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Comparative evaluation of antibacterial activity of silver nanoparticles synthesized using *Rhizophora apiculata* and glucose

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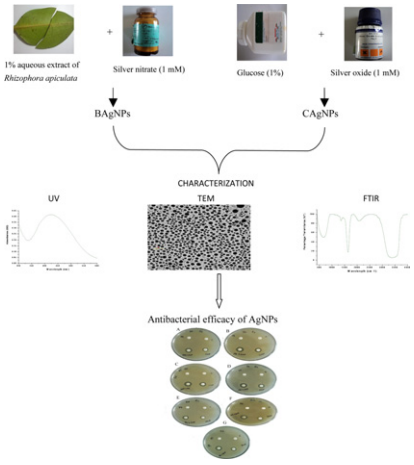
HIGHLIGHTS

- Synthesis of silver nanoparticles using *Rhizophora apiculata* and glucose.
- Characterization of nanoparticles by UV, FTIR and TEM.
- Comparison of antimicrobial efficacy of the silver nanoparticles of biological and chemical origin.

GRAPHICAL ABSTRACT



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ABSTRACT

The focus of the study is to compare the antibacterial efficacy of silver nanoparticles (AgNPs) fabricated by exploiting biological (a mangrove plant, *Rhizophora apiculata*) and chemical means (Glucose). The synthesized nanoparticles were characterised using UV–visible absorption spectrophotometry (UV–vis), Fourier transform Infra-red Spectroscopy (FTIR) and Transmission electron microscopy (TEM). Biologically synthesized silver nanoparticles (BAgNPs) were observed at 423 nm with particle sizes of 19–42 nm. The chemically synthesized silver nanoparticles (CAgNPs) showed a maximum peak at 422 nm with particle sizes of 13–19 nm. An obvious superiority of the antibacterial potency of BAgNPs compared to the CAgNPs as denoted by the zone of inhibition (ZoI) was noted when the nanoparticles were treated against seven different Microbial Type Culture Collection (MTCC) strains. The current study therefore elucidates that the synthesized AgNPs were efficient against the bacterial strains tested.

1. Introduction

Particles of nanorange have been synthesized without aggregation using chemical methods for its simplicity and the added advantage of high yield for large scale production. The chemical methods follow electrochemical, thermal, laser, microwave, polyol,

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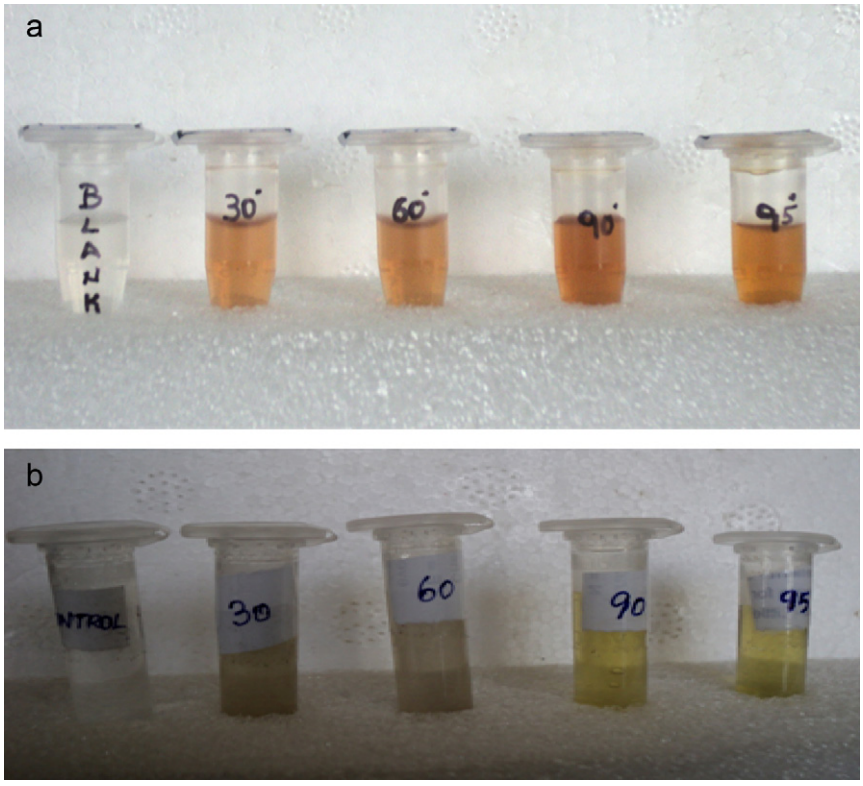

