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Antibacterial activity of green synthesized silver nanoparticles using *Citrullus lanatus* fruit rind extract

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Abstract

The increased resistance of microbes to current antibiotics calls for development of new effective antimicrobial agents. Metal nanoparticles such as silver nanoparticles have continued to attract increased research interest in the recent past because of their wide range of applications such as in the areas of chemical sensing, nanomedicine and electronics. In this work, spherical AgNPs (17.96 ± 0.16 nm in diameter) synthesized via a green method using *Citrullus lanatus* (water melon) fruit rind extract were evaluated for antimicrobial activity against clinical isolates of *Escherichia coli (E.coli)* and *Salmonella typhi* (*S.typhi)* bacteria using the disc-diffusion method. The Minimum Inhibition Concentration (MIC) value of the nanoparticles was found to be 45.00 ± 0.01 μg/ml for *S.typhi* and 38.50 ± 0.00 μg/ml for *E.coli* while the Minimum Bactericidal Concentration (MBC) value was found to be 60.00 ± 0.05 μg/ml for *S.typhi* and 50.00 ± 0.00 μg/ml for *E.coli.* This study demonstrated that these two bacterial strains were susceptible to the green synthesized AgNPs.

Keywords: Green synthesis; Antimicrobial activity; Silver nanoparticles; *Citrullus lanatus*.

1. Introduction

Pathogenic microorganisms have led to antimicrobial resistance and are termed as a major threat to human health (Nanda and Saravanan 2009, Morens and Fauci 2013, Kotcherlakota, Das *et al*. 2018). A number of antibiotics are currently available to treat microbial infections but the development of antibiotic resistance and their toxicity has limited their use (Kotcherlakota *et al*., 2018). The emergence of nanotechnology which includes the design, characterization, production and application of materials, devices and systems in the nanometre (10^{-9} m) scale (Ndikau *et al*., 2017); promises an alternative treatment method to cure of microbial infections (Kotcherlakota *et al*., 2018). Nanotechnology normally studies the manipulation of matter on atomic and molecular levels linking atoms and molecules to produce materials known as nanoparticles or nanomaterials with exceptional physical, chemical and biological properties in comparison to their bulk counterparts (Gatebe E. 2012, Bagherzade *et al*., 2017, Noah 2018). Due to these unique properties, it has found a wide range of applications especially in the field of biotechnology, biomedical, optical, medical imaging, catalysis and electronics (Makarov *et al*. 2014, Verma and Mehata 2016, Bagherzade *et al*., 2017, Usman *et al*., 2019).

Metal nanoparticles such as silver nanoparticles are known to show remarkable potential applications. They are among the most extensively used nanoparticles due to their exceptional optical, electrical, thermal properties, and their profound role in high sensitivity biomolecular detection, catalysis biosensors and medicine (Ahmed *et al*., 2016). They have shown a broad spectrum of antibacterial activity and are known to prevent replication of the Auto Immune Deficiency Syndrome (AIDS) virus (HIV) (Bagherzade *et al*., 2017). The strong antimicrobial activity of AgNPs make them potentially capable of being applied in water treatment against waterborne pathogens such as *Vibrio cholerae*, *Escherichia coli* and *Salmonella typhi* especially at the point-of- use in homes (Pradeep, 2009). Several investigators have reported remarkable antimicrobial effects of green synthesized silver nanoparticles. For example, silver nanoparticles synthesized by Singh *et al.* using the culture supernatant of endophytic fungus (*Raphanus sativus*) were found to have antibacterial effect on Gram-positive (methicillin-resistant *Bacillus subtilis*, MTCC 441, *Staphylococcus aureus*, MTCC 740) and Gram-

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negative (*Escherichia coli*, MTCC 443, and *Serratia marcescens*, MTCC 97) bacterial pathogens(Singh *et al*., 2017). Other studies have also demonstrated that silver nanoparticles synthesized using the leaf extract of *Acalypha indica* have a high antimicrobial activity (Krishnaraj *et al*., 2010). Silver nanoparticles synthesized using leaf extract of *Moringa oleifera* were reported to have potent antimicrobial activity (Mubayi *et al*., 2012). Although numerous plant extracts have been shown to synthesize silver nanoparticles, very few studies have been done to investigate the potential of discarded agricultural wastes to synthesize silver nanoparticles. The *Citrullus lanatus* (Watermelon) rind which is normally thorwn away as an agricultural waste has been reported to contains phytochemicals such as polyphenols, tannins, alkaloids, flavonoids and saponins (Johnson *et al*., 2012) which can be used as a reductant and stabilizing agent in the synthesis of silver nanoparticles. In this work, we therefore report the antibacterial activity of silver nanoparticles synthesized via green method using *Citrullus lanatus* fruit rind extract.

