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Original Research Article

Biogenic synthesis of gold nanoparticles from waste watermelon and their antibacterial activity against *Escherichia coli* and *Staphylococcus epidermidis*

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ABSTRACT

Background: Globally, large quantities (tonnes) of diverse sources of food wastes derived from horticulture are produced and offer a valuable renewable source of biochemical compounds. Developing new recycling and food waste utilisation strategies creates unique opportunities for producing gold (Au) nanoparticles with desirable antibacterial properties. The present study used an eco-friendly procedure for biologically synthesizing gold (Au) nanoparticle shapes from waste *Citrullis lanatus var* (watermelon).
Methods: The green chemistry-based procedure used in this study was straightforward and used both red and green parts of waste watermelon. The generated Au nanoparticles were subsequently evaluated using several advanced characterization techniques. The antibacterial properties of the various extracts and synthesised nanoparticles were evaluated using the Kirby-Bauer sensitivity method.
Results: The advanced characterization techniques revealed the Au particles ranged in size from nano (100 nm) up micron (2.5 µm) and had a variety of shapes. The red watermelon extract tended to produce spheres and hexagonal plates, while the green watermelon extract tended to generate triangular shaped nanoparticles. Both red and green watermelon extracts produced nanoparticles with similar antibacterial properties. The most favourable response was achieved using a 5:1 green watermelon-based mixture for *Staphylococcus epidermidis*, which produced a maximum inhibition zone of 12 mm. While gram-negative bacteria *Escherichia coli* produced a maximum inhibition zone of 10 mm for the same mixture.
Conclusions: The study has shown both red and green parts of waste watermelon can be used to produce Au nanoparticles with antibacterial activity towards both *Escherichia coli* and *Staphylococcus epidermidis*. The study has also demonstrated an alternative method for producing high-value Au nanoparticles with potential pharmaceutical applications.

Keywords: Antibacterial, Food waste, Gold nanoparticles, Green chemistry, Watermelon

INTRODUCTION

Studies have shown the unique physiochemical and biological properties of gold (Au) nanoparticles makes them ideal for applications such as in cancer therapeutics, drug delivery, antimicrobials and medical imaging.[1-7] Au nanoparticle properties are dependent on their size and shape which makes them different from their bulk equivalent.[8] Because of these unique properties, researchers have investigated the use of Au nanoparticles to combat antibiotic resistant strains of bacteria and fungal species. Currently, several bacterial and fungal species have developed immunity against routinely used antibiotics. Thus, there is a critical need to develop new and more effective antimicrobial agents to fight antibiotic resistant bacteria and fungal species. Interactions occurring between Au nanoparticles and microorganisms have been found to result in cell membrane damage via bio-sorption and toxicity via cellular uptake.[9,10] The precise mechanisms involved in these interactions is not fully understood. But, studies have shown the nanoparticle size, shape and surface reactivity can have a significant bearing on antimicrobial properties.[11,12] Thus, prospective antimicrobial agents like Au nanoparticles can be used against many microorganism and deliver an important and immediate health benefit. [13,14]

Conventional physical and chemical methods are used to manufacture Au nanoparticles with a wide range of sizes and shapes. However, carcinogenicity, cytotoxicity and environmental toxicity concerns related to the chemical compounds and solvents used during these methods in an ongoing problem.[15]

In recent years, more eco-friendly manufacturing methods using alternative approaches were investigated by several researchers.[16,17] Biological synthesis, where plants, bacteria, fungus and similar organisms are used as bio-factories to produce metal nanoparticles is one such approach. Studies have shown nanoparticles produced by plant extracts are stable and formation rates are fast compared to other biological entities such as bacteria and fungus.[18] Horticultural wastes, like other plant sources contain a large selection of biomolecules that include alkaloids, amino acids, enzymes, phenolics, proteins, polysaccharides, saponins, tannins, terpinoids and vitamins.

