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Novel Compatible Silver Nanoparticles Functionalized by Vitamin C and its Derivatives: Characterization and their Antibacterial Activity against *Escherichia coli* and *Staphylococcus aureus*

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Abstract: Silver nanoparticles (AgNPs) in this study are prepared in one step using vitamin C (VC) and kojic acid (KA) as stabilized ligands in the presence of sodium borohydride (NaBH4). As known, the Food and Drug Administration (FDA, USA) approved the use of KA for dermatological treatment purposes. Also, vitamin C as an antioxidant has reduced cancer diseases. The AgNPs are sufficiently stabilized by these biomolecules to remain stable for up to 12 weeks. The AgNPs were characterized using UV-Visible absorption spectroscopy (UV-Vis), transmission electron microscopy (TEM), dynamic light scattering (DLS), and attenuated Fourier transform infrared (ATR-FTIR). The produced AgNPs were spherical and monodispersed with a size diameter range of 11- 15 nm. The AgNPs were tested against *Escherichia coli* (*E. coli*) and Streptococcus aureus (*S. aureus*) at different concentrations (4, 8, 15, and 30 µg/ml). The AgNPs significantly reduced bacteria growth, especially at the highest concentration (30 µg/ml). The VC-AgNP was highly active on both kinds of bacteria, even at a lower concentration (4 µg/ml). VC /or KA-AgNPs provided more encouraging results than when VC and KA were used alone, which would eventually allow VC dosages to be reduced, which could be more effective against many diseases in the future.

Keywords: vitamin C; ascorbic acid; kojic acid; AgNPs; stability; aggregations.
Abbreviations: AgNPs: Silver nanoparticles; VC: Vitamin C; KA: Kojic acid; UV-Vis: UV-Visible absorption spectroscopy; TEM: Transmission electron microscopy; DLS: Dynamic light scattering; ATR-FTIR: Attenuated Fourier transform infrared; *E. coli*: Escherichia coli; *S. aureus*: Streptococcus aureus; ROS: Reactive oxygen species.

1. Introduction

AgNPs play a significant role in nanotechnology and nanoscience, particularly in nanomedicine [1]. In recent years, AgNPs have been shown several applications, where various of their applications depend on their size, shape, and dielectric environment [2,3]. Although numerous noble metals have been used for different purposes, AgNPs have been focused on

potential applications in cancer therapy [1] and diagnostic and therapeutic applications in medicine [2]. For example, AgNPs are being used as successful qualified antimicrobial agents worldwide and have shown an important antimicrobial activity [2].

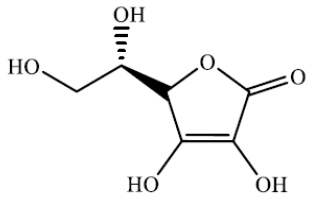
In addition, the antimicrobial properties of AgNPs apply to a significant target in enhancing the activity of drugs such as Amphotericin B, Nystatin, and Fluconazole, and composite scaffolds in order to control drug release and targeted drug delivery as a result of their biocompatibility and low toxicity [3]. Different applications require the preparation of AgNPs with good stability. For this reason, the green synthesis of stable silver nanoparticles (AgNPs) using vitamin C (ascorbic acid) and kojic acid as stabilized ligands are used in this study. In addition, it is essential to find non-toxic and natural products for functionalizing metal NPs in an aqueous medium [4]. For instance, the VC is a biomolecule readily available in nature and was used as a stabilized ligand for SnO_2 NPs. It showed an advantageous effect on the body weight of neonatal rats, as mentioned in the literature [4]. VC is used with SnO_2 NPs as both a reducing and capping agent through the synthesis of SnO_2 NPs [4]. Herein, NaBH4 was employed as a reducing agent to produce AgNPs with small sizes and good long-term stability.

It is worth mentioning that in the early 1970s, the two-time Nobel Prize-winning chemist Linus Pauling reported that vitamin C in high doses reduced cancer by acting as an antioxidant [5,6]. Also, it is demonstrated that intravenous administration of vitamin C, followed by oral use, increases the survival rate of cancer patients. [7, 8]. Conversely, other clinical studies have shown that vitamin C offers a low antitumor effect [5, 9,10]. Therefore, from our perspective, combining vitamin C with AgNPs could increase its efficacy.

