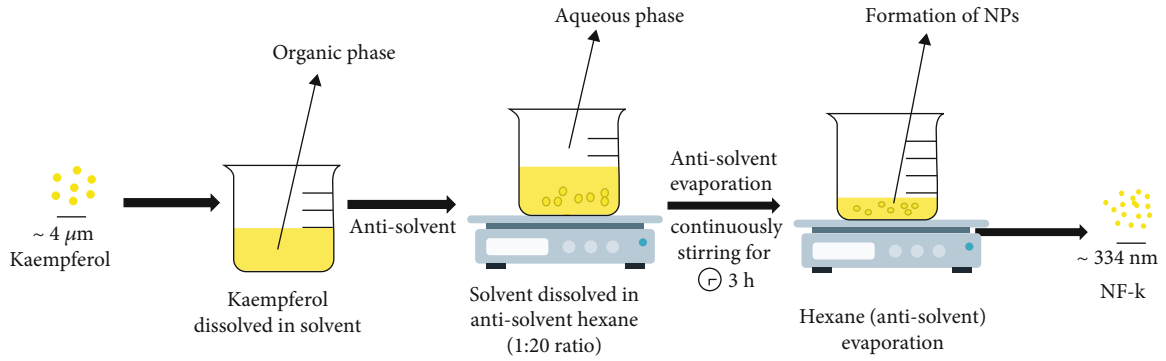

Figure 1: Schematic representation of synthesis of NF-k.

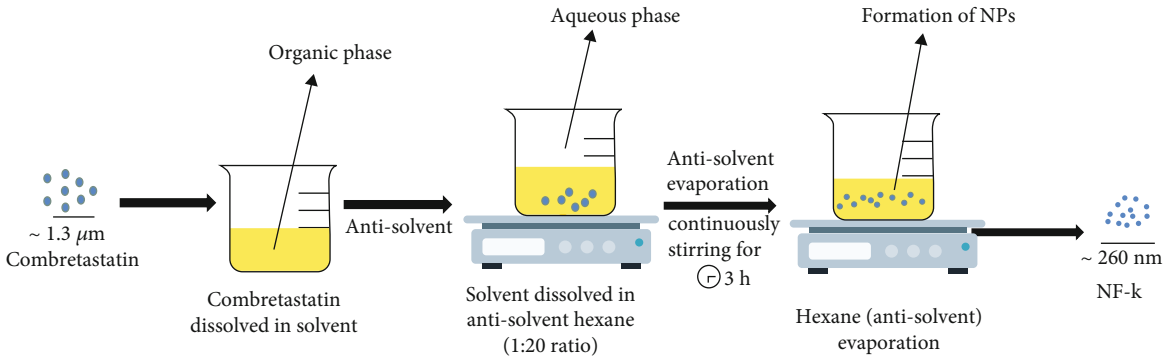

Figure 2: Schematic representation of synthesis of NF-c.

in a beaker containing 100 ml of phosphate-buffered saline (PBS). The beaker was kept on a magnetic stirrer at a speed of 250 rpm. In specified time intervals, an appropriate quantity of the samples were withdrawn from the beaker (replaced by fresh PBS medium). It was analyzed using a UV-vis spectrophotometer at 373 nm for kaempferol [15] and 289 nm for combretastatin [16]. The improvement in the absorption value was directly influenced by the quantity of drug released to the PBS solution.

2.7. Antimicrobial Activity. The antibacterial activity of NF-k and NF-c against gram-positive *Staphylococcus aureus* was assessed using UV-vis spectrophotometer. 10 ml of an overnight culture of *Staphylococcus aureus* strain was prepared. Afterward, to the 5 ml of LB broth, 0.3 ml of overnight culture was added. To the above bacterial culture, different concentrations (5 μl, 10 μl, 20 μl, 50 μl, and 100 μl) of NF-k and NF-c were added and incubated for 24 h, 48 h, and 72 h at 37°C. After the incubation period, the optical density (OD) measurements were recorded at 600 nm. The experiments were performed in triplicate, and the statistical analysis was carried out using Excel. SEM images were recorded to assess the morphology of *Staphylococcus aureus* before and after treatment with nanoformulated drugs. The NF-k and NF-c were mixed with the culture of *Staphylococcus aureus* separately and were incubated overnight at 37°C. After incubation, the samples were centrifuged, and the pellet was collected and washed thrice with PBS. After PBS wash, the samples were fixed with 2.5% glutaraldehyde solution for 2 h. Finally, samples were dropped on a sterile coverslip and air-dried at room temperature. All samples were coated with gold before recording the microscopic images.

3. Results

The kaempferol and combretastatin phytochemicals were nanoformulated through the solvent evaporation method and are subjected to various studies. The solubility of NF-k and NF-c prepared through the solvent evaporation method was evaluated by dispersing it in Milli-Q water. When nanoformulated drugs were added to the water, they were easily distributed, ensuring a hydrophilic nature (Figure 3(a)). In contrast, when pristine kaempferol and combretastatin were added to water, the drugs are found insoluble. They were floating over the surface of the water, validating the hydrophobic nature of the drug (Figure 3(b)). Therefore, it was substantiated that the nanoformulation converts the wetting behavior of the drug from hydrophobic to hydrophilic in nature.

To confirm the nano regime of prepared NF-k and NF-c compared to the micron-sized pristine drugs, DLS measurements were recorded. The nanoformulated drugs were ultrasonicated for 1 h before recording DLS measurement. The average particle size distributions of control kaempferol and NF-k were found as 5193 nm and 334.3 nm, respectively, having a polydispersity index (PDI) of 0.497 (kaempferol) and 0.207 (NF-k) (Figures 4(a) and 4(b)). Likewise, the particle size distributions of control combretastatin and NF-c were

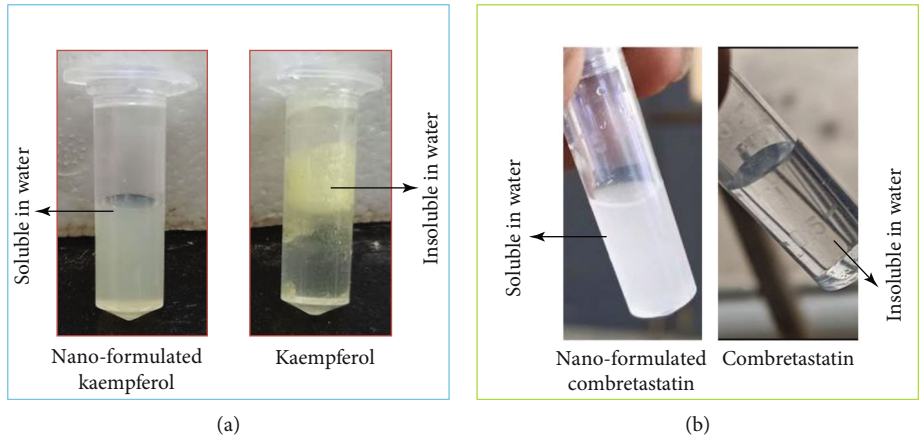

Figure 3: Photographs of drugs suspended in Milli-Q water. (a) Kaempferol and NF-k. (b) Combretastatin and NF-c.

