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Optimizing the Production of Silver Nanoparticles Using Mimosa Diplotricha Leaf Extract

A.G.B Aruggoda[1], S.R Weerakoon[2*], J.A.S Chathurika[3], M.G.W.K Weerasinghe[4]

[1, 4]Department of Agricultural and Plantation Engineering,

[2]Department of Botany, The Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka

[3]Department of Urban Bio-resources, University of Sri Jayawardhanapura Kotte, Ganagodawila, Nugegoda, Sri Lanka

ABSTRACT: Plant extracts are applicable over the other biological sources due to the availability and ability to act as stabilizing agent during the green nanoparticle synthesise. The present study evaluates the possibility of synthesising silver nanoparticles (SNPs) using plant leaf extracts of invasive weed *Mimosa diplotricha*. Biological and physical properties of synthesising Silver NPs were also evaluated using the UV-Vis spectroscopy (UV-Vis) and Scanning Electron Microscopy (SEM). To determine the best volume ratio of leaf extract, salt and temperature condition, an experiment was carried out 1:1 and 1:9 (V/V) ratios reacted with (2 mM) of Silver Nitrate under room temperature and 60-80 ^{0}C temperature condition. The colour-change conformed formation of Silver NPs. The broad peak obtained at 420-460 nm with UV–Vis surface plasmon resonance studies confirmed that the synthesised nanoparticles were Silver NPs. SEM microscopic studies revealed that spherical shape nanoparticles were formed with the average sizes 28.20 nm. This study shows that *M. diplotricha* mediated SNPs production is dependent on the concentration, incubation time and temperature of the reaction mixture. The best ratio of plant extract and $AgNO_3$ is 1:1 to optimize the production of SNPs. The temperature range 60-80^{o}C promotes higher SNPs production comparative to the room temperature. Green synthesised SNPs are poly-dispersed without forming agglomeration. Based on the observations, it is possible to conclude that *M. diplotricha* leaf extract is an effective source for SNPs production. This study is useful to optimize protocols for biological synthesis of SNPs using other plants.

KEY WORDS: Silver nanoparticles, Metallic Nanoparticle, Bio synthesis, Mimosa diplotricha

INTRODUCTION

Production, characterization, and application of nanoparticles is one of an escalating field in current research. Bio-nanotechnology is another branch of nanotechnology which integrate the biological precursors to generate the nano scale particles with unique functions. These protocols are based on the green chemistry principles, so they are environmentally friendly, cost effective, simple, and easy procedure, low energy consumption, and easy to apply in commercial level. Synthesise of nanoparticles using biological methods using plant or microbes as a biological agent. According to Keat *et al.,* (2014) reported that there are three main concerns during the green bio synthesis. Selection of reaction medium, selection of suitable reducing agent, and selection of appropriate stabilizing agent. Silver nanoparticle (SNPs) have wider range of application in number of fields such an agriculture, medicine, chemistry, industries, environmental application, sensors and optical biosensors, drug, and gene delivery (Ramasamy,2016), human health care such as coating contact lenses, cardiovascular implants, wound dressing, and bone cement (Gharibshahi et al., 2017) etc. According to the keat., *et al* (2014) SNPs is prominently applied in agriculture as an antibacterial, antifungal application. Furthermore, identified lethal effects on bacterial species and even yeast. Prabhu and Poulose (2012) reported, chemical and physical method are not so favoured as they are often expensive, utilize lethal chemicals, and are comparatively complex. Many of researchers suggested that green synthesise of nano particle can reduce the drawbacks of chemical and physical methods.

Mimosa diplotricha belongs to the family Fabaceae, with invasive growing habit which is native to Brazil. The plant spreads rapidly to the other countries and now it's considered as one of invasive species worldwide (Naima *et al.,* 2018). Seed produced are extremely difficult to control effectively using mechanical or chemical method. According to the phytochemical screening of *Mimosa diplotricha,* it contains several phytochemicals such as, alkaloids, carbohydrates, saponins, glycosides, phytosterols, phenols, flavonoids, proteins, and lipids (Naima *et al.,* 2018). M. *diplotricha* causes negative impact on number of ecological systems including agricultural, livestock, and biodiversity conservation. *M. diplotricha* is one of the most invasive species and has not yet identified a successful control and therefore introduced mitigation methods such as erosion control, nitrogen fixation, hedges, and barriers, and as a source of honey (CABI). Present investigation is an attempt to synthesise SNPs through green protocol using

M. diplotricha and identify the optimizing method of nanoparticle formed by optizing the volume ratio of the Silver Nitrate, temperature of the reaction mixture, and incubation time.

