Group 1-03

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# Green synthesis of silver nanoparticles with plant and bacteria extracts and testing its antimicrobial and antioxidant properties

*Introduction:*

Silver nanoparticles are nanoparticles of silver of between 1 nm and 100 nm in size (Graf *et al.*,2003). Silver nanoparticles are highly useful substances that can be incorporated into products, mainly due to its ability to conduct electricity, optical properties and antimicrobial, antifungal properties. Commercial methods involve reducing silver nitrate with trisodium citrate in the presence of polyvinylpyrrolidone (PVP), in which trisodium citrate acts as a reducing agent and PVP acts as a capping agent to stabilize the nanoparticles. (Sun et al.,2002). Other chemicals such as ethylene glycol is also used as an alternative reducing agent. (Wiley et al.,2004).

However, commercial methods of synthesis require chemicals that are relatively expensive and not eco-friendly. For example, the price of PVP is currently about $93 per 100 grams. Thus, to provide a more affordable way of producing silver nanoparticles, we aim to find a cheaper and more environmentally friendly way to synthesise silver nanoparticles. The objectives of the study include synthesis of nanoparticles using plant and bacterial extracts and to compare their properties to commercial nanoparticles. For the hypothesis, nanoparticles synthesised with plant and bacteria extracts will have a better antibacterial and antifungal effect

than commercial nanoparticles; nanoparticles will also be successfully synthesised using Bacillus subtilis ATCC 19659, Escherichia coli and Citrus sinensis, due to the presence of capping agents within the plant and bacteria.

## MATERIALS AND METHODS

To synthesise Silver nanoparticles, several steps were taken, and different tests were conducted to see the difference between the effects of the Silver nanoparticles. These are the materials used for the whole experiment: The bacteria used were *Bacillus subtilis ATCC 19659, Bacillus cereus ATCC 11778 , Escherichia coli ATCC 25922* and *Staphylococcus epidermidis ATCC 12228*. Plants used to make extracts were: Citrus sinensis(common orange), Syzygium cumini (Java plum). The fungus stock used was *Aspergillus niger*. Chemicals used were silver nitrate, Methanol, and DPPH.

### *Preparation of plant extracts:*

To prepare the plant extracts, peels were dried and grinded into powder. 25 g of powder was then soaked in 250 ml of water for 7 days. The mixture is then centrifuged, filtered, and the extract is obtained.

### *Preparation of bacterial extracts:*

Extracellular:

To prepare the bacterial extracts, the bacteria was cultured overnight in Nutrient Broth. The broth was then centrifuged to obtain the supernatant, which is then filtered and the extract is obtained.

Intracellular:

To prepare the bacterial extracts, the bacteria was cultured overnight in Nutrient Broth. The cell membrane of the bacteria was destroyed using a sonicator and examined under a microscope. The resulting mixture was then obtained as the extract.

*Preparation of nanoparticles:*

To synthesise the nanoparticles, 30 ml of extract was added to 30 ml of 0.001mol/$dm^3$ of silver nitrate and stirred overnight.

*Concentrating silver nanoparticles*

To enhance the effect shown in the tests, Silver nanoparticles produced were concentrated. Nanoparticles were centrifuged using the microcentrifuge at 13200 rpm. Supernatant was then removed to obtain the nanoparticles. The nanoparticles were added to sterile water to make aqueous and concentrated Silver nanoparticles.

*Antibacterial Test*

To investigate the antibacterial properties of nanoparticles synthesised, a zone of inhibition test was carried out on 2 different strains of bacteria: a gram-negative and a gram-positive bacteria, E.coli and S.epidermidis respectively (Morones, Jose Ruben, et al. 2005). Sterile water and 10% bleach solution was used a negative and positive controls respectively. The diameter of a clear ring around the well is then measured. To carry out this test, the bacteria was firstly cultured overnight in Nutrient Broth. Then, the bacteria was spread on Mueller-Hinton agar. Wells were punched into the agar and 80 µL of concentrated Silver nanoparticles were added into the wells. Finally, the plates were incubated overnight at 35℃ and the diameter of the zone of inhibition was measured the following day.

*Antifungal Test*

To investigate the antifungal activity of the nanoparticles synthesised, an antifungal test was carried out Only sterile water was used as a control. The diameter of fungal growth was then measured.Firstly, Potato dextrose agar (PDA) was prepared and 10 ml of concentrated Silver nanoparticles were added to 100 ml of PDA. Then, a small block of *A. Niger* stock culture was cut and placed onto PDA plates. Lastly, the plates incubated at 30℃ and observations were recorded on the 3rd and 5th day.