Fig. 1. Colour change at varying temperatures (from left to right): blank, 30 °C, 60 °C, 90 °C, 95 °C (A) BAgNPs (B) CAgNPs.

radiolytic, sonochemical and various other techniques [1,2]. Hazardous reducing agents used for chemical procedures mounts a bias for an eco-friendly and feasible approach for the synthesis of nanoparticles. Hence, plants are used as an alternative trigger for the green synthesis of nanomaterials [3,4]. Major parameters for synthesis of nanoparticles are the selection of solvent, reducing agent and non-toxic substances for synthesis [5]. Hence, biological means of synthesizing nanoparticles provides an edge over chemical means as it is cost effective, does not involve physical barriers with regard to reducing agents and eliminates the toxic effects of chemicals used for the synthesis [6,7].

Microbial resistance towards the available antimicrobial agents has been a major factor for the development of novel microbe-inhibitory agents. Silver has been known to possess antimicrobial effects [3,8–10] with distinctive properties of conductivity, stability and activity [11]. Therefore, the antimicrobial endurance of AgNPs is slowly finding its avenue in various health related applications [12]. The reason is that, the possibility of the microbes becoming resistant to AgNPs is slim as the nano-formulation acts on a broad range of targets in the microorganism [13]. AgNPs known to possess inhibitory and bactericidal effects have a high surface area to volume ratio along with high fraction of surface atoms that elicits elevated antimicrobial activity compared to the silver metal as a whole [14]. Though the bactericidal activity of AgNPs has been established by past studies, the mode of action still stays unclear. Researchers predict that silver species have an effect at the molecular, metabolic or membrane level of the microorganism [15]. Taking these studies as an initiative, we have synthesized AgNPs for analyzing their antibacterial activity. Mangroves are plants of the coastal ecosystem used in traditional medicine for their antibacterial, antiviral and anti-ulcer properties [16]. This attempt is hence an effort to revitalize the use of ethical knowledge of plants for ailments that can be treated with traditional medicine applying modern techniques at nano-scale.

To our knowledge this is the first report considering three different aspects. This is with regard to exploration of the biological reduction performed by the leaf extract of *Rhizophora apiculata*, chemical synthesis mediated by glucose using silver oxide as the precursor, and the comparison of antibacterial efficacy of the biologically and chemically synthesized nanoparticles. The synthesized nanoparticles were characterized and their antimicrobial activity against seven different pathogens, two of Gram positive and five of Gram negative origin was determined. The CAgNPs are used as comparative materials, a positive control to implicit the reliability and efficacy of BAgNPs.

2. Materials and methods

2.1. Preparation of silver organosol

Shade dried leaves of *R. apiculata* collected from the Mangroves of Pichavaram, India, were powdered and used for the study. Briefly, 1% aqueous *R. apiculata* extract was prepared with Millipore water and incubated at 60 °C for 5 min and used for the bioreduction.

2.2. Synthesis of silver nanoparticles

The procedure followed for the preparation of the nanoparticles was the protocol used by Song and Kim [7]. 5 ml of the organosol was added to 95 ml of 1 mM aqueous Silver nitrate (Qualigens – 99.8%) solution and the mixture was gradually heated in a water bath at varying temperatures ranging from 30 to 95 °C. To ensure better separation from free entities, the brown organosol was centrifuged at 10,000 rpm for 20 min at 4 °C and repeated thrice to remove undispersed residues. 1 mM Silver oxide (LOBA Chemie – 97%) was used as a substrate for the synthesis of AgNPs using 1% Glucose (Qualigens – 98.9%) as the reductant. The procedure was

