Category 1: Experimental Research

Group 1-49

Antibacterial properties of Green synthesised Silver nanoparticles

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Abstract

The ability of certain extracts to synthesise silver Nanoparticles as well as it is antibacterial and antioxidant effects of it were tested in this research. Extracts were obtained from Chinese herbs, Dang Gui, Huai Shan and Da Huang. As for fruit peels, banana peel and watermelon rind was selected as extracts. Extracts were also obtained from *Lactobacillus* and *E.coli*. The extracts would need to be first prepared. After which, Silver nitrate would have to be added to the extract, and waterbathed to obtain the silver nanoparticles. Da Huang, *E.coli, Lactobacillus* and Banana peel were able to successfully synthesise Silver nanoparticles while the other extracts were not able to. The SEM and UV-Vis spectroscopy showed that the Silver nanoparticles synthesised were indeed nanoparticles less than 100nm and spherical in shape. It also showed that the silver nanoparticles synthesised had a peak wavelength of between 390-420 nm, which falls in the range for successfully synthesised silver nanoparticles. Silver nanoparticles synthesised with *Lactobacillus* was the most effective in killing *E.coli*, as the zone of inhibition formed around it was the largest. As for the antibacterial test against *Bacillus Cereus*, all the silver nanoparticles exhibited similar amounts of antibacterial activity. As for the antioxidant test, all the Silver nanoparticles were able to exhibit antioxidant properties, with Silver nanoparticles synthesised with Lactobacillus exhibiting the most. In conclusion, all the successfully synthesised Silver nanoparticles displayed antibacterial and antioxidant properties.

1. Introduction

Nanoparticles are particles which are smaller than 100nm in size. In recent times, there has been an increase in the use of silver nanoparticles and has been widely used in many different fields. Silver nanoparticles have been used for medicinal, industrial purposes etc. , thanks to it possessing many desirable properties. To synthesise silver nanoparticles, one can either synthesise it chemically or through green synthesis, also known as biological synthesis. However, chemical synthesis of silver nanoparticles has neither been very friendly to both the environment (due to the large amounts of chemicals used) nor cost-effective. Silver nanoparticles that are synthesised using chemicals are also found to be less consistent and dependable. （

Xi-Feng Zhang et al. 2016). Green synthesis, on the other hand, has been Environmentally friendly, less costly, simple and dependable, as the silver nanoparticles obtained are often more consistent in size. This research is aimed at researching and finding out the extracts that are able to successfully green synthesise these extremely useful and sought after silver nanoparticles, as well as the antibacterial and antioxidant properties they exhibit.

To undergo the green synthesis of Silver nanoparticles, extracts obtained from organic matter like bacteria, fruit peels, and herbs are required to have reducing agents (Liang et al.2010), such as phenols and flavonoids, which are biomolecules that can provide silver ions electrons to reduce the silver ions to stable silver atoms. Besides, the biomolecules also slow down the silver atoms from agglomerating, which can prolong its effectiveness in killing bacteria (Liang et al.2010). In this research, Banana peel and watermelon rind extract were chosen to synthesise silver nanoparticles. The extracts chosen were Dahuang, Danggui and Huaishan for Chinese herbs, *Lactobacillus* and *E.coli* for bacteria.

2. Our Objectives and Hypotheses

Objectives of this research are to

1. Successfully synthesise Silver nanoparticles with different types of extracts, coming from herbs, fruit peels and bacteria extracts.

2. Evaluate how effective the successfully synthesised silver nanoparticles are in killing bacteria and exhibiting antioxidant activities

3. Compare which is the most effective in killing bacteria, and which exhibits the most antioxidant activity.

Hypotheses of this research are

1. The extracts chosen would be able to successfully synthesise silver nanoparticles.

2. The successfully synthesised silver nanoparticles are effective in killing bacteria and exhibiting antioxidant activities, such that it is comparable, if not better than the control setup.

