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Optimizing the Bio-Synthesis of Silver and Ferrous Oxide Nanoparticles Using *Marsilea quadrifolia* (L.) Leaf Extract

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Abstract:

Bio-mediated nanoparticle synthesis is a 'green' environmental friendly process. The present study investigates bio-synthesis of silver (Ag) nanoparticles (NPs) and Ferrous Oxide (FeO) nanoparticles using leaf extract of *Marsilea quadrifolia,* a noxious weed in rice fields worldwide. Optimal synthesis of AgNPs and FeONPs with desirable concentrations of Silver Nitrate and Ferrous Nitrate and different quantities of the plant extract was investigated under room temperature. Characterization of NPs was carried out using ultraviolet–visible spectroscopy, (SEM) Scanning Electron Microscope. To determine the appropriate concentrations for AgNPs and FeONPs synthesis, different dilution series of Silver Nitrate and Ferrous Nitrate were reacted with *M. quadrifolia* leaf extract at 1:1, 1:2, 1:3 ratios respectively, under room temperature condition. For the extraction, 8g of *M. quadrifolia* dried leaf samples were used and extraction was done by using deionized water. The results showed that plant extract and salt mixed ratios have a significant effect on yield of synthesized AgNPs and FeONPs. The best ratio of plant extract and $AgNO_3$ is 1:1 to optimize the production of AgNPs. The temperature range 60-80°C promotes higher AgNPs production comparative to the room temperature. Similar observations were recorded with FeONPs. *M. diplotricha* leaf extract is an effective source for AgNPs and FeONPs production.

Keywords: *Silver Nanoparticles, FeO Nanoparticles, Bio-synthesis, UV-Vis spectrum, Marsilea quadrifolia.*

Introduction

Nanotechnology is widely applicable worldwide due to its advanced features. Nanoparticles (NPs) are preferred in many applications because of their large surface area. Currently, one of the most fascinating research areas is synthesis of nanoparticles *via* the green route. Researchers show an immense interest in this

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area due to the border range of application of nanoparticles in the field of agriculture, medicine, biology, physics, and chemistry (Ali et al., 2015) energy science, mechanics, electronics (Amjad et al., 2021). Many studies have carried out on bio synthesis of copper, zinc, gold, iron, and silver nanoparticles.

Silver nanoparticles (AgNPs) are gaining a considerable attention due to its strong inhibitory and bactericidal effects with anti-fungal activities at low concentrations (Amjad et al., 2021, Yugandhar et al., 2015), high surface area ratio, catalytic properties (Ali et al., 2015) and hepatoprotective properties (Yugandhar et al., 2015). Several methods have been used to synthesize AgNPs via chemical reduction, sol gel, etc. However, many of these available conventional methods are expensive, have significant adverse consequences due to the usage of hazardous chemicals like sodium borohydride ($NaBH_4$). Green synthesis of nanoparticles provide advancement over other methods as they are simple, one step, safer, cost-effective, environment friendly and relatively reproducible and often results in more stable materials and are sustainable (Yugandhar et al., 2015, Ahmjad et al., 2021, Ali et al., 2015). Previously, bio synthesis of Ag NPs using stem bark extract of *Syzygium alternifolium* (Yugandhar et al., 2015), leaf extract of *Salmalia malabarica* (Krishna et al., 2015) were reported.

Unique properties such as super magnetism is of interest in the extraction of Ferrous Oxide nanoparticles (FeONPs). Therefore, FeONPs are used for various application fields in science and technology. Literature is available on synthesized FeONPs using different plant parts such as stem, roots, and leaves. In the bottom-up approach, number of methods have been developed to synthesize FeONPs such as chemical, physical and biological methods. However, chemical and physical methods including sol-gel process, chemical co-precipitation, and chemical methods such as vapour deposition are highly expensive and are being used as stabilizing agents like Sodium Dodecyl Sulphate, which produces very hazardous by products (Hamdy et al., 2022). Several reports are available of FeONPs synthesis through green mode. Razack et al., (2019) reported that synthesis of ferrous oxide NPs using *Hibiscus rosa-sinensis,* synthesis of iron nanoparticles using aqueous extract of *Musa* and further investigated the resistances against the pathogenic bacteria. Synthesis of iron nanoparticles using aqueous extract of *Musa Ornate* is reported by Saranya et al., (2017).