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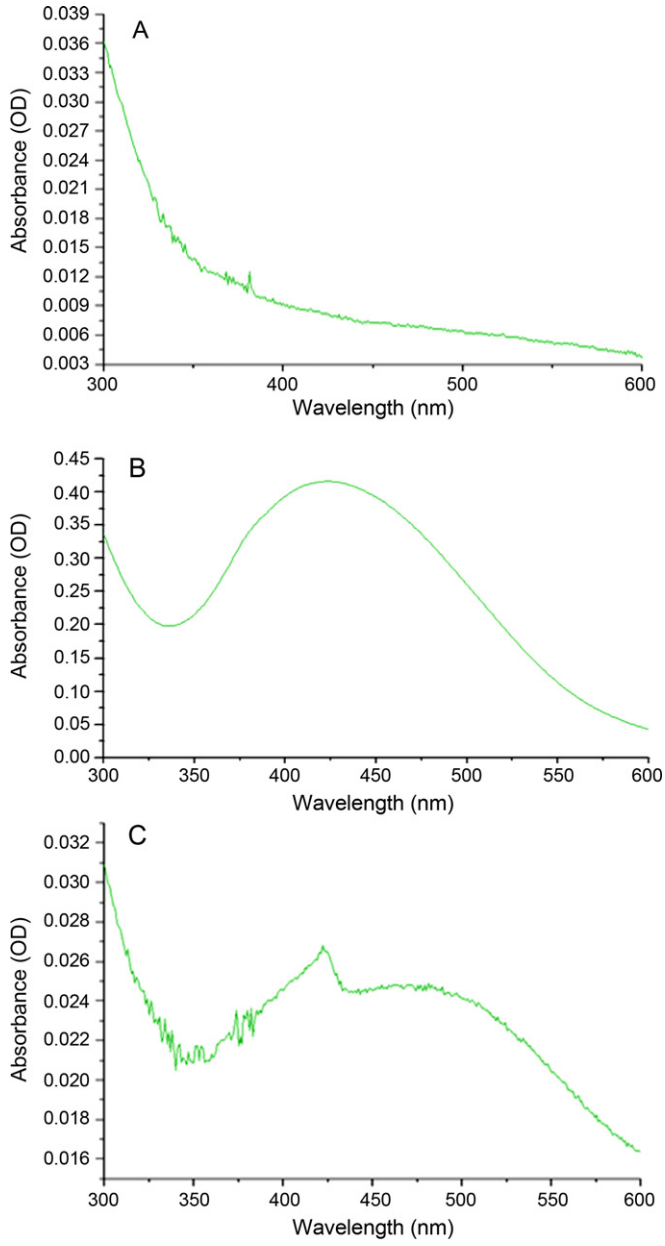

Fig. 2. UV Spectra of Silver nitrate without a reductant (A), BAgNPs (B) and CAgNPs (C).

identical to the synthesis protocol followed by Song and Kim with variations in the reactants involved [7].

2.3. Characterization of silver nanoparticles

The synthesized nanoparticles were stored at 4 °C. Apart from identifying phase transfer in the organosol observed by colour change, UV–visible spectrometric measurements were carried out on Hitachi double beam equipment (Model Lambda 35), in the 200–600 nm range. AgNPs were analyzed by FTIR on a Spectrum RX 1-One instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets and the spectra were recorded in the wavelength interval of 4000 and 400 nm^{-1}. For comparison, leaf broth was mixed with KBr powder and pelletized after drying properly and subjected to measurement. TEM measurements were performed on a Tecnai 10 instrument operated at an accelerating voltage of 120 keV.

