Preparation, characterization, and antibacterial competence of silymarin and its nano-formulation

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ABSTRACT
Nowadays, antibiotic resistance is a serious problem, especially in countries that have unregulated use of antibiotics. We aimed to prepare and characterize Sily-Nano from silymarin to present its antimicrobial activities in comparison to silymarin and gentamycin and provide natural antibacterial from natural sources. Silymarin NPs (Sily-Nano) were prepared using a high-pressure homogenization and characterized using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), ultraviolet-visible (UV-visible) spectrum, and scanning electron microscopy (SEM). The antibacterial monitoring of Sily-Nano and silymarin was compared against Escherichia coli UN75 (E.coli-UN75), Enterobacter aerogenes SL3 (E.aerogenes-SL3), Staphylococcus aureus-PS41 (S.aureus-PS41), and Klebsiella pneumoniae-BD29 (K.pneumoniae-BD29). Sily-Nano was effectively prepared and confirmed using FTIR. SEM, with even distribution, and significant bacterial zone inhibition, compared with silymarin. In conclusion, Sily-Nano preparation is an efficient agent, and effective against certain clinical antibiotic-resistant bacterial strains. Sily-Nano has a novel significant inhibitory effect against the antibiotic-resistant strain of K. pneumonia-BD29, Ent. Aerogenes-SL3 and E. coli UN75 when compared with silymarin and the common antibiotic gentamycin. Therefore, sily-Nano is potentially used as an antibacterial and natural sanitizer agent.

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ABBREVIATIONS:
ATP: adenosine triphosphate; BHI: brain heart infusion broth; DMSO: dimethyl-sulfoxide; FTIR: Fourier-transform infrared spectroscopy; MICs: minimal inhibitory concentrations; SEM: scanning electron microscopy; Sily-Nano: Silymarin NPs; UV-visible: ultraviolet-visible; XRD: X-ray diffraction

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Introduction

Antibiotic resistance and medicinal efficiency is significant problem, challenging healthcare in several countries. Recently, novelties in nanotechnological strategies offer a great opinion to enhance the assimilation, solubility, and bioavailability within the cells so, provide a method to potentiate the therapeutic action and provide sustained silymarin release of the active herbal extract at the absorption sites via nanoencapsulation [1].

The number of scientific literature demonstrating silymarin-based formulations with improved solubility, stability, enhanced bioavailability, and effective medicinal roles is continually expanding. The formulations schemes rationally followed stage by stage the development of the nanosystems and nanotechnologies [2].

Antibacterial activity is one of the vital applications in the current studies in biomedical specialties. Numerous infectious species as *S. aureus*, *E. coli*, *Staphylococcus aureus*, *P. aeruginosa*, and *K. pneumoniae* induce several serious diseases in humans, so, we need to find out a prospective antibiotic to treat these infections. Sometimes, the antibiotics cannot inhibit the harmful bacterial progression.

Natural and synthetic antibiotics that are leaked in water resources or utilized in food preservation accelerate the development of bacterial resistance and reduce the medication efficiency which, in the judgment of specialists, will have permanent consequences and may become fatal. Antibiotics in uncontrolled amounts not only induce bacterial resistance but also may cause an ecological danger. The expansion of antibacterial resistance for several pathogenic bacteria has one of the riskiest issues in the management of contagious diseases (WHO) [3].

Antibiotic resistance, especially for the latest generation of antibiotics developed by a pharmacist, is one of the main challenges in the recent therapeutic approaches. Therefore, the discovery of innovative medication with a new mode of action is a great task for biomedical investigators and industries. The blend of biomolecules derived from medicinal plants and formulating into nanomaterial offer major possible sources of novel antibacterial agents to regulate bacterial diseases and their resistance.