3. Materials and Methods

3.1 Materials

The silver nitrate used to synthesise the silver nanoparticles were purchased from VMR. The banana and watermelon peels were obtained from local fruit stalls. The Chinese herbs were obtained from local TCM halls and supermarkets. As for the bacteria, they were obtained from ATCC.

The extracts chosen for Chinese Herbs were Dang Gui, Huai Shan and Da Huang. As for fruit peels, banana peel and watermelon rind was selected as extracts. Extracts were also obtained from Bacteria strands, namely *Lactobacillus* and *E.coli.* These extracts were chosen as they contained reducing agents such as phenols and flavonoids, which are biomolecules that can provide silver ions electrons to reduce the silver ions to stable silver atoms. Besides, the biomolecules also slow down the silver atoms from agglomerating, which allows the silver nanoparticles to be more effective, due to its smaller size and not clumped together. (Liang et al.2010)

3.2 Preparation of bacteria extract

First prepare overnight bacteria broth culture (inoculate the bacterias, namely *Lactobacillus* and *E.coli*), then adjust the overnight broth culture to optical density using Spectrophotometer to 0.8. After doing so, filter the broth culture using 0.45 μm sterile syringe filter to remove bacteria, to obtain the Cell free extract (filtered broth).

3.3 Extraction of extract from fruit peel/ Chinese herbs

To obtain the extracts required to synthesise the silver nanoparticles, 50g of the extracts, namely would have to be dried (if necessary, some might already be dried). After which, the dried extracts would be blended in a blender to obtain a powdery substance. 10g of this powdered substance would be added to 50 ml of deionised water and water bathed for 10 minutes at a temperature of 70°C. After which, the mixture would be centrifuged at 10,000 rpm for 10 minutes, to obtain the supernatant.

3.3 Synthesis of silver nanoparticles

5ml of extract(supernatant) was added to 50 ml of silver nitrate(1mM). The mixture was then water bathed for 15 minutes, at 75°C. After the waterbath, the solution would be incubated at room temperature for 24 hours to observe color change. Successfully synthesised silver nanoparticles should be brownish. Through this test, it was determined that only Dahuang, E.coli, Lactobacillus and banana peel were able to successfully synthesise silver nanoparticles.

3.4 Characterisation of Silver nanoparticles

The silver nanoparticles were also put into the UV–vis spectroscopy machine to read its peak wavelength, as a successfully synthesised silver nanoparticle should have a wavelength peak between 390nm and 420nm (D Das 2012). The silver nanoparticles were also sent to be observed under a Scanning Electron Microscope to observe the silver nanoparticles size. Aqueous solution of Silver nanoparticles was analysed in the UV-Vis spectrophotometer, while the solid silver nanoparticles were analysed under the Scanning Electron Microscope.

3.5 Anti-bacterial test (Zone of inhibition)

To ensure the reliability of the test, the silver nanoparticles would have to be micro centrifuged and washed three times with sterile water (replacing the leftover extracts(supernatant) and silver nitrate with sterile water). During the last round, add sterile water and ultrasonicate it. This was to ensure that the concentration of the silver nanoparticles is consistent throughout.

After that, the zone of inhibition test can be carried out. Firstly, the agar has to be swabbed with the bacteria our silver nanoparticles against, namely *Bacillus Cereus* and *e.coli*, a gram positive and gram negative bacteria respectively.

4 wells would then be punched in the agar, and 800µl of Silver nanoparticle would be dripped into the wells using a micropipette. Apart from the silver nanoparticles, positive and negative control would also be dripped into the wells, namely bleach and sterile water respectively.

This would have to be done 5 times, as there are 5 duplicates to ensure reliability of the results.

After leaving the agar plates in the incubator for a day, the zone of inhibition around the well was measured using a ruler.

3.6 Anti-oxidant test (DPPH assay)

To ensure the reliability of the test, the silver nanoparticles would have to be micro centrifuged and washed three times with sterile water (replacing the leftover extracts(supernatant) and silver nitrate with sterile water). During the last round, add sterile water and ultrasonicate it. This was to ensure that the concentration of the silver nanoparticles is consistent throughout.