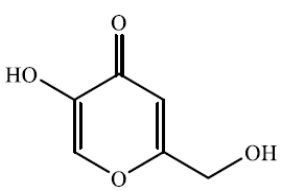
It is known that VC has been shown to inhibit the production of free radicals and reduce oxidative stress caused by reactive oxygen species (ROS). Since many chronic diseases, including cancer, diabetes, inflammation, atherosclerosis, neurodegenerative diseases, and aging, are linked with oxidative stress [5], it has also been found that ROS can lead to cell death because several cancer cells have lower levels of antioxidant enzymes in comparison with normal cells [5].

The properties of vitamin C may have dramatically changed when it is used as a functionalized ligand for nanomaterials, such as being more effective compared to vitamin C alone [4]. A lower vitamin C dose might be more beneficial against numerous diseases in the future due to the promising outcomes of vitamin C-stabilized AgNPs.

The second ligand used in this work is kojic acid, also considered an antioxidant agent [11]. Kojic acid has two different hydroxyl groups, and its chemical structure is (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one), as shown in Scheme 1.



Vitamin C (Ascorbic acid)



Kojic acid [5-Hydroxy-2-(hydroxymethyl)-4 H-pyran-4-one]

Scheme 1. Shows vitamin C and Kojic acid chemical structures.

It possesses antioxidant activity and protects the skin from harmful UV radiation and sunlight by scavenging free radicals generated by the reactive oxygen species [12]. Moreover, it is also considered one of the main drugs used in treating skin whitening, anti-browning, and

antibacterial agents [13, 14]. In addition, KA is widely used as a food additive to reduce food browning [15]. In Japan, for example, KA is used to produce traditional foods such as sake, shoyu, and mirin, where it is also used as a food preservative [16]. Furthermore, the Food and Drug Administration (FDA, USA) approved using KA associated with other compounds for dermatological treatment purposes [16].

The silica nanoparticle was used as a drug delivery system after being functionalized by kojic acid due to its interesting characteristics, such as large surface area, high volume, and good biocompatibility [13]. On the one hand, KA solid lipid nanoparticles (KA-SLNs) were produced and showed that KA-SLNs dispersion enhanced percutaneous delivery of KA as a promising and potential novel topical preparation which might open new paths for the treatment of hyperpigmentation disorders [17]. According to Wu, Y *et al*. [18], KA has antibacterial properties against gram-negative bacteria due to the presence of the free CH_2OH group at C-2 [18]. Kojic acid could offer more effective if linked with NPs. Biocompatible AgNPs with these stabilized ligands offered high stability and could be stored for long at normal conditions in this study.

2. Materials and Methods

Over the last decade, several efficient synthetic approaches have been developed that lead to good control of AgNPs' size and shape [19]. Different conditions of preparation methods of NPs lead to distinctive properties and a significant broadening of applications [20]. Good coating ligands are necessary to produce compatible AgNPs with high stability.

2.1. Materials and reagents.

Vitamin C, kojic acid, and sodium borohydride were purchased from Sigma-Aldrich and were used as received without further purification.

2.2. Characterization.

The characterization of AgNPs was carried out weekly by surface plasmon resonance band via using UV-Visible spectroscopy (Shimadzu 2400), UK. An Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) instrument was used to observe the surface capping of the AgNPs. Size and size distribution were evaluated by both TEM and DLS techniques.

2.2.1. Ultraviolet-visible absorption spectroscopy (UV-visible).

UV-visible spectroscopy was obtained on an Evolution 300 spectrophotometer with a double beam principal system and data recording using the Vision software version on Windows XP/2000. For greater accuracy, three measurements were made.

UV-Vis was used to confirm AgNPs production and their stability over time. The AgNPs were monitored weekly for more than 12 weeks. The surface plasmon resonance (SPR) band of AgNP samples was recorded in the 200-800 nm range. In this investigation, all AgNP samples displayed a distinctive SPR band at 406 nm and 399 nm for VC-AgNP and KA-AuNP, respectively.

2.2.2. Attenuated total reflectance–Fourier transform infrared (ATR-FTIR) spectroscopy.

ATR-FTIR spectroscopy (Bruker Tensor 27, Germany) was used to examine the AgNPs and coated ligands. ATR-FTIR spectra of these samples were recorded in the range of 400 to 4000 cm^{-1}. Origin software (Version 7.5) equipped with a Peak-Fitting Module (PFM) was used to analyze the spectra of AgNPs.

2.2.3. Transmission electron microscopy (TEM).

The best method for Figuring out NP morphology is TEM, which is mostly used to evaluate the size, shape, and aggregation state of NPs. A JEOL2100 field emission gun transmission electron microscope (FEG TEM) set at 100 KV was used in this study to obtain the TEM micrographs. The size distribution was obtained by counting and measuring about 160 AgNPs.

