Synthesis of Bio-Silver Nanoparticles using Leaf Extract of Cymbopogon nardus and Examination of Their Physical and Anti-Bacterial Properties

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Abstract: The production of silver nanoparticles using biological methods has become the preferred choice of experts over chemical and physical methods because it is cost-effective, rapid, requires minimal energy, and is environmentally friendly. In this study, antibacterial activities of bio-silver nanoparticles synthesized using leaf extract of Cymbopogon nardus against Gram-positive Staphylococcus aureus (*S. aureus*) and Gram-negative Escherichia coli (E. coli) were assessed. Using TEM it was found that most of the nanoparticles are spherical, and their diameters vary from 7.0 nm to 31.0 nm with the mean diameter calculated to be 15.7 ± 4.60 nm. The UV-VIS spectrum shows 435 nm as the wavelength of localized surface plasmon resonance while the FTIR spectrum indicates the presence of the extract on the surface of the particles, suggesting the action of extracts as the reducing as well as capping agents. Antibacterial assessment was done using both diffusion disc and spectrophotometric methods, and the results show that the silver nanoparticles can inhibit the growth of both gram-positive (*S. aureus*) and gram-negative (E. coli) bacteria.

Keywords: Antibacterial Activities; Cymbopogon nardus; Bio-Silver Nanoparticles; Surface Plasmon Resonance

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I. INTRODUCTION

Silver nanoparticles (AgNPs) have been the focus of many studies in recent times. This is due to the facts that they have been used in many applications, i.e., in medicine and biosafety [1], in cosmetics products [2], in textile [3], and in water treatment [4], wherein these applications AgNPs are used because of their antibacterial properties. Other than that AgNPS are also used in electronic products [5], in biosensing and photocatalytic applications [6], microstrip antenna [7], and many more.

AgNPs can be produced using physical, chemical, and biological methods [8]. However, due to the high cost and energy of physical methods, and the issue of toxicity in chemical methods, biological methods become popular methods of choice. Using biological methods, AgNPs can be produced quickly and easily, and the methods are economically and environmentally friendly. In biological methods, AgNPs can be produced using plant extracts and microorganisms (fungi and bacteria) as reducing agents. The involvement of cell culture in using microorganisms to produce AgNPs makes the methods more complicated compared to the one using plant extracts.

AgNPs have been produced using plant extracts and their physical, chemical, and biological properties have been examined. Examples of plant extracts used are leaf extracts of *Graptophyllum pictum* [9], *Syzygium aromaticum* [10], *Anredera cordifolia* [11], of *Ficus variegate* [12], stem extract of *Anredera cordifolia* [13], tuber extract of *Manihot esculenta* [14], seed extract of *Sauropus androgynus* [15], flower extract of *Hemigraphis colorata* [16], and fruit extract of *Annona squamosa* [17]. In all these studies, the physical, chemical, and antibacterial properties of AgNPs were studied, and in some of these studies, Indonesian traditional plants, such as *Graptophyllum pictum*, *Syzygium aromaticum*, *Anredera cordifolia*, *Ficus variegate*, *Manihot esculenta*, and *Sauropus androgynus*, were used. All of these studies showed that AgNPs can be synthesized using plant extract and have a potential as an antibacterial agent.

In this present study, for the same purpose, leaf extract of another Indonesian traditional plant, Cymbopogon nardus is used to synthesize AgNPs and to examine their physical and antibacterial properties. The choice of this extract was due to the results of studies showing their antibacterial properties. Studies have shown the potential use of Cymbopogon nardus in medicine by evaluating its essential oil antibacterial and antifungal activities [18, 19]. Other studies also show that the major compound of oils of Cymbopogon nardus leaves is Citronellal [20, 21], which has been shown to have antibacterial activity [22].

The objectives of this study are to synthesize silver nanoparticles using leaf extract of Cymbopogon nardus, to

characterize their physical properties, and to assess their antibacterial activity against Gram-positive *S. aureus* and Gram-negative *E. coli*.

II. METHODOLOGY

A. Synthesis of Silver Nanoparticles

Before the preparation of leaf extract, which follows the protocol described in the previous study [10], leaves of Cymbopogon nardus were collected from a local garden in Ambon, Indonesia. Tap water and distilled water were consecutively used to clean the leaves. 20 grams of the leaves were cut into small pieces, dropped into 200 ml distilled water, and the mixture was heated for 20 minutes. The mixture was cooled and filtered through a Whatman filter paper No.1 to get the extract. Then, the leaf extract was mixed with 1 mM $AgNO_3$ (silver nitrate) solution (1:2 vv) to obtain AgNPs.