1217 nm and 260.5 nm, respectively, with PDI 0.503 and 0.125 (Figures 4(c) and 4(d)). The obtained results showed that the particle size of the drugs reduced significantly with nanoformulation and have lower PDI below 0.25.

The FTIR spectra of the pristine kaempferol and synthesized NF-k were shown in Figure 5(a). The spectrum of pristine kaempferol exhibited bands at 3314 cm^{-1} and 3420 cm^{-1}, confirming the presence of the O-H stretch. The aromatic groups and C-O, C-H, C=C, and C=O were observed at 818 cm^{-1}, 1177 cm^{-1}, 1381 cm^{-1}, 1607 cm^{-1}, and 1666 cm^{-1}, respectively. The FTIR spectrum of NF-k also shows similar peaks at 822 cm^{-1}, 1177 cm^{-1}, 1381 cm^{-1}, 1615 cm^{-1}, and 1661 cm^{-1} ascribed to the aromatic groups and C-O, C-H, C=C, and C=O functional groups, respectively. Even though similar bands were observed in the spectrum of both pure kaempferol and NF-k, the transmittance intensity originated from NF-k was lower than the pristine form. This phenomenon was due to the presence of amorphous constituents in the prepared NF-k [17]. The presence of amorphous constituents improves the solubility of the drug in water than pristine kaempferol [18]. Moreover, the peaks obtained at 2918 cm^{-1} and 2851 cm^{-1} ascribed to C-H stretching vibration originated from the long-chain C of SDS substantiate that the SDS molecules were sorbed with kaempferol while nanoformulation [19].

The FTIR spectra of the pristine combretastatin and synthesized NF-c were shown in Figure 5(b). Likewise, the FTIR spectrum of control combretastatin exhibits a band at transmission peak 3510 cm^{-1}, representing the O-H stretch. C=C, C-H, and C-O were also observed at 1584 cm^{-1}, 1329 cm^{-1}, and 1125 cm^{-1}, respectively. The NF-c also showed similar peaks at 3509 cm^{-1}, 1580 cm^{-1}, 1329 cm^{-1}, and 1125 cm^{-1}, corresponding to O-H, C=C, C-H, and C-O, respectively, with lesser intensity. Here also, the reduction in peak intensity indicates an amorphous structure, which improves the solubility and dissolution rate of NF-c than pristine combretastatin. As with NF-k, NF-c possesses peaks at 2918 cm^{-1} and 2849 cm^{-1} originated from the C-H stretching of SDS that was sorbed along with NF-c during the preparation process.

The particle size and morphology of the NF-k and NF-c were assessed through SEM micrographs (Figures 6(a) and 6(b)). From the SEM image, it was inferred that the size of the NF-k was ranged within ~270 nm with spherical-shaped structures, whereas NF-c also shows near-spherical-shaped structures with a size range within ~250 nm. The nanoformulated drugs have a certain degree of monodispersity, which is an important prerequisite for drug development.

The drug release kinetics assay for the pristine and nanoformulated drugs was performed to elucidate the superiority of the nanoformulated drugs over pristine drugs (Figures 7(a) and 7(b)). The obtained results displayed a whopping 52% release of NF-k drug in 50 h, whereas the kaempferol releases only 25%. Likewise, 47% NF-c was released in 50 h, whereas combretastatin releases only 24% of the drug. From the results, it was inferred that the nanoformulated drugs release more of their constituent molecules than their pristine forms. Further, the drug release kinetic of NF-k was better than NF-c.

The antibacterial activity of NF-k and NF-c at different concentrations was assessed by recording the OD value at 600 nm against gram-positive *Streptococcus aureus* and was compared with the control (without drug). At concentrations 5 μl, 10 μl, 20 μl, 50 μl, and 100 μl, NF-k displays OD values as 0.4, 0.39, 0.38, 0.32, and 0.25, respectively, and NF-c displays OD values as 0.49, 0.47, 0.43, 0.45, and 0.36, respectively, after 24 h of incubation, whereas the control shows OD value of 0.52. Likewise, after 72 h, NF-k and NF-c display OD values of 0.3 and 0.45 for 5 μl concentration and OD values of 0.24 and 0.35 for 100 μl concentration (Figures 8(a) and 8(b)). The obtained results substantiated that the NF-k and NF-c displayed prominent antibacterial activity showing a considerable reduction in the OD value attributed to the inhibition in bacterial growth.

The SEM micrographs of the bacterial cells were recorded before and after treating with nanoformulated drugs and were shown as Figures 9(a)–9(c). From the micrographs of the control specimen, it was clear that the *Staphylococcus aureus* with spherical morphology having a high degree of growth (Figure 9(a)). However, after treating with nanoformulated drugs, the bacterial population was found to be declined ascribed to the antibacterial activity of the nanoformulated phytochemicals (Figures 9(a) and 9(b)).

