ECO-FRIENDLY SYNTHESIS OF SILVER NANOPARTICLES USING *ACALYPHA WILKESIANA* (RED ACALYPHA) LEAF EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

Abiaziem, Chioma & Oguntade, Bukunola
chioma.abiaziem@federalpolyilaro.edu.ng;
bukunola.oguntade@federalpolyilaro.edu.ng

Abstract

In this study, simple method was applied for synthesis of silver nanoparticles using *Acalypha wilkesiana* aqueous leaf extract. The plant extract acts as bioreducing agent and capping agent. The effects of extract contact time, temperature and concentration on the AgNPs formation were investigated. The data revealed that the rate of formation of the silver nanoparticles increased significantly with increasing time and temperature; and at lower concentration. The nature of AgNPs synthesized was analysed by UV-Visible spectroscopy which showed absorbance peak in range of 450-540 nm. The silver nanoparticles at 0.001 M concentration showed a better inhibition of bacterial growth against *Escherichia coli and Staphylococcus aureus* than 0.01 M. The results confirmed this procedure as simple, eco-friendly, cost effective and an alternative to conventional physical/ chemical methods without the immersion of any toxic chemical. Thus, AgNPs from extract of *Acalypha wilkesiana* showed broad spectrum antibacterial activity at lower concentration and may be a good alternative therapeutic approach in future as a substitute for commercial antibacterial therapeutics.

Keywords: *Acalypha wilkesiana*, *Escherichia coli*, Silver nanoparticles, *Staphylococcus aureus*

Introduction

Nanotechnology is a science that configures matters into a wide surface area that has unique properties which has attracted the attention of several researchers (Dada et al., 2015). The field of nanotechnology is distinct as a discipline in the design, synthesis, characterisation and application of nanoscale materials. It has opened the door of innovations in different fields including but not limited to medicine, catalysis, texture, engineering and micro-electronics among others (Ahmed et al., 2016). An important aspect of nanotechnology concerns the development of experimental process for the synthesis of nanoparticles of different sizes, shapes and controlled dispersity with the development of new chemical of physical methods. Nowadays nanotechnology owes to the tremendous improvement in human life and it has a multidisciplinary research area.

Amid the various fields of nanotechnology, it has provided more effective nanoparticles synthesis with expected product and economical manner (Vanja et al., 2013). Hence, a material particle or an object with at least a size less than 100 nm (a nanoparticle) which is made up of atoms in form of a single or poly-crystalline arrangement is referred to as a nanocrystal (Burt, 2005). Nanoparticles exhibits completely new or improved properties based on specific characteristics such as sizes, distribution and morphology. The silver nanoparticles have various and important applications. 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 (AgNPs) are non-toxic to human and most effective against bacteria, virus and other eukaryotic microorganisms at low concentration without any side effect (Jeong et al., 2005). Moreover, several set of silver and its derivatives are commercially manufactured as anti-microbial agents (Krutyakov et al., 2008). Green synthesis of silver nanoparticles is an eco-friendly method of avoiding harmful effect in medical application of silver nanoparticles synthesized by physical, chemical, photochemical such as reverse micelles, thermal decomposition of silver compounds and radiation assisted process (Renal & Iruthayakalai, 2015). 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. In particular, nanoparticle such as silver and copper (AgNPs) and (CuNPs), have been applied in wide variety of field including medicine, agriculture, bioengineering among others (Erico et al., 2017), because they have been proven and recognized as antibacterial and biocide agents.

Unethical human activities have greatly increased the presence of pathogenic microorganisms in our environment, most especially bacteria. More so, cases of antibiotic resistant bacterial strains are also on the increase that requires alternative solution rather than new antibiotics. Therefore, this study was aimed at preparing a cost effective, eco-friendly, non-toxic and potent green synthesis of silver nanoparticles from extract of *Acalypha wilkesiana* as antibacterial agent.

Experimental Methods of the Study

All chemicals used in this study were of analytical grades. The chemicals used are silver nitrate ($AgNO_3$), Ethanol (C_2H_5OH) and Nutrient agar. Red acalypha (*Acalypha wilkesiana*) leaves were collected from the premises of The Federal Polytechnic Ilaro campuses (East and West campus).

Preparation of Plant Extracts

The samples were washed with distilled water, cut into small sizes and air-dried for 3 days. According to method by Shakeel et al. (2016), 20 g proportion of the air-dried *Acalypha wilkesiana* leaves were mixed with 100 ml of distilled water and exposed to heat for 5 mins to subdue the active plant extracts. The solution was then filtered in hot condition. The clear filtrate which is the extract of *Acalypha wilkesiana* leaf was used for the synthesis of silver nanoparticle.

Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesised by adding 50 ml of the 0.001 and 0.01 M silver nitrate ($AgNO_3$) into 10 ml of *Acalypha wilkesiana* leaf extract. Reduction of Ag^+ to Ag^o was confirmed by colour change from yellow to brown and the solution was centrifuged using (Ohaus centrifuge-model: FC5718) at 300 rpm for 20 mins. 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 the capping agents. The suspension was dried and stored as a crystalline powder (Sangeetha et al., 2016).

Different parameters such as concentrations of aqueous solution of silver nitrate ($AgNO_3$) ranging from 0.001 M to 0.01 M were added to each bottle containing *Acalypha wilkesiana* leaf extract under dark condition at different temperature; 30, 40, 50 and 60^{o}C at different timing; 20, 40 and 60 mins were optimised.

Each heated solution of *Acalypha wilkesiana* leaf extract, containing the silver nanoparticles at concentrations of 0.001 M and 0.01 M were observed by Jenway UV-Visible Spectrophotometer Model 6305 to monitor the rate of reduction of silver from Ag^+ to Ag^o. The absorbance of the sample was measured at 400 to 700 nm.

Antibacterial Activity of Silver Nanoparticles

Laboratory culture of *E.coli* and *Staphylococcus aureus* was obtained from the Biological Sciences Laboratory of Science Laboratory Technology Department, Federal Polytechnic Ilaro, Ogun State, Nigeria. The antibacterial tests were done on human pathogen. *E. coli* and *S. aureus* were cultured on nutrient agar using swab stick and were incubated for 37^{o}C for 24 h. The synthesised nanoparticle was smeared on the cultured bacteria according to the method by Henry et al. (2019) and the antibacterial activity was measured based on the inhibition zone (Renal & Iruthayakalai, 2015).

Results and Discussion

The factors; time, temperature and concentration involved in the reduction of $AgNO_3$ to AgNPs in the presence of *Acalypha wilkesiana* were studied. The reacted mixture of *Acalypha wilkesiana* (red acalypha) leaf extract and silver nitrate solution is presented in Fig. 1, as a function of reaction time. A visible colour change from yellow to light brown and to dark brown as the time increases indicates formation of silver nanoparticles (AgNPs) which was confirmed by UV-visible spectral at a wavelength between 450-540 nm. This result is similar to that reported by Bagyalakshmi and Haritha (2017). The silver nanoparticle exhibited a yellowish brown colour in water which was due to Surface Plasmon Vibration (SPV) (Mahmudin et al., 2015). Thus, an increase in reaction time from 20 to 60 mins resulted in increase in formation of silver nanoparticles.

Temperature is another factor that affects the synthesis of nanoparticles significantly. Fig. 2 revealed the absorption spectra of silver nanoparticles at different temperatures in the range of 30-60^oC. As the temperature increases, reduction of silver nanoparticles also increases rapidly, which was indicated by a colour change of the solution. The peak absorption wavelength shifted from 450 to 540 nm as the temperature varied from 30-60^oC. The shift in the band maximum was due to localization 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).

Different concentrations of silver nitrate; 0.001 M and 0.01 M were added to the extract of *Acalypha wilkesiana*, respectively, colour changed was observed from yellow to brown and finally to dark brown. There was a rapid colour change observed in 0.001 M than in 0.01 M. The colour change of the solution was due to the formation of silver nanoparticles reduction to silver ion. Thus, lower concentration was required for the synthesis of silver nanoparticles. And this has been confirmed by the UV- visible spectral as shown in Fig. 3. This can be attributed to the presence of large amount of reductance in the reaction medium which causes the rapid reduction of the silver ions (Ag^+). The fast reduction of silver ions typically facilitates further growth of nanoparticles by a phenomenon called Ostwald ripening which leads to increase in size of nanoparticles (Muhammed et al., 2018). However, in this study, *Acalypha wilkesiana* has produced silver nanoparticles at a very low concentration.

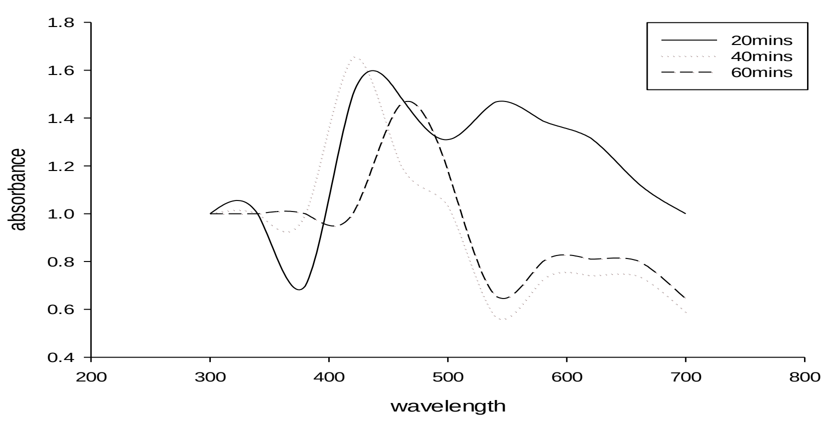

Fig. 1: UV-Visible absorption spectra of *Acalypha wilkesiana* at different time interval

