Green synthesis, characterization and biological activities of silver nanoparticles from alkalinized Cymbopogon citratus Stapf

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Abstract
Silver nanoparticles (AgNPs) have been synthesized from the alkalinized leaf extract of Cymbopogon citratus, also known as lemon grass (LG), and characterized for their size and shape using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The total formation of the AgNPs was observed visually with a color change from yellow to brownish-black. Fourier transform infrared spectroscopy (FTIR) and energy dispersive x-ray spectroscopy (EDS/EDX) were conducted to determine the various functional groups and the concentration of metal ions in the nanoparticles. The data analysis showed spherically shaped nanoparticles with a size of 10–33 nm, as revealed by TEM, thereby complementing the result for SEM. FTIR identifies the ethylene group as a reducing and capping agent for the formation of the nanoparticles. The x-ray diffraction pattern confirmed the presence of silver crystallites as well as their size, further confirming the result of the TEM. AgNPs do not exhibit very good potential as free radical scavengers when compared to the standards. The synthesized AgNPs in suspension showed activity against both gram-positive and gram-negative bacteria, with minimum inhibitory concentrations (MICs) in the range of 31.25–62.5 µg ml⁻¹. In summary, the synthesized AgNPs possessed an acceptable size and shape.

Keywords: carbon nanotubes, coating, corrosion, mechanical properties
Classification numbers: 4.02, 5.08

1. Introduction

Many researchers have embarked on the subject of nanotechnology with the sole mission of improving the living standards of man. Nanotechnology is the branch of technology that deals with dimensions and tolerances of less than 100 nm; it is science, engineering, and technology conducted at the nanoscale (about 1–100 nm). The synthesis of metal nanoparticles is a growing area of research in material science because they exhibit a unique size and shape, and have dependent characteristics that are different from the bulk metals [1, 2]. Nanoparticles are considered to be the fundamental building blocks of nanotechnology [3]. Nanoparticles obtained from plants can successfully replace chemical reduction processes and are considered to be eco-friendly; the plants are easily available, safe to handle and possess a broad variety of metabolites that may aid reduction [4–8]. In a chemical reduction process, the nanoparticles obtained come with toxic residuals which are undesirable for any sort of biomedical application. This phenomenon significantly limits the medical applications of AgNPs [8]. Silver nanoparticles have also been synthesized using microorganisms, which have the capacity to reduce metal ions via resistance and detoxification mechanisms [8]. The use of plants for the synthesis of nanoparticles has laid the
foundation for environmentally benign green nanotechnology, and this method could be advantageous over other biological processes as it eliminates the elaborate process of maintaining cell culture [5, 9]. Nanoparticles have also been synthesized from a seaweed extract, Padina tetrastrumatica, and their cytotoxicity against the breast cancer cell line was evaluated [10]. Silver nanoparticles, owing to their powerful bioactivity against bacteria, fungi, protozoa and viruses, are considered to be the most promising of any antimicrobial agent [3, 11]. The Cymbopogon genus (lemon grass) is a member of the family of Gramineae, and is a herb that is known worldwide for its high essential oil content. It is widely distributed in the tropical and subtropical regions of Africa, Asia and America. Traditional applications of Cymbopogon citratus in different countries show its diversity as a common tea, medicinal supplement, insect repellant, insecticide, and as an anti-inflammatory and analgesic. Its applications in Nigeria include cures for stomach upset, malaria therapy and as an antioxidant (in tea consumption) [12].

Although several works have extensively reported on C. citratus, using various solvents to obtain extracts from the plant, there is a deficiency of information regarding the variation in pH of the extraction medium. Our aim was to establish the fact that a variation in the pH of the extraction medium alters the chemical composition of the extracts and makes them behave differently compared to when conventional methods are used. With a holistic approach, it can be concluded that the extract obtained from the alkaline solvent is pure and suitable for everyday use. The basic reason for this is that 5% NaHCO₃, when used as an extracting solvent, is a legal food component. Sodium bicarbonate, referred to as baking soda, is primarily used in cooking (baking) as a leavening agent. In the medical world, sodium bicarbonate mixed with water can be used as an antacid to treat acid indigestion and heartburn. An antacid is a substance which neutralizes stomach acidity. It is used as a medicinal ingredient in gripe-water for infants. Gripe water is a liquid given to infants with colic, gastrointestinal discomfort, teething pain, reflux and stomach ailments. In the present study, alkalinized C. citratus is used to synthesize silver nanoparticles, which were then characterized by various analytical instruments.