Studies have shown these compounds assist in the formation of nanoparticles by acting as reducing agents and capping agents.[19-21] The green chemistry-based method is straightforward and begins by producing an aqueous extract from the plant material. An aqueous metal salt solution is then added to the plant extract (forming the reaction mixture). During the reaction, Au (III) ions bio-reduce to their metallic form (Au0) initiating nanoparticle nucleation.[22] Progressively, smaller neighbouring particles cluster to former larger thermodynamically stable nanoparticles.[23] During growth period nanoparticles form their most favourable and stable shape, which can include cubes, spheres, triangles, hexagons, pentagons, rods and wires.[23] During this biosynthesis, factors like plant extract concentration, metal salt concentration, reaction time, reaction solution pH and temperature all influence the properties of the resulting nanoparticles.[24] To date only a small number of studies have *reported* using renewable horticultural wastes to generate high-value products like Au nanoparticles. The present study reports the use of waste *Citrullis lanatus var* (watermelon) to produce Au nanoparticles with antibacterial properties towards *Escherichia coli* and *Staphylococcus Epidermidis*. The aqueous-based process individually used red and green parts of the watermelon to produce Au nanoparticles. The procedure is straightforward and did not require specialised equipment. The generated Au nanoparticles were characterized using UV–visible spectroscopy, X-ray diffraction analysis, energy dispersive spectrometer (EDS) analysis and scanning electron microscopy (SEM). Furthermore, antibacterial activity towards *Escherichia coli* and *Staphylococcus Epidermidis* were evaluated using the Kirby-Bauer sensitivity method.[25]

METHODS

Materials

The source of Au+ ions used in this study came from aqueous solutions containing of gold chloride (HAuCl4, (99.99%)). The gold chloride was supplied by Sigma-Aldrich (Castle Hill, NSW Australia) and used without any further purification. All aqueous solutions were made using Milli-Q® water produced by a Barnstead Ultrapure Water System D11931 (Thermo Scientific Dubuque IA: 18.3 MΩ cm-1).

Preparation of red and green watermelon parts

The watermelon has three parts. The first is the inner red part, the second is the outer green part, and the third is the outer skin. The red and green parts were removed and separated, while the outer skin was disregarded. Then 100 g of red part (Red WM) were cut up into small pieces and place into a glass beaker.

Similarly, 100 g of green part (Green WM) were cut up and placed into a glass beaker. Then 50 mL of Milli-Q® water (equates to a mass ratio of 1:2) was added to each glass beaker. Then each beaker in turn was homogenized for 10 minutes. Following homogenization, each beaker was subjected to 45 s of microwave heating (1100 W at 2450 Hz, LG® Australia). After the thermal treatment each batch was filtered using standard Whatman filter paper to remove debris & pulp from the mixtures.

Following initial filtration a 0.22µm syringe filters were used to screen the respective extracts before the first centrifugation. After 30 minutes of centrifugation the extracts were screened using 0.22µm syringe filters. This was followed by a second centrifugation and subsequent 0.22µm syringe filtering.

A final centrifugation (third) was carried out before final filtering was carried out again using 0.22µm syringe filters. After centrifugation and filtering, the resulting extracts were visibly clear with a slight yellowish-green tint. A schematic presentation of the preparation procedure is shown in (Figure 1).

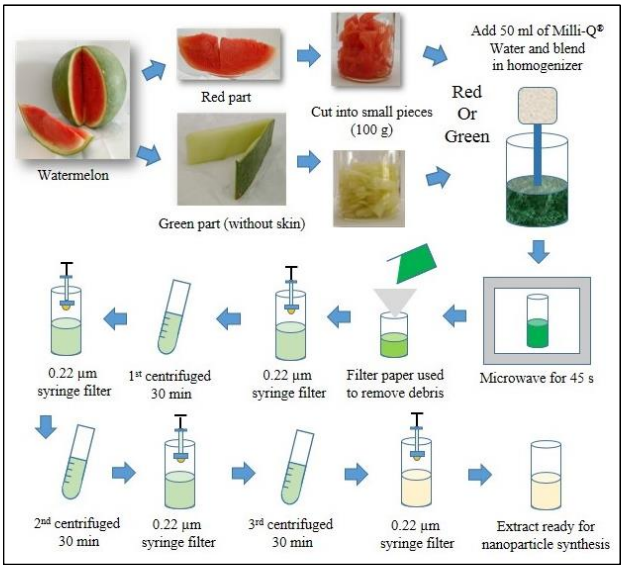

Figure 1: A schematic representation of the green and red watermelon extract preparation procedure.