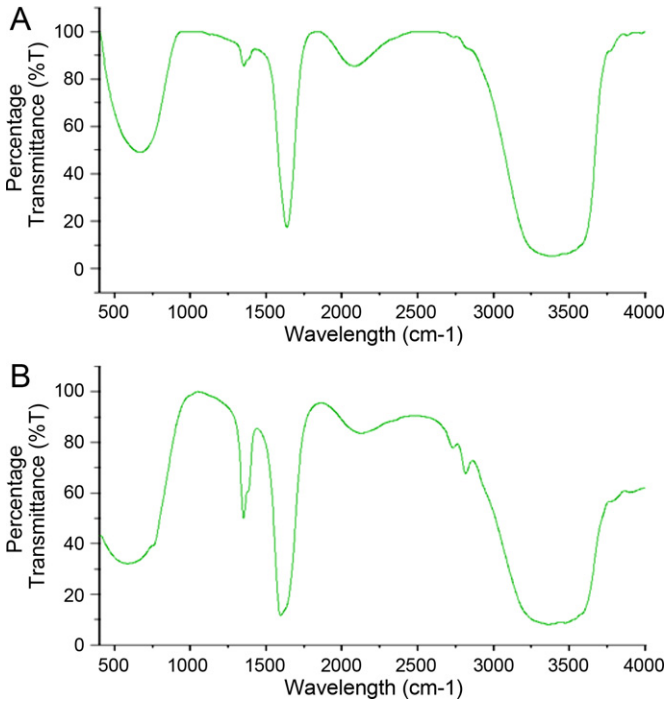

Fig. 3. FTIR spectrum of BAgNPs (A) and CAgNPs (B).

2.4. Evaluation of antimicrobial activity

Bacillus cereus (MTCC 1272), *Escherichia coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 530), *Proteus mirabilis* (MTCC 425), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella typhii* (MTCC 531) and *Staphylococcus aureus* (MTCC 96) were obtained from MTCC, Indian Institute of Microbial Technology (IMTECH), Chandigarh, India and used for analysis of bactericidal activities.

A disk diffusion method was adopted to assay the nanoparticles for bactericidal activity against the test strains on Luria Bertani (LB) agar plates. This was performed by determining the ZoI, which is rapid and inexpensive to determine the susceptibility of a particular antigen to the bactericidal agent applied [17]. This was executed by measuring the diameter (mm) of the area that stays clear of microbial growth using a vernier calliper. To accomplish this method, standard antibiotic disks were purchased from HIMEDIA Laboratories, India. Each disk had a diameter of 6 mm and was loaded with 50 μL of the test sample and air dried. Bacterial cultures about 10^6 CFU/mL were spread plated on LB agar, impregnated with the sample loaded disks and incubated at 37 °C for 18 h. The diameters of ZoI were measured and the assays were performed in triplicate.

3. Results

3.1. Characterization of BAgNPs

Blending silver nitrate with *R. apiculata* extract changed the colour of the solution to dark brown at varying temperatures with 90 °C indicating a prominent colour change (Fig. 1A). Before thermal reduction, the UV–visible spectrum indicated no absorption in the range of 260–800 nm. SPR peaks corresponding to AgNPs were not observed when silver nitrate was incubated at varying temperatures without any reductant. The silver nitrate solution alone was not thermally reduced without the presence of any reductant as by the procedure followed for the synthesis (Fig. 2A).

The UV–visible spectrum of BAgNPs showed a well observable peak at 423 nm (Fig. 2B), the characteristic wavelength