2. Experimental

2.1. Materials

The collection of the plant and the preparation of the alkalinized Cymbopogon citratus were undertaken as follows. Fresh stems of C. citratus harvested from a greenhouse were identified and authenticated by professor D Grierson of the Botany Department, University of Fort Hare. The voucher specimen (GRM-K72) was prepared and deposited in the Giffen herbarium of the University. Matured leaves were collected, air-dried and milled into powdered form. The powdered material (100 g) was extracted in 500 ml (5%) sodium bicarbonate (NaHCO₃) for 48 h. The extract was filtered under vacuum using Whatman No. 1 filter paper. The filtrate obtained was lyophilized for 48 h using a freeze dryer (VirTis BenchTop K, VirTis Co., Gardiner, NY, USA). The resulting extract was kept till further use.

2.2. Synthesis of silver nanoparticles of alkalinized C. citratus

Twenty grams (20 g) of alkalinized C. citratus was weighed into a sterile 250 ml conical flask, and 100 ml of deionized water was added. The mixture was stirred at 60 °C continuously for 10 min in a water bath. It was allowed to cool and filtered with Whatman Filter Paper No. 1. The filtrate obtained was stored at 4 °C for further experiments. Then, 15 ml of the alkalinized extract was added to 45 ml of aqueous AgNO₃ (0.1 M solution) at room temperature. The mixture was stirred nonstop for 15 min with a magnetic stirrer. The resultant solution was kept in the dark to prevent the auto-oxidation of the silver. The reduction reaction was completed after 24 h with the appearance of a brownish-black color, confirming the formation of silver nanoparticles by alkalinized C. citratus (figure 1). The silver nanoparticles were centrifuged at 3000 rpm for 10 min, and the resulting pellets were dried in an oven at 100 °C for 24 h. The dried silver nanoparticles of alkalinized C. citratus were stored in a tight container away from light until further use.

2.3. Characterization of alkalinized C. citratus silver nanoparticles

2.3.1. UV–visible spectrometric analysis of silver nanoparticles. The UV–visible spectra of the synthesized silver nanoparticles were recorded as a function of wavelength using a UV–vis spectrophotometer (UV-3000 PC spectrometer) operated at a resolution of 0.5 nm. The reduction of silver was measured periodically at 300–700 nm. A spectrum of silver nanoparticles was plotted with the wavelength on the x-axis and absorbance on the y-axis.

2.3.2. Fourier transform infrared (FTIR) analysis of silver nanoparticles. FTIR measurements of the silver nanoparticles were carried out to identify the major functional groups of the synthesized compounds. FTIR measurements were carried out using a JASCO FT-IR 4100 by employing the KBr disc technique. The FTIR spectra were collected from 50 scans at a resolution of 4 cm⁻¹ in the transmission mode (4000–440 cm⁻¹).

2.3.3. Scanning electron microscopy (SEM) energy dispersive x-ray spectrometry. For SEM and elemental analysis, the sample was prepared by mounting the nanoparticles of alkalinized C. citratus on a stub using double-sided tape. They were later coated with Au/Pd using an Eiko IB 3 Ion Coater. They were observed using JOEL-JSM-6390 LVSEM at a rating voltage of 15–20 kV at different magnifications, as indicated on the SEM images.

2.3.4. Transmission electron microscopy (TEM). The TEM technique was used to visualize the morphology of the synthesized silver nanoparticles. The TEM micrographs were obtained using a Zeiss LIBRA® 120 TEM operating at 80 kV.
A drop of the silver nanoparticles in methanol was loaded on a carbon-coated copper grid, allowed to dry at room temperature and later analyzed.

2.3.5. X-ray diffraction analysis of silver nanoparticles. The x-ray diffraction of the nanoparticles was recorded on a Brucker D8 Advanced, equipped with a proportional Cu-Kα radiation counter (λ = 1.5405 Å, nickel filter) operated at a voltage of 45 kV and a current of 30 mA.