2.2.4. Dynamic light scattering (DLS).

The size and size distribution of NPs were measured using the Dynamic Light Scattering (DLS) technique and used to monitor any changes in the size/stability of AgNP. The gathered information was combined into a size distribution curve.

2.3. Synthesis of silver nanoparticles functionalized by vitamin C and kojic acid.

The novel AgNPs used in this study follow the literature's methodology [21] with a tiny proportion of chemically modified. The chemical structures of VC and KA are shown in Scheme 1.

In brief, (0.0015g, 0.008 mol) and (0.0022 g, 0.015 mol) from VC and KA, respectively, dissolved in 20 ml of distilled water with vigorous stirring for 20 minutes. After adding 10 ml of the stabilized ligand solutions, add to AgNO3 solutions (0.004 g, 0.023 mol/ in 20 ml of distilled water) and keep stirring for 2 hours at RT. The color of the solutions changed from colorless to light-yellow and greenish-yellow when a reducing agent (2 ml of freshly prepared NaBH4) (0.15 g in 10 ml of distilled water) was added dropwise to water mixture solutions, respectively. This color change indicates that there has been a reduction in Ag (+1) to Ag (0). The mixtures were left to stir for an extra 2 hours to ensure completed reactions. UV-Vis was done directly after the end of the reaction, which confirms the successful synthesis of all AgNP samples. Weekly UV-Vis measurements were taken to track the stability of AgNPs in this work. The samples were kept in a typical laboratory environment for three months.

2.4. Antimicrobial assay.

In this assay, the ability of the AgNPs to inhibit the growth of microorganism colonies is evaluated. The microorganisms used in this investigation were *Staphylococcus aureus* (MRSA) and *E. coli* (FA321). All experiments were made in triplicate, and the assay was performed in 96well plates, as previously illustrated by an earlier study [13].

The optical density (OD) measurement method was used to investigate the antibacterial effects of VC-AgNP and KA-AgNP against the mentioned bacteria above. The same procedure in our later study was done [22]. The samples' optical density at 600 nm (OD600) was determined at varied incubation times ranging from 0, 1, 2, 3, 4, and 6 hours by using a UV-Vis spectrophotometer (Jenway model 6305, UK). The antibacterial effects of AgNPs and the

antibiotic imipenem10 µg were compared. Bacteria alone (without NPs) is used as a reference (control). The data was analyzed by using GRAPHPAD PRISM V5.00 software (the ANOVA test ($p < 0.05$).

2.4.1. Minimum inhibitory concentration.

The lowest concentration of an antibiotic that will kill the vast majority (99.99%) of bacterial inoculums is known as the minimum inhibitory concentration (MIC). The MIC in this study was evaluated by the standard broth dilution method (CLSI M07-A8) to study the antibacterial activities of AgNPs in this study. Different AgNPs concentrations, such as 4, 8, 15, and 30 µg/ml, were utilized with adjusted bacterial concentrations (108 CFU/ml, 0.5 McFarland's standard), which were used to estimate the MIC in LB broth. The blank control included only inoculated broth and was incubated for 24 h at 37 °C. Each sample was measured three times, and the samples' inhibition rates were calculated as shown in the earlier literature [23].

3. Results and Discussion

3.1. Silver nanoparticle's stability.

Stability studies of the AgNPs functionalized by VC and KA were performed at room temperature for a period of 12 weeks. The stability of AgNPs was monitored by using UV-Vis to determine the SPR, and physical appearance, such as a change in the color of the produced AgNPs, as well as the formation of agglomeration/ aggregation of AgNPs, was considered. As known, UV-Vis is an important analytical technique to ascertain the formation and stability of metal nanoparticles in an aqueous solution [24].

It is worth mentioning that the control of surface chemistry is a key parameter to enable the use of NPs in several applications, and a broad range of approaches have been investigated for their subsequent surface functionalization [21]. For example, some studies used other ligands with VC, such as sodium dodecyl sulfate, through AgNP preparation to increase the stability of AgNP [24]. Furthermore, AgNPs functionalized by VC gave a size ranged of 10 - 175 nm and remained stable for just one month, as mentioned in the previous study [21]. However, in this work, VC efficiently stabilized AgNPs for a long time due to the control of procedure steps, as shown in Figure 1.