Synthesis of Au nanoparticles

Two types of extract were prepared, the first was Red WM and the second was Green WM as mentioned previously. Nanoparticle synthesis was the same for both extract types and consisted of mixing varying amounts of extract with a fixed amount of AuC l4- (500ppm). The varying amounts of extract (1, 3 and 5 mL) were added to an aqueous 1mL solution of AuC l4 - (500ppm) to form the respective reaction mixtures of 1:1, 3:1, and 5:1. The reaction mixtures were then allowed to stand for two hours at room temperature.

Advanced characterisation

A Varian Cary 50 series UV-Visible spectrophotometer version 3, over a spectral range from 200 to 1100 nm (spectral resolution of 1 nm), operating at a room temperature of 24 °C was used to determine the surface plasmon resonance (SPR) peak.

A Bruker D8 series diffractometer, with flat plane geometry scanning over a 2θ range from 15° to 80° (incremental step size of 0.04°) was used to detect the presence of crystalline metal Au in the samples. The diffractometer operated at 40 kV and 30 mA (Cu Kα = 1.5406 Å radiation source), with 2 second acquisition period. A JEOL JCM-6000, Neo Scope TM electron microscope produced images that were used to determine particle size and shape. While the energy dispersive spectroscopy (EDS) attachment was to determine the compositional analysis of the samples.

Before electron microscope analysis, dried samples were attached to SEM holders (carbon tape) and sputter coated (Cressington 208HR) with a 2 nm layer of platinum to prevent charge build up.

Antibacterial activity of synthesized Au nanoparticles

The sensitivity method of Kirby-Bauer was used to investigate the antibacterial properties of the synthesised Au nanoparticle against two bacterial strains (*Escherichia coli;* gram-negative and *Staphylococcus epidermis*; gram-positive).[25] The sub-cultured bacteria were swabbed evenly over a nutrient agar medium contained in several Petri dishes (90 mm Dia.) using a sterile cotton swab. Nanoparticle solution samples (50 µL) produced from both Red WM and Green WM reaction mixtures were deposited on sterile disks (6 mm Whatman® AA 2017-006) using a micropipette. After drying in air for 20 minutes, the disks were placed on respective bacteria treated agar plates using sterile forceps. The plates were then incubated at 37 ºC for 48 hours. After incubation, the inhibition zone diameters were measured. Sample testing was carried out in triplicate and the mean inhibition zone diameters were used in the subsequent data analysis.

RESULTS

The formation of Au nanoparticles in the respective reaction mixtures was monitored using UV-Visible spectroscopy. The resulting brown colour seen in the samples was the result the surface plasmon resonance (SPR), which occurred at 560 nm for both the Red WM and Green WM samples. A representative UV-visible spectrum for both Red WM and Green WM samples (3:1 ratio) is shown in (Figure 2).

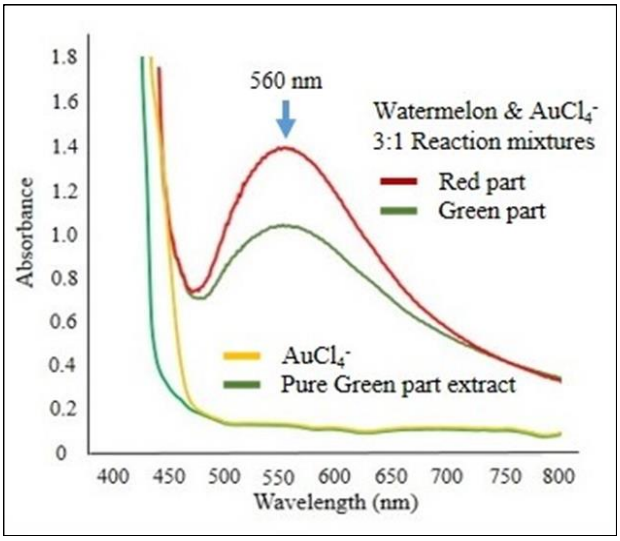

Figure 2: UV-visible spectroscopy analysis of Au nanoparticles synthesised from Red WM and Green WM extracts (3:1 ratio: watermelon: AuCl4-).