Silymarin is virtually water-insoluble, upon oral administration, because of its remarkably non-ionizable and hydrophobic structure, so represents minimal biological accessibility where their dissolution is a rate-limiting stage for the expression of bioactivity [4]. Its rate of absorption in the digestive tract is small, offering only about 20–40% bioavailability [5]. To improve silymarin bioavailability, complexation with a phospholipid [6, 7], β-cyclodextrin, and hydroxypropyl-β-cyclodextrin; solid dispersion with a hydrophilic polymer, such as PEG 6000; self-micro emulsifying systems; co-precipitates; and a complex with crosslinked polymers are required. The antimicrobial activity and control of the growth of food microorganisms of silymarin may be effectively improved by nanoformulation, therefore, we formulated Sily-Nano to challenge these issues.

The recognized effects of silymarin are anti-lipid peroxidation; anti-inflammatory, anti-angiogenic, and anticarcinogenic, and the ability to stimulate hepatic regeneration. Though, there are inadequate records about its antibacterial activity [8–10]. Natural extracts have large quantities of bioactive substances, mostly polyphenols which prevent the microorganisms’ growth, particularly bacteria. Their actions are not completely understood however, they may be linked to their biochemical structures. Silymarin revealed antibacterial action versus the gram-positive bacteria *Bacillus subtilis* and *Staph. epidermidis*. This was mainly due to the inhibition of RNA expression and the synthesis of protein. Also, its antiviral action is played through the inhibition of viral transmission and diffusion [11].
There is a shortage in the research dealing with natural antibacterial as a sanitizer and its use as nanoformulation, that consider a research gap that leads to our study. Because of the antioxidant and antibacterial activity of phenolic composites, plant extracts offer a novel substitution to the chemical additives used in the industry of meat, particularly nitrates. They can prevent the development of spoilage and pathogenic microflora, inhibit oxidation of meat components, like carbohydrates, proteins, and lipids, and inhibit staining and discoloration. Therefore, the current study aimed to prepare and characterize Sily-Nano from silymarin to present its antimicrobial activities in comparison to silymarin and gentamycin.

Materials and methods

Materials

Silymarin powder was attained commercially from MADAUS GmbH Cologne (51101), Germany (reg no. 4-1089). Silymarin (Figure 1 derived from pubchem.ncbi.nlm.nih.gov/compound/Silymarin) is a combination of flavonoids obtained from seeds of the MILK THISTLE, Silybum marianum. It is composed of silybin and its isomers, silichristin and silidianin. Silymarin shows antioxidant and protects several tissues against biochemical damage, and act as a hepatoprotective agent.

Silymarin and Sily-Nano as antibacterial agents

The initial silymarin stock solution was prepared at 10 mg/mL concentration in one mL of dimethyl-sulfoxide (DMSO). The concentration of DMSO didn’t exceed the value of 2% to avoid its detrimental effects on the tested bacteria. This concentration was used as follows: the two tested composites were diluted in 1 mL of sterile distilled H2O to attain concentrations of 8, 12, 16, 24, 32, 48, 64, 96, 128, 192, and 256 μg/mL.

The antibacterial activity of silymarin and its derivative was evaluated at the concentration of the initial stocks by measuring the resulting inhibition zones (mm) in seeded Muller-Hington agar by a formerly adapted method of Beecher and Wong [12] against selected clinical antibiotic-resistant bacterial strains of E. coli UN75, Staph. aureus PS41, Ent. aerogenes SL3, and K. pneumoniae BD29. Those strains were obtained from the culture collection of the microbiology lab of Basic and Applied Scientific Research Center, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. The Muller–Hington agar consists of beef extract (30%), casein hydrolysate (1.75%), starch (0.15%), and agar (1.7%). It was prepared then autoclaved at 121 °C for 15 min thereafter, cooled to 47 °C, and sowed with the examined bacteria under aseptic conditions. Subsequent to solidification, 5 mm diameter holes were pressed by a sterilized cork-borer. The examined compounds with reference to the antibiotic gentamycin, were then added in the holes (only 100 μL) after being dissolved in the DMSO at 10⁻⁴ M. Then, these culture plates were incubated for 18 h at 37 °C. The diameter of the inhibition zones in mm was measured to determine the antibacterial activity.