MATERIAL AND METHODOLOGY

PLANT EXTRACT PREPARATION

Mimosa diplotricha plant leaves were collected form an arable land at Balangoda, Sri Lanka in July 2022. Surface sterilised with running tap water followed by 30 min to remove debris and other contaminated matter and thoroughly washed with deionized water for 30 min and dried at room temperature. 05 gm of leaves were ground into a fine powder in a 100 ml Erlenmeyer flask along with 50 ml deionized water. Mixture was kept at a room temperature around 1 hour. Then the extract was filtered with Whatman filter paper no.1 thrice and stored at 4 ^{0}C for further use.

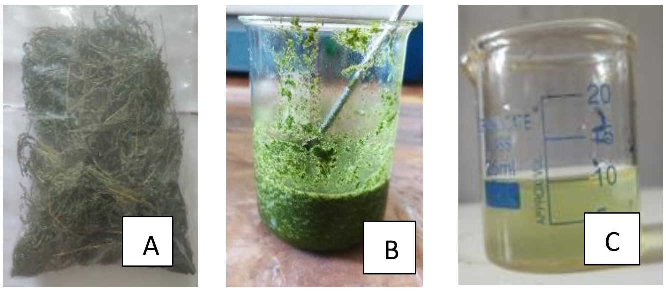

Figure1; (A): *M. diplotricha* leaf dried under room temperature, (B): leaf extraction as a grinded paste, (C): filtered leaf extraction.

SILVER NANOPARTICLE SYNTHESIS

Silver nitrate ($AgNO_3$) (98%) was purchased from Sigma Aldrich. A 50 mL aqueous solution (2 mM) of Silver Nitrate was prepared. The plant extract was mixed with $AgNO_3$ in different proportions under different temperature conditions.

Mimosa leaf extract was mixed with $AgNO_3$ solution at 1:9, 1:1 v/v ratio at room temperature (30^{0}C) and stored in dark at room temperature. The leaf extract was mixed with Silver Nitrate solution at 1:9, 1:1 v/v ratio at 60-80^{0}C for 3 hours and finally stored in dark condition at room temperature. Each reaction mixture was centrifuged at 14000 rpm at 20 min and removed the supernatant and remaining pellet was washed with deionized water and ethanol. Observations were done using Uv- Vis spectrophotometer at 24 hrs and 48 hrs.