1ml of an 0.1mM solution of DPPH in methanol was added to 3 ml of the extracts in methanol at different concentration (50,100,200,400 & 800µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes in ambient temperature and in darkness. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Lower absorbance values of the reaction mixture indicate higher free radical scavenging activity /antioxidant activity.

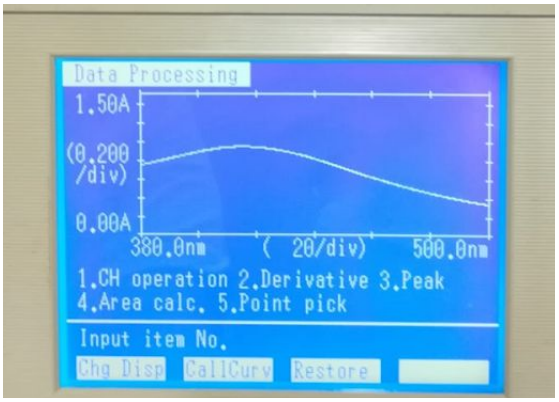
4. Result and Discussions

4.1 Characterisation of the Silver nanoparticles

4.1.1 UV VIS spectrum

This is a wavelength peak of a successfully synthesised silver nanoparticle. Figure 1 is the wavelength peak of the silver nanoparticles synthesised with *Lactobacillus*, with an absorption peak of 411nm, which falls under the wavelength of successfully synthesised silver nanoparticles.

Figure 1. Image of nanoparticle synthesised with Lactobacillus peak



Silver nanoparticles synthesised with	**Wavelength peak (nm)**
Banana peel extract	399
Dahuang extract	415
Lactobacillus extract	411
E.coli Extract	405

Figure 2. Table showing the wavelength peaks of the Silver nanoparticles synthesised

Figure 2 is the table for the wavelength peak of the successfully synthesised silver nanoparticles and all of them falls under the wavelength of successfully synthesised silver nanoparticles, which is between 390nm-420nm (D Das 2012) and the extracts have successfully synthesised silver nanoparticles.