Moreover, the action of silymarin and Sily-Nano was tested in a plate culture of the bacterium Staph. aureus PU41 growing on Baird-Parker specific medium. This medium consists of (g/l), the pancreatic digest of casein (10), glycine (12), sodium pyruvate (10), lithium chloride (5), beef extract (5), yeast extract (1), egg yolk tellurite enrichment, 60 mL, and agar (20) with pH adjusted to 7.0 and incubation was done at 37 °C. The
upper wells of the plate culture were inoculated with 40 μl of Sily-nano at a concentration of 40 μg/mL, while the lower wells were inoculated with silymarin.

**MIC preparation**

The minimal inhibitory concentrations (MICs) were determined for both compounds. The following culture media were utilized for the bacteria; brain heart infusion broth (BHI) at 10% as showed by the constructor; Acumedia Manufacturers Inc.) and heart infusion agar (HIA) Difco Laboratories Ltda). All culture media were equipped following the producer’s guidelines. Cultures of bacteria were kept at 4°C in HIA. The strains were passaged, before the assessments, utilizing the aforementioned media then incubated for 24 h at 37°C. The strains that plated were injected into BHI broth and once more incubated for 24 h at 37°C. A minor aliquot part of the cultured inoculum was detached and diluted in sanitary saline to provide turbidity equal to 0.5 on the McFarland scale, equivalent to 10^5 CFU/mL [13].
The indicator for the bacterial growth was sodium resazurin reagent. It was attained from Sigma Company, and kept at 4°C far away from the daylight. Concerning the interpretation of the test, bacterial growth was indicated by changing the color from blue to pink owing to the reduction of resazurin [14].

The MICs of silymarin and its prepared derivative regarding the antibiotic gentamycin were measured by the microdilution analysis. 100 μL of 10% BHI medium was tallied to every well of the microplate then 100 μL of the bacterial suspension was inoculated to all wells, thence, 100 μL of the examined product was used to achieve concentrations in the average of 2:512 μg/mL. The negative control consists of 100 μL (10% BHI medium) and 100 μL (test product without inoculation of bacteria, while adding sterile distilled water instead). In The Meantime, the positive control only had the bacterial suspension and 10% BHI.

The microplates were reared at 37°C for 18 h [15]. Bacterial growth was measured utilizing resazurin. The analyses were applied in triplicate. MIC was described as the smallest concentration upon which no bacterial growth was detected in agreement with NCCLS [9]. Ultracentrifugation for the preparation and characterize the particles with SEM.

**Silymarin NPs preparation**

Three capsules of Silymarin (Legalon forte) were ruptured and the obtained powder was transferred into a separating funnel followed by the addition of methanol (100 mL). The mixture was shaken very well and filtered. The obtained filtrate was evaporated under reduced pressure and obtained powder was stored at 4°C. To prepare the Sily-Nano, 50 mg of Silymarin powder was dispersed in 25 mL of methanol in a beaker, the solution was transferred into a high-pressure homogenizer and treated at 1200–1500 psi using 3 cycles. The solvent was evaporated using a rotary evaporator and the obtained powder was stored at 4°C for additional studies.

**Morphology and characterization of the Sily-Nano**

Sily-Nano with diverse amounts was equipped by solvent evaporation and nano-precipitation methods. X-ray diffractometer (XRD, Rigaku, Japan) (Figure 2), was employed to study the phases of Sily-Nano in the range of 10°–80° with 0.9°/minute scanning speed.
The morphology and size of Sily-Nano were determined by a scanning electron microscope (FEI, Czech Republic). The sample was spread uniformly on the sample holder having carbon tape on it and images were captured using different magnifications. FTIR (PerkinElmer) was used to evaluate the functional groups of Sily-Nano using ATR accessory. The background was recorded with an empty ATR accessory and then a small amount of sample was put on the ATR crystal and a spectrum was recorded using 4 cm\(^{-1}\) resolution and 32 scans in the range 500–4000 cm\(^{-1}\). UV-visible spectrophotometer (UV-Vis, JASCO V-750) was used to record the UV-visible spectra of Sily-Nano in methanol in the range 200–800 nm, before the analysis baseline was recorded with blank methanol.