2. Materials and Methods

2.1 Materials

Nine ripe water melon fruits of 1.5 kilogrammes each were randomly selected and purchased from nine fruit vendors at Wakulima Market, Muthurwa Market and Githurai Market within Nairobi County. Three water melon fruits were selected from each market, one fruit from each of the three sampling sites per market. All the water melon fruits were then transported to Kenyatta University Chemistry research laboratory. The following reagents were purchased from Sigma-Aldrich (USA); Analytical grade Silver nitrate (99.5% Purity), Sodium hydroxide (NaOH), standard silver nanoparticles, Analytical grade trisodium citrate dihydrate ($C_6H_5O_7Na_3.2H_2O$, Purity >99%), Mueller Hinton Agar. Clinical isolates of *E.coli* and *S.typhi* bacteria were obtained from the Microbiology laboratory of Kenyatta University while ciprofloxacin and vancomycin were used as positive controls.

2.2 Green Synthesis of Silver Nanoparticles (AgNPs) using the water melon rind extract (WMRE)

Silver nanoparticles were prepared and characterized as described in literature (Ndikau *et al*., 2017). Briefly, 250 g/L watermelon fruit rind extract (used as a reductant and stabilizing agent) was reacted with 0.001 M silver nitrate solution at 80^{o}C and a pH of 10 which was adjusted using sodium hydroxide (NaOH). UV-Visible spectroscopy was used to confirm the formation of AgNPs. In order to achieve that, the WMRE-AgNPs sample was diluted and then 4 mL of the diluted supernatant placed in a quartz cuvette with a 1cm path length and inserted in a UV-Vis spectrophotometer in the wavelength range of 300-700 nm to obtain the UV-Visible spectra of the sample (Ndikau *et al*., 2017). Transmission electron microscopy (TEM) was used to determine the sizes of the synthesized nanoparticles. This was done by drop-coating the WMRE-AgNPs, citrate-AgNPs and standard reference AgNPs (20 nm diameter) samples on carbon-coated copper grids which had been placed on blotting paper and then allowed to dry in air for five minutes (Ndikau *et al*., 2017).

2.3 Antimicrobial Activity of green synthesized AgNPs and standard AgNPs

The antimicrobial sensitivity tests were done using the disc-diffusion method. Mueller Hinton Agar was purchased from Sigma Aldrich (U.S.A). Briefly, 100 µL of suspension containing 10^6 colony forming units per mL of bacteria was streaked on the agar plates. Sterilized paper discs (6.0 mm diameter) were impregnated with 10 µL of the green synthesized AgNPs colloidal dispersion and placed on the agar plates streaked with clinical isolates of *E.coli* and *S.typhi* bacteria. The inoculum streaked plates were incubated in an oven at 37 ^{0}C for 24 hours. This procedure was repeated using standard reference- AgNPs (20 nm diameter). Negative controls were prepared using the same reactants (0.001 M silver nitrate and 250 g/L water melon rind extract (WMRE) and the solvent (water) used to synthesize the WMRE- Ag NPs and evaluated against each clinical isolate of *E.coli* and *S.typhi* bacteria.

Ciprofloxacin (10 µg per disc) and vancomycin (10 µg per disc) were used as positive reference standards to determine the sensitivity of one strain per isolate for each microbial species tested as shown. The inoculum streaked plates were incubated in an oven at 37 ^{0}C for 24 hours. The antimicrobial activity was evaluated in triplicate by measuring the zone of inhibition.