4.1.2 SEM images of silver nanoparticles

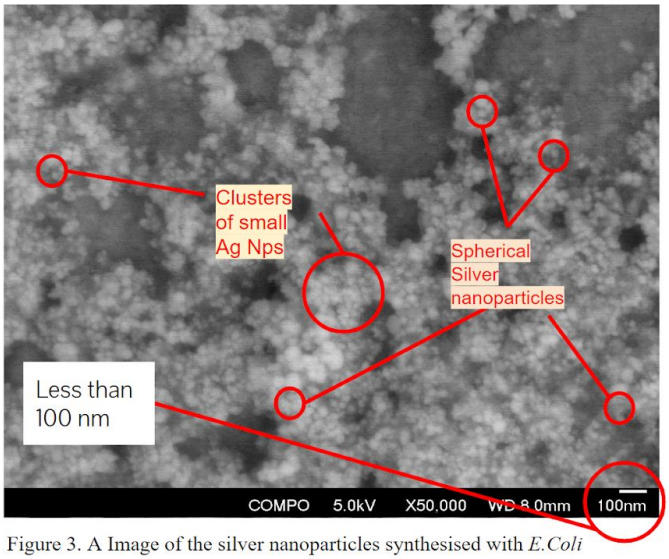


Figure 3. A Image of the silver nanoparticles synthesised with *E.Coli*

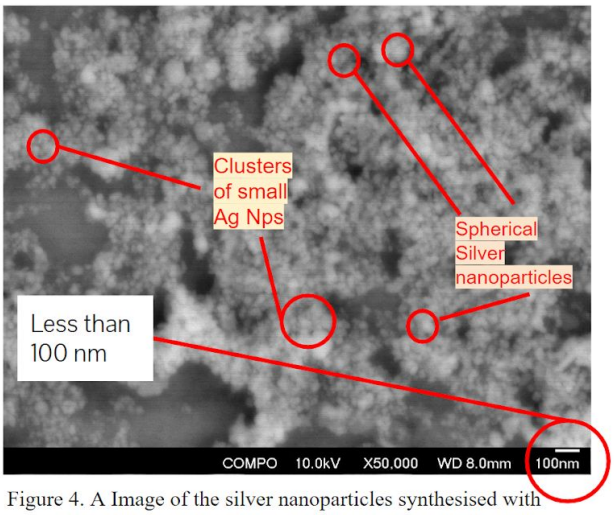


Figure 4. A Image of the silver nanoparticles synthesised with *Lactobacillus*

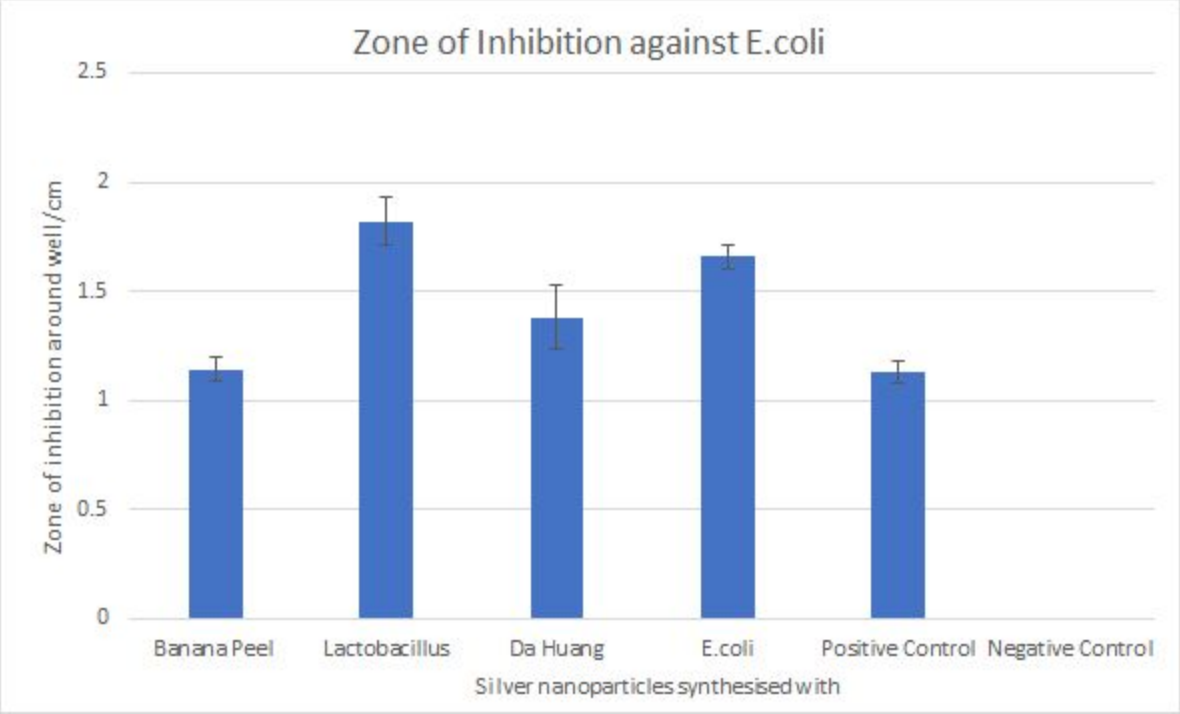
Silver nanoparticles synthesised using *E.coli* were spherical in shape and less than 100nm in size, as seen in Figure 3. Silver nanoparticles synthesised with *Lactobacillus* were also spherical in shape and less than 100nm in size.