B. UV-VIS and FTIR Spectroscopies

To identify the wavelength of maximum absorption of Cymbopogon nardus AgNPs, UV-VIS spectroscopy was used. The wavelength is related to the localized surface plasmon resonance [23]. In this experiment, UV-VIS spectrophotometer UV-1700 PharmaSpec Shimadzu was used. For the measurements, the AgNPs sample (3.5 ml) was contained in a 10×10 mm optical path cuvette, and 3.5 ml extract was used as the standard. The wavelength used from the light source varied from 600 nm to 300 nm.

To characterize chemical bonds, thus to confirm the presence of the extract on the surface of Cymbopogon nardus AgNPs, FTIR spectroscopy was used. In this experiment, FTIR spectrophotometer 8201 PC Shimadzu was used. Before the measurements, the sample was centrifuged for 20 minutes (12.000 rpm) and a 2 mg pellet was taken and mixed with 200 mg KBr. The wavenumbers used varied from 4000 cm1 to 400 cm^{-1}.

C. Determination of Size and Concentration of Silver Nanoparticles

To identify the shape and size distribution of Cymbopogon nardus AgNPs, Transmission electron microscopy (TEM) was used. In this experiment, TEM JEOL JEM 1400 owned by Departemen Kimia Gadjah Mada University was used. In the measurements, a small drop of the sample was put onto a Cu-substrated grid and was left to dry at room temperature.

The molar concentration of *Cymbopogon nardus* AgNPs, c, was calculated using the formula

$$C = \frac{N_T}{NVN_A} \tag{1}$$

where N_T and V are the total amount of Ag atoms used (Found from the concentration of 1 mM AgNO3) and the volume of water used, respectively, while N is the number of Ag atoms and N_A is Avogadros number [24]. N can be calculated using the formula

$$C = \frac{\pi \rho D^3}{6M} N_A \tag{2}$$

where, ρ, D, and M are the density of FCC silver, the mean diameter of the particle, and silver atomic mass, respectively.

D. Antibacterial Assay

Antibacterial activities of *Cymbopogon nardus* AgNPs against both Gram-positive *S. aureus* and Gram-negative *E. coli*, were examined using disc diffusion and spectrophotometric methods. The procedure for these two methods follows the protocol used previously [10].

For bacterial culture preparation, a loop of each bacterial culture was suspended in the nutrient broth (500 ml), and each suspension was put in a shaker at room temperature overnight. Each of the overnight cultures was diluted with distilled water until it reached an inoculum size of about 1.5×10^8 CFU/ml (Optical Density (OD) 620 = 0.1).

In a disc diffusion method, a nutrient agar (NA) plate was prepared, and about 200 μl of the diluted culture for each bacterial suspension was spread on the NA surface using a spreader. The solution of AgNPs was washed with distilled water by centrifugation (12,000 rpm) 2 times. Sterile paper discs were infused with the solution of AgNPs (20 μl/disc) and were left in a laminar airflow to dry for about 30 minutes. The discs were placed into the NA surface, 3 discs per plate for 3 replicates. The plates were then incubated at 37°C in an incubator for 24 hours followed by measurements of inhibition zones.

In a spectrophotometric method, OD values at 620 nm of samples with and without AgNPs were measured to see the effect of AgNPs on the growth of bacteria. The first sample was the mixture of 500 l diluted culture bacterial suspension, 5 ml nutrient broth, and AgNPs, while the second sample was the mixture of 500 μl of each diluted culture bacterial suspension, 5 ml nutrient broth, and 5 ml distilled water. OD 620 was chosen since it is far enough from the maximum absorption of 435 nm. The OD 620 values were measured after incubation times of 0, 2, 4, 6, 8, 12, 16, 20, and 24 hours. A colorimeter (Smart 2 LaMotte) was used to measure OD 620, where for sample 1 (with AgNPs) solution of AgNPs was used as a standard, and for sample 2 (without AgNPs), distilled water was used as a standard.