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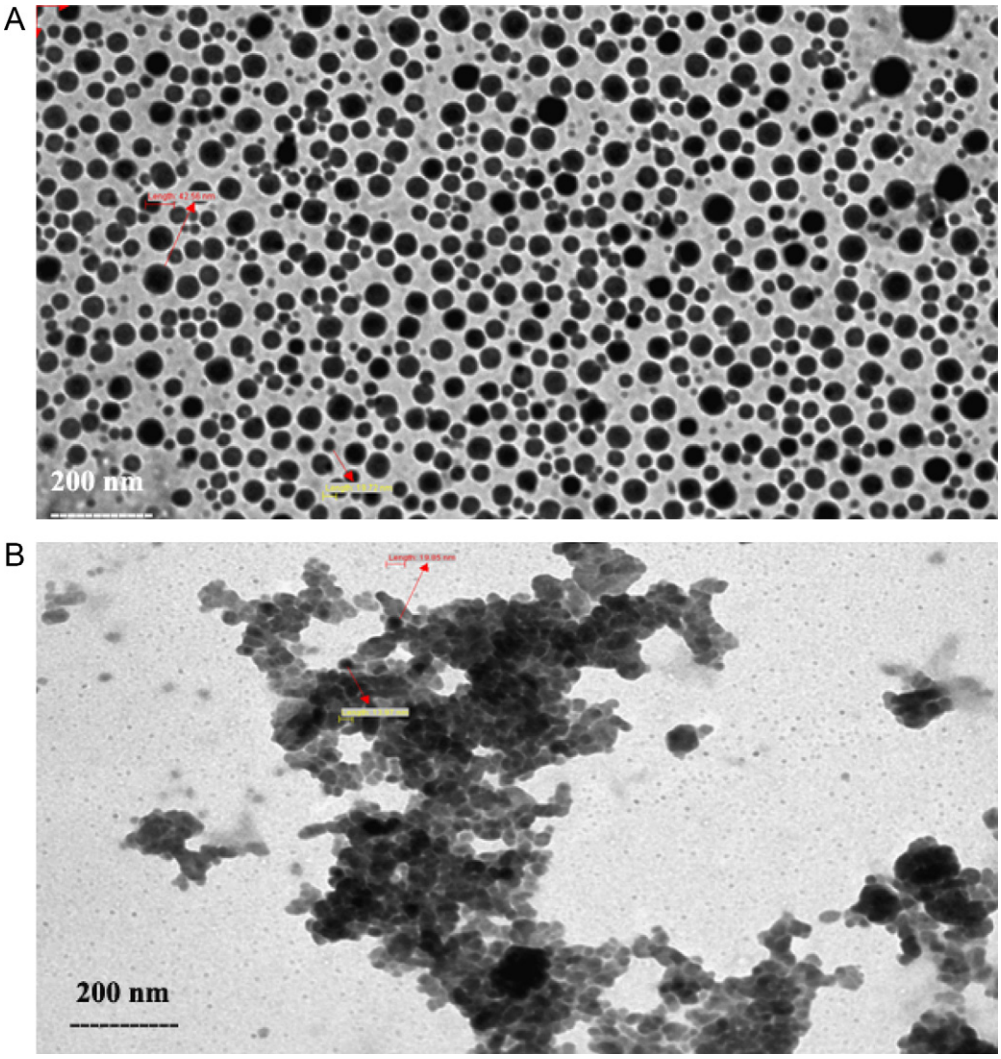

Fig. 4. TEM observation of BAgNPs (A) and CAgNPs (B) at a scale bar of 200 nm.

range of AgNPs [12]. Intense FTIR bands of BAgNPs were inferred at 3397 cm^{-1}, 2082 cm^{-1}, 1635 cm^{-1}, 1350 cm^{-1} and 668 cm^{-1} (Fig. 3A). TEM micrographs revealed monodisperse nanoparticles of spherical shape with varying sizes from 19 to 42 nm (Fig. 4A).

3.2. Characterization of CAgNPs

Pale brown colour developed with increasing temperatures when incubated with glucose (Fig. 1B). The UV–visible spectra showed a notable peak at 422 nm (Fig. 2C). FTIR predictions elucidate intense bands at 3363 cm^{-1}, 2815 cm^{-1}, 2731 cm^{-1}, 2126 cm^{-1}, 1595 cm^{-1}, 1349 cm^{-1} and 583 cm^{-1} (Fig. 3B). TEM observations elucidated that the average particle size varied from 13 to 19 nm (Fig. 4B).

3.3. Bactericidal activity of the AgNPs

After 18 h of incubation, the bacterial growth inhibition of BAgNPs was significant compared to CAgNPs and the plant extract alone. ZoI used as a tool for bactericidal activity determination

Table 1
Mean zone of inhibition (mm) of silver nanoparticles synthesized using aqueous extract of *Rhizophora apiculata* (1%), glucose (1%) and the plant extract (1%) itself alone against 7 different bacterial species. Disk diameter was 6 mm.