Limitations and Difficulties

However, we did not have enough Silver nanoparticles synthesised with Da Huang, nor did we have enough Silver nanoparticles synthesised with Banana Peel extract to send for SEM. In spite of this, we can still conclude that the silver nanoparticles synthesised with Banana Peel and Da Huang were less than 100nm in size, as the UV-Vis Spectrophotoscopy showed us that the peaks of the Silver nanoparticles were roughly the same(within the range of 30nm). The wavelength peak is directly affected by the size of the nanoparticles, the lower the absorption spectrum (shorter wavelength), the smaller the particle size.(E Saion - 2013)

4.2 Antibacterial Activity of Silver nanoparticles

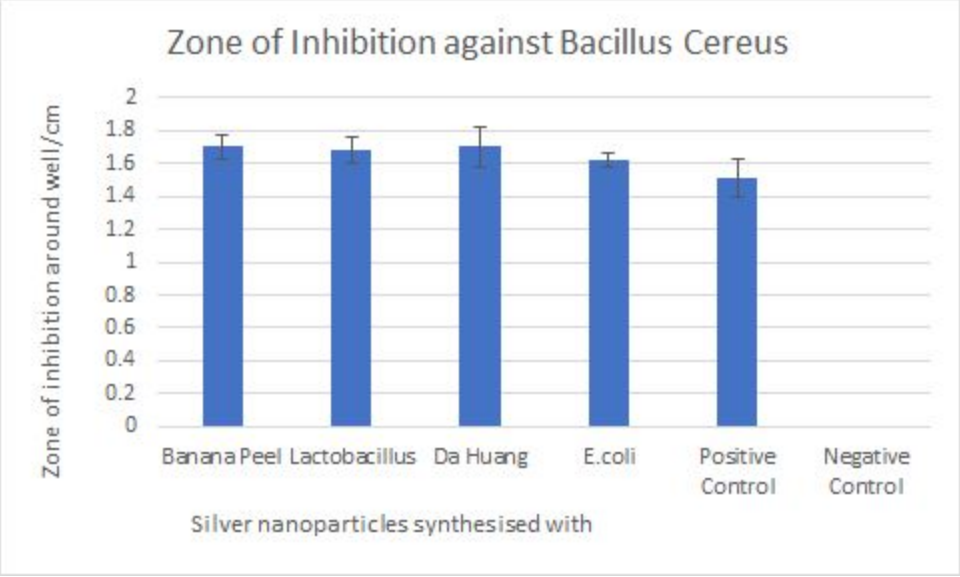
4.2.1 Antibacterial Activity of Silver nanoparticles against *E.coli*



As seen in the graph, all of the silver nanoparticles synthesised were able to exhibit antibacterial properties, as in comparison to the negative control, all of them had a zone of inhibition around them. Almost all of the Silver nanoparticles synthesised with the extracts chosen were proven to be more effective in killing bacteria (*E.coli*) as compared to the positive control(bleach). They had larger zones of inhibition around their wells as compared to the positive control. Only silver nanoparticles synthesised using Banana peel extract exhibited similar amounts of antibacterial activity compared to the positive control.