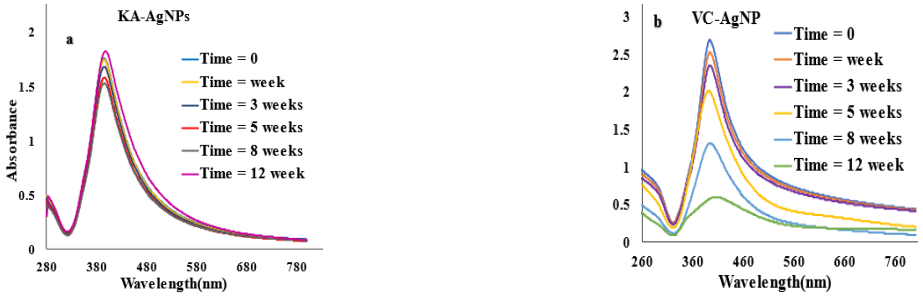


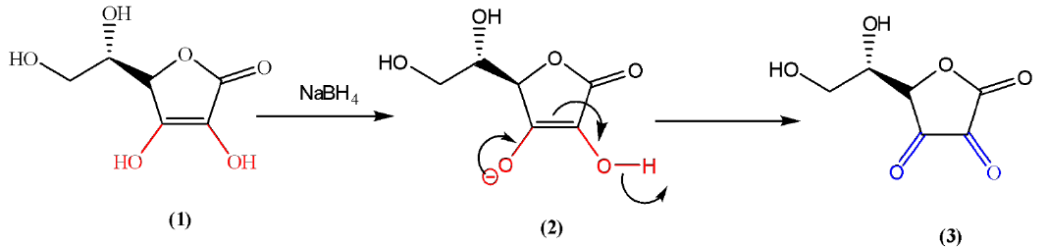
Figure 1. UV-Vis spectra of Kojic acid-AgNP **(a)** and Vitamin C-AgNPs; **(b)** at wavelengths 406 nm and 399 nm at the initial time, respectively. Which changed to 416 nm and 408 nm after 12 weeks of preparation. After a 12-week period of preparation, they shifted to 416 and 408 nm due to an increase in their sizes with time.

Producing biocompatible AgNPs and offering better control over their sizes are the key benefits of using VC and KA, which motivates researchers to use them in the medical field.

Smaller AgNPs, for example, have been shown to have higher bioactivities than larger particles [25].

The mixed solutions of $AgNO_3$ with VC and KA turned dark yellow or light yellow after adding $NaBH_4$, respectively. This color change indicated the successful synthesis of AgNPs (see Figures 1 and 3). The plasmon peaks for the AgNPs synthesized by VC and KA were observed at 406 and 399 nm, respectively.

The occurrence of plasmon peaks between 400 nm and 500 nm was good evidence to confirm the presence of AgNPs as previously described [25, 26]. The formation of AgNPs was increased with an increase in incubation time. Slight differences were observed between the two types of AgNPs. For example, VC-AgNP showed quick synthesis compared with KA-AgNP. In contrast, VC-AgNP lost its stability faster than KA-AgNP. This is due to the fact that VC, as known, contains a lactone ring with an electron-rich 2-en-2,3-diol-1-one moiety (see Scheme 2, compound 1). So, VC undergoes deprotonation of both hydroxyl groups due to the effect of a reducing agent as shown in Scheme 2, leading to the formation of dehydroascorbic acid (compound 3), as well as compound 2 is resonance stabilized as suggested by Macan *et al* [5] which converted to compound 3 that perhaps have less affinity toward AgNPs surface. That is why the stability of AgNP stabilized by VC was less than AgNP stabilized by kojic acid in the present study.



Scheme 2. Deprotonation Reaction mechanism of Vitamin C in the presence of $NaBH_4$.

3.2. Transmission electron microscopy (TEM) results.

According to Wang Y. *et al*., the size of the particle was significantly affected by the topology and end groups of ligands functionalized Nanoparticles [27]. Both VC and KA are used as capping ligands, which are responsible for preventing uncontrolled growth of AgNPs and then preventing NP aggregation as well as controlling growth rates and sizes. TEM micrographs confirm the presence of AgNPs in all the samples (Figure. 2).

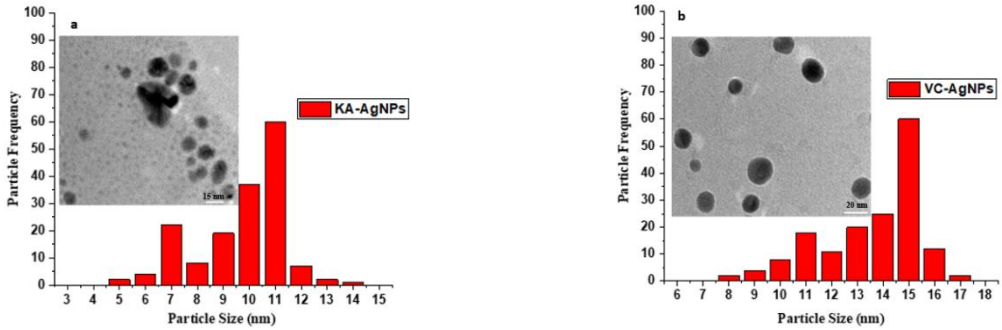


Figure 2. Demonstrative TEM micrographs of AgNPs synthesized by KA **(a)** and VC **(b)** as protecting ligands in aqueous solution and corresponding particle size histograms.

