Antimicrobial activity of Phytofabricated Silver Nanoparticles against Escherichia coli and Staphylococcus aureus: Effect of Plant Extract Concentration on the Antimicrobial activity

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Background: ***Background:*** Plant Mediated Synthesis uses phytochemicals (amino acids, proteins, vitamins) present in plants as both reducing and capping agent in metallic nanoparticles synthesis. Compared to conventional chemical methods, it is a one-pot synthesis approach, avoiding separate reducing agents, capping agents and toxic chemicals. As such, plant mediated synthesis offers an environmentally friendly and sustainable approach to metallic nanoparticle synthesis. Silver (Ag^+) is a well-established antimicrobial; several studies have shown its ability to inhibit the growth of fungi, some viruses and bacteria. There exists an increasing limitation of therapeutic options for bacterial infections. Hence, there is a need for novel treatment options due to antibacterial drug resistance, which is considered a major global health burden. AgNPs are suitable candidates as they possess antimicrobial activity, which occurs through multiple bacteria-killing mechanisms compared to conventional drugs. Various parameters influence plant-mediated synthesis of silver nanoparticles (AgNPs), including the Plant Extract Concentration (PEC). Notably, the PEC and its influence on the fabrication of nanoparticles to effect antimicrobial activity is unknown. This study aimed at determining the influence of different concentrations of *Spinacia Oleracea* leaf extract on the size and antimicrobial activity of Phyto-fabricated AgNPs

Methods: The work detailed here investigated the influence of *Spinacia Oleracea* leaf Extract (SLE) concentration on the size of AgNPs synthesised and their antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus.* Spherical AgNPs were synthesised via plant-mediated synthesis at 4,7 and 10% SLE concentration with silver nitrate. AgNPs nanoparticles generated are referred to as AgNPs-4 AgNPs-7 and AgNPs-10. The synthesised AgNPs were characterised spectrophotometrically using ultraviolent-visible spectroscopy (UV-VIS), morphology and size characterisations were conducted using scanning electron microscopy (SEM) and dynamic light scattering (DLS), then chemical composition investigations were conducted using Fourier transform infrared (FTIR) spectroscopy. Antimicrobial activity was determined used the Resazurin broth method***.*** Results confirmed the successful synthesis of monodispersed AgNPs well in the nano-range and spherically shaped. The experiments showed the clear influence of SLE and biosynthesis methodology on the particle size as well as antimicrobial activity.

Results: The synthesised AgNPs ranged from 109 – 148nm. The trend observed was size reductions with increased SLE concentration; AgNPs-4 (148nm), AgNPs-7 (120nm) and AgNPs-10

(109nm). All the concentrations synthesised showed limited activity in gram negative bacteria *Escherichia Coli* and only 10% had limited activity in gram positive bacteria *Staphylococcus Aureus*

Conclusions: In conclusion, the work detailed here highlighted the significance of PEC in the facilitation of silver nanoparticle synthesis, particle size reductions and antimicrobial activity. It established a concentration-dependent effect of *Spinacia Oleracea* leaf extract where increased amounts led to size reductions of particles synthesised. Additionally, all the synthesised nanoparticles showed varied antimicrobial activity. All the concentrations demonstrated limited activity against gram positive bacteria *Escherichia coli* and only the 10% having limited activity against gram negative bacteria *Staphylococcus Aureus*.