3. Results and Discussion

3.1 Characterization of the green synthesized silver nanoparticles using the water melon rind extract

They synthesized silver nanoparticles (WMRE-AgNPs) were characterized using UV/Vis spectroscopy and the results obtained were as shown in figure 1. The results indicated a narrow absorption peak at 404 nm for the WMRE-AgNPs sample which was very close to the peak obtained from the commercial standard AgNPs. This when compared with the broad absorption peak for sample SD_1 (Citrate-AgNPs) which was obtained at 437 nm (Ndikau, *et al*. 2017) indicated that the WMRE-AgNPs were of a relatively smaller size than those in sample SD_1 because small size AgNPs absorb and scatter electromagnetic radiation at shorter wavelengths than larger size AgNPs (Alzoubi and Bidier 2013). Also, the results indicated that the maximum absorption peaks of the synthesized AgNPs and SD_1 were not due to unreacted watermelon rind extract and unreacted $AgNO_3$ 0.001 M solution.

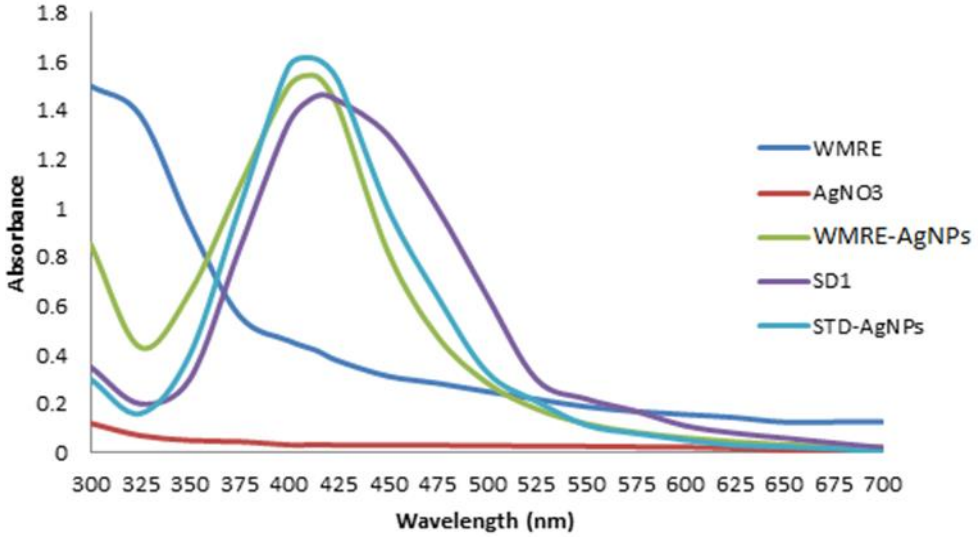

Figure 1: UV-Vis Spectra of watermelon rind extract (WMRE), $AgNO_3$ (0.001 M) solution, STD-AgNPs, WMRE-AgNPs and sample SD_1 (Citrate-AgNPs) (Ndikau *et al*., 2017)

The size and shape of the WMRE-AgNPs were determined using TEM and as reported by Ndikau, *et al*., they were found to be spherical in shape with an average diameter of 17.96 ± 0.16 nm while the citrate- AgNPs were spherical with an average diameter of 36.96 ± 0.51 nm as shown in Figure 2 below. Various researchers have reported similar results where different green methods have been used to synthesize spherical shaped AgNPs with an average diameter ranging between 20 – 34nm (Ahmed *et al*., 2016, Ibrahim 2015, Jyoti *et al*., 2016). This was a clear indication that *Citrullus lanatus* fruit rind served as a good reductant and a capping agent in the synthesis of the AgNPs (Ndikau *et al*., 2017).

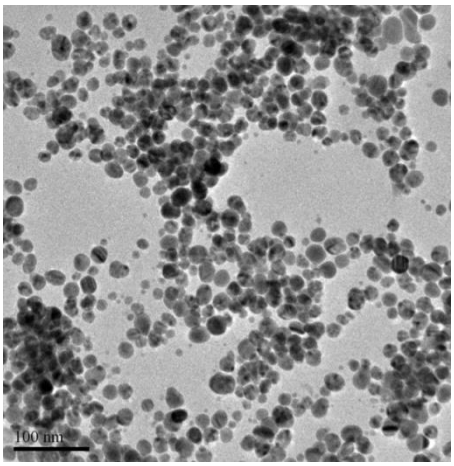

Figure 2: TEM Micrograph of WMRE-AgNPs (Ndikau et al., 2017)

3.2 Antimicrobial Activity of the WMRE rind extract as well as the positive and negative controls

When the negative controls of Silver nitrate and water melon solution prepared as described before were evaluated against each clinical isolate of *E.coli* and *S.typhi* bacteria the results were as shown in Figure 3 (a) and (b) for the inhibition zones of each. The results as seen did not show significant inhibition of the *E.Coli* and *S. typhi* as is expected for positive controls.