The scanning electron microphotograph of Sily-Nano was displayed in Figure 3. It indicated that Sily-Nano has a distinct circular structure without accumulation. There were no drug crystals noticed on the exterior of the NPs.

**Statistical analysis**

Data were statistically assessed by one-way analysis of variance (ANOVA), followed by the Tukey-Kramer method for post-hoc analysis. Results showed as mean ± SEM, and the values were considered significant when \(p<0.05\) and very highly significant when \(p<0.001\) in the tables. The dissimilar superscript letters (a, b, c) considered significant changes at \(p<0.05\) in the tables. The statistical examination was achieved by the GraphPad Prism 6 software (San Diego, CA, USA). The statistical examination was achieved by the GraphPad Prism 6 software (San Diego, CA, USA).

**Results and discussion**

Table 1 showed that the tested bacteria including, *E. coli* UN75, *Staph. aureus* PU41, *Ent. aerogenes* ST3 has a statistically significant difference between the inhibition zones in silymarin, Sily-Nano, and the antibiotic *gentamycin* groups \((p \leq 0.05)\).
Comparative data of MIC of silymarin, its nano-derivative, and the antibiotic gentamycin, where the nano-derivative induced 15.25 mm inhibition zone diameter with MIC of 48 μg/mL with Ent. Aerogenes-SL3. While the maximum resistance was exerted by K. pneumoniae-BD29, where the nano-derivative just induced 10.25 mm inhibition halos with MIC of 96, 48 μg/mL (Tables 1 and 2).

XRD pattern of Sily-NPs indicates amorphous nature of Sily-NPs (Figure 2). SEM image of Sily-NPs, showed that, Sily-Nano particles are discrete spherical structures without aggregation with an average size of 500 nm. Furthermore, there were no crystals of drug observed on the surface of NPs (Figure 3). Figure 4 presented the FTIR spectra of Sily-NPs. Figure 4 displayed the UV absorption spectrum (288 nm) and stability of silymarin-NPs. The antibacterial activity of silymarin and its nano derivative with reference to the antibiotic gentamycin were presented in Table 1.

24 hr plate culture showing the characteristic black growth of the bacterium Staph. aureus PU41 grown on Baird-Parker agar specific medium (Figure 6). The upper plate wells that inoculated with Sily-nano and showed high inhibition zone (Figure 6a). Moreover, the lower wells inoculated with silymarin and demonstrated lower inhibition zone compared with Sily-nano (Figure 6b). The characteristic black growth of the bacterium Staph. aureus PU41 in the absence of tested compounds shown in (Figure 6c). This type of bacteria that grows on Baird-Parker agar specific medium is distinguished from all known bacteria by its black color showed that Sily-Nano inhibits the growth of bacteria compared to silymarin.

For all the tested bacteria, there was a statistically significant difference between the inhibition zones due to the silymarin, Sily-Nano, and the antibiotic gentamycin as established by one-way analysis of variance (ANOVA).

The test of homogeneity of variances ($p = 0.31, 0.87, 0.28, 0.45 > 0.05$ respectively cleared that data have equal population variances then the Tukey test indicated that the original and Sily-Nano are the most significantly effective on inhibition zone for E. coli UN75, Ent. aerogenes-ST3, and K. pneumoniae-L29, while the ANOVA test ($p = 0.0002, 0.001, 0.001 < 0.05$ respectively (assure that the Sily-Nano scored higher inhibition zone than other groups. Thus, the Sily-Nano is the most significantly effective on inhibition zone for E. coli-UN75, Ent. aerogenes-ST3, and K. pneumoniae-L29.