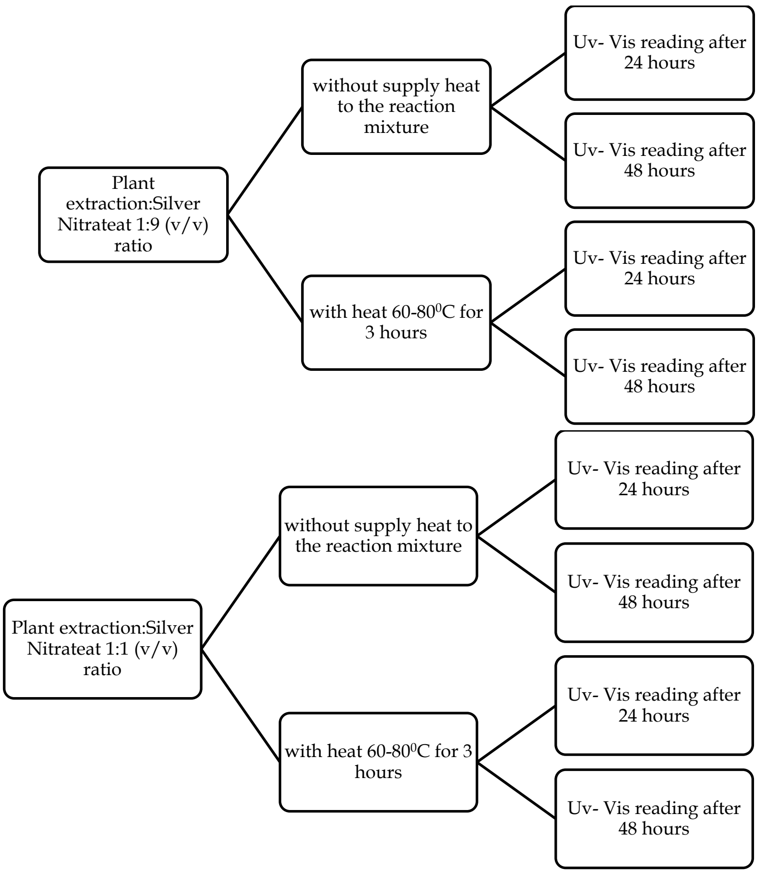

Figure 2: Outline of the synthesis of AgNPs under different conditions

CHARACTERIZATION OF SYNTHESISED SILVER NANOPARTICLES

UV–vis spectrometry analysis was done by using CT-2600 UV-vis Spectrometers (BioTek©) with a resolution of 1 nm between 300 and 600 nm. The resulting SNPs pellets were suspended in deionized water and used for characterization. 1 ml of Ag nanoparticles were added to the cuvettes by pipetting and dilute the ¾ of cuvettes with deionized water. Scanning Electron Microscope (SU6600 -HITACHI) was used for the morphological analysis of the synthesised NPs. Microscopic structural characteristics was observed at 50.00 KV and under multiple (KX) magnifications.

RESULTS AND DISCUSSION

PRELIMINARY TEST

The formation of nanoparticles was confirmed with colour changes in the reaction mixture. It was appeared in the Figures 3, and 4. Light green colour leaf extract was reacting with $AgNO_3$ turned to pale cream colour and the colour turned brown within 24 hours. Over the next 24 hours, the brown colour turned darker. Similar results were observed by the Ali *et al*., (2015).

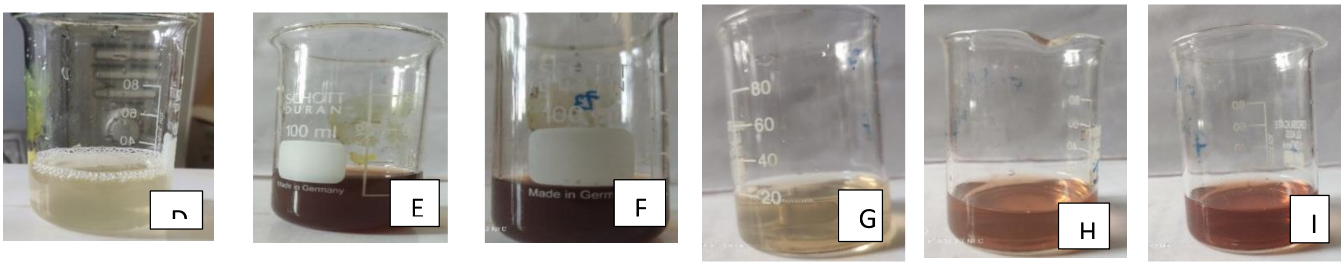

Figure 1; (D): *M. diplotricha* leaf extraction with Silver Nitrate solution (1:9 v/v), (E): colour change after 24 hrs, (F): colour change after 48 hrs, (G): *M. diplotricha* leaf extraction with Silver Nitrate solution (1:9 v/v) with heat 60-80^{0}C, 3 hrs, (H:) colour change after 24 hrs, (I): colour change after 48 hrs.

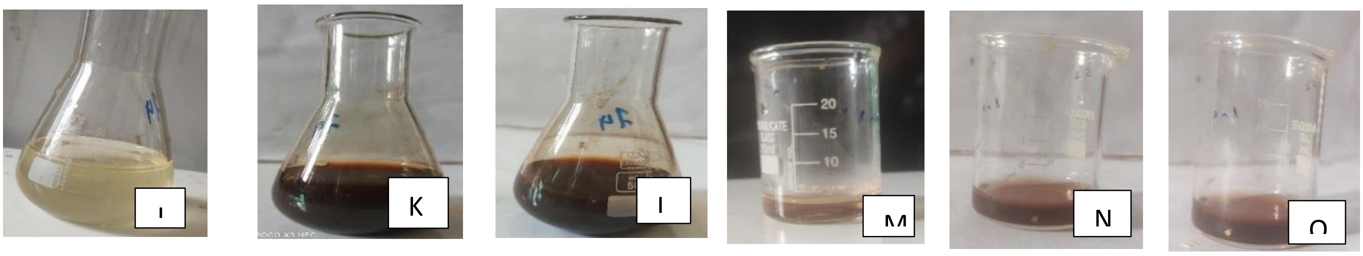

Figure 2: (J) : *M. diplotricha* leaf extraction with Silver Nitrate solution (1:1 v/v), (K): colour change after 24 hrs, (L): colour change after 48 hrs, (M): *M. diplotricha* leaf extraction with Silver Nitrate solution (1:1 v/v) with heat 60-80^{0}C, 3 hrs, (N): colour change after 24 hrs, (O): colour change after 48 hrs.