*Antioxidant test*

Finally, to investigate the antioxidant properties of the nanoparticles, wavelength of the spectrophotometer was adjusted to 517 nm and the difference between the reading of the control and the reading of the nanoparticles was measured, with the control blank being 3 ml of Methanol and control test being 2 ml of methanol with 1 ml DPPH. For each test for antioxidant test, the blank was 2 ml of methanol mixed with 1 ml of silver nanoparticles, while the test was 1 ml of methanol, 1 ml of DPPH and 1 ml of silver nanoparticles. They are incubated for 15 min under no light before testing.

## RESULTS AND DISCUSSION

*UV-vis spectrophotometer*

To test for formation of Silver nanoparticles and to characterise them, samples were taken and scanned with the spectrophotometer from the range of 350-600 nm. The results are shown in the table below:

**Table 1:**

| Nanoparticle | 2 hours of stirring(nm) | Overnight stirring(nm) |
|---|---|---|
| *E.coli* extract | 400.2, 403.2 | 422.7 |

| | | |
|---|---|---|
| *B.subtilis* extract | 400.2, 403.2 | 411.1 |
| *B.cereus* extract | 400.2, 403.2 | No peak detected |
| Orange extract | 496.8 | No peak detected |
| *E.coli* (Intracellular) extract | 402.5 | |
| *B.subtilis* (Intracellular) extract | 413.0 | |
| *B.cereus* (Intracellular) extract | 409.0 | |
| Plum extract | Small peak at $\approx 410$ | |
| Commercial Silver nanoparticles | 427.5 | |

**Table 1**, Peak detected by spectrophotometer.

The presence of Silver nanoparticles is shown with peaks between 400 nm and 430 nm, and the ideal peak is at 422 nm. For *E. Coli, B. Subtilis*, both intra and extracellular, *B.subtilis* intracellular, and commercial silver nanoparticles, it is evident that there is Silver nanoparticles present. However, *B. Cereus* did not show a peak after overnight stirring, but one possible explanation is due to the fact that the concentration of nanoparticles was too low. For Plum extract, the concentration might also be too low as not enough light is being absorbed. For

Orange extract, the peak detected in the first 2 hours of stirring might be due to presence of other compounds present that absorbs strongly at 496 nm.

To confirm the findings, the mixtures were sent to be looked under a Scanning Electron Microscope. The results are shown below:

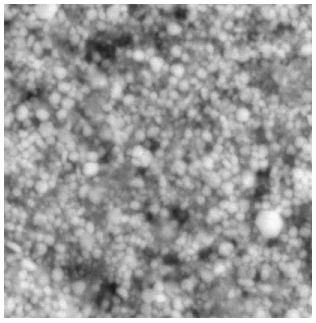

**Figure 1**: *E. Coli* extract

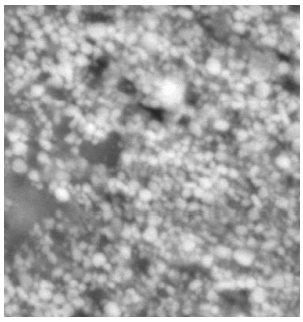

**Figure 2**: *B. Subtilis* extract

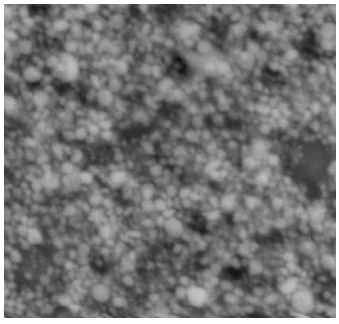

**Figure 3**: *B. Cereus* extract

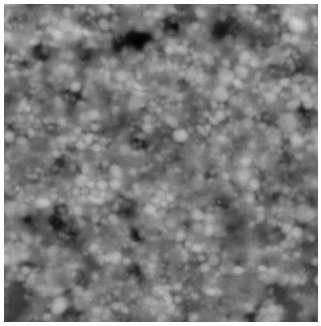

**Figure 4**: Orange extract

As seen from the figures 1, 2 3 and 4, all of the mixtures contains Silver nanoparticles, and we have successfully synthesised Silver nanoparticles with all of the extracts.