An optical image of a represent sample of Green WM (3:1) is presented in (Figure 3a insert). The image shows the initial clear mixture with a slight yellowish-green tint and the final dark brown colour of the mixture after bio-reduction. XRD spectroscopy was used to determine if crystalline Au was present in the samples. Analysis of the respective diffraction patterns revealed the presence of phase peaks that were consistent with results reported in the ICDD (International Centre for Diffraction Data) databases. A typical XRD pattern for a Red WM (3:1) sample is presented in (Figure 3a). Inspection of the pattern reveals four intense peaks located at 38.4°, 44.6°, 64.5° and 77.8°. The peaks were identified as the main (hkl) indices for pure crystalline Au, which also confirmed the nanoparticles had an fcc lattice structure. Further confirmation was provided by EDS compositional analysis which revealed the presence of metallic Au in the samples. A representative EDS analysis for a Green WM (3:1) sample is presented in (Figure 3b) and shows peaks confirming the presence of Au in the sample. SEM analysis of the early stages of synthesis (~15 min), revealed nanoparticles were sphere-like in shape before growing into larger nanoparticles and micrometre scale particles with different shapes. Typically, Red WM the particle aggregates contained spherical nanoparticles ranging in size from 100 up to around 350 nm. Also present in the aggregates were large smooth hexagonal plate-like structures. With the largest plate size reaching 2.5 µm as seen in (Figure 3c). The thickness of the hexagonal plates varied between 100 and 200 nm. Whereas, green WM sample aggregates contained many smooth sided triangular pyramid type particles that ranged in size from 200 up to around 500 nm as seen in (Figure 3d).

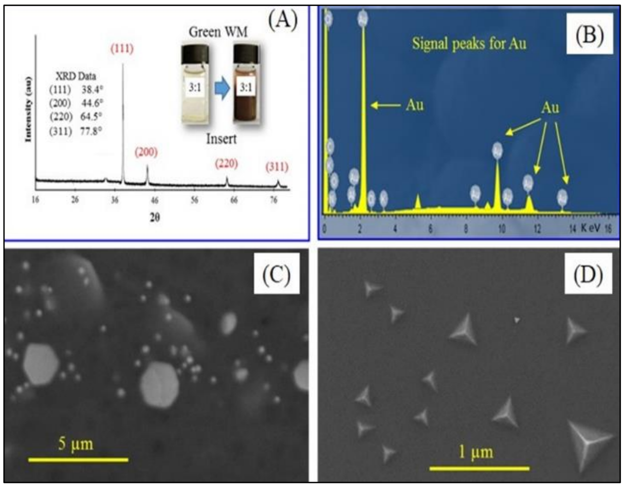

Figure 3: (A) XRD pattern showing the presence of crystalline Au and insert showing colour change of a 3:1 reaction mixture, (B) EDS compositional analysis showing metallic Au in a typical sample, (C) representative SEM image showing various Au nanoparticle shapes produced by the Red WM, and (D) image of Au triangular nanoparticles generated by Green WM.

The antibacterial study appraised the performance of the Au nanoparticles against *Escherichia coli* and *Staphylococcus Epidermidis*. Initially, both bacterial strains were evaluated against test disks individually treated with either pure by Green WM or Red WM extracts. The results revealed neither extract had any antibacterial properties towards *Escherichia* coli or *Staphylococcus Epidermidis*. In both cases the inhibition zone measurements produced a null result. The study also found varying the ratios of Green WM or Red WM extracts to AuCl4- produced differing degrees of bacterial susceptibility. For Green WM based mixtures, it was found that the 5:1 ratio produced the best response against *Escherichia coli* with an inhibition zone of 10 mm and Staphylococcus Epidermidis with an inhibition zone of 12 mm. Whereas, for Red WM based mixtures, the 3:1 ratio produced the best response against *Escherichia coli* with an inhibition zone of 10 mm and Staphylococcus Epidermidis with an inhibition zone of 10 mm. Represent inhibition zone results are presented in (Figure 4 c & d) and clearly shows the influence of varying amounts of extract have on bacterial susceptibility.