2.4. Antioxidant assay

2.4.1. Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl radical. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity evaluation is a standard assay in antioxidant activity studies. It is regarded as a rapid method for screening the radical scavenging activity of specific compounds [13]. The method in [14], using a microtiter plate, was used for the determination of DPPH radical scavenging activity. First, 100 μl of methanol was added to the wells with the exception of the second (B) and third (C) rows. Then, 200 μl of silver nanoparticles (0.5 mg ml\(^{-1}\)) and standard drugs prepared in methanol were added in triplicate to the third row (starting with the first column), and 100 μl was transferred into the second well of the same column. The procedure was repeated up to the seventh well of the same column, and the last 100 μl from the seventh well was discarded. Various concentrations of nanoparticles and standards ranging from 0.5–0.01 mg ml\(^{-1}\) were prepared in the wells, following a two-fold dilution method. A solution of 0.135 mM DPPH radical was prepared in methanol, and 100 μl of this solution was added to all the wells. The reaction mixture was then vortexed thoroughly and left in the dark at room temperature for 30 min; the absorbance was then measured spectrophotometrically at 517 nm. The actual decrease in absorbance was measured against that of the control. The scavenging ability of the nanoparticles was calculated using the equation

\[
\text{DPPH scavenging activity (\%)} = \left(\frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{control}}}\right) \times 100,\]

where \(A_{\text{control}}\) is the absorbance of DPPH + methanol and \(A_{\text{sample}}\) is the absorbance of DPPH + sample (nanoparticles/standards).

2.4.2. The 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid radical scavenging activity assay. The modified method, as described in [15], was employed for the determination of the scavenging activity of the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS). The stock solutions, comprising 7 mM ABTS solution and 2.4 mM potassium persulfate solution, were prepared. The working solution was later obtained by mixing the two stock solutions (1:1 v/v) and allowing them to react for 12 h in the dark at room temperature. The solution was then diluted by mixing 1 ml ABTS\(^+\) solution with 60 ml of methanol to obtain an absorbance of 0.708 ± 0.001 units at 734 nm using the spectrophotometer. Then, 100 μl of methanol was added to all the wells with the exception of the second (B) and third (C) rows. Following this, 200 μl of the nanoparticles (0.5 mg ml\(^{-1}\)) or the standard drugs prepared in methanol were added in triplicate to the third row (C). A two-fold serial dilution was done by mixing the contents in each well of the third row (starting with the first column) and transferring 100 μl into the second well of the same column. This procedure was repeated up to the seventh well of the same column and the last 100 μl from this well was discarded. Various concentrations of nanoparticles and standards ranging from 0.5–0.01 mg ml\(^{-1}\) were prepared in the wells, following the two-fold dilution method. The silver nanoparticles (100 μl) and the control were allowed to react with 100 μl of the ABTS\(^+\) solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS\(^+\) scavenging capacity of the extract was then compared with that of the standard drugs. The percentage inhibition was calculated as follows:

\[
\text{ABTS scavenging activity (\%)} = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right] \times 100,\]

where \(A_{\text{control}}\) is the absorbance of the ABTS radical + methanol and \(A_{\text{sample}}\) is the absorbance of the ABTS radical + sample (nanoparticles/standard drugs).

2.5. Antibacterial assay

2.5.1. Bacteria samples and culture preparation. The four reference strains of bacteria used in this study were chosen based on their pathological effects on humans and the
deterioration of food products. The gram positive bacteria Enterococcus faecalis (ATCC: 29212) and Bacillus cereus (ATCC: 10702), and the gram negative bacteria Escherichia coli (ATCC: 25922) and Shigella flexneri (KZN) were obtained from the Microbiology Unit of the MPED Research Centre, Botany Department, University of Fort Hare. The bacteria isolates were sub-cultured on nutrient agar (SAARCHEM, Gauteng SA) plates and incubated at 37 °C for 24 h. A loopful of bacterial cells from the nutrient agar plates was inoculated into 50 ml of nutrient broth (DIFCO, California, USA) in a 250 ml side-arm Erlenmeyer flask and incubated at 37 °C for 16 h with vigorous shaking (Orbital incubator, S150, UK). After incubation, the culture was diluted with fresh media to give an OD600nm of 0.1, then 100 µl of cell culture was added to the plate and spread into an agar lawn using a sterile glass spreader.