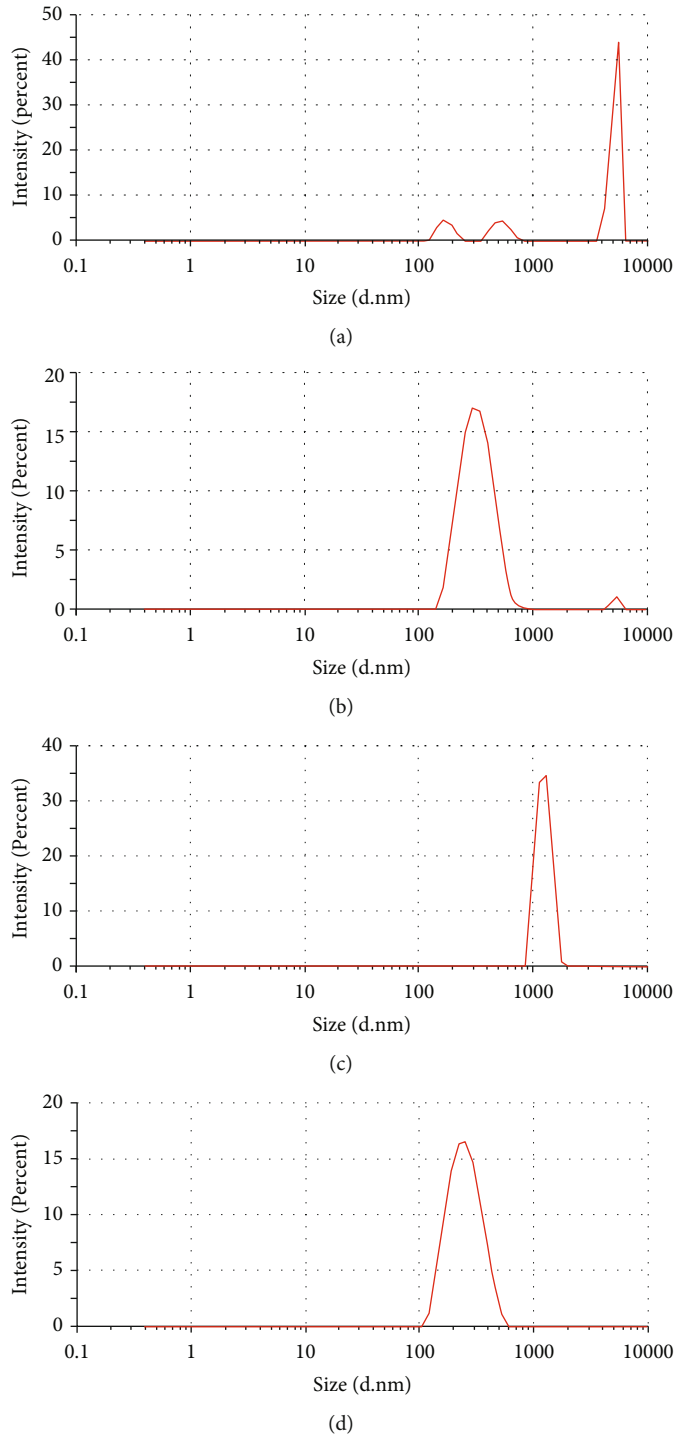

Figure 4: DLS measurements of (a) kaempferol, (b) NF-k, (c) combretastatin, and (d) NF-c.

4. Discussion

Both the kaempferol and combretastatin were insoluble in water [17] and were soluble in organic solvents like DMSO and ethanol. However, it has been reported that the poorly aqueous soluble drugs displayed better solubility through nanoformulation [18, 19]. Bhawana et al. [20] improved the solubility of the curcumin in water through nanoformulation that has more effective antibacterial activity than the curcumin suspended in DMSO. The improved activity was due to the reduction in particle size. The size was 40 nm for nanoformulated curcumin, which was much lower than the curcumin dissolved in DMSO, showing 800 nm. A 20-fold decline in the particle size resulted in improved diffusion and higher uptake by the cells, leading to better antibacterial efficacy of the nanoformulated drug.

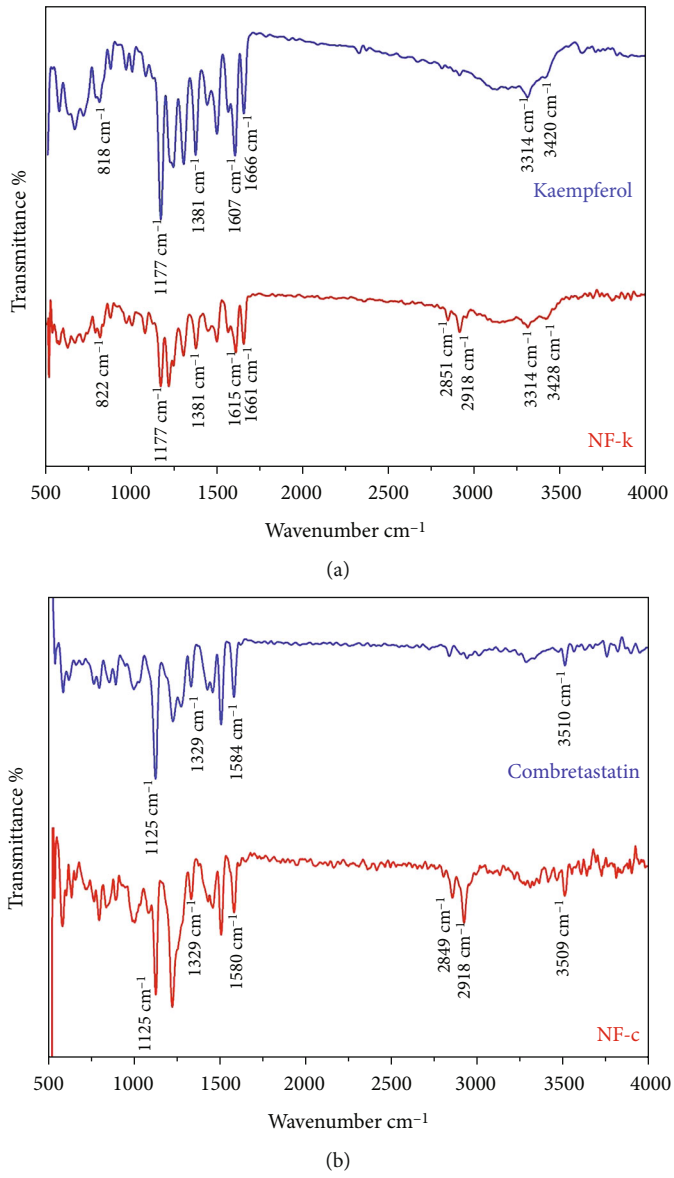

Figure 5: FTIR spectra of (a) kaempferol and NF-k and (b) combretastatin and NF-c.

Qian et al. [17] improved the bioavailability of kaempferol by 2.92 times that of pristine kaempferol through the preparation of nanosuspension. In the present study, the size of kaempferol and combretastatin wasreduced significantly through the solvent evaporation technique. From the obtained results, it was cleared that the sizes of kaempferol and combretastatin show ~15-fold and ~5-fold reduction, respectively, while nanoformulation. In the present work, the nanoformulated drugs displayed better solubility in water than their pristine forms.