Because of these problems, researchers are finding an environmentally friendly and sustainable method for nano manufacturing. Use of various natural material for synthesizing NPs is called bio-nanotechnology. Algae, microbes, and plants are frequently used in the synthesis of nanoparticles. They can act as a capping and reducing agent during the stabilizing phase of nanoparticle synthesis. Plant-mediated nanoparticle synthesis is faster than microorganism-mediated nanoparticle synthesis (Ahmjad et al., 2021). Same as microbe-mediated method, plant-mediated method is so far not an industrially applicable at commercial level. Plants offer a highly desired system for the synthesis of nanoparticles as they can produce a broad range of secondary metabolites with high reduction potential.

Marsilea quadrifolia L. belongs to the family Marsileaceae, commonly known as four leaf clover, water clover, pepperwort, and water shamrock. It is an herbaceous, perennial fern grows in aquatic environments. It requires moist soil and can be found in slow-moving water environments. According to a quantitative analysis reported by Gopalakrishnan and Udayakumar (2017), tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, carbohydrates, proteins, phenolic compounds and phytosterol are the main phytochemicals contain in *M. quadrifolia* plant. These bio-active compounds have medicinal values for curing several ailments like cough, bronchitis, diabetes, psychiatric diseases, eye diseases, diarrhoea, and skin diseases. In many Asian and European counties, *M. quadrifolia* is recognized as a serious weed in rice fields. There are no known biological or physical control techniques available, only chemical control methods are available with Bensulfuron Methyl herbicide that resists *M. quadrifolia.*

Bio-active compounds contain in *M. quadrifolia* plant are used in bio-conversion of silver iron or ferrous iron into NPs. Because many of the bio-active compounds in the plants can act as reducing and capping agents (Ali et al., 2015).

Nucleation, aggregation or coarsening and bio-reduction are the three main steps in nanoparticle synthesis process. During the nucleation, proton activates to form various functional groups from metallic iron or silver. During growth phase or aggregation, nanoparticles form into large groups and into different irregular shapes. During the termination phase, they stabilize into metallic nanoparticles (Amjad et al., 2021**).** Production of smaller sized nanoparticles are promoted by the plant, such as alkloids, flavonoids, which are active bio-compounds. In addition to the plant phytochemicals, various physical parameters such as temperature, pH of the reaction mixture, substrate metal iron concentration, light, enzymes also effect on the nanoparticle production (Ali et al., 2015).

In the present study silver and iron Oxide nanoparticles are synthesized using *Marsilea* q*uadrifolia* (L.) leaf extract, used as both capping and reducing agent and the optimum salt concentration and *M. quadrifolia* leaf extract to optimize the production.

Materials and Methods

Plant-Extract Preparation

Marsilea q*uadrifolia* plants (Figure 1) were collected form the paddy fields at Balangoda, Sri Lanka in November 2022. Plants were surface sterilized with running tap water to remove debris and other contaminated organic contents, followed by deionized water and dried in air direr at 42 ^{0}C temperature overnight. Five grams (5 g) of finely ground leaf powder were kept in a beaker containing 80 ml de-ionized water and boiled for 30 min. The extract was cooled down and filtered with Whatman filter paper no.1 thrice and the extract was stored at 4 ^{0}C for future use.



Figure 1. *Marsilea quadrifolia* (L.)

Synthesis of Silver Nanoparticles (AgNPs)

Silver Nitrate (98% - Sigma Aldrich) was used to prepare 100 ml of 2 mM Silver Nitrate in an Erlenmeyer flask. Then 5 ml of plant extract was added to three separate test tubes. Silver Nitrate was added 5, 10 and 15 ml in the ratio of 1:1, 1:2, 1:3. Observations were made at 24 hrs. The reactions were allowed to progress at room temperature. Each reaction mixture was centrifuged at 14000 rpm at 20 min and removed the supernatant and remaining pellet was washed one time with de ironized water and ethanol.

Synthesis of Ferrous Oxide Nanoparticle (FeONPs)

FeONPs were prepared by adding 1 M (100 ml water) of Ferrous Nitrate. Then 5 mL of plant extract was added to three separate test tubes. Then 5ml and 10ml Ferrous Nitrate was added in the ratio of (plant extract: salt) 1:1, 1:2, 2:1 respectively. The solution was maintained at 80 °C for hours and then reactions were allowed to progress at room temperature. During this time, the colour of the extract reaction changed from translucent pale to black and light green to black, indicating the formation of FeONPs. Observations were done at 24 hrs. Each reaction mixture was centrifuged at 14000 rpm for 20 min. and removed the supernatant and remaining pellet was washed with de ironized water.