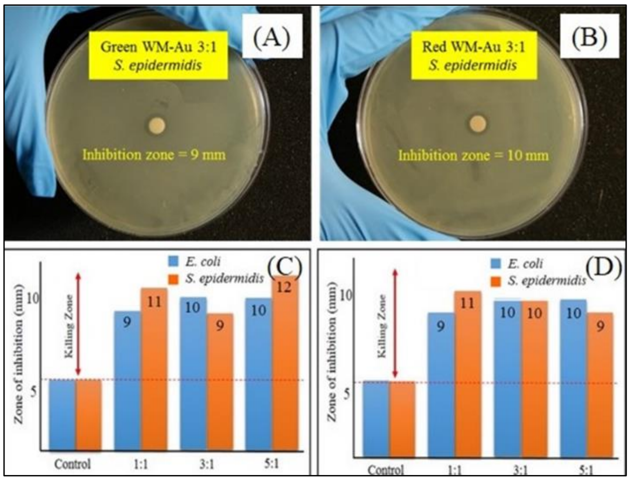

Figure 4: Representative images of antibacterial challenge: Staphylococcus epidermidis challenged by (a) Green WM (3:1) and (b) Red WM (3:1) based extracts samples; and typical mean inhibition zone diameter measurements for (c) Green WM-based extracts and (d) Red WM-based extracts.

DISCUSSION

Horticultural food wastes are currently being investigated as a possible renewable source of biochemical suitable for producing high-value Au nanoparticles.[26] The bio-reduction of Au (III) ions to their metallic form (Au0) using plant-based food waste has several advantages compared to conventional chemical-based methods. Recent review articles have reported the advantages of using green chemistry-based procedures for producing a variety of metal nanoparticles and their prospective applications.[18,27] The present study has evaluated the viability of using waste *Citrullis lanatus var* (watermelon) to bio-reduce Au nanoparticles with antibacterial properties towards *Escherichia coli* and *Staphylococcus Epidermidis.* The natural water-soluble compounds found in both Green WM and Red WM were found to be effective chemical agents for reducing Au (III) ions to their metallic form (Au0). Furthermore, the extracts were capable of modelling growth and proficient at producing highly stable nanoparticles.

During bio-reduction the reaction mixture turned brown colour as seen in (Figure 3a insert). The resulting UV-Visible spectroscopy of the samples revealed a maximum absorbance peak occurred at 560 nm (Figure 2) and is similar to peaks reported by other green synthesis studies. [9, 28] The 560 nm peak is broad and suggests the generated Au nanoparticles have an anisotropic nature. The nature was confirmed by the presence of nanoparticles of varying size and differing shapes for both Red WM and Green WM samples as seen in SEM images (Figure 3 c & d). XRD studies were carried out to detect the presence of crystalline Au nanoparticles in the samples. A representative analysis is presented in (Figure 3a) and confirms the presence of Au nanoparticles with a face centred cubic structure. The XRD analysis was found to be similar to the results reported by Singh et al. [29] and Pasca et al, for the biosynthesis of Au nanoparticles using plant extracts.[30] EDS compositional analysis was used to confirm the presence of elemental Au in the samples. A representative analysis of a Green WM (3:1) sample is present in (Figure 3 b) and clearly shows strong signal peaks for Au in the sample. As mentioned above, SEM imagery was used to examine the physical properties of the Au nanoparticles. Representative images are presented in (Figure 3 c & d) and reveal a variety of particle sizes and shapes were produced during biosynthesis. (Figure 3 c) is a typical image of Au nanoparticles produced by a Red WM based extract. The Red WM based extracts tended to produce spherical and hexagonal plate-like particles that ranged from the nanoscale to micron scale. The spheres ranges in size from 100 to 350 nm, while the hexagonal plate-like particles ranged from 500 nm to 2.5 µm, and the thickness varied between 100 and 200 nm. The Green WM based extracted tended to produce more triangular pyramid type particles that where characterised by their smooth sides. Image analysis revealed these nanoparticles ranged in size from 200 to 500 nm. Similar studies using plant extracts have also produced Au nanoparticles with a variety of shapes other than spherical.[31,32] For instance, Poinern et al, found leaf extracts from the Australian plant Eucalyptus macrocarpa produce smooth sided hexagonal and truncated triangular Au plates with side lengths ranging from 4 to 6 µm.[33] While Narayanan and Sakthivel have used Coriandrum sativum (coriander) leaf extracts to produce decahedral, triangular and spherical shaped Au nanoparticles ranging in size from 7 to 58 nm. [34] The exact mechanism deriving the biosynthesis of Au particles is not fully understood. However, Wang et al, have proposed a possible mechanism that begins with the spontaneous self-assembly of Au particles along particular crystallographic orientations. Further particle assembly at the planar interface reduces its surface energy. Thus, orientating and promoting particle deposition along the planar interface.[35] Other researchers have suggested the type of plant extract and the competitive nature between crystallographic surfaces as being mechanisms for growth orientations.[36, 37]