As the particle size reduces, the surface energy increases, leading to the higher wetting ability of the nanoformulated drugs in the aqueous medium resulting in better solubility.

The solvent evaporation technique facilitates the formulation of drugs in a nanoregime compared to other literature techniques. Qian et al. [17] nanoformulated kaempferol through high-pressure homogenization and reduced the particle size from 1737 nm to 424 nm. Likewise, Zhao et al. [21] prepared the combretastatin nanoparticles. They reported the particle size of combretastatin nanoparticles which is 153 nm in diameter. Zhang et al. [22] formulated the combretastatin nanoparticles and noted the size of 55 nm in diameter. Shen et al. [16] prepared the PLGA-loaded combretastatin nanoparticles using the nanoprecipitation method and found that the size reduced to 208 nm. Therefore, the solubility of NF-k and NF-c in an aqueous medium can be attributed to its nanosized regime prepared through solvent precipitation technique, where SDS was used as an ionic stabilizer. Further, the PDIs of both NF-k and NF-c were less than 0.25, substantiating that the particles were uniformly distributed, which validates the solvent evaporation technique [23].

FTIR analysis was carried out to elucidate whether kaempferol and combretastatin's molecular structure has

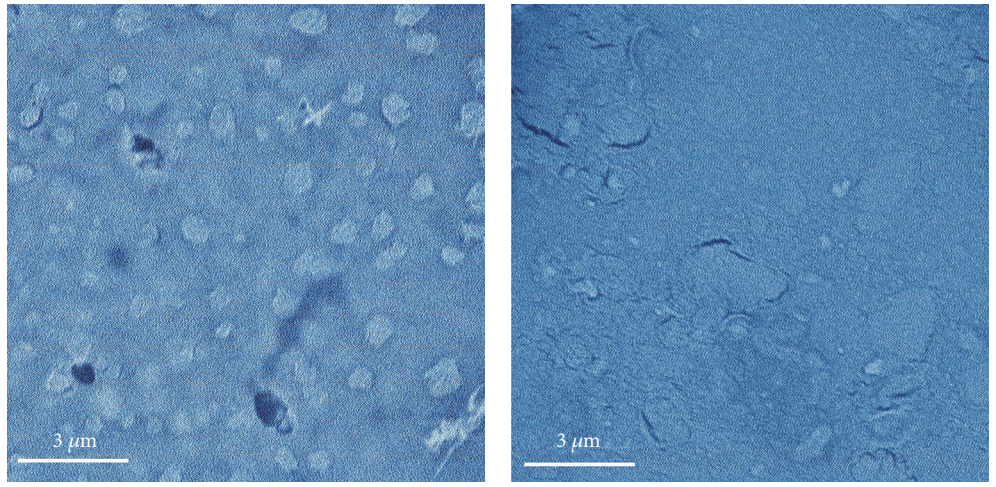

Figure 6: SEM images of (a) NF-k and (b) NF-c.

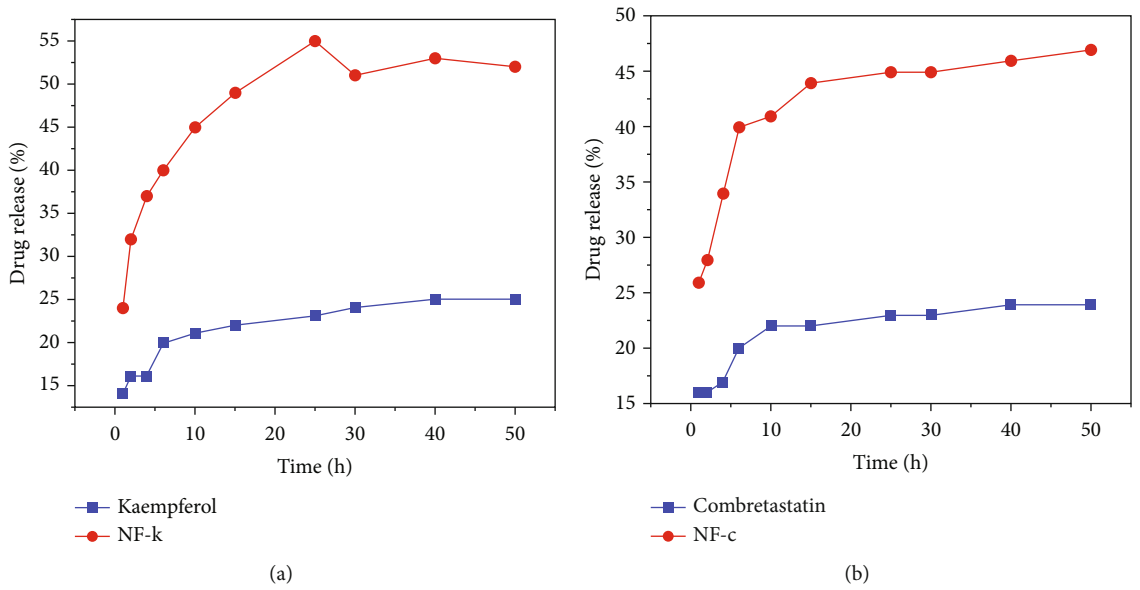

Figure 7: Drug release kinetics of (a) NF-k and kaempferol and (b) NF-c and combretastatin.

changed while nanoformulation. Previously, few investigations were reported on the FTIR results of NF-k. Qian et al. [17] reported the FTIR characterization of pure kaempferol and NF-k. The results showed lower intensities and no major shifts in the FTIR peaks of NF-k compared with pure kaempferol. Telange et al. [24] reported the FTIR characterization of pure kaempferol and kaempferol-phospholipid complex. The results showed that the interaction of kaempferol with phospholipid showed novel peaks when compared to pure kaempferol. The present study results can be agreed with the earlier reports. There are no studies reported on the FTIR spectra of NF-c and combretastatin.