The AgNPs were monodispersed and had narrow size distribution with a spherical shape. Calculating averages and standard deviations yielded the mean particle diameters. The

average particle sizes for AgNPs synthesized by vitamin C and its deviate (kojic acid) were found to be 15±1 nm and 11± 0.9 nm, respectively. At least 150 particles per sample from several photos captured were analyzed to determine the particle sizes for samples KA-AgNP and VC-AgNP. The AgNPs were stable for more than three months without any evidence of particle agglomeration. Consequently, this capping provided high stability for AgNPs.

3.3. Dynamic light scattering (DLS) results.

The prepared AgNPs were characterized by using dynamic light screening as well. The advantages of DLS include quick measurement when the sample is in a colloidal state and does not require sample preparation, also the good statistical significance of the results [28].

DLS results confirmed the formation of AgNPs with a particle size distribution of around 22 ± 4 nm and 20 ± 3 nm for VC-AgNP and Kojic acid-AgNP, respectively (see Figure. 3).

These results are in good agreement with the particle size measured using the UV and TEM images in this study, indicating that all the results of AgNPs are confirmed with each other. As known, DLS is often used to investigate the aggregation of nanoparticles [29]. Some molecules contain carboxylic acid or alcohols or amines are used as capping ligands to prevent the NPs from aggregation because they affect the nucleation and growth rates [29]. The one-step method in this study is found to be effective in producing spherical AgNPs in an aqueous solution with high stability.

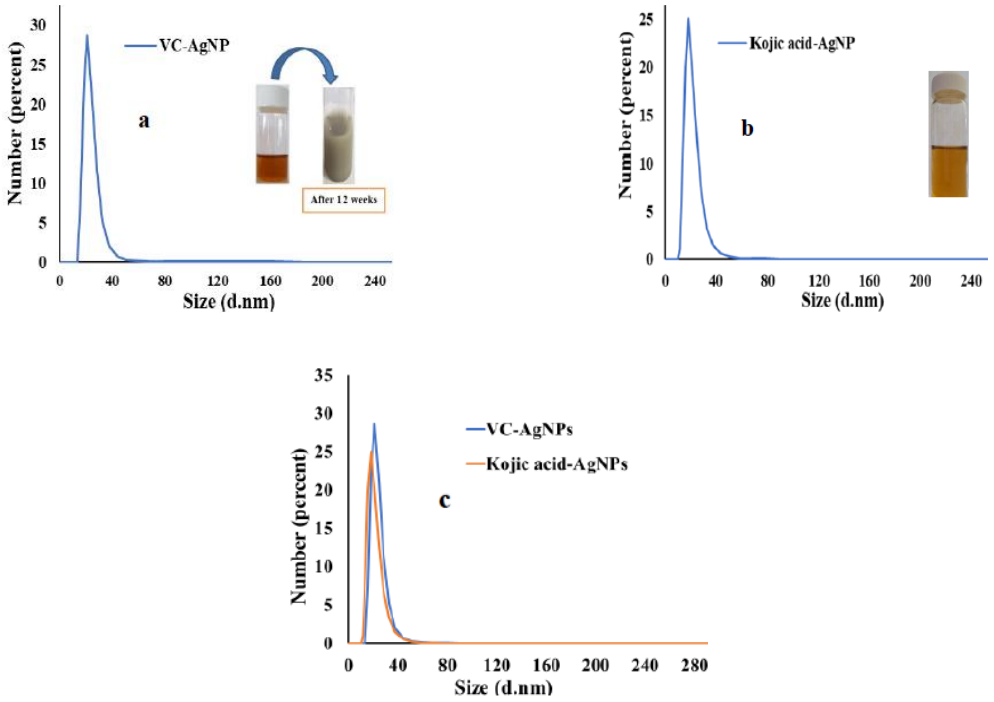


Figure 3. Size distribution of VC-AgNP **(a),** KA-AgNP **(b),** and comparison between them **(c)** measurement by using DLS. Sizes are 22 ± 4nm, and 20 ± 3 nm for VC-AgNP and Kojic acid-AgNP, respectively.