2.5.2. Minimum inhibitory concentration (MIC) determination. The MIC of the silver nanoparticles was determined using the agar dilution method as described [16]. The bacterial strains were grown at 37 °C overnight and maintained on nutrient agar. Inoculums of the test organisms were prepared in normal saline (9 mg ml⁻¹) compared with the 0.5 McFarland standard to achieve 5 × 10⁵ CFU ml⁻¹. A stock solution of various synthesized silver nanoparticles was prepared in DMSO (Sigma) and further diluted in molten MHB agar at 50 °C to give a final concentration ranging from 0.015625–0.25 mg ml⁻¹. After pouring it into the plates and allowing the agar to set, the plates were inoculated with standardized inocula of the test bacteria. The plates were further incubated at 37 °C for 24 h under aseptic conditions. The MIC was recorded as the lowest concentration at which no visible growth was observed.

2.6. Statistical analysis

Where applicable, the results are expressed as the mean ± standard deviation (SD) of three replicates and subjected to an analysis of variance (ANOVA) using Minitab release version 12, Windows 95. Significant levels were tested at P < 0.05.

3. Results and discussions

The alkalinized extract of C. citratus possesses a broad variability of metabolites that may help in the reduction of silver nitrate. The preliminary confirmation for the formation of AgNPs was the visual observation of the color change of the reaction mixture. The color, noted by visual observation, increased in intensity resulting in a brownish-black color from the original yellow after 24 h of incubation (figure 1). The color change was noticed some few minutes after the addition of AgNO₃, but the intensity increased with time. Similar changes in color have been observed in previous studies [17, 18], thereby confirming the completion of the reaction between the alkalinized extract and AgNO₃.

The synthesis of silver nanoparticles by the reduction of aqueous metal ions during the exposure of alkalinized C. citratus leaf extract was easily monitored by using UV–vis spectrophotometry. In this study, the formation of AgNPs was monitored by measuring the UV–vis spectra at different time intervals (figure 2). As the time increased, the intensity of the absorbance increased, indicating an increase in the amount of AgNPs produced by the mixture. A UV absorption spectrophotometric analysis of the LG-AgNPs showed absorbance spectra at 435 nm, suggesting the bioreduction of silver nitrate into silver nanoparticles. The broad absorption band at 435 nm is due to surface plasmon resonance typical of silver nanoparticles. The sizes of the AgNPs are in the range of other reports [5, 20, 21].

FTIR measurements were carried out to identify the major functional groups present in the LG-AgNPs. The representative spectra of the synthesized silver nanoparticles are shown in figure 3. The FTIR analysis spectrum showed sharp absorbance between 440 and 4000 cm⁻¹ for the synthesized nanoparticles. There are other peaks in the spectrum at 811, 905, 1092, 1216, 1229, 1365, 1737, 2946, 2970, 3016 and 3456 cm⁻¹ which could be esters, ethers, carbonyl or aromatic compounds. The very strong absorption peak at 1365 cm⁻¹ represents the presence of NO₂ which may be from the AgNO₃ solution, which is the metal precursor involved in the silver nanoparticle synthesis process. Ethylene groups (–C–H, C = C) detected by FTIR have been reported [22], and these groups are capable
of acting as reducing or capping agents. The two sharp absorption peaks at 1737 cm$^{-1}$ and 2970 cm$^{-1}$ indicate the possible interaction between proteins and AgNPs. The absorption peak at 1737 cm$^{-1}$ could be due to an amide bond coming from the carbonyl group of a protein [23] and the peak at 3456 cm$^{-1}$ may be due to the OH groups present in alcohols and phenolics [24]. The strong interaction between water and the surface of the silver could be the reason for the O–H stretching mode peaks at 3456 cm$^{-1}$ [25]. It has already been reported that the lemongrass leaf extract contains a lot of biomolecules, such as terpenes, alcohols, ketones, aldehydes and esters, as well as phytoconstituents, such as flavonoids and phenolic compounds, possessing antioxidant and antimicrobial activities [26]. Some chemical functional groups, such as the hydroxyl and phenolic groups, act as reducing agents, while carboxyl groups can possess shape-directing functionality [8]. In the FTIR spectrum, the absorption peaks at around 3435 and 1720 cm$^{-1}$ are attributed to the hydroxyl group and binding of the C=O functional group with the AgNPs [10]. The FTIR results suggest that the presence of the functional group in the extract serves as the reducing and capping agent of the AgNPs (figure 3).