On the other hand for Staph.aureus-PU41, Tukey test indicated that the antibiotic gentamycin is the most significantly effective on inhibition zone but Sily-Nano is still better than silymarin according to the t-test ($p = 0.00 < 0.05$).

| Tested bacterium                  | Inhibition zone (mm) | Silymarin | Sily-Nano | Gentamycin |
|----------------------------------|----------------------|-----------|-----------|------------|
| E. coli UN75                     | 13.20 ± 0.10 a       | 14.90 ± 0.07 b | 3.25 ± 0.03 c |
| Staph. aureus PU41              | 9.30 ± 0.01 a        | 11.00 ± 0.02 b | 27.5 ± 0.51 c |
| Ent. aerogenes ST3              | 13.95 ± 0.02 a       | 15.25 ± 0.27 b | 8.75 ± 0.20 c |
| Klebsiella pneumoniae L29       | 8.85 ± 0.12 a        | 10.25 ± 0.06 b | 6.50 ± 0.15 c |

These values represent means and standard errors. The different superscript letters indicated a significant difference at $p < 0.05$. }

Comparative data of MIC of silymarin, its nano-derivative, and the antibiotic gentamycin, where the nano-derivative induced 15.25 mm inhibition zone diameter with MIC of 48 μg/mL with Ent. Aerogenes-SL3. While the maximum resistance was exerted by K. pneumoniae-BD29, where the nano-derivative just induced 10.25 mm inhibition halos with MIC of 96, 48 μg/mL (Tables 1 and 2).

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Bacteria as E. coli-UN75, Staph. aureus-PS41, Ent. aerogenes-SL3, and K. pneumoniae-BD29 causing infections have a high incidence and are accountable for the increase in worldwide morbidity and mortality of infections [16]. Reasons included in this increase differ from inadequate sources of antibacterial activities, particularly in poor countries, to the rate of antibiotic resistance. Therefore, in the past eras, the common utilization of
medicinal plants and their byproducts has been improved for the treatment of diseases caused by bacteria [17]. Many research on the estimation of the antibacterial activities of natural medicinal plants has been directed to increase the range of antimicrobial treatment. Nevertheless, it was significant to indicate that the technique of microdilution, working in the present study, presently signifies the method most frequently used for this bioassay [18].

Medicinal plants are widely used because of their safety, are well tolerated with minimal side effects, and could be used as dietary supplements. *Silybum marianum* (L.)

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**Table 2.** A comparative data of MIC of silymarin, its nano-derivative, and the antibiotic gentamycin.

| Tested bacterium                      | MIC (µg/mL) | Silymarin | Sily-Nano | Gentamycin |
|---------------------------------------|-------------|-----------|-----------|------------|
| *E. coli* UN75                        | 64          | 48        | 256       |            |
| *Staph. aureus* PU41                  | 96          | 64        | 4         |            |
| *Ent. aerogenes* ST3                  | 64          | 48        | 64        |            |
| *Klebsiella pneumoniae* L29           | 96          | 96        | 256       |            |

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**Figure 4.** FTIR spectra of Silymarin NPs.

**Figure 5.** UV spectra of Silymarin NPs recorded in different days.
Gaertn known as the milk thistle has been applied for a long time ago to treat hepatic and gallbladder illnesses, comprising hepatic inflammation, cirrhosis, jaundice, and to defend the hepatic tissues against chemical poisons and environmental toxins [19]. The active component obtained from a standardized extract of S. marianum seeds is silymarin, it includes about 70:80% flavonolignans of silymarin and about 20:30% is a chemically indefinite fraction, including frequently oxidized polyphenolics and polymeric compounds [20].