UV–VIS SPECTROMETRY ANALYSIS

The absorption spectra were recorded on UV–visible spectrophotometer 300-600nm wavelength and under 25^{0}C temperature condition. Figure 5 shows the UV–vis spectrum of the M. *diplotricha* leaf extraction before adding $AgNO_3$ solution. Figures 6 and 7 show the UV-Vis absorption spectrum of the synthesized SNPs. Synthesise of SNPs display on peak range 420- 460 nm. Figure 6 compares the UV-Vis spectroscopic analysis of 1:9 ratio (v/v) at room temperature and at temperatures of 60-80^{O}C. Figure 7 shows the UV-Vis spectroscopic analysis of 1:1 ratio (v/v) at room temperature and at temperatures of 60-80^{O}C to investigate the effect of temperature on the production of SNPs. Peaks indicate the characteristics of the surfaces Plasmon resonance of SNPs. SNPs have free electrons, due to the vibration of free electron with light waves result in Surfaces Plasmon resonance (SPR). Anandalakshm., *et al* 2015 have observed characteristic surfaces of SPR display in the peak range of 424 – 430 nm. According to the study of Njagi *et al*. (2011) synthesis of colloidal nanoparticle depicts the in the range of 400-450 nm due to the excitation of surface plasmon vibration. Akashraj *et al* ., (2014) reported absorption peak between 430-460 nm confirm the presences of Ag NPs. Uv-Vis spectral readings show that the ingredients in the *M. diplotricha* leaf extract are capable of stabilizing Ag nanoparticles.

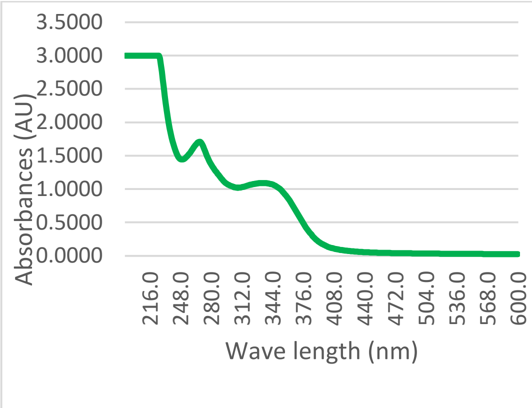

Figure 3; UV–vis spectrum of the *M. diplotricha* leaf extraction

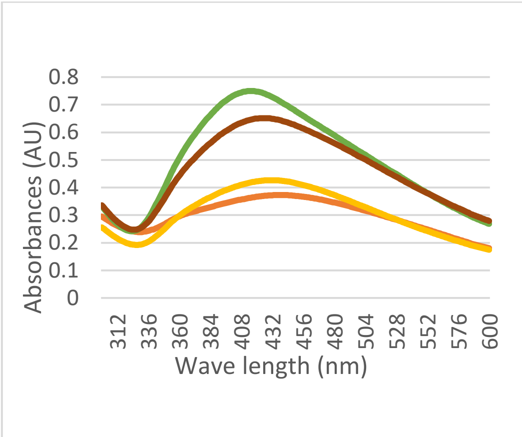

Figure 4

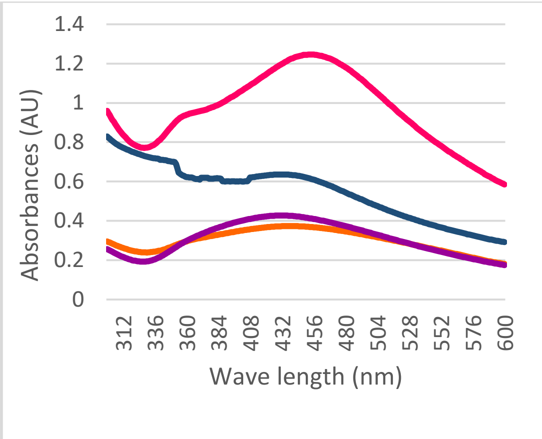

Figure 7

Figure 5; UV–vis spectrometry analysis 1:9 ratio (v/v)

	Concentration, Mimosa leaf extraction: Silver Nitrate solution	Incubation Period (hr)	provided temperature.
	1:9	24	30^{0}C
	1:9	48	30^{0}C
	1:9	24	60-80^{0}C
	1:9	48	60-80^{0}C

Figure 6; UV–vis spectrometry analysis 1:1 ratio (v/v)