The shape of the synthesized silver nanoparticles was analyzed by SEM. Figures 4(a) and (b) show the results of surface morphological and nanostructural studies using SEM and EDX images. The result (figure 4(a)) showed monodispersed spherical silver nanoparticles of varying sizes and shapes. Overall, the synthesized LG-AgNPs are spherical in shape, and well dispersed with low agglomeration. EDX analysis gives a qualitative as well as quantitative status of the elements that may be involved in the formation of nanoparticles. The elemental profiles of the synthesized nanoparticles for the LG-AgNPs, showing a higher count at 3 keV due to the silver, confirm the formation of silver nanoparticles (figure 4(b)). In general, metallic silver nanocrystals show a typical optical absorption peak at approximately 3 keV due to their surface plasmon resonance [27, 28]. The elemental
analysis of the silver nanoparticles shown in figure 4(b) reveals the highest proportion of silver (Ag) followed by O, Na, K, C, and Cl. A few other elements, such as Fe, Mg, P and Ca, were also present in trace amounts.

The shape and size distribution of the AgNPs were characterized by TEM. The sample was processed by dropping 3 µl of the sample onto a copper grid, followed by drying at room temperature for 15 min. Figure 5 shows the TEM image of the LG-AgNPs, which appears as a spherical shape, with loosely distributed nanoparticles. The spherical shapes confirmed the results obtained by SEM. The data analysis showed sizes of silver nanoparticles in the range of 10–33 nm with a spherical shape, which may confer their ability to penetrate cells/microbes and execute their bactericidal properties. Similar size ranges of AgNPs have been reported [5, 17, 18, 29].

An XRD pattern of LG-AgNPs was measured in the scan range of 50–120°. The crystalline sizes obtained by the x-ray diffractograms of the silver nanoparticles supported the results for TEM. The XRD spectrum confirmed the crystalline structure of the precipitate as silver (Ag) (figure 6). The XRD data for the LG-AgNPs shows diffraction peaks at 2θ = 64.88°, 77.68° and 81.95°, which can be indexed to the (2 2 0), (3 1 1) and (2 2 2) reflection planes, confirming the face-centered cubic crystalline structure of nanosilver [5, 7, 30]. In addition to the Bragg peaks representative of silver nanocrystals, additional peaks were also observed, although they were not assigned to the spectrum and may have been due to organic compounds present in the extracts, responsible for silver ion reduction and the stabilization of the resultant nanoparticles [31].

The free radical scavenging activity of the synthesized silver nanoparticles of C. citratus was studied by its ability to reduce the DPPH, which is a stable free radical. Any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby bleach the DPPH absorption. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, and this can be measured quantitatively by changes in absorbance [32]. The LG-AgNPs nanoparticles showed a maximum activity of 70.12% at a concentration of 500 µg ml⁻¹, whereas ascorbic acid and rutin at the same concentration exhibited 95.50% and 93.02% inhibition, respectively. The silver nanoparticles exhibited considerable DPPH free radical scavenging activity, as indicated by their half maximal inhibitory concentration (IC₅₀) values as shown in table 1. IC₅₀ indicates the potency of scavenging activity: the lower the IC₅₀, the higher the potency. Standard rutin and ascorbic acid were both found to have an IC₅₀ of 15.63 µg ml⁻¹, whereas LG-AgNPs showed an IC₅₀ of 30.60 µg ml⁻¹. The synthesized nanoparticles exhibited the undesired potential to act as free radical scavengers, with an IC₅₀ for DPPH inhibition comparable to rutin and ascorbic acid, which are known free radical scavengers.