Micro organism	*A*	*B*	*C*	Percentage increase (%)	
				A–B	*B–C*
Bacillus subtilis	11	10	10	10	40
Escherichia coli	14	12	12	16.66	16.66
Klebsiella pneumoniae	14	12	10	16.66	40
Proteus vulgaris	14	12	13	16.66	7.69
Pseudomonas aeruginosa	12	9	8	33.33	50
Salmonella typhii	14	11	8	27.27	37.5
Staphylococcus aureus	14	11	8	27.27	75

A – BAgNPs; B – CAgNPs; C – plant extract.
Percentage increase (%) of bacterial inhibition between BAgNPs and CAgNPs was calculated using the formula $(A - B)/B \times 100$, which indicated an overall 21.12%.
An overall 38.1% increase was observed between *Rhizophora apiculata* extract and AgNPs synthesized using the same, calculated by the formula $(A - C)/C \times 100$.

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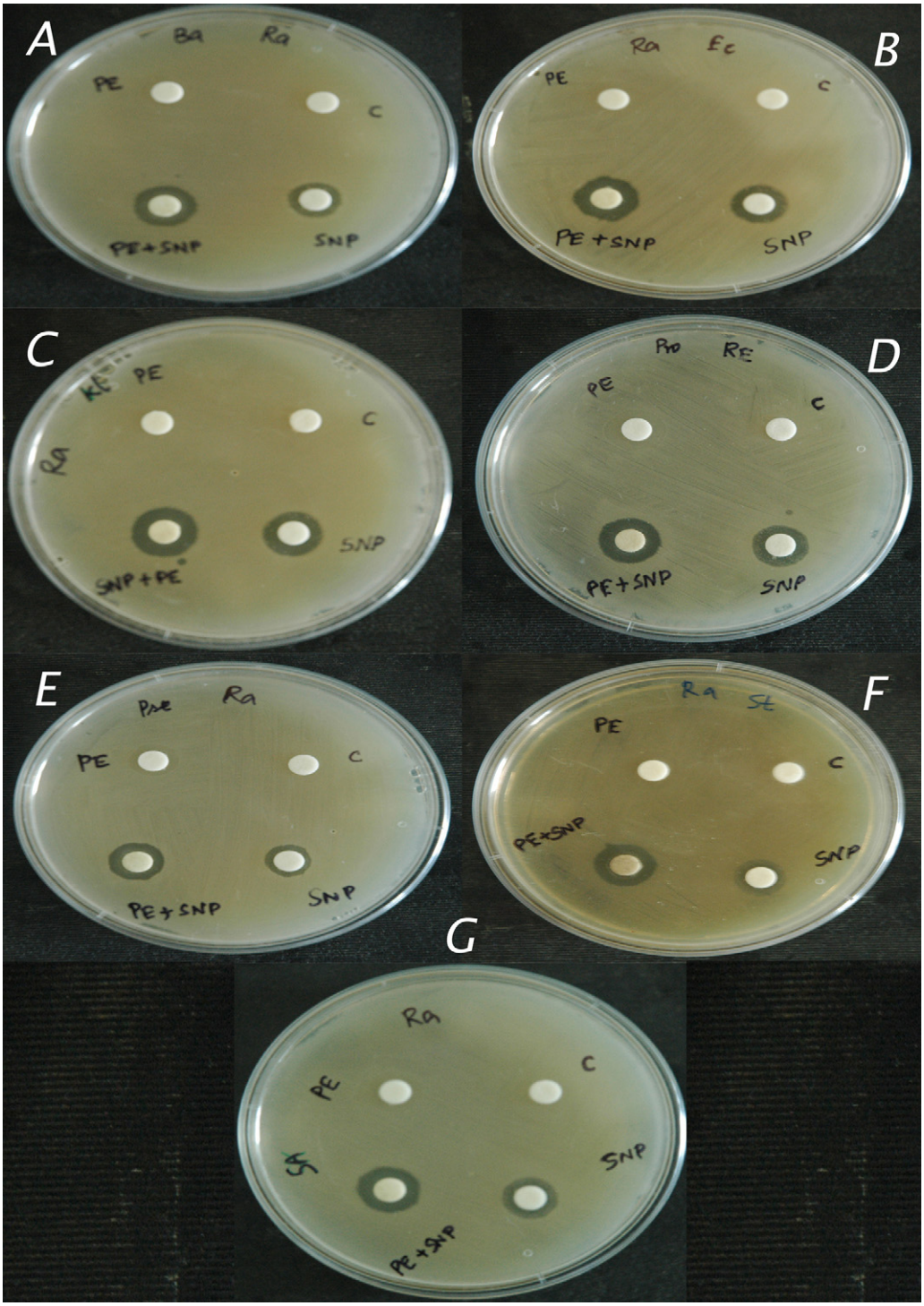