	Concentration, Mimosa leaf extraction: Silver Nitrate solution	Incubation Period (hr)	provided temperature.
	1:1	24	30^{0}C
	1:1	48	30^{0}C
	1:1	24	60-80^{0}C
	1:1	48	60-80^{0}C

Several factors affect optimizing the nanoparticle formation. Mainly the concentration of $AgNO_3$ and, volume/concentration of plant leaf extraction, temperature, autoclaved-time etc. Anandalakshmi *et al.* (2015) reported that increasing the concentration of leaf extract can optimize SNPs formation. Through this, increases the quantity of phytochemicals needed to reduce metallic iron. According to Figure 6 the production of NPs is optimized under thermal conditions and with increasing incubation time. Figure 6 and 7 show that SNPs quantity is always higher with the increasing incubation time. Graph shows higher absorption value in 48 hrs Uv-Vis reading compared to 24 hrs reading. By comparing the absorption values given in Figures 6 and 7, it is possible to conclude that, 1:1 (V/V) is the most appropriate concentration to optimize the SNPs production.

SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS OF AG NPS

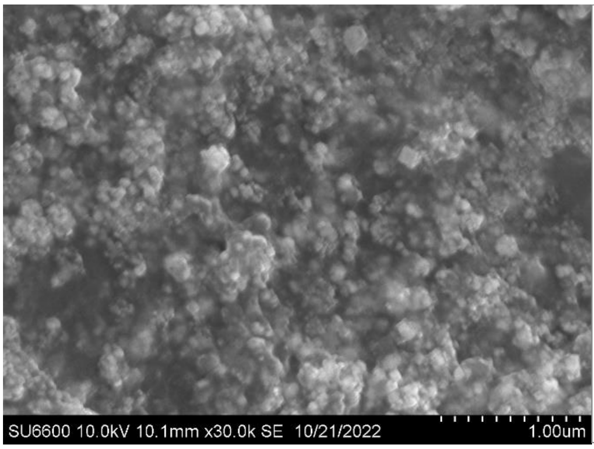

Figure 7: SEM images of Ag NPs.

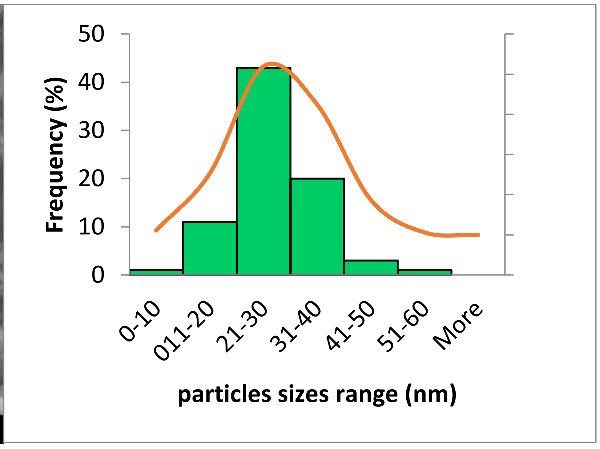

Figure 8: Particles sizes distribution of Ag NPs.

Morphological features analysed through SEM are given in Figure 8. Surfaces morphology of SNPs was observed under the 500 nm resolution. It shows spherical shape and particles are poly-disperse with wide range with unique spherical shape without forming agglomerations, and the average particle size is 28.20 nm. *M. diplotricha* plant extract contains number of phytochemicals. SNPs are visible as particles and were stick together with thin placenta like structures, due to the presences of gummy like phytochemicals. Yugandhar *et al*. (2015) reported that SEM images are spherical shape in SNPs and Anandalakshi *et al*. (2015) reported the similar observations.
Silver nanoparticles have been applied in a wide range of fields for a long time. Several reports related to the biosynthesis of nanoparticles using plant-based products have excellent antibacterial and antifungal properties. Surfaces plasmon resonance indicates the formation of SNPs and indirectly shows the ability of plant phytochemicals found in *M. diplotricha* are able to convert metallic silver into iron Nano form.

CONCLUSION

In conclusion, this study shows that *Mimosa diplotricha* mediated SNPs production is dependent on the concentration, incubation time and temperature of the reaction mixture. According to the results, the best ratio of plant extract and $AgNO_3$ is 1:1 to optimize the production of SNPs. The temperature range 60-80°C promotes higher SNPs production comparative to the room temperature. Green synthesised SNPs are poly-dispersed without forming agglomeration. Based on the observations, it is possible to conclude that *M. diplotricha* leaf extract is an effective source for SNPs production. This study is useful to optimize protocols for biological synthesis of SNPs using other plants.

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