*Zone of inhibition test*

**Figure 5:**

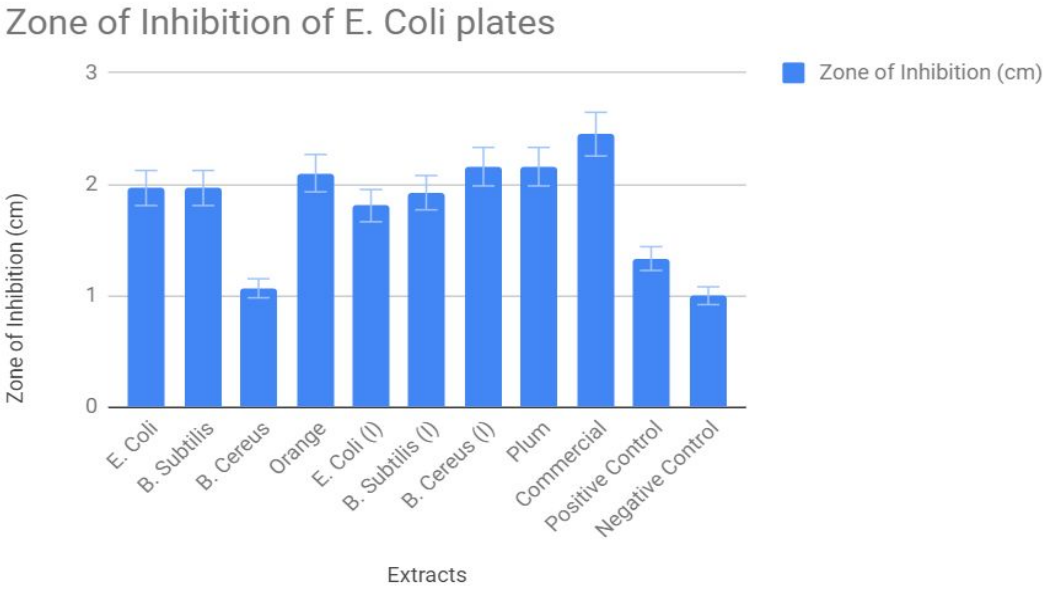

**Figure 5**, the antibacterial test results for *E. Coli*

As seen from figure 5, commercially produced silver nanoparticles still had the best antibacterial effect on *E. Coli* , and is proved with the Kruskal Wallis test to be of significant difference with Plum extract, *B.cereus* (Intracellular) extract and Orange extract. We have also done a Mann–Whitney U test to compare *B.cereus* (Extracellular) extract with the negative control, and the difference is significant, and thus proving there was still some form of antibacterial effect on *E. Coli* with the nanoparticles synthesised with *B.cereus* (Extracellular) extract. Note that the well is of diameter 1 cm.