Fig. 5. Comparison of antimicrobial activity of BAgNPs and CAgNPs. (A) *Bacillus cereus*, (B) *Escherichia coli*, (C) *Klebsiella pneumoniae*, (D) *Proteus mirabilis*, (E) *Pseudomonas aeruginosa*, (F) *Salmonella typhii*, (G) *Staphylococcus aureus*.

indicated that BAgNPs showed more than 10 mm zone against all applied strains [18]. CAgNPs had a similar yet considerably limited activity (Figs. 5 and 6A). There was an overall 21.12% increase between BAgNPs and CAgNPs mediated bacterial inhibition and 38.1% to the plant extract alone (Table 1, Fig. 6B).

4. Discussion

The colour change observed indicates the transition of silver nitrate to AgNPs [19]. The colour change that was not observed in the silver nitrate solution when incubated alone indicates that the change has occurred by the reductants involved in the procedures followed. Surface Plasmon peak at 423 nm of BAgNPs and 422 nm of CAgNPs corresponds to the SPR of AgNPs. An intense spectrum in the range of 335 and 560 nm for BAgNPs was not observed as they denote nanoparticle aggregation [12,20]. Multiple peaks in the UV–vis spectra of CAgNPs are due to the formation of nanoparticles of varying shapes and sizes [21]. BAgNPs did have a single peak determining that the particles were synthesized with uniform sizes and shapes. The peaks that were not observed for silver nitrate

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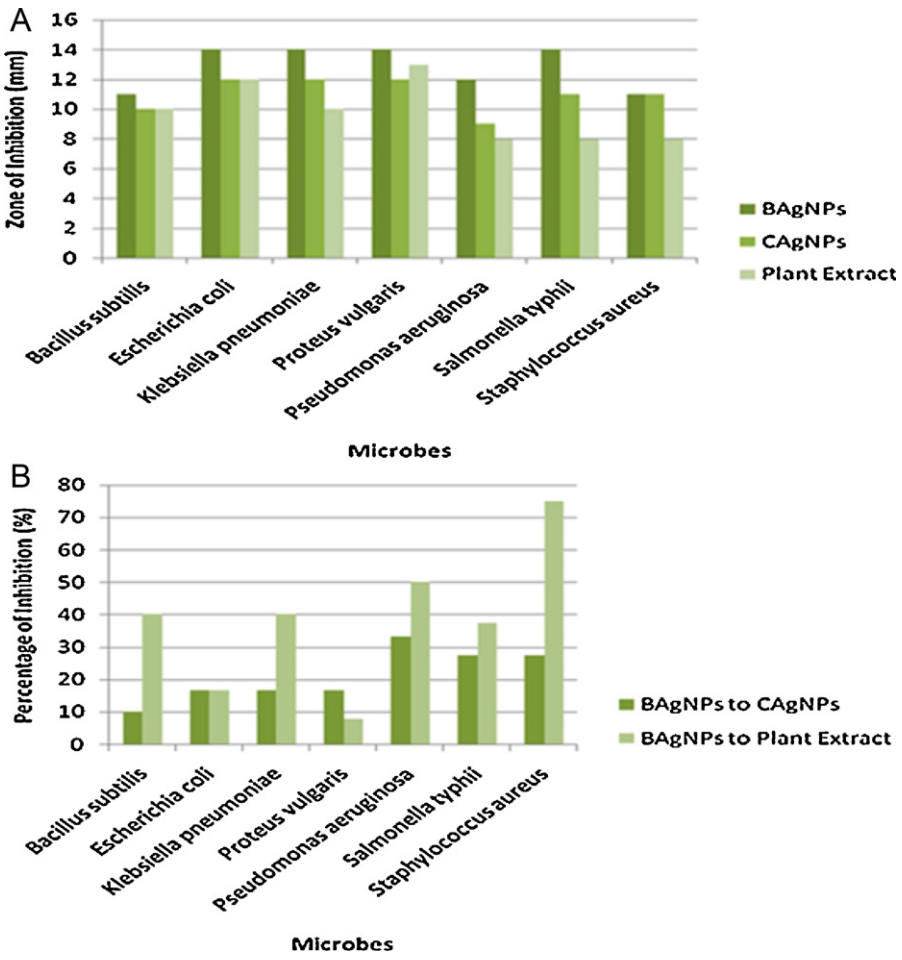