ABTS is a blue chromophore generated by the reaction of potassium persulphate with ABTS for 12–14 h in the dark. It is often used in phytomedicine research to measure the antioxidant properties of hydrogen-donating and chain-breaking antioxidant agents. In this study, the synthesized silver nanoparticles, standard rutin and vitamin C scavenged the ABTS radical in a concentration-dependent manner. The LG-AgNPs efficiently scavenged the ABTS radicals generated by the reaction between the radicals and ammonium persulphate (table 1). The data obtained for the LG-AgNPs showed potent antioxidant activity, although it was markedly lower in comparison to the standard drugs. The study demonstrated the ability of the synthesized nanoparticles to scavenge radicals, thus suggesting their usefulness as a therapeutic agent for the treatment of diseases related to free radicals. The antioxidant assays are shown in figure 7.

Nanomaterials are a leading requirement in the developing field of nanomedicine and biotechnology. Nanoparticles normally have better qualities than the bulk material of the same element, having an immense surface relative to volume. Silver has been used for centuries for its antibacterial qualities; however, silver nanoparticles have proven to demonstrate better antibacterial activities than silver [33].

Table 1. The IC₅₀ values of silver nanoparticles, LG-AgNPs in DPPH and ABTS scavenging assays.

| Nanoparticles/ standard drug | DPPH assay IC₅₀ (µg ml⁻¹) | ABTS assay IC₅₀ (µg ml⁻¹) |
|-----------------------------|---------------------------|---------------------------|
| LG-AgNPs                    | 30.60                     | 123.89                    |
| Rutin                       | 15.63                     | 23.64                     |
| Vitamin C                   | 15.63                     | 19.58                     |

Figure 6. The x-ray diffraction pattern of the synthesized silver nanoparticles of the LG-AgNPs.
As well, with equal MIC values of 62.5 µg ml⁻¹ were sensitive to the alkalinized lemongrass nanoparticles for the nanoparticles ranged between 31.25 – 62.5 µg ml⁻¹.

Varying concentrations of the nanoparticles released silver ions in order to cause an imbalance in the cell and generate amplified biocidal effects [37].

The agar dilution method was used to provide evidence for the antibacterial activity of the synthesized LG-AgNPs against some selected gram positive and gram negative pathogens. This method was employed because all the microorganisms are exposed equally to the tested samples at different concentrations. The synthesized silver nanoparticles displayed antibacterial activity against the studied pathogenic microorganisms, with varying degrees as summarized in table 2. Varying concentrations of the nanoparticles were tested in order to determine their MICs. The MICs of the synthesized nanoparticles are presented in table 2. The synthesized nanoparticles were active on all the organisms tested. The highest activity against the tested bacteria was obtained with the MIC of 31.25 µg ml⁻¹ on B. cereus, gram positive bacteria. The bacterial strains of E. faecalis, E. coli and S. flexneri were sensitive to the alkalinized lemongrass nanoparticles as well, with equal MIC values of 62.5 µg ml⁻¹. The MICs for the nanoparticles ranged between 31.25–62.5 µg ml⁻¹ for all the studied organisms, while for the ciprofloxacin, it ranged from 31.25–15.63 µg ml⁻¹. These nanoparticles are therefore known to be biologically active. The antibacterial studies with LG-AgNPs showed a profound antibacterial effect against both gram positive and gram negative strains. The results of the present study suggest that plants and silver in their nano form possess certain constituents with antibacterial properties that may be used as antibacterial agents in new drugs against common bacterial pathogens. Several underlying mechanisms on how silver nanoparticles work against microbes have been documented. Firstly, silver nanoparticles attach themselves to negatively charged cell surfaces, thereby altering the physical and chemical properties of the cell membranes and cell wall. This action is known to destabilize important functions such as electron transport, osmoregulation, permeability and the respiration of the cell [34–36]. Secondly, silver nanoparticles can permeate the cell effectively as a result of their nano-size, thereby interacting with DNA, proteins and protein-containing components [34, 37]. Thirdly, silver nanoparticles release silver ions in order to cause an imbalance in the cell and generate amplified biocidal effects [37].

4. Conclusion

The silver nanoparticles synthesized from alkalinized lemon-grass leaf extract by a bio-reduction method exhibit all the characteristic features of nanoparticles. Most importantly, the AgNPs demonstrate strong antibacterial activity against drug-resistant isolates of B. cereus, E. faecalis, E. coli, and S. flexneri, which make them a potent source of antibacterial agents. These studies would certainly be useful for the development of AgNPs as an effective antimicrobial agent against drug-resistant micro-organisms.

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