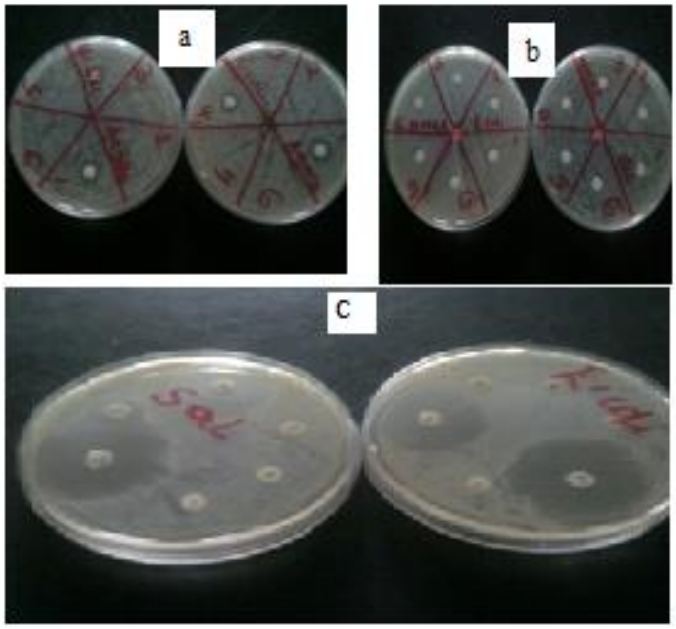

Figure 3: Inoculated plates showing the Inhibition zone against *E. Coli* and *S. typhi* bacteria (a) silver nitrate (0.001 M), (b) WMRE (250g/L) (c) Ciprofloxacin (10 μg per disc) and Vancomycin (10 μg per disc)

3.3 Antibacterial Activity of WMRE-AgNPs and the STD-AgNPs against *E. Coli* and *S. typhi*

Figure 4 shows the zones of inhibition for the ApNPs standards and the WMRE-AgNPs against *E. Coli* and *S. typhi.* From the results, it can be seeing that there is inhibition of the inoculates indicating that the standard AgNPs and the WMRE-AgNPs had an effect against the *E. Coli* and *S. typhi*.

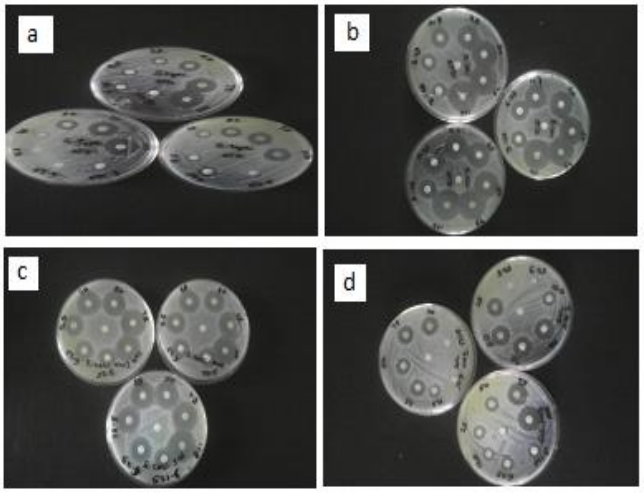

Figure 4: Inoculated plates showing zones of inhibition (a) STD-Ag NPs against *S.typhi* (b) STD-Ag NPs against *E.coli*, (c) WMRE-AgNPs against *E.coli* and (d) WMRE-Ag NPs against *S.typhi*.

The antimicrobial activity was evaluated in triplicate by measuring the zone of inhibition for the test organisms. The mean values for the WMRE-AgNPs are shown in table 1 while those for the STD-AgNPs were as shown in table 2. From the results, it is clear that the inhibition of the bacterial growth is concentration dependent indicating that the concentration of the nanoparticles plays a big role in inhibiting the growth of the bacteria. Table 3 shows a comparison of the inhibition of the bacteria by the controls, WMRE-AgNP, standard antibiotics and the STD-AgNPs.