III. RESULTS AND DISCUSSION

A. Formation of Cymbopogon nardus Silver Nanoparticles

Fig. 1(a) shows the sample of *Cymbopogon nardus* AgNPs a few hours after mixing the extract and silver nitrate solution. Once mixed, the color of the sample was transparent-light yellow (not shown) and with time, it changed to become

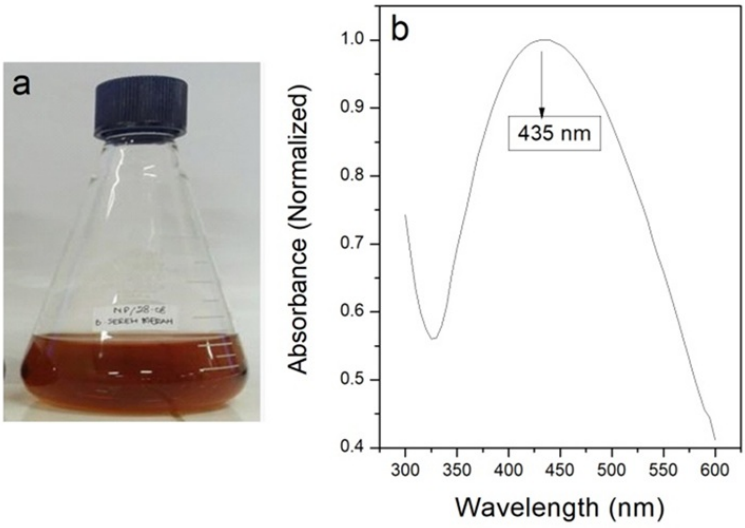

FIG. 1: (a) The sample of Cymbopogon nardus AgNPs and (b) the UV-VIS spectrum of Cymbopogon nardus AgNPs.

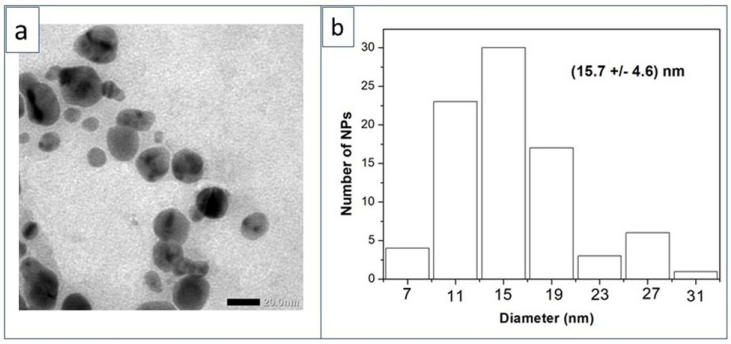

FIG. 2: (a) TEM photograph and (b) the particle size distribution of Cymbopogon nardus AgNPs.

yellowish-brown as shown in the figure. The color of the sample is due to a phenomenon known as localized surface plasmon resonance (LSPR). Electrons on the surface of a particle oscillate with a unique frequency known as a plasmon, where the frequency depends on the size, shape, and dielectric properties of the particle [23]. When light with the same frequency as the frequency of plasmon impinges on the particle, the light is absorbed and LSPR takes place. The change of the color of the sample from transparent to yellowish-brown is one of the indicators of the formation of AgNPs.

Fig. 1(b) shows the UV-VIS spectrum of *Cymbopogon nardus* AgNPs, where the sample was impinged with light wavelength varying from 600 nm to 300 nm. The figure shows maximum absorption at the wavelength of 435 nm. The 435 nm wavelength is then the wavelength (or frequency) of LSPR. Yellowish-brown color of the sample observed is due to the fact that the blue color (435 nm) was absorbed, and the color observed is usually the complement of the color absorbed [25]. The 435 nm wavelength of LSPR is consistent with the results from previous studies showing that the wavelength of LSPR for AgNPs ranges from 415 - 459 nm [26].