The Red WM and Green WM extracts were initially tested against the two bacterial strains to determine if there was any antibacterial property present. Testing revealed there was no antibacterial property present in either extract. However, nanoparticles produced by all three extract ratios (1:1, 3:1 & 5:1) derived from Red WM and Green WM extracts did display antibacterial properties against both *Escherichia coli* and *Staphylococcus Epidermidis.* As seen in (Figure 4 c & d) the various extracts had varying degrees of effectiveness against the bacterial strains. The 5:1 ratio for Green WM based extracts produced the most favorable response against and *Staphylococcus Epidermidis* with an inhibition zone of 12 mm. While the response to Escherichia coli, was an inhibition zone of 10 mm. Similarly, the best response for Red WM based mixtures was the 3:1 ratio, which produced an inhibition zone of 10 mm for both *Escherichia coli* and *Staphylococcus Epidermidis.* Interestingly, the 1:1 ratio produced inhibition zones of 9 mm for *Escherichia coli* and 11 mm for Staphylococcus Epidermidis. Similar studies have shown Au nanoparticles can inflict cellular damage which leads to the death of the bacteria.[38] It is the cellular damage caused by Au nanoparticles that makes them an ideal antibacterial agent with the potential to overcome the immunity of several bacterial strains to conventional antibiotics.[39] However, further research is needed to elucidate the interactions taking place between bacteria and Au nanoparticles, and the mechanisms occurring within bacteria when Au nanoparticles cross the cell membrane. In the light of increasing antibiotic resistance by several bacterial strains, it is important to develop new types of antibiotics that can protect humanity in the future. The present study offers an alternative method for producing Au nanoparticles with antibacterial properties towards *Escherichia coli* and *Staphylococcus Epidermidis.*

CONCLUSION

In the present study, aqueous extracts obtained from red and green parts of waste watermelon were assessed for their ability to reduce Au (III) ions to their metallic form (Au0) and create stable Au nanoparticles. Formation was established by UV–visible spectroscopy which revealed a SPR peak at 560 nm and XRD analysis confirmed the crystalline nature of the Au nanoparticles. The presence of Au in the samples was also confirmed by EDS and SEM images revealed a variety of particle shapes. Besides spherical, the Red WM-based extracts also produced large smooth hexagonal plate-like structures (up to 2.5 µm in size), while the Green WM-based extracts tended to produce smooth sided triangular pyramid particles (200 to 500 nm). Importantly, the Kirby-Bauer sensitivity method indicates the Au nanoparticles have antibacterial properties towards both *Escherichia* coli and *Staphylococcus Epidermidis*. Moreover, the results emphasise the effectiveness of the low-cost green chemistry-based technique for producing Au nanoparticles from a renewable food waste that is both eco-friendly and nontoxic in nature. The antibacterial properties of the Au nanoparticles makes them a potential candidate for incorporation into new types of antibiotic pharmaceutical products.

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