From Figure 3, it was clear that the FTIR of NF-k and NF-c showed peaks the same as that of their pristine forms, substantiating that the kaempferol and combretastatin molecules present in NF-k and NF-c do not lose their functional groups. Further, the intensities of the peaks werefound to be decreased with nanoformulation, which validates the formation of nanosized particles. Moreover, after nanoformulation, extra peaks emerged from the SDS molecules sorbed along with NF-k and NF-c. SDS presence also improves the solubility of the nanoformulated drug in water through its hydrophilic tail. Therefore, it is understood that the solubility of NF-k and NF-c is because of the nanosized effect and the presence of SDS molecules.

The particle size and morphology of NF-k and NF-c were determined from the SEM images. As seen in the previous literature data, the NF-k and NF-c have spherical-shaped structures [15, 25]. This may be due to the presence of SDS molecules where it may facilitate the formation of micelles while the drug may be encapsulated within the hydrophobic compartment. Similar spherical-shaped structures were

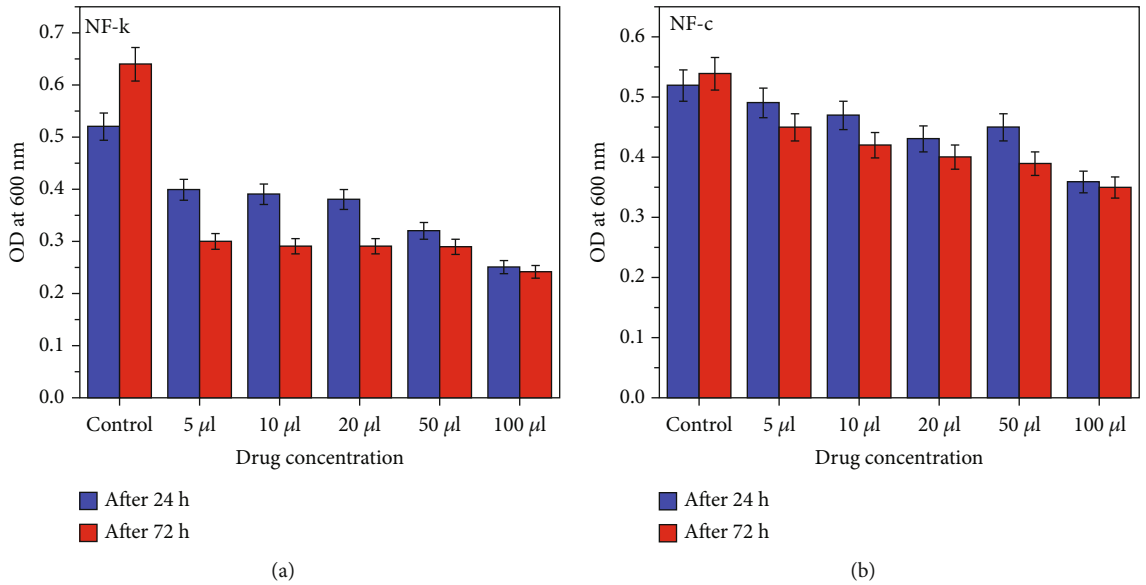

Figure 8: Antibacterial activity of (a) NF-k and (b) NF-c assessed through optical density values.

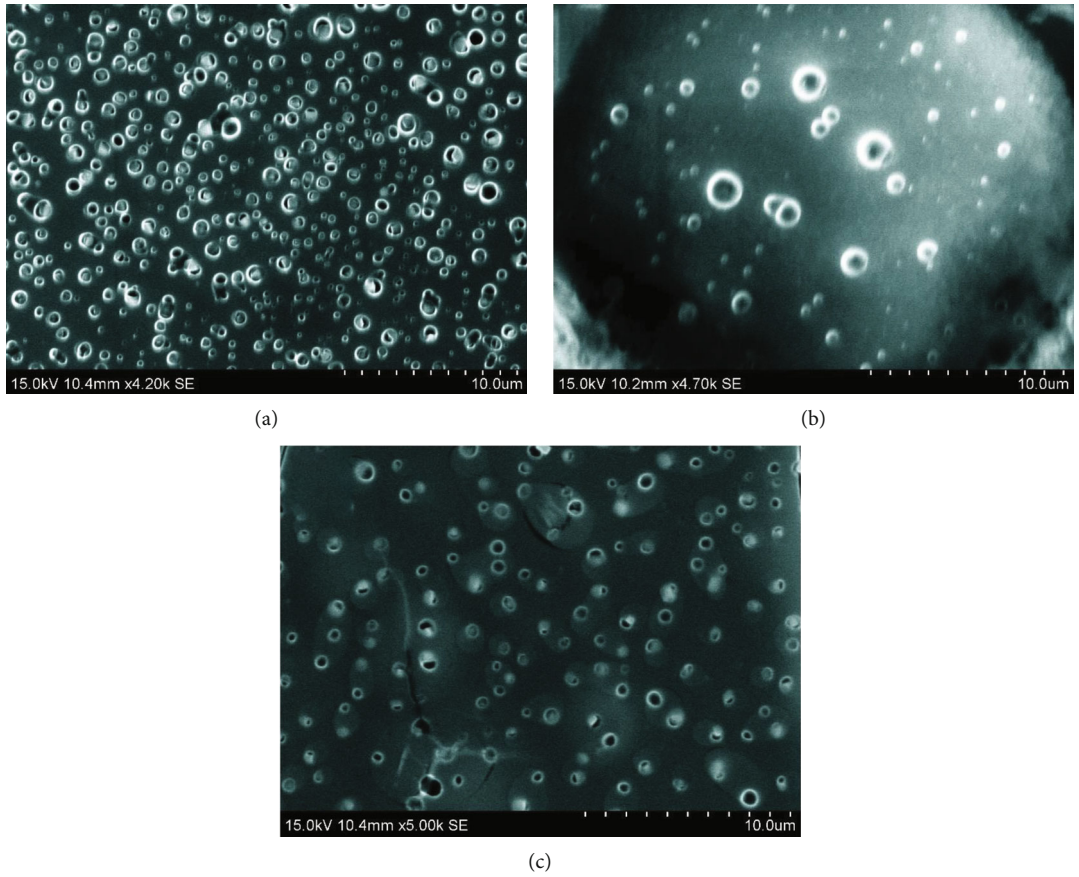

Figure 9: SEM images of bacterial cells (a) before and after treatment with (b) NF-k and (c) NF-c.

