GREEN SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICLES USING EXTRACT FROM *LAWSONIA INERMIS* (HENNA) LEAF AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Nanoparticles compared to bulk materials, exhibit improved characteristics due to their size, distribution and morphology and are widely used in numerous scientific fields. Plant extract from Lawsonia inermis was used for the synthesis of silver nanoparticles which was used as an antibacterial agent. The effect of contact time, temperature and concentration was studied on the extract of Lawsonia inermis. The silver nanoparticle was synthesized using silver nitrate and Lawsonia inermis leaf extract until a colour change was observed indicating a reduction from Ag^{+} to Ag^{o}. The synthesised AgNPs were characterised using uv-visible spectrophotometry. The formation and stability of the reduced AgNPs in the colloidal solution were monitored by uv-visible spectroscopy. The effect of concentration showed that the concentration of 0.001 M was the most suitable for the synthesis of AgNPs. Effect of contact time and temperature revealed that the synthesis of AgNPs required a greater time and a temperature of 40^{o}C. The antibacterial activity of the synthesised AgNPs inhibited the growth of bacterium (E.coli and Staphylococusaureus). The outcome of this study showed that synthesised AgNPs had a positive effect on inhibiting the growth of E.coli and Stapylococcusaureus.

Keywords: Silver nanoparticle, antibacterial, *Lawsonia inermis, synthesis, E.coli, Stapylococcusaureus.*

1.0 INTRODUCTION

Nanotechnology is an emerging rapidly expanding field with its application in science and technology for the goal of devising new materials at the nanoscale level (Albreeht, Evans & Rason, 2006). The concern for environmental pollution are also upheaved as the chemical procedures involved in the synthesis of nano-materials initiate a large amount of hazardous by-product. Thus, there is need for green chemistry that comprises of a clean, harmless and environmentally friendly method of nanoparticle synthesis (Morone, Alimuhammadu, Shamei & Rahimi, 2015). Nanotechnology has opened the door of innovations in different fields including but not limited to medicine, catalysis, texture, engineering and micro-

electronics (Ahmed, Scafullah, Ahmad, Swami & Ikram, 2016). Nowadays nanotechnology owes to the tremendous improvement in human life and it has a multidisciplinary research area. Among the various fields of nanotechnology provides more effective nanoparticles synthesis with expected product and economical manner (Vanja, Rajeshkumar, Gnanajobitha, Paulkumar, Malarkodi & Annadurai, 2013). Nanoparticles exhibit completely new or improved properties based on specific characteristics such as sizes, distribution and morphology. Decreasing the size of any materials to nanoscale may change its intrinsic properties. Thus, properties of manufactured material can be quite dissimilar from those of the bulk materials making it suitable for different applications, thus, because they have been proven and recognized as antibacterial and biocide agents (Erico, Noelia, Tanya & Gonzalo, 2017).

Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicine to culinary items. It has been reported that silver nanoparticles (SNPs) are nontoxic to human and most effective against bacteria, virus and other eukaryotic microorganisms at low concentration without any side effect (Jeong and Yeo, 2005). Moreover, several set of silver and its derivatives are commercially manufactured as anti-microbial agent (Krutyakov & Kudrynskiy, 2008). Bacterial infections poses serious threat to human and the environment, especially in view of the fact that, the emergence of resistant bacterial and adverse side effects associated with prolonged use of antibacterial therapeutics. There is need to prepare a cheap antibacterial therapeutics of silver nanoparticles from extract of *Lawsonia inermis* as an inhibitor against bacterium (E.coli and staphylococcus aureus). Therefore, this study was to prepare silver nanoparticles from *(Lawsonia inermis)* leaf extract as an antibacterial agent for the inhibition of bacterium (E.coli and staphylococcus aureus).

2.0 Materials and Method

2.1 Materials

All chemicals used in this study were of analytical grades. The chemicals used are silver nitrate ($AgNO_3$), Ethanol (C_2H_5OH) and Agar.

2.2 Sample Collection

Henna leaf *(Lawsonia inermis*) was collected around Ilaro. The sample was washed with distilled water, placed in a clean container and air-dried for two days.

2.3 Preparation of Plant Extracts

20 g proportion of the air-dried *Lawsonia inermis* leaves were cut into pieces mixed with 100 ml of distilled and boiled for 15 min to subdue the active plant extracts. The solution was allowed to cool and filtered through layers of muslin cloth and Whatman No.1 filter paper and stored at 4°C in the refrigerator. The clear filtrate which is the extract of *Lawsonia inermis* leaf was used for the synthesis of silver nanoparticles (Shaniba & Kumar**,** 2017).

2.4 Synthesis of Silver Nanoparticles

Silver nanoparticles was synthesised using method described by Sangeetha, Pavithra and Dhanalakshmi (2016). 50ml of 0.001 and 0.01 M silver nitrate ($AgNO_3$) was added into 10 ml of *Lawsonia inermis* leaf extract. Colour change from yellow to brown was observed and the solution was centrifuged at 300 rpm for 20 min. The silver nanoparticles were isolated and concentrated by repeating centrifugation of the reaction mixture. The supernatant was placed with distilled water each time. The nanoparticles were washed with mixture of acetone and methanol to remove any residue particles that were not capping agents. The suspension was dried and stored as a crystalline powder for further characterisation.

2.5 Effect of Silver Nitrate Concentration

Different concentrations of aqueous solution of silver nitrate ($AgNO_3$) ranging from 0.001 M to 0.1 M were added to each bottle containing *Lawsonia inermis leaf* extract under dark condition at different temperature.

2.6 Effect of Temperature and Contact Time

The *Lawsonia inermis* leaf extract was extracted at different temperatures; 30^oC, 40^oC, 50^oC and 60^oC and different contact time; 20, 40 and 60 min.

2.7 UV- Visible Spectrophotometer

UV spectrophotometer was employed to monitor the rate of reduction of silver from Ag^+ to $Ag^{o.}$. The UV-Visible spectral of the synthesised silver nanoparticle was taken in a wavelength range of 400-700 nm.

2.8 Preparation of Bacterium Culture (E.coli and Staphylococcus aureus)

20 g of nutrient agar was weighed into a conical flask and 100 ml of distilled water was added. The mixture was placed in an autoclave until gel was formed and brought out from the autoclave to cool. After cooling, the prepared agar was spread in a petri-dish. The bacterium (E.coli and staphylococcus aureus) were cultured on the agar using swab stick. The synthesised nanoparticle was applied on the cultured bacterium (E.coli and staphylococcus aureus).

3.0 RESULTS AND DISCUSSION

3.1 Results

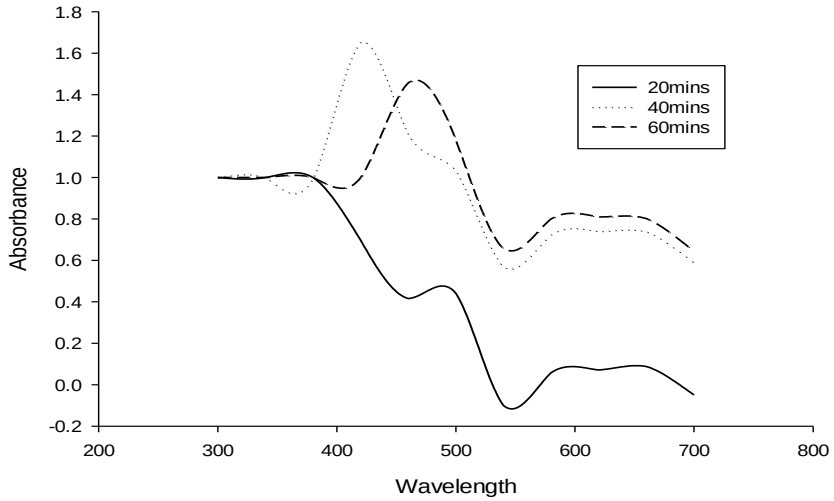

Figure 4.1: UV-Visible absorption spectra of *Lawsonia inermis* at different timing

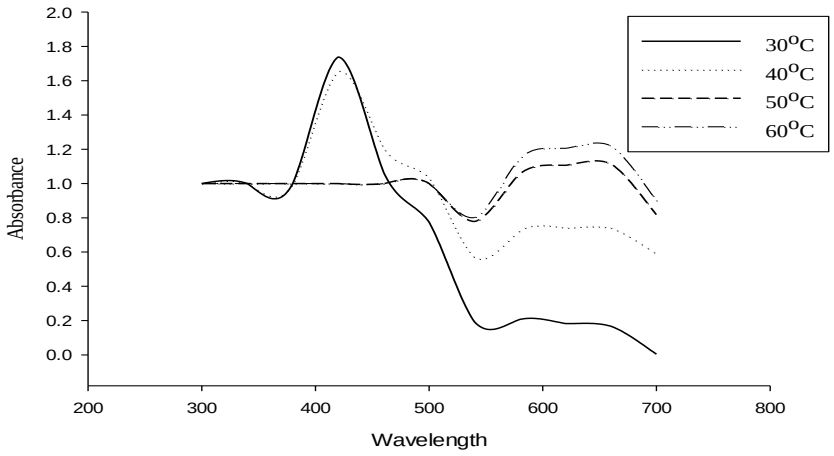

Figure 4.2: UV-Visible absorption spectra of *Lawsonia inermis* at different temperature

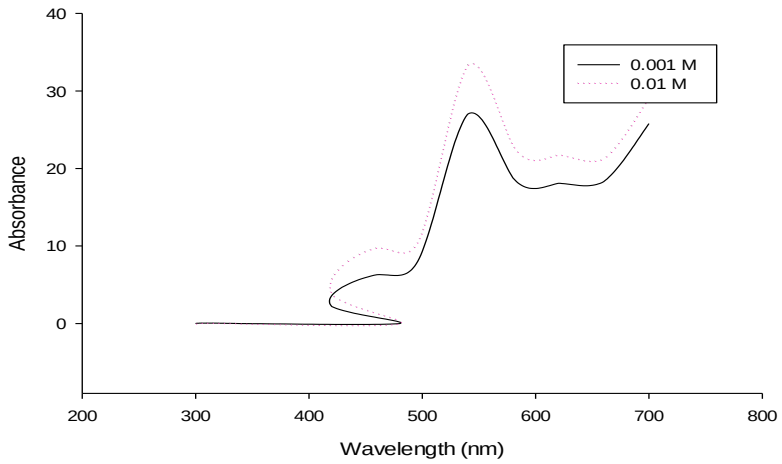

Figure 4.3: UV-Visible absorption spectra of *Lawsonia inermis* at different concentrations

3.2 DISCUSSION

3.2.1 UV-Visible Spectroscopy (Effect of Reaction Time)

The reacted mixture of *Lawsonia inermis* leaf extract and silver nitrate solution as shown in Figure 4.1 as a function of reaction time. A visible colour change from yellow to light brown then to dark brown as the time increases indicated formation of silver nanoparticles (AgNPs) which was confirmed by UV-Visible spectral showing a peak between wavelength 450-540 nm which is similar to that reported by Bagyalakshmi and Haritha (2017). As it is well known that silver nanoparticles exhibit yellowish brown colour in water due to surface plasmon vibration (Mahmudin, Suharyadi, Utomo & Abraha, 2015). Thus, an increase in reaction time from 20 to 60 min results in increasing synthesis of silver nanoparticles.

3.2.2 UV-Visible Spectroscopy (Effect of Temperature)

Temperature is another factor that affects the synthesis of nanoparticles significantly. Figure 4.2 showed the absorption spectral of silver nanoparticles at different temperature range of 30^{o}C – 60^{o}C. Increase in temperature enhances reduction of silver nanoparticles as indicated by change in colour of the solution. The peak absorption wavelength shifted from 450 nm to 540 nm as the temperature varies from 30^{o}C-60^{o}C. The shift in the band maximum was due

to localisation of surface plasmon resonance of the silver nanoparticles (AgNPs). At high temperature, the kinetic energy of the molecule increases and the silver ions get consumed faster, thus leaving less possibility for particle size growth (Verma & Mehata, 2015).

3.2.3 UV-Visible Spectroscopy (Effect of Concentrations)

0.001 M and 0.01 M of silver nitrate were added to the extract of *Lawsonia inerm*is leaf, colour change from yellow to brown and finally to dark brown was observed. But changes occurred faster in 0.001 M than in 0.01 M. The change in colour of the solution was due to the formation of silver nanoparticles by reduction of silver ion. This can be attributed to the presence of large amount of reductance in the reaction medium which cause the rapid reduction of the silver ions (Ag^{+}). The fast reduction of silver ions facilitated further growth of nanoparticles by a phenomenon called Ostwald ripening which leads to increase in size of nanoparticles (Mohammed *et al.*, 2018). This was confirmed by the UV-Visible spectral presented in Figure 4.2. However, in this study, Lawsonia inermis produced silver nanoparticles at a very low concentration.

3.3 Antibacterial Activity

There has been an increasing demand for eco-friendly synthesis of nanoparticles that do not have any toxic effect so as to avoid adverse effects in medical application. The current method of biosynthesis of silver nanoparticle has a time-related advantage over conventional methods (Bhati-kushwaha & Malik, 2013). In this study, antibacterial activity of the synthesised silver nanoparticle (AgNPs) against bacterium (E.coli and staphylococcus aureus) was investigated. Silver nanoparticle from extract of Lawsonia inermis leaf was found to inhibit the growth of the bacterium (Ecoli and staphylococcus aureus). According to Bagyalakshmi & Haritha (2017), electrostatic interaction may be possible reasons for the antibacterial activities of the silver nanoparticles. However, both concentrations of 0.001 M and 0.01 M inhibited the growth of (E.coli and Stapylococcusaureus).

4.0 CONCLUSION

An increasing attention towards green chemistry and utilization of plant extract for synthesis of silver nanoparticle has led to the development of an eco-friendly material. Silver nanoparticle from plant extract is economical, energy efficient and cost-effective. The present

study described a green and simple way to synthesize silver nanoparticle from Lawsonia inermis extract characterised by UV-Visible spectroscopy, which indicates a characteristic absorption peak between 450 and 540 nm. Effect of concentration, reaction time and temperature showed production efficiency and formation rate of silver nanoparticles, making it have positive effect on the inhibition of the bacterium (E.coli and Staphylococcus aureus).

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