3.4. Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) Spectroscopy analysis.

The samples of AgNPs were characterized by using different techniques that demonstrate success in the functionalization and loading process, as can be seen from the

results obtained by DLS, UV-Vis, and TEM. ATR-FTIR also confirmed the presence of VC and KA in the produced AgNPs surface, as shown in Figure. 4.

Changes within the intensity of IR peaks and small shifts were observed in the spectra of the VC, KA, and therefore the AgNPs, IR spectrum of KA-AgNP showed absorption bands around 3386, 2939.68, 1635,1258, and 1033 cm^{-1} corresponding to the stretching of the –OH, –CH2, C=O, –CH, and C–O–C, respectively. In contrast, the IR spectrum of KA illustrates the absorption peaks at 1651, 1581, and 1064 cm^{-1} referring to the stretching vibration of C=O, C=C, and C–O–C in kojic acid, respectively. As a previous study pointed out [23], the absorption peak of the -OH group in KA was shifted to a high wave number from 3363 cm^{-1} to 3386 cm^{-1} in the case of KA-AgNP as seen in Figure. 4 indicating that the hydroxyl group of KA reacted on the surface of AgNP and confirming the production of AgNP. This confirmation was also supported by the band that was observed at 1643 cm^{-1}, which was broadened band in KA, and shifted to the sharp band at 1635 cm^{-1} of AgNP that could be due to covalent bonding of the KA onto AgNP [23].

Furthermore, the IR spectrum of VC and VC-AgNP showed that the intensity of the IR spectrum absorption peak was weakened, and the peak shape was narrowed in the VC compared with the VC-AgNP (see Figure 4 b). The absorption peak of the –OH group was shifted from 3471 cm^{-1} in VC and changed to a high wave number to 3494 cm^{-1} in the VC-AgNP, indicating the production of AgNP. In addition, the C=O stretching frequency of VC at 1697 cm^{-1} is present in VC-AgNP as well. However, the hydroxyl stretching of enediol of VC at 1319 cm–1 became absent in the VC-AgNP, which indicates that vitamin C was conjugated with AgNPs through the enediol, which confirms what was mentioned in the previous literature [30].

Additionally, the newly observed absorption peak in AgNP at 1234 cm−1 was the C–O–C vibration group [31] on the AgNP after being conjugated with vitamin C. This modification suggests that the AgNP and VC molecule were linked by a covalent bond, confirming that the OH group was the site of the VC's reaction with the NPs [32].

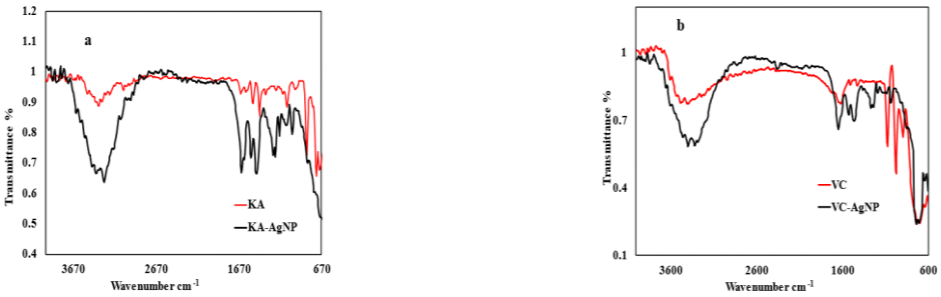


Figure. 4 ATR-FTIR spectra of KA-AgNP compared with free kojic acid (curve **(a),** and VC-AgNP (black line, curve **(a)** in comparison to free vitamin C (red line) in the wavelength region 4000–400 cm^{-1} with a resolution of 4cm^{-1} and 32 scans.

3.5. Antibacterial activities of AgNPs.

Nanoparticles have been widely used today, especially in the latest developed science materials. Silver nanoparticles, one of the most popular antimicrobial materials, have been generally utilized in the textile industry and medical engineering. Different silver nanoparticle fabrication methods will produce different physical or chemical properties [20,33-35]. The AgNPs in this study exhibited good antimicrobial activity, where *E. coli* and *S. aureus* were used as the models for gram-negative and gram-positive bacteria, respectively. An

antimicrobial activity of AgNPs was applied to *E. coli* bacteria for 6 hours and showed excellent results, as shown in Figures 5 a and b. It can be seen that the bacteria continue to grow in the presence of AgNPs at the beginning of incubation (1-3 h). However, this growth is greatly reduced at 3-6 h due to the increase in the effect of VC-AgNP and penetrating bacterial cells with time.