The results shown in tables 1, 2 and 3 reveal that both clinical isolates of *E.coli* and *S.typhi* bacteria were susceptible to the antimicrobial activity of WMRE–AgNPs, standard antibiotics and the standard-AgNPs. The minimum inhibitory concentration (MIC) value of the nanoparticles was found to be 45.00 ± 0.01 µg/ml for *S.typhi* and 38.50 ± 0.00 µg/ml for *E.coli* while the minimum bactericidal concentration (MBC) value was found to be 60.00 ± 0.05 µg/ml for *S.typhi* and 50.00 ± 0.00 µg/ml for *E.coli*. These results are in close agreement to those demonstrated by (Ruparelia, *et al*. 2008) that applied ANOVA and reported a MIC of 40 µg/ml and MBC of 60 µg/ml for *E.coli* as well as results reported for antibacterial effects of silver nanoparticles (Kaviya *et al*., 2011).

Table 1: Effect of WMRE-AgNPS concentration on *E. Coli* and *S. typhi* bacteria

WMRE –Ag NPs Concentration (%)	*E.coli* Zone of Inhibition Diameter (mm) Mean±SE	*S.typhi* Zone of Inhibition Diameter (mm) Mean±SE
3.125	13.00±0.00[a]	8.00±0.00[a]
6.25	15.33±0.33[b]	10.33±0.33[b]
12.5	17.33±0.33[c]	13.33±0.33[c]
25	19.33±0.33[d]	17.00±0.00[d]
50	22.67±0.33[e]	18.67±0.33[e]
75	25.00±0.00[f]	20.33±0.33[f]
100	29.90±0.00[g]	23.50±0.00[g]
p-value	<0.001	<0.001

Mean values followed by different small letter within the same column are significantly different from one another (one way ANOVA, SNK-test)

Table 2: Effect of STD-AgNPS concentration on *E. Coli* and *S. typhi* bacteria

STD–Ag NPs Concentration (%)	*E.coli* Zone of Inhibition Diameter (mm) Mean±SE	*S.typhi* Zone of Inhibition Diameter (mm) Mean±SE
3.125	13.50±0.00[a]	7.67±0.33[a]
6.25	14.33±0.17[b]	8.33±0.33[a]
12.5	18.33±0.33[c]	13.00±0.00[b]
25	20.33±0.33[d]	17.17±0.17[c]
50	23.50±0.00[e]	19.00±0.58[d]
75	24.50±0.00[f]	20.83±0.17[e]
100	29.50±0.00[g]	23.00±0.00[f]
p-value	<0.001	<0.001

Mean values followed by different small letter within the same column are significantly different from one another (one way ANOVA, SNK-test)

Table 3: Comparison of the zones of inhibition diameter (mm)

Antibiotics and control	*E.coli* Zone of Inhibition Diameter (mm) Mean±SE	*S.typhi* Zone of Inhibition Diameter (mm) Mean±SE
0.001M $AgNO_3$	13.83 ± 0.17^{c}	13.17 ± 0.17^{c}
250g/L WMRE	10.17 ± 0.17^{b}	6.50 ± 0.00^{b}
WMRE-Ag NPs 40 µg/mL	29.90 ± 0.00^{e}	23.50 ± 0.00^{e}
std- Ag NPs 40 µg/mL	29.50 ± 0.00^{e}	23.00 ± 0.00^{e}
10µg Ciproflaxacin	35.17 ± 0.17^{f}	33.17 ± 0.17^{f}
10µg Vancomycin	24.17 ± 0.17^{d}	22.17 ± 0.17^{d}
Sterile distilled water	6.00 ± 0.00^{a}	6.00 ± 0.00^{a}
p-value	<0.001	<0.001

Mean values followed by different small letter within the same column are significantly different from one another (one way ANOVA, SNK-test).

4. Conclusion

The antimicrobial activity of the WMRE-Ag NPs against clinical isolates of *E.coli and S.typhi* bacteria were evaluated using the disc-diffusion method. The MIC value of the nanoparticles was found to be 45.00 ± 0.01 µg/ml for *S.typhi* and 38.50 ± 0.00 µg/ml for *E.coli* while the MBC value was found to be 60.00 ± 0.05 µg/ml for *S.typhi* and 50.00 ± 0.00 µg/ml for *E.coli*. The study has also demonstrated that the WMRE-Ag NPs synthesized were effective in inhibiting the growth of *E.coli* and *S.typhi* bacteria.

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