obtained for kaempferol-loaded lecithin/chitosan nanoparticles (KAE-LC NPs) when tocopheryl polyethylene glycol succinate was used as the surfactant by Sedef et al. [15]. Likewise, Paramita et al. [25] prepared near-spherical-shaped hesperetin nanoparticles using SDS as a surfactant. Further, Zaid et al. [26] used PVA as a surfactant to prepare spherical-shaped combretastatin A4-loaded poly(L-lactide-co-glycolide) (CA4-loaded PLGA NPs). Therefore, it is evident that

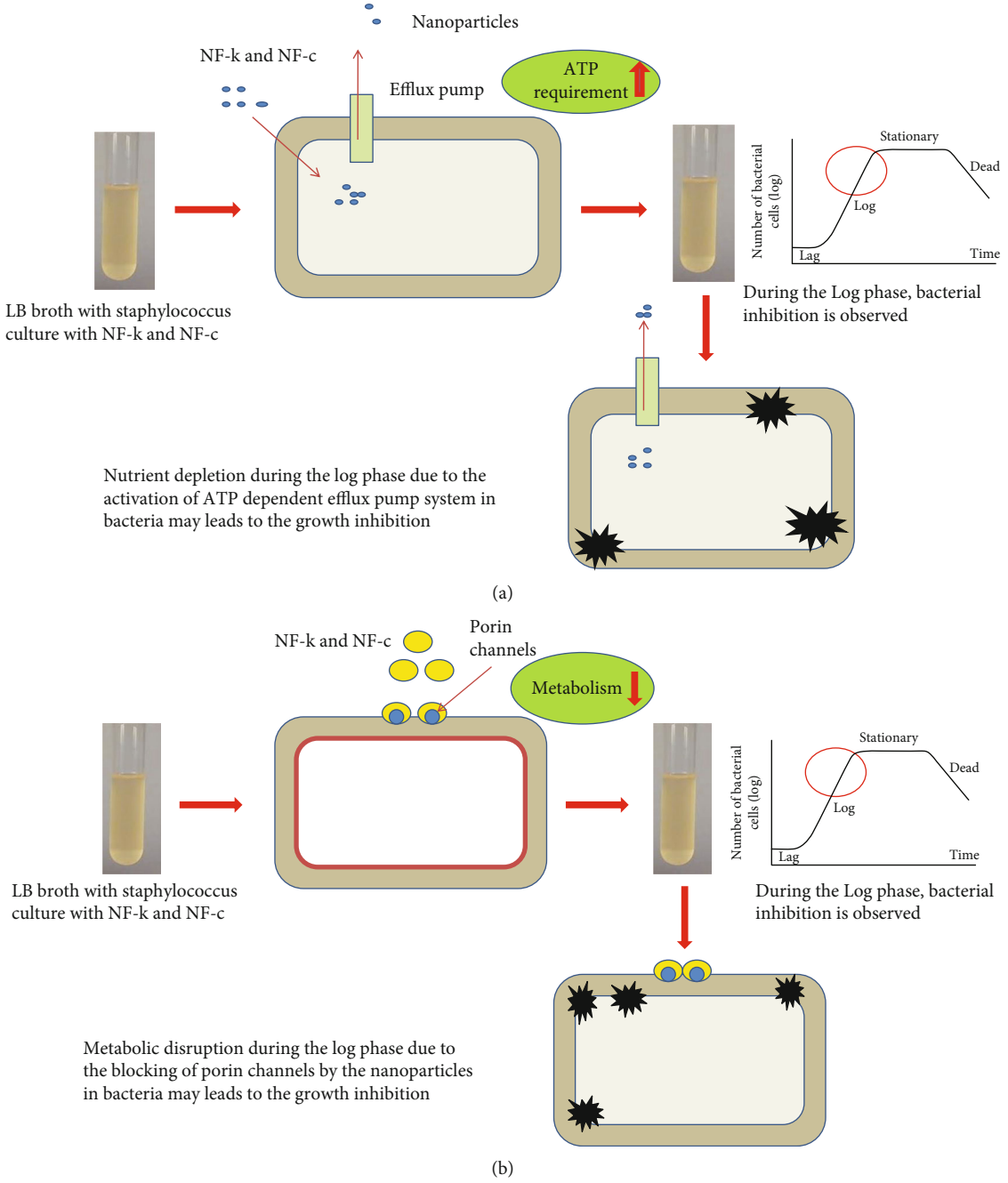

Figure 10: Possible mechanisms of antibacterial activity of nanoformulated drug through (a) blocking of porin channels and (b) activation of ATP-dependent efflux pump system.

the presence of SDS surfactant molecules promotes the formation of spherical-shaped NF-k and NF-c particles. Also, it was inferred that the sizes of NF-k and NF-c were in the nano regime, validating the superiority of the solvent precipitation technique.

The drug release kinetic assay was carried out using the dialysis membrane method. Previously, Sedef et al. [15] reported the drug release kinetics of KAE-LC NPs (200 nm to 350 nm), where the results revealed that the KAE-LC NPs released 82% of the drug in 24 h (25°C). Likewise, Zaid et al. [26] reported the drug release kinetics of CA4-loaded PLGA NPs revealed the release of 87% drug in 17 days (37°C). In the present investigation, the obtained results showed that the kaempferol and combretastatin suspended in DMSO release 20% and 21% drug, respectively, in the first 6 h after that release rate is slow down up to 50 h reaching 25% and 24%, respectively. However, in the case of NF-k and NF-c, a higher percentage of drugs, i.e., 52% and 47%, respectively, was released in a sustained manner which was found higher than the pristine drugs and lower than the reported nanoformulated drugs. The difference in the release kinetics for nanoformulated medicines with their pristine

forms and literature data was influenced by particle size and composition changes. The improved release kinetics of NF-k and NF-c than their pristine forms increase their antibacterial activity. In contrast, the declined release rate than the literature data will allow the safe circulation of nanoformulated drugs in the bloodstream without releasing a lethal amount to the healthy tissue.

The antibacterial activity of NF-k and NF-c was analyzed by evaluating the OD values after incubation with bacterial strain. For both the nanoformulated drugs, the concentration-dependent antibacterial activity trend was elucidated. Also, it was clear that the bactericidal action was higher in the first 24 h of incubation. The activity sustains for 72 h may be due to the sustained release of the nanoformulated drugs. The presence of SDS does not take part in the antibacterial activity since their percentage in the nanoformulation preparation procedure was found to be very low (0.2%) [27]. As the literatures dealing with the antibacterial activity of the NF-k and NF-c were very scarce, the mechanism behind the bacterial growth inhibition is not yet revealed. However, through previous studies on the antibacterial activity of nanoformulated phytochemicals, it is clear that the possible mechanisms behind the bactericidal activity of NF-k and NF-c are either activation of ATP-dependent efflux pump system or blocking of porin channels.