B. The Size and Concentration of Cymbopogon nardus Silver Nanoparticles

The shape and size distribution of *Cymbopogon nardus* AgNPs were identified using TEM and Fig. 2(a) shows one of the TEM photographs. From all the TEM photographs taken, most of the particles were found to be spherical. 100 of the particles in the photographs were chosen randomly to measure the particle diameters, where they were found to vary from the minimum value of 7.0 nm to the maximum value of 31.0 nm, and the mean diameter was found to be 15.7 ± 4.6 nm. Fig. 2(b) shows the particle size distribution. The range of diameters of *Cymbopogon nardus* AgNPs found are typical diameters for bio-AgNPs. For instance, diameters of *Graptophyllum pictum* AgNPs ranging from 5.4 to 50.6 nm [9], *Syzygium aromaticum* AgNPs from 2.9 to 33.6 nm [10], *Ficus variegate* AgNPs from 10.0 to 40.5 nm [12], and *Manihot esculenta* AgNPs from 5.5 to 68.0 nm [14].

The number of Ag atoms in one nanoparticle (N) was then determined using Eq.(2), where the mean diameter D = 15.7 nm, the density of FCC silver ρ = 10.5 g/cm^3, and silver atomic mass M = 107.868 g, and was found to be N = 118,678.

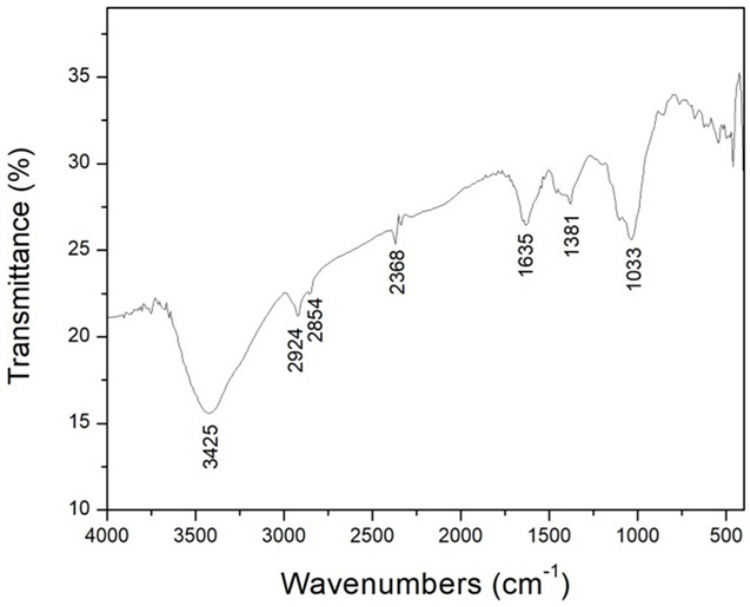

FIG. 3: FTIR spectrum of Cymbopogon nardus AgNPs.

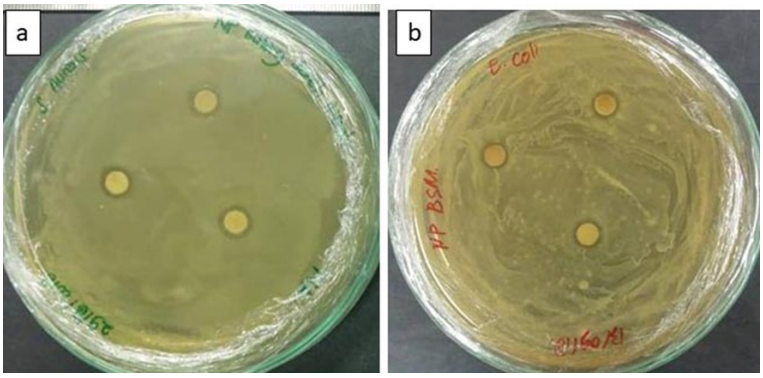

FIG. 4: The inhibition zones on the NA surface containing (a) *S. aureus* and (b) *E. coli* after applying Cymbopogon nardus AgNPs.

The molar concentration (c) of the AgNPs was determined using Eq.(1), where N_T = 0.001 $\times$ 6.02 $\times$ 1023, V = 9 ml, and N = 118,678, and the value was found to be c = 936 nM. This concentration is in the same order of magnitude (hundreds of nM) as that of other green AgNPs previously studied [27].

C. FTIR Spectrum of *Cymbopogon nardus* Silver Nanoparticles

FTIR spectrum of *Cymbopogon nardus* AgNPs is shown in Fig. 3. The peak of a broad band at 3425 cm^{-1} indicates a hydrogen-bonded O-H stretching, while two consecutive minor peaks at 2924 and 2854 indicate CH_2 asymmetric and symmetric stretches, respectively. The peak at 1635 is indicative of a C=O stretch and the peak at 1033 is contributed from an asymmetric C-C-O stretch. The band with a peak at 1381 is likely due to O-H bending. All of these results are indicative of the presence of the extract on the surface of the particle. This suggests the involvement of the extract as a reducing agent in the formation of *Cymbopogon nardus* AgNPs as well as a capping agent in stabilizing the AgNPs.