Fig. 6. Comparison between BAgNPs, CAgNPs and plant extract: (A) zone of inhibition and (B) percentage inhibition.

solution when incubated without a reductant indicate that the plant extract was responsible for reduction of silver nitrate to AgNPs. The formation of AgNPs is attributed to hydrophilic-hydrophobic interactions resulting in clusters which prevent the particles from aggregation by intermolecular forces [22]. Intense FTIR bands for BAgNPs indicated alcohols and phenols with free O–H stretches at 3397 cm^{-1} demonstrating the presence of polyphenols as capping agents on the surface. The major FTIR bands for CAgNPs indicate aldehydes and ketones with C–H stretches at 2731 cm^{-1} and 2815 cm^{-1} signifying the presence of glucose as the stabilising and capping agent. Monodisperse nanospheres of BAgNPs stayed apart from each other to a certain degree due to repulsive forces between themselves preventing the formation of clusters and thus remained oriented. The sizes did vary with a maximum at 42 nm. CAgNPs were found as groups with 19 nm as a maximum particle size.

Taking advantage of the microbicidal activities of the silver compounds and the ability to stay as a drug without microbial resistance, chemical drugs are slowly taken over by bioorganic nanoparticles. Smaller particles with a larger surface area possess higher antibacterial effects compared to the larger particles [23]. The antibacterial activity of the nanoparticles may be centred on permeability of bacterial cells due to cell wall layers or its charges [17,24]. Studies have illustrated that nanoparticles may infiltrate the cells causing intracellular loss leading to cell death and this inhibition depends on the concentration of the AgNPs [25]. AgNPs had a considerably minimal microbicidal activity on Gram positive bacteria compared to Gram negative bacteria. This is due to the high lipopolysaccharide and thick peptidoglycan layer of the microorganisms. The negatively charged AgNPs can bind to Gram negative cell wall better. The Gram positive bacteria are made up of rigid peptidoglycan layer and thus are more stable with minimal binding sites for AgNPs [19]. It was evident from the observations that Gram negative bacteria are killed more rapidly than Gram positive bacteria.

In spite of the studies conducted on the antibacterial activity and efficacy of some chosen mangroves, little is known about the antibacterial activity of the nanoparticles synthesized using the species [25]. *R. apiculata* possesses alcohols, ketones, phenols, furan and pyran derivatives of which polyphenols have been reported to possess anti-carcinogenic, anti-mutagenic, antioxidant and antibacterial activities [26,27]. The percentage fold increase of BAgNPs was higher by 21.12% to CAgNPs and 38.1% to the plant extract as indicated by the ZoI. Plant extracts did indicate unclear zones at 1% concentration. The increase in bacterial impediment between the BAgNPs and plant is highly probable due to the polyphenols and other phytochemical compounds of the plant determining that they are capped on the surface of nanoparticles [27]. Therefore, it can be suggested that the phytochemicals were coated on the surface of the AgNPs synthesized using plants and the combination has elevated inhibition of bacteria compared to CAgNPs. Among the strains tested, outcomes have indicated *P. mirabilis* as the most and *B. cereus* to be least susceptible to the AgNPs as indicated by the consequent ZoI on LB agar.

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5. Conclusions

To conclude, the antimicrobial activity of AgNPs fabricated using a biosynthetic method was evaluated and compared with chemically synthesized AgNPs. In this analysis, the AgNPs displayed antimicrobial activity against all seven cultures tested. BAgNPs revealed higher microbicidal activity compared to CAgNPs indicating that they are not alone eco-friendly, but also yield an enriched turnover compared to chemically synthesized particles. Consequently, this is a future prospect for novel antimicrobial agents raised from plants as nanofactories.

Acknowledgements

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