Compared with pristine drugs, the nanoformulated drugs have a small size which makes the drugs more comfortable to enter through the bacterial cell wall. The efflux pump, which is present on the surface of the cell wall, allows the bacteria to control the internal environment by removing the toxic substances, including metabolites, antibacterial agents, and quorum sensing signal molecules [28]. Previous studies revealed that the efflux pump present in *Staphylococcus*was NorA [29–31]. However, the efflux pump requires more ATP to remove the nanoformulated drugs present inside the cell wall. Due to the continuous extrusion of nanoformulated drugs, nutrient depletion occurs for the *Staphylococcus aureus* strain during the log phase leading to bacterial inhibition (Figure 10(a)).

Another possible mechanism is that the NF-k and NF-c block the porin channels on the cell wall's surface [32, 33]. Achouak et al. [34] revealed that the porins channels that regulate the nutrients into the cell for metabolism in gram-positive bacteria. Choi and Lee [35] examined the role of porins for antibiotic resistance in gram-positive bacteria. They revealed that the porins would affect the resistance to antibiotics in different classes and maintain cellular integrity. In the present work, the nanoformulated drugs may block the porin channels and cause metabolic disruption in the log phase leading to bacterial inhibition (Figure 10(b)). However, further studies are required in the future to know the exact mechanism of antibacterial activity of NF-k and NF-c.

Further, the inhibition of bacterial growth is inferred from the SEM images recorded for control and nanoformulated drugs treated bacterial culture. From the obtained results, the control shows the round-shaped, highly populated bacterial structures confirming the presence of *Staphylococcus aureus* with a smooth surface (Figure 9(a)). However, after treating with NF-k and NF-c, the bacterial population declines with distorted surfaces substantiating the antibacterial activity of the nanoformulated drugs (Figures 9(b) and 9(c)). As discussed earlier, the bacterial growth inhibition might be due to the activation of the ATP-dependent efflux pump system or/and the blockage of porin channels. Also, it is to be noted that the bacterial population is lower for NF-k than the NF-c, which is by the obtained OD values.

5. Conclusions

In the present study, NF-k and NF-c were synthesized using the solvent evaporation method and were characterized using DLS, FTIR, and SEM. The drug release kinetic assay confirmed that the NF-k and NF-c release the drugs significantly than the control up to 50 h. The present research validates the bacterial inhibition property of nanoformulated drugs. The possible mechanism behind the bacterial growth inhibition by the nanoformulated drugs may be due to the activation of ATP-dependent efflux pump system in bacteria or/and blocking of porin channels by nanosized drugs that disrupt the metabolism causing bacterial inhibition. Further, well-deliberated studies are needed in the future to elucidate the antibacterial properties of NF-k and NF-c.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

The authors would like to thank Prof. Dr. T. Balasubramanian, Honorable Vice-Chancellor, Smt. S. Jeyendra Saraswathi, Registrar, and the management of Chettinad Academy of Research and Education (Deemed to be University) for providing facilities to perform this study. This research was supported by the Department of Science and Technology-Science and Engineering Research Board (DST-SERB), Government of India, New Delhi (grant number: EMEQ/2018/000882).

References

[1] P. R. Hsueh, "New Delhi metallo-β-lactamase-1 (NDM-1): an emerging threat among Enterobacteriaceae," *Journal of the Formosan Medical Association*, vol. 109, no. 10, pp. 685–687, 2010.

[2] K. Pooled, "Mechanisms of bacterial biocide and antibiotic resistance," *Journal of Applied Microbiology*, vol. 92, pp. 55S–64S, 2002.

[3] R. Jayaraman, "Antibiotic resistance: an overview of mechanisms and a paradigm shift," *Current Science*, vol. 96, pp. 1475–1484, 2009.

[4] M. L. W. Knetsch and L. H. Koole, "New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles," *Polymers*, vol. 3, no. 1, pp. 340–366, 2011.

[5] J. M. Calderon-Montano, E. Burgos-Moron, C. Perez-Guerrero, and M. Lopez-Lazaro, "A review on the dietary flavonoid kaempferol," *Mini Reviews in Medicinal Chemistry*, vol. 11, no. 4, pp. 298–344, 2011.

[6] Y.-B. Huang, M.-W. Lin, Y. Chao, C.-T. Huang, Y.-H. Tsai, and P.-C. Wu, "Anti-oxidant activity and attenuation of bladder hyperactivity by the flavonoid compound kaempferol," *International Journal of Urology*, vol. 21, no. 1, pp. 94–98, 2014.

[7] P. Rajendran, T. Rengarajan, N. Nandakumar, R. Palaniswami, Y. Nishigaki, and I. Nishigaki, "Kaempferol, a potential cytostatic and cure for inflammatory disorders," *European Journal of Medicinal Chemistry*, vol. 86, pp. 103–112, 2014.

[8] R. A. Escandón, M. del Campo, R. López-Solis, E. Obreque-Slier, and H. Toledo, "Antibacterial effect of kaempferol and (−)-epicatechin on Helicobacter pylori," *European Food Research and Technology*, vol. 242, no. 9, pp. 1495–1502, 2016.

[9] D. Piyush and V. Kiran, "Kaempferol attenuates diabetic nephropathy by inhibiting RhoA/Rho-kinase mediated inflammatory signaling," *Biomedicine & Pharmacotherap*, vol. 109, pp. 1610–1619, 2019.

[10] M. Imran, B. Salehi, J. Sharifi-Rad et al., "Kaempferol: a key emphasis to its anticancer potential," *Molecules*, vol. 24, no. 12, p. 2277, 2019.

[11] N. Martini, D. R. P. Katerere, and J. N. Eloff, "Seven flavonoids with antibacterial activity isolated from Combretum erythrophyllum," *South African Journal of Botany*, vol. 70, no. 2, pp. 310–312, 2004.

[12] N. Martini, D. R. P. Katerere, and J. N. Eloff, "Biological activity of five antibacterial flavonoids from Combretum erythrophyllum (Combretaceae)," *Journal of Ethnopharmacology*, vol. 93, no. 2-3, pp. 207–212, 2004.