D. Antibacterial Activities of *Cymbopogon nardus* Silver Nanoparticles

Fig. 4(a) and 4(b) show the inhibition zones on the NA surfaces containing *S. aureus* and *E. coli*, respectively, 24 hours after introducing *Cymbopogon nardus* AgNPs. For *S. aureus*, the mean diameter ($\pm$ standard deviation) of the inhibition zones is 8.3 $\pm$ 0.3 mm, while for *E. coli*, it is 6.3 $\pm$ 0.3 mm. The results of statistical analysis (t-test, $\rho < 0.05$) suggest no significant difference between the two, which implies that *Cymbopogon nardus* AgNPs inhibit the growth of both *S. aureus* and *E. coli* equally.

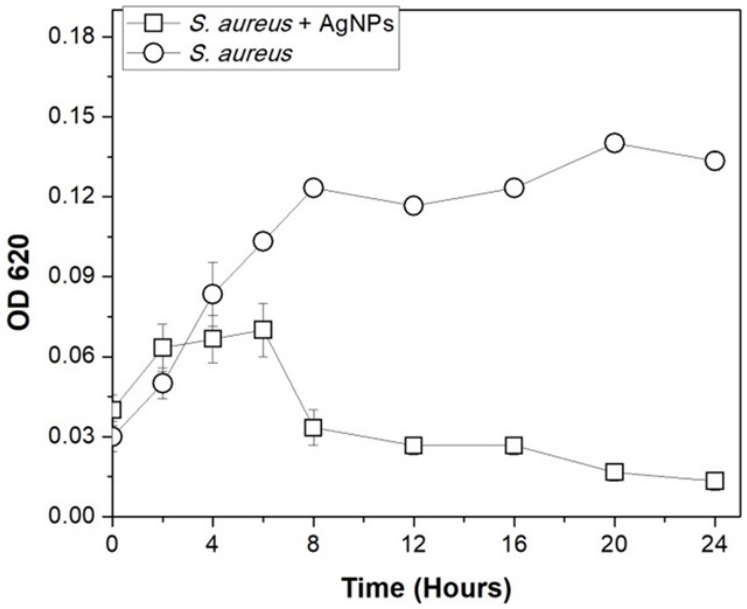

FIG. 5: OD 620 of *S. aureus* and *S. aureus* with Cymbopogon nardus AgNPs.

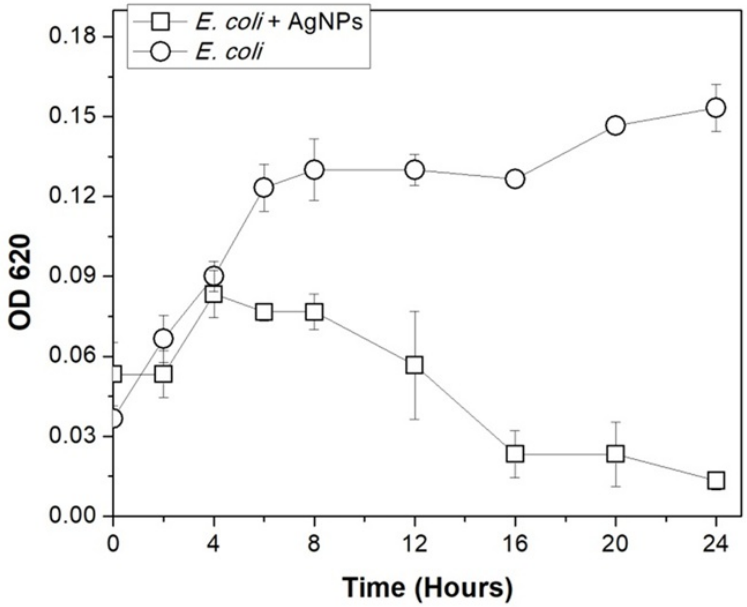

FIG. 6: OD 620 of *E. coli* and *E. coli* with Cymbopogon nardus AgNPs.