Studies exploring the biological activity of silymarin has improved, including several pharmacological roles accompanied with these substances, in addition to the safe use of silymarin, while there were few records of the contrary effects, the contrary effect is few side effect which may be, diarrhea, nausea, heartburn and itching, the possible reason is that milk thistle can trigger allergic reactions [21, 22]. Various methods have been used to improve its efficacy and solubility of medicines/drugs. Herein, we prepare the Sily-Nano to improve its antibacterial activities in comparison to silymarin.

XRD pattern of Sily-NPs is presented in Figure 2, a wide peak between 10–35 degrees was observed indicating the amorphous phase of Sily-NPs.

SEM image of Sily-NPs, specified that, Sily-Nanoparticles are distinct spherical structures without accumulation with 500 nm size (Figure 3). Also, there were no crystals of drug observed on the surface of NPs. This data confirms the validity and homogeneity of Sily-Nano for the antibacterial agent.

FTIR spectra of Sily-NPs indicated that the peak was observed at 3300–3400 cm\(^{-1}\) which is assigned to the OH (hydroxyl) group of silymarin while the peak at 2900 cm\(^{-1}\) is due to the presence of CH group. The carbonyl group (C=O) of silymarin showed a peak at 1638 cm\(^{-1}\). Almost, similar FTIR pattern was observed in the case of silymarin (Figure 4). The UV absorption spectrum of silymarin-Nano was reordered in methanol and it showed an absorption spectrum around 288 nm (Figure 5). To study the stability of

![Figure 6](image-url)
Sily-NPs, spectra of Sily-NPs were recorded in methanol for three consecutive days, as it is evident from the spectra (Figure 5), there is no degradation of Sily-NPs and in all spectra, peak was observed around 288 nm. Moreover, no extra peak was observed in all spectra indicating that Sily-NPs are stable.

The results in this study demonstrate a comparative antibacterial activity of silymarin and its nano-derivative in comparison with the antibiotic gentamycin. Where the activities of the nano-derivative against all the tested bacteria were better than the original silymarin (Table 1). The best results were obtained against Ent. Aerogenes-SL3 as the nano-derivative induced 15.25 mm inhibition zone diameter with MIC of 48 μg/mL. While the maximum resistance was exerted by K. pneumoniae-BD29, where the nano-derivative just induced 10.25 mm inhibition halos with MIC of 96 μg/mL and 48 μg/mL (Tables 1 and 2).

The highest significant inhibition zone for antibiotic resistance strain of K. pneumonia, Ent. aerogenes-SL3 and E. coli-UN75, was recorded with Sily-Nano compared with gentamycin. These results indicated the best effect of Sily-Nano on the inhibition zone. MIC showed that Sily-Nano’s inhibitory concentration is minimal compared with both silymarin and gentamycin signifying its efficiency in the inhibition of bacterial growth. This may be due to the large surface area of NPs to provide its effect on bacterial growth. The antibacterial action of silymarin as a natural medicinal plant extract is based on the presence of flavonoids.

In addition, for most bacteria, the antibacterial activity of gentamycin was inferior in comparison with the modified drug except for the bacterium Staphylococcus aureus PU41. This may be attributed to the lower amounts of phospholipids in the cell wall of this gram-positive bacterium. Whereas, the cell walls of other tested bacteria are rich in lipids.

The plate culture that inoculated with Sily-nano and silymarin (Figure 6a and 6b) showed a high inhibition zone in Sily-nano compared with original silymarin confirming the efficiency of Sily-nano as an antibacterial agent. Figure 6(c) displayed the characteristic black growth of the bacterium Staph. aureus-PU41 on Baird-Parker agar, declaring that Sily-Nano inhibits the growth of bacteria compared to silymarin, indicating its efficiency as a specific antibacterial agent against Staph. aureus.