**Figure 6:**

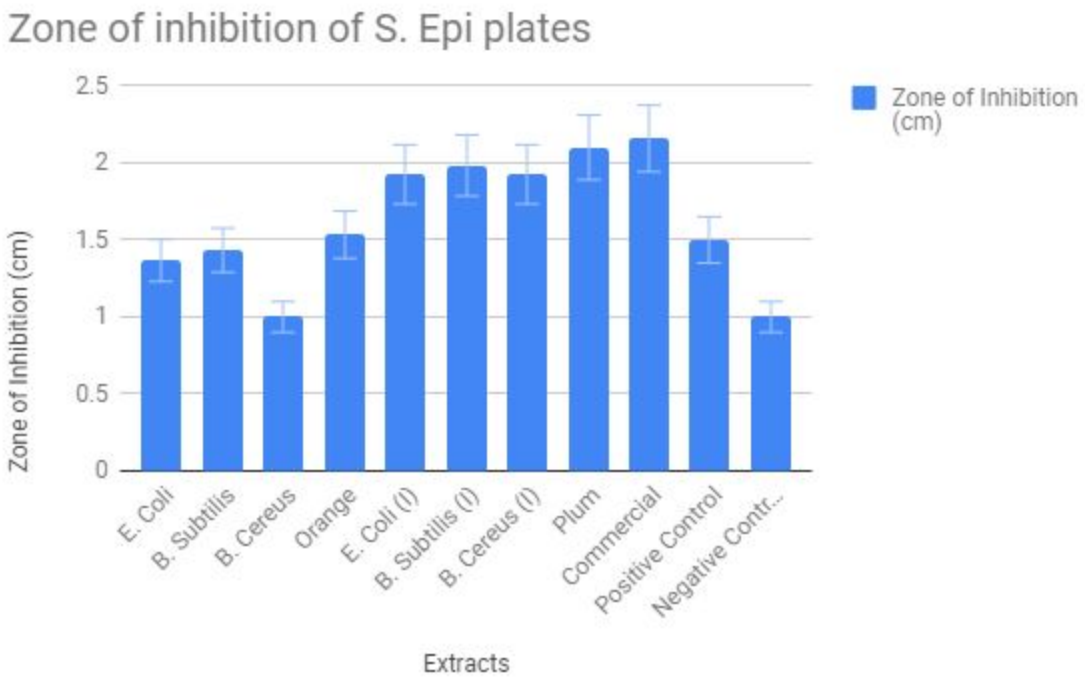

**Figure 6**, the antibacterial test results for *S.epidermidis*

As seen from figure 6, commercially synthesised Silver nanoparticles has the highest antibacterial effect. However, the difference between the commercially produced Silver nanoparticles, Plum extract produced nanoparticles have no significant difference while the difference between Plum extract, *B.cereus* (Intracellular) extract, *E.coli* (Intracellular) extract, and *B.subtilis* (Intracellular) extract produced Silver nanoparticles have significant differences. All of the extracts displayed a weaker antibacterial effect on *S.epidermidis* than *E. Coli.*

Conclusion:

Commercially synthesised Silver nanoparticles has the strongest antibacterial effect on *E. Coli*, and has the same antibacterial effects as Silver nanoparticles synthesised from plum extract on *S.epidermidis*. All of the nanoparticles had antibacterial effects, but weaker antibacterial effects on gram positive bacteria. For the Silver nanoparticles produced using *B.cereus* (Extracellular) had little but significant effect on *E. Coli,* but no antibacterial effect on *S.epidermidis,* suggesting that the concentration of the Silver nanoparticles in the mixture is too low. For Silver nanoparticles synthesised using plum and orange extracts, the compounds present in the extract itself might have antibacterial properties, thus enhancing the antibacterial effect of the mixture. Therefore, we conclude that our synthesised nanoparticles does not have better antibacterial properties than commercially synthesised Silver nanoparticles.

*Antifungal Test*

**Figure 7:**

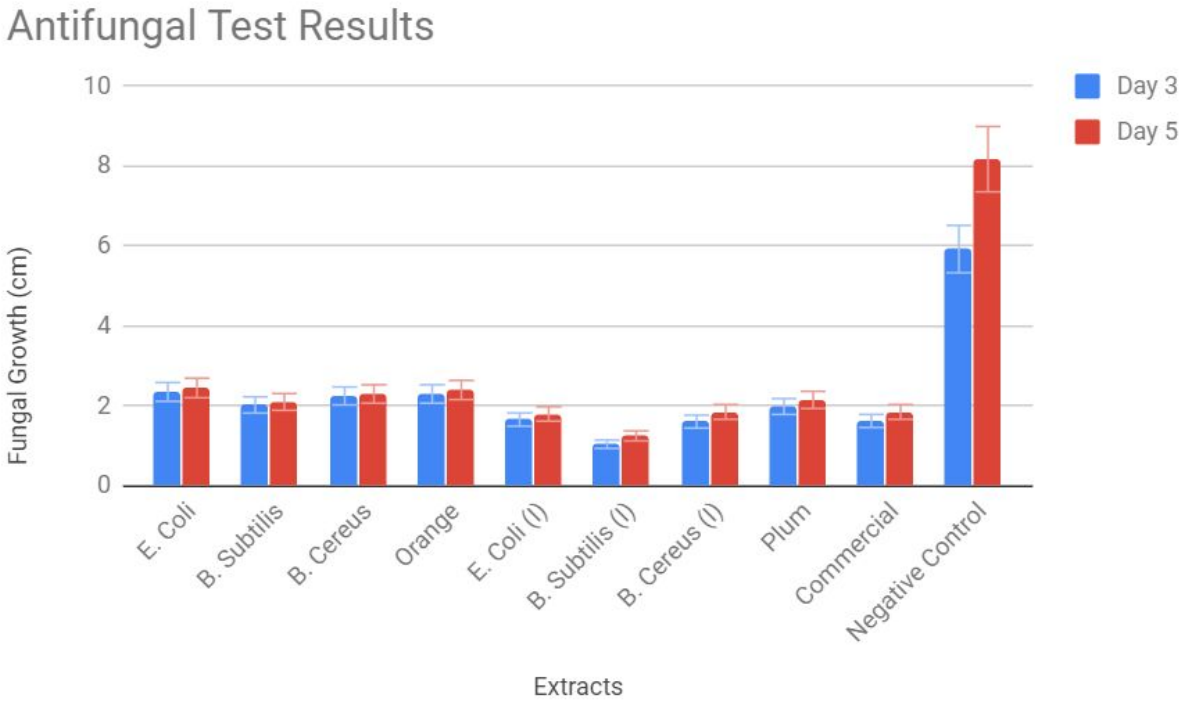


**Figure 7**, the diameter of fungal growth after 3 and 5 days of following incubation. The lower the diameter of fungal growth, the better the antifungal properties are.