Fig. 5 shows an OD 620 of *S. aureus* samples with and without the AgNPs 24 hours after the application of the AgNPs, thus denoting the growth of *S. aureus* with and without the application of the AgNPs. OD 620 of *S. aureus* sample increases in 24 hours denoting *S. aureus* growth, while OD 620 of *S. aureus* mixed with the AgNps levels off, denoting the inhibition of *S. aureus* growth by the AgNPs. The statistical analysis of the two data shows a significant difference (t-test, $\rho < 0.05$) of OD 620, thus the growth of *S. aureus*, between samples with the AgNPs and those without the AgNPs, at 6 hours and more after introducing the AgNPs. The comparison of these two implies that *Cymbopogon nardus* AgNPs inhibit the growth of *S. aureus*.

Fig. 6 shows an OD 620 of *E. coli* samples with and without the AgNPs 24 hours after the application of the AgNPs, thus denoting the growth of *E. coli* with and without the application of the AgNPs. Similar to the data of *S. aureus*, OD 620 of samples without the AgNPs increases, while of samples without the AgNPs levels off, denoting the inhibition of *E. coli* growth by the AgNPs. The statistical analysis of the two data shows a significant difference of OD 620 (t-test, ¡0.05) between the two at 6 hours and more after introducing the

AgNPs. The comparison of these two data implies that *Cymbopogon nardus* AgNPs inhibit the growth of *E. coli*.

For comparison of the ability of the AgNPS in *S. aureus* and *E. coli*, data of OD 620 of samples with the AgNPs between *S. aureus* (Fig. 5) and *E. coli* (Fig. 6) 6 hours after introducing the AgNPs were analyzed, and the results show no significant difference in the effect of inhibition on *S. aureus* and *E. coli* (t-test, ¿0.05). These facts suggest that the AgNPs inhibit the growth of *E. coli* as well as they inhibit the growth of *S. aureus*, which is consistent with the results from the disc diffusion method.

The results of this study show that AgNPs synthesized using *Cymbopogon nardus* leaf extract can inhibit the growth of both *S. aureus* and *E. coli*. One of the factors contributing to this effect is the size of the particle. The mean diameters of 15.7 nm of *Cymbopogon nardus* AgNPs make them easy to adhere to the surface of the cell membrane of *S. aureus* and *E. coli*. To disrupt the cell membrane, the particles need to adhere first. The small size of the particles also benefits them in reaching the location in the cell. Another factor is the ability of AgNPs to release Ag^{+} ions [28]. The Positif ion of Ag can interact electrostatically with the negatively charged membrane of the cell, which penetrates inside the membrane changes the DNA structure of bacteria, and may cause the death of the cell. Another researcher suggests that the AgNPs can stimulate the formation of reactive oxygen species that may lead to oxidative stress in the cell [29].

The result showing that AgNPs inhibit the growth of *E. coli* as well as the growth of *S. aureus* is consistent with previous studies using *Syzygium aromaticum* and *Phaleria macrocarpa* AgNPs [10,30]. However, this is in contrast to the previous studies showing that AgNPs are more effective in inhibiting the growth of *E. coli* than of *S. aureus* [12,31-34], suggesting that it is because the cell membrane of *E. coli* is thinner than that of *S. aureus*. Furthermore, Some studies show that AgNPs inhibit the growth of *S. aureus* more effectively than they inhibit the growth of *E. coli* [35,36]. These three cases suggest that the thickness of the cell membrane is not the only successful factor for AgNPs to inhibit the growth of bacteria.

IV. CONCLUSION

These studies show that *Cymbopogon nardus* leaf extract can facilitate the formation of silver nanoparticles. The color of the silver nanoparticle sample, yellowish-brown, is due to a localized surface plasmon resonance phenomenon, which absorbs the blue light of 435 nm shown by the UV-VIS spectrum. The results from FTIR measurements show the presence of the extract on the surface of the particle. The nanoparticles are mostly spherical, and their diameters vary from 7.0 nm to 31.0 nm with mean diameters of 15.7 ± 4.60 nm. *Cymbopogon nardus* silver nanoparticles can inhibit the growth of *E. coli* and *S. aureus* shown by the antibacterial assessment using both disc diffusion and spectrophotometric methods, and the inhibition ability in *E. coli* is as good as in *S. Aureus*.

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