In contrast, KA-AgNP showed high effectiveness through the beginning of incubation (1-3 h) and lost its effect after this time. This may suggest that it needs to be more concentrated (more than 30 µg/ml) (see Figure. 5 b). The results indicate that this VC-AgNP has more antimicrobial activity than KA-AgNP. Incorporating vitamin C into the AgNPs improves their effectiveness as an antimicrobial on bacteria growth. This effect was noteworthy, as shown by the declining growth curves (see Figure. 5 a) below. KA-AgNP has a lower bactericide effect than VC-AgNP (see Figure. 5 b).

Based on the inhibition results presented in this study, it is possible to confirm that VC and KA can be good therapeutic candidates for developing drugs to treat patients with different diseases when they are occupied with AgNPs.

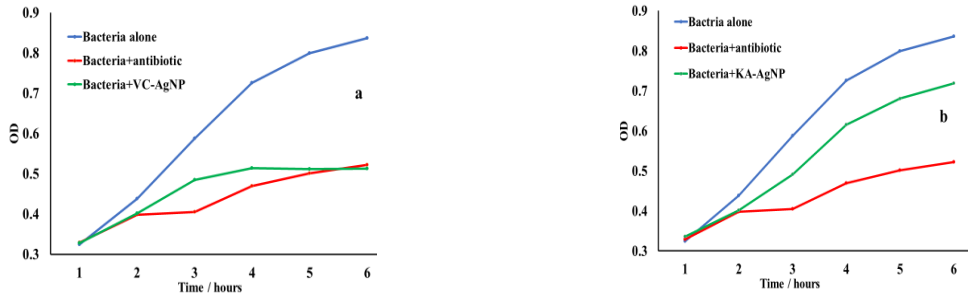


Figure.5 Shows Growth curves of *E. coli* bacteria treated with **(a)** VC-AgNP and **(b)** KA-AgNP with antibiotic (imipenem10 ug) used as a comparison with AgNPs ($P < 0.05$) at various times (1-6 hours).

3.5.1. Minimum inhibitory concentration (MIC) of KA-AgNP and VC-AgNP.

The MBC is known as the lowest concentration of any antibacterial agent that kills 100% of the bacterial population. The minimum inhibitory concentration (MIC) of KA-AgNP and VC-AgNP against *S. aureus* and *E. coli* was found to be 15 µg/ml and 4 µg/ml, respectively. *S. aureus* was found to be more sensitive than *E. coli*, with a MIC value of 15 μg/ml (see Figure 6 (a to f)).

At concentrations of 30 µg/ml, KA-AgNP, and VC-AgNP inhibit the growth of 80% of both types of bacterial stains. While at concentrations of 4 µg/ml (the lowest concentration), both of AgNPs offer a little bit of effect against *E. coli*. Herein, VC-AgNPs showed satisfactory results against *S. aureus* and *E. coli* in comparison to the case when using vitamin C alone, according to the previous study [36]. Furthermore, it was found that the gold nanoparticles provided good chemical stability for vitamin C. Consequently, vitamin C was delivered in its intact chemical structure without any oxidation. The gold nanoparticles conjured with VC were found to be more effective for cell delivery, especially at low concentrations [30], which encourages us to use AgNP functionalized by VC as an antibiotic agent.

It is well known that the biological activities of vitamin C result from its enediol structure, which displays a strong electron-donating ability. However, as previously reported [37], its low stability is a significant limitation. Because it is found to be easily oxidized, particularly under aerobic conditions and light exposure [37], for this reason, the chemical modification of ascorbic acid via its combination with NP has led to a more stable compound.

According to the literature, the KA-functionalized silica nanoparticles had higher antibacterial activity than free kojic acid against *Staphylococcus aureus* and *Candida albicans* [13].

In this study, the high bioactivity of AgNP samples was achieved. The main reason behind this result is the excellent properties, such as high stability and small particle size, which are potentially promising results. Indicates it may also enable the controlled release of drugs in the future.

As mentioned, AgNPs functionalized by VC showed the highest antimicrobial susceptibility against both types of bacteria, with the highest concentration of 30 µg/ml compared to KA-AgNPs. This is because they already have different affinities with bacterial cells and, according to the literature, different structures that allow them to easily penetrate [38, 39].

Finally, our results confirmed that the long-term stability of the colloidal AgNPs contributed to stronger biological effects against *E. coli* and *S. aureus*. This is due to their small size and the high surface-to-volume ratio [40, 41]. The results also confirm that KA and VC are indeed required to achieve sufficient stability of AgNPs, and AgNPs with these results could help to develop new and more potent antibacterial for the treatment of several diseases caused by bacteria resistance.