With reference to literature, studies have revealed that several natural compounds including silymarin exert their antibacterial activity by changing the permeability of the cell membrane [23] and can produce morphological alterations in the bacteria, destruct bacterial cell membrane, or interact with DNA topoisomerase that modifies the enzyme binding activity [24]. Moreover, polyphenols affect bacterial protein synthesis, modify the metabolic processes in bacterial cells, and suppress ATP and DNA biosynthesis. In this perception, the antimicrobial action of these substances might also be connected to the existence of hydroxyl phenolic groups that inhibit the enzymatic activities in the bacterial synthetic processes [25–27]. For this, medicinal plant types rich in active ingredients as flavonoids merit consideration [28, 29].

The superiority of NPs bulk counterparts may be explained based on (1) their capacity to hook up thiol groups (–SH groups) located on the bacterial cell membranes, leading to cell cracking and lysis, (2) their ability to induce the production of high amounts of reactive oxygen species, and (3) the release of NPs tracked by the production of highly reactive oxygen species (OH\(^{-}\), H\(_2\)O\(_2\) and O\(_2^{2-}\)). Thereafter, holes split up water molecules into hydroxyl ions and proton ions. Then dissolved O\(_2\) is converted to superoxide radical anions (\(^{\cdot}\)O\(_2^{2-}\)), that react with protons to generate HO\(_2^{-}\) radicals, which strike electrons to form hydrogen peroxide anions (HO\(_2^{-}\)). Therefore, they react with protons to form H\(_2\)O\(_2\) that penetrates the bacterial cell membrane and suppresses the intracellular metabolic pathways of bacteria [30].
As a conclusion, this study specifies the possibility of using Sily-Nano as a basis of a new medication as adjuvants in the antibiotic treatment versus several antibiotic-resistant bacteria. The mechanism of Sily-Nano as an antibacterial agent is due to several bioactive components, Silybin, a flavonoid with a smaller surface area [31, 32]. Mostly NPs, due to smaller particle sizes, cause an increase in adhesiveness and actively penetrate the cell membrane through small pores in the microbial cell, leading to disrupting the bacterial membrane and exhibiting higher antibacterial activity. Moreover, Sil-Nano may cause the imbalance of minerals and leakage of intracellular proteins and enzymes, resulting in cell growth inhibition and death. It also prevents the accumulation of the bacterial strain that causes antibiotic resistance and failure in treatments.

**Conclusion**

Sily-Nano as an organic compound could be prepared and characterized with stability. Both Sily-Nano and silymarin displays antibacterial activities against particular typical strains of bacteria which could be valued as a nutritional supplement or a medication. The use of Sily-Nano could be effective as an antibacterial agent via a significant increase in inhibition zone and decrease in MIC against the antibiotic-resistant strain of *Staph. aureus* PU41, *Ent. Aerogenes*-SL3 and *E. coli* UN75 when compared with silymarin, so prevent the development of antibiotic resistance for bacteria with suitable applied methods. Moreover, Silymarin and Sily-Nano could be used as natural sanitizers without bleaching and in the fresh-cut food industry to certify its microbiological safety. The outcomes of this work may be used in the novel industrial field to improve the nanoformula for topical application and to deliberate encapsulation of conservative antibiotics to improve the germicide activity. Further research may be applied to explore the bioavailability improvement via nanoformulation of silymarin.

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**Authors’ contributions**

The authors’ FSA, EK, MN, and KAA designed, conducted the experimental work, biochemical and statistical analysis, interpretation, and discussion of the findings, and wrote the paper related to their portion of the work. SA drafted the work and substantively revised it. Also, she approved the modified submitted version. Help in the revised version. All authors read, revised, and approved the final manuscript.

**Availability of data and material**

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

**Disclosure statement**

The authors declare no competing interests.
Ethics approval and consent to participate

The investigations have complied with the National Institutes of Health guidelines. We confirm that this study was carried out in agreement with local ethical committee standards, with the consent of Imam Abdulrahman University’s Institutional Review Board (IRB).

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