As seen in Figure 7, nanoparticles synthesised from *B.subtilis* (extracellular) extract showed the best antifungal property by both the third and fifth day. There was contamination in the plates of *E. Coli, B. Subtilis, B. Cereus* and Orange extracts. We have eliminated the cause of it to be that the Silver nanoparticles to be not strong enough as there was no contamination in the rest of the

plates, which were done on a separate day. Therefore, the contamination was due to mishandling of the plates.

Conclusion:

Silver nanoparticles synthesised with *B. Subtilis* (intracellular) extract had the best antifungal properties, and have a significant difference compared to *B. Cereus* (intracellular) extract and *E. Coli* (intracellular) extract. Compared to commercially produced Silver nanoparticles, *B. Cereus* (intracellular) extract has no significant difference but *E. Coli* (intracellular) extract have a significant difference. Therefore, we can conclude that silver nanoparticles synthesised with *B. Subtilis* (intracellular) extract and *E. Coli* (intracellular) extract has better antifungal properties than commercially produced silver nanoparticles.

*Antioxidant test*

**Figure 8:**

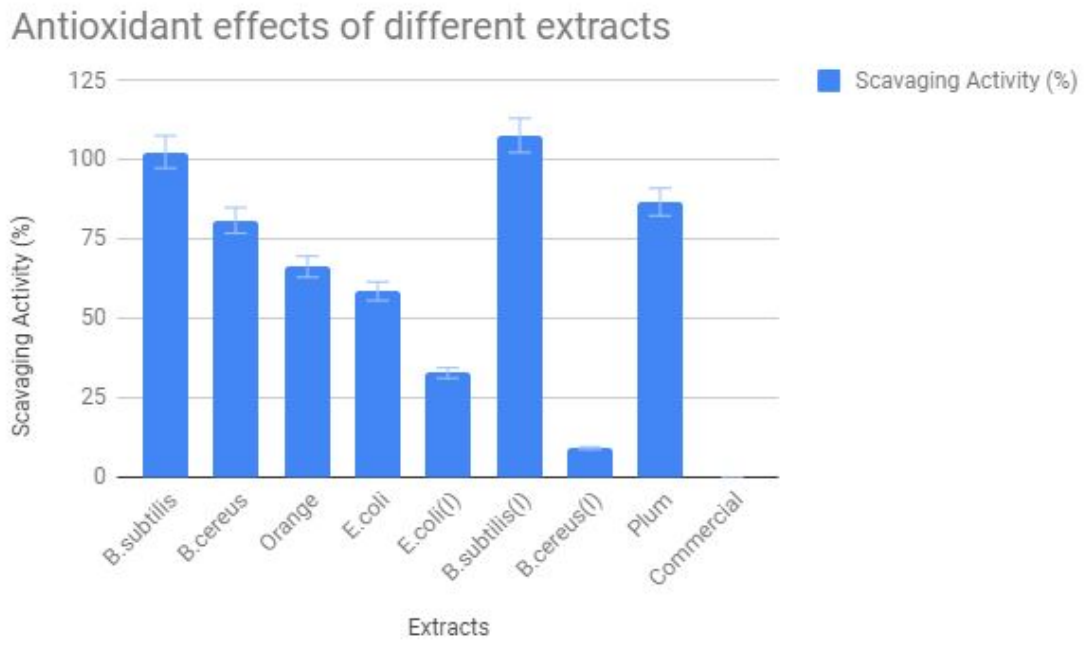


**Figure 8,** the scavenging activity of nanoparticles in %.

As seen in Figure 4, nanoparticles synthesised from *B.subtilis* (intracellular) extract has the strongest antioxidant properties, but has no significant difference with the nanoparticles synthesised from *B.subtilis* (extracellular) extract. Commercially produced nanoparticles had no effect on the DPPH, thus having a higher reading than the control, and we have shown the Scavaging Activity to be 0% instead of a negative percentage.

Conclusion:

Since there was no antioxidant effects of the commercially synthesised Silver nanoparticle, we can conclude that all our nanoparticles have better antioxidant properties than the commercially

synthesised Silver nanoparticle, and Silver nanoparticles synthesised from *B.subtilis*, both intra and extracellular has the best antioxidant properties.

*Suggested future work:*

Colony count test to visualise bactericidal effects over time can be conducted to further confirm the antibacterial effect of nanoparticles. The effect of pH value on the size of nanoparticles formed can also be investigated.

*References:*

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