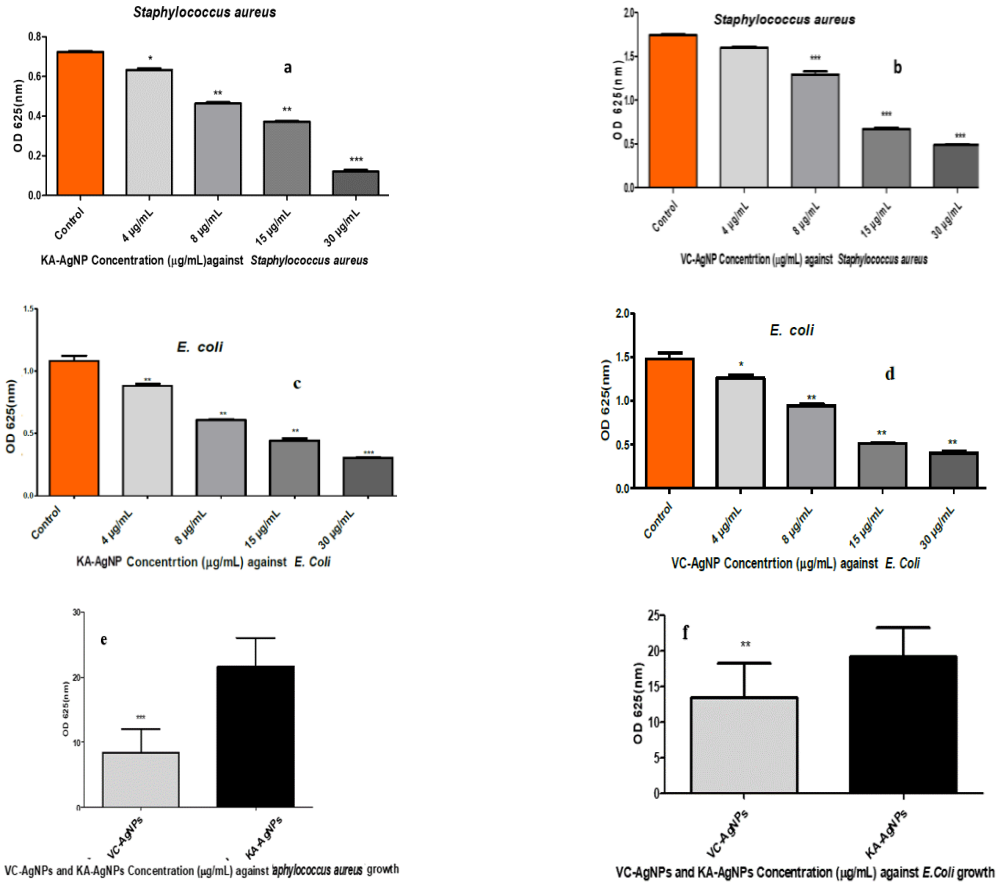


Figure 6. Results of minimum bactericidal concentration of KA-AgNPs and VC-AgNP against *S. aureus* bacteria (**a**, **b**) and *E. coli* bacteria (**c**, **d**), and controls at different concentrations (4 µg/ml, 8 µg/ml, 15 µg/ml, and 30 µg/ml) respectively. Percentage of inhibition of *S. aureus* (e) and *E. coli* (f), which are treated by VC-AgNPs, and KA-AgNPs, respectively. Where (*** represents p-value ≤ 0.0001), (** represents p ≤ 0.001), (*represents p ≤ 0.05).

4. Conclusions

In summary, we have developed a fast and inexpensive method to synthesize AgNPs. Biosynthesis of AgNPs was performed for the first time using two different stabilizers, including vitamin C and kojic acid. Experimental demonstrations are performed with single nominally spherical AgNPs with sizes ranging from 11 nm to 15 nm.

The results of the present study indicate that AgNPs strongly inhibit bacterial growth. We investigated the dose-dependent effects of AgNPs on the *S. aureus* and *E. coli* strains. The growth was monitored at different time intervals via measuring absorbance at 600 nm using a microplate reader. The treatment with AgNPs to all the bacterial strains was given in four dilutions, and the wells without AgNPs were used as a control. The MIC value was 15 µg/ml and was found to be inhibitory to more than 80% of the bacteria. Herein, we conclude that AgNP can be a better option for delivering vitamin C and kojic acid than the other nanoparticles.

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Conflicts of Interest

The authors declare no conflict of interest.

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