[13] A. Das, K. C. Samal, A. B. Das, and G. R. Rout, "Quantification, antibacterial assay and cytotoxic effect of combretastatin, an anticancer compound from three Indian Combretum species," *International Journal of Current Microbiology and Applied Sciences*, vol. 7, no. 1, pp. 687–699, 2018.

[14] S. Kakran, N. G. Sahoo, I.-L. Tan, and L. Li, "Preparation of nanoparticles of poorly water-soluble antioxidant curcumin by antisolvent precipitation methods," *Journal of Nanoparticle Research*, vol. 14, no. 3, p. 757, 2012.

[15] S. Ilk, N. Saglam, and M. Özgen, "Kaempferol loaded lecithin/chitosan nanoparticles: preparation, characterization, and their potential applications as a sustainable antifungal agent," *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 45, no. 5, pp. 907–916, 2017.

[16] N. Shen, J. Wu, C. Yang et al., "Combretastatin A4 nanoparticles combined with hypoxia-sensitive imiquimod: a new paradigm for the modulation of host immunological responses during cancer treatment," *Nano Letters*, vol. 19, no. 11, pp. 8021–8031, 2019.

[17] Y. S. Qian, S. Ramamurthy, M. Candasamy, S. Md, R. H. Kumar, and V. S. Meka, "Production, characterization and evaluation of kaempferol nanosuspension for improving oral bioavailability," *Current Pharmaceutical Biotechnology*, vol. 17, no. 6, pp. 549–555, 2016.

[18] B. C. Hancock and M. Parks, "What is the true solubility advantage for amorphous pharmaceuticals?," *Pharmaceutical Research*, vol. 17, no. 4, pp. 397–404, 2000.

[19] P. Arnnok and R. Burakham, "Retention of carbamate pesticides by different surfactant-modified sorbents: a comparative study," *Journal of the Brazilian Chemical Society*, vol. 25, no. 9, pp. 1720–1729, 2014.

[20] R. K. B. Bhawana, R. K. Basniwal, H. S. Buttar, V. K. Jain, and N. Jain, "Curcumin nanoparticles: preparation, characterization, and antimicrobial study," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 5, pp. 2056–2061, 2011.

[21] B. Zhao, Z. Dong, W. Liu et al., "Co-administration of combretastatin A4 nanoparticles and anti-PD-L1 for synergistic therapy of hepatocellular carcinoma," *Journal of Nanobiotechnology*, vol. 19, no. 1, pp. 1–18, 2021.

[22] Y. Zhang, X. Liu, X. Wang et al., "Nanoparticles composed of PEGylated alternating copolymer-combretastatin A4 conjugate for cancer therapy," *Macromolecular Bioscience*, vol. 21, no. 8, article 2100077, 2021.

[23] B. Feng, M. A. Ashraf, and L. Peng, "Characterization of particle shape, zeta potential, loading efficiency and outdoor stability for chitosan-ricinoleic acid loaded with rotenone," *Open Life Sciences*, vol. 11, no. 1, pp. 380–386, 2016.

[24] D. R. Telange, A. T. Patil, A. M. Pethe, A. A. Tatode, S. Anand, and V. S. Dave, "Kaempferol-phospholipid complex: formulation, and evaluation of improved solubility, in vivo bioavailability, and antioxidant potential of kaempferol," *Journal of Excipients and Food Chemicals*, vol. 7, no. 4, p. 89, 2016.

[25] P. Paramita, S. N. Sethu, N. Subhapradha et al., "Neuro-protective effects of nano-formulated hesperetin in a traumatic brain injury model of Danio rerio," *Drug and Chemical Toxicology*, vol. 44, no. 6, pp. 1–8, 2020.

[26] A. N. Zaid, M. Hassan, N. Jaradat et al., "Formulation and characterization of combretastatin A4 loaded PLGA nanoparticles," *Materials Research Express*, vol. 6, no. 12, 2019.

[27] M. A. Díaz De Rienzo, P. Stevenson, R. Marchant, and I. M. Banat, "Anti-bacterial properties of biosurfactants against selected Gram-positive and-negative bacteria," *FEMS Microbiology Letters*, vol. 363, no. 2, p. 224, 2016.

[28] D. Gupta, A. Singh, and A. U. Khan, "Nanoparticles as efflux pump and biofilm inhibitor to rejuvenate bactericidal effect of conventional antibiotics," *Nanoscale Research Letters*, vol. 12, no. 1, 2017.

[29] S. Zimmermann, M. Klinger-Strobel, J. A. Bohnert et al., "Clinically approved drugs inhibit the Staphylococcus aureus multidrug NorA efflux pump and reduce biofilm formation," *Frontiers in Microbiology*, vol. 10, p. 2762, 2019.

[30] N. Kahzad and A. Salehzadeh, "Green synthesis of CuFe2O4@Ag nanocomposite using the Chlorella vulgaris and evaluation of its effect on the expression of norA efflux pump gene among Staphylococcus aureus strains," *Biological Trace Element Research*, vol. 198, no. 1, pp. 359–370, 2020.

[31] N. Shokoofeh, Z. Moradi-Shoeili, A. S. Naeemi, A. Jalali, M. Hedayati, and A. Salehzadeh, "Biosynthesis of Fe3O4@Ag nanocomposite and evaluation of its performance on expression of norA and norB efflux pump genes in ciprofloxacin-resistant Staphylococcus aureus," *Biological Trace Element Research*, vol. 191, no. 2, pp. 522–530, 2019.

[32] L. Liao, Y. Li, and S. Tjong, "Bactericidal and cytotoxic properties of silver nanoparticles," *International Journal of Molecular Sciences*, vol. 20, no. 2, p. 449, 2019.

[33] J. D. Prajapati, U. Kleinekathöfer, and M. Winterhalter, "How to enter a bacterium: bacterial porins and the permeation of antibiotics," *Chemical Reviews*, vol. 121, no. 9, pp. 5158–5192, 2021.

[34] W. Achouak, T. Heulin, J.-M. PagÃ¨s et al., "Multiple facets of bacterial porins," *FEMS Microbiology Letter*, vol. 199, no. 1, pp. 1–7, 2001.

[35] U. Choi and C. R. Lee, "Antimicrobial agents that inhibit the outer membrane assembly machines of Gram-negative bacteria," *Journal of Microbiology and Biotechnology*, vol. 29, no. 1, pp. 1–10, 2019.