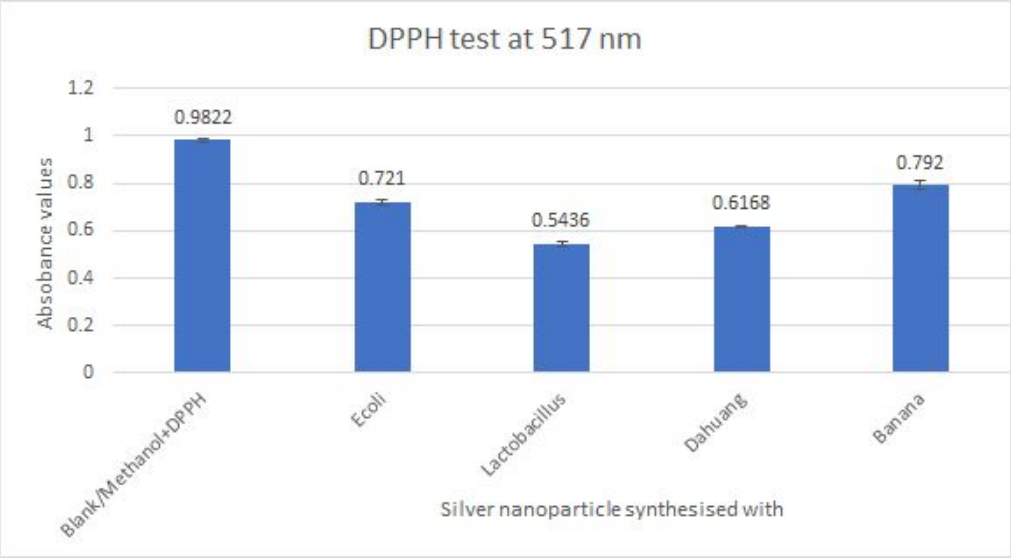
It is also clear in the graph that silver nanoparticles synthesised with *Lactobacillus* and *E.coli* were the most antibacterial, and were able to have a larger zone of inhibition around them as compared to the rest. The difference between them is rather small.

4.2.2 Antibacterial Activity of Silver nanoparticles against *Bacillus Cereus*



The antibacterial properties of these nanoparticles synthesised were comparable/similar to one another, as the error bars overlap each other. The antibacterial properties of the silver nanoparticles synthesised with the four extracts were also comparable to that of the control, which is bleach, having a similar zone of inhibition as compared to it.

4.2 Antioxidant Activity of Silver nanoparticles



All the silver nanoparticle (methanol and extract used as blank sample, methanol, extract and DPPH used as test sample) had significantly lower absorbance levels as compared to the blank (methanol used as blank sample while methanol with DPPH used as test sample), with lactobacillus having the least. The lower the absorbance level, the more antioxidant the sample is. It goes to show that silver nanoparticles synthesised with *Lactobacillus* is the most effective in exterminating free radicals, and it is very antioxidant. It also shows that all the silver nanoparticles synthesised were more antioxidant than the blank setup, and is in fact very effective in exterminating free radicals.

5. Conclusion

Overall, the silver nanoparticles were able to be synthesised with Banana Peel, Da Huang, *E.coli* and *Lactobacillus*. On the other hand, Watermelon rind, Dang Gui and Huai Shan were not able to synthesise silver nanoparticles. The synthesis was eco-friendly, simpler, cheaper and even better in some ways (consistent in size etc.). They were also generally found to be more effective in killing *Bacillus Cereus* than *E.coli*. When compared to the negative control in the antibacterial test, sterile water, all the silver nanoparticles synthesised were able to exhibit antibacterial properties. In the well diffusion test against *E.coli*, silver nanoparticles synthesised using *Lactobacillus* is the most effective in killing *E.Coli.* In the well diffusion test against *Bacillus*

Cereus, all the silver nanoparticles managed to exhibit similar antibacterial properties, while others were similar, if not more antibacterial than the positive control, bleach. As for the antioxidant test, All the silver nanoparticles exhibited large amounts of antioxidant activities, with Silver nanoparticles synthesised with *Lactobacillus* exhibiting the most, and having the lowest absorbance value. In conclusion, Banana Peel, Da Huang, *E.coli* and *Lactobacillus* are able to synthesise silver nanoparticles, and it has been very successful, as they have indeed been shown to possess pronounced antibacterial, and antioxidant effects.

6. Future work

Studies are being carried out to determine how to incorporate AgNPs into biopolymeric membranes (Ellison et al., 2014; Kanmani, Rhim, 2014; Rhim, Wang, Hong 2013), due to the biocompatibility of these polymers (Okamoto, John, 2013) with multiple biological systems and their enhanced antimicrobial activity (Guo et al., 2013; Madhumathi et al., 2010).Many techniques have been employed to prepare nanofibers from polymers (Soyekwo *et al.*, 2014). In future, the nanoparticles could be incorporated with these membranes and be used as an antibacterial filter.

7. References

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