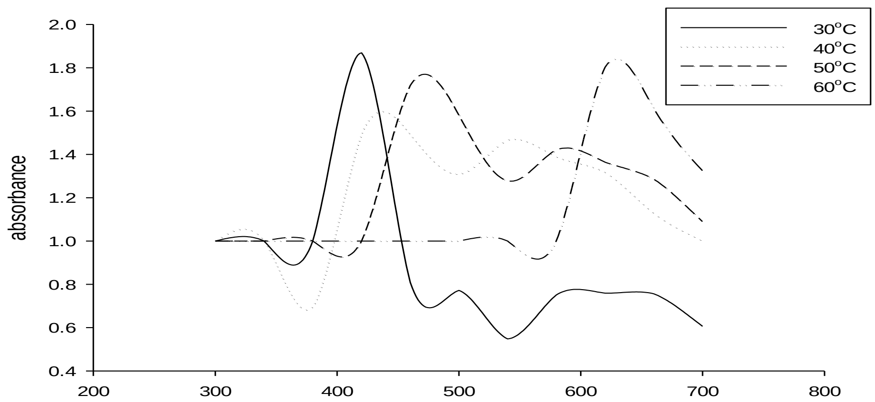

Fig. 2: UV-Visible spectra of *Acalypha wilkesiana* at different temperature

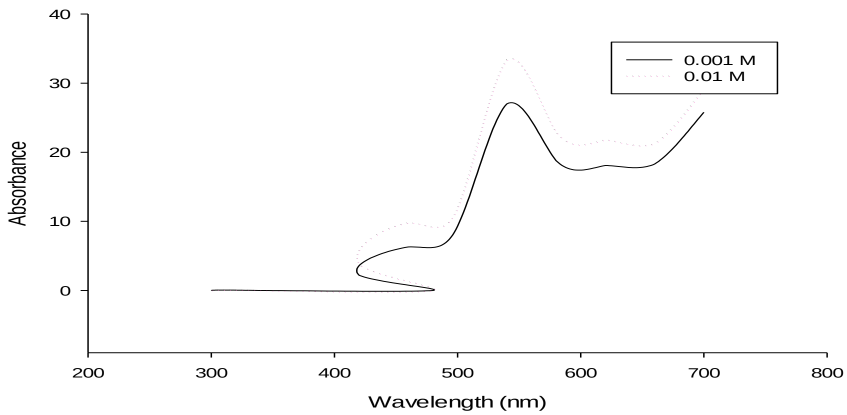

Fig. 3: UV-Visible absorption spectral of *Acalypha wilkesiana* at different concentration

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 nanoparticles (AgNPs) against bacteria (*E. coli* and *S. aureus*) was investigated. Silver ion was found to be the most toxic species to inhibit the growth of the bacteria (*E. coli and S. aureus*). Though, both concentrations of 0.001 M and 0.01 M inhibited the growth of *E.coli* and *S. aureus*. The concentration of 0.001 M showed a better against E. *coli* and *Staphylococcus aureus*. Previous studies reported that the electrostatic interaction may be possible reasons for the antibacterial activities of the silver nanoparticles. According to Ahmed *et al.* (2016), silver nanoparticles showed efficient antimicrobial property likened to others due to their exceedingly large surface area providing enhanced contact with cell wall of microorganisms.

Conclusion

Aqueous extracts of fresh *Acalypha wilkesiana* leaf acts as bioreduction agents to produce Ag nanoparticles. Synthesis was found to be efficient in terms of reaction time, temperature and concentration of $AgNO_3$ to AgNPs. The formation of AgNPs in the extract was observed by colour change of *Acalypha wilkesiana* extract from yellow to brown. It has proved to be an eco-friendly, cost effective and an efficient way for the synthesis of silver nanoparticles. Due to the increasing growth of microbial organisms in the environment, which poses serious threat to human health and the environment, especially in view of the emergence of resistant bacteria and side effects associated with prolonged use of commercial antibacterial therapeutics, AgNP contained in extract of *Acalypha wilkesiana* has proved to be an excellent inhibitor against *E.coli and S. aureus*. Therefore, this study was aimed at preparing a cost effective, eco-friendly, non-toxic and potent green synthesis of silver nanoparticles from extract of *Acalypha wilkesiana* as an inhibitor against bacterium (*E.coli and S. aureus*).

Data Availability

The data used to sustain the verdicts of this study are available from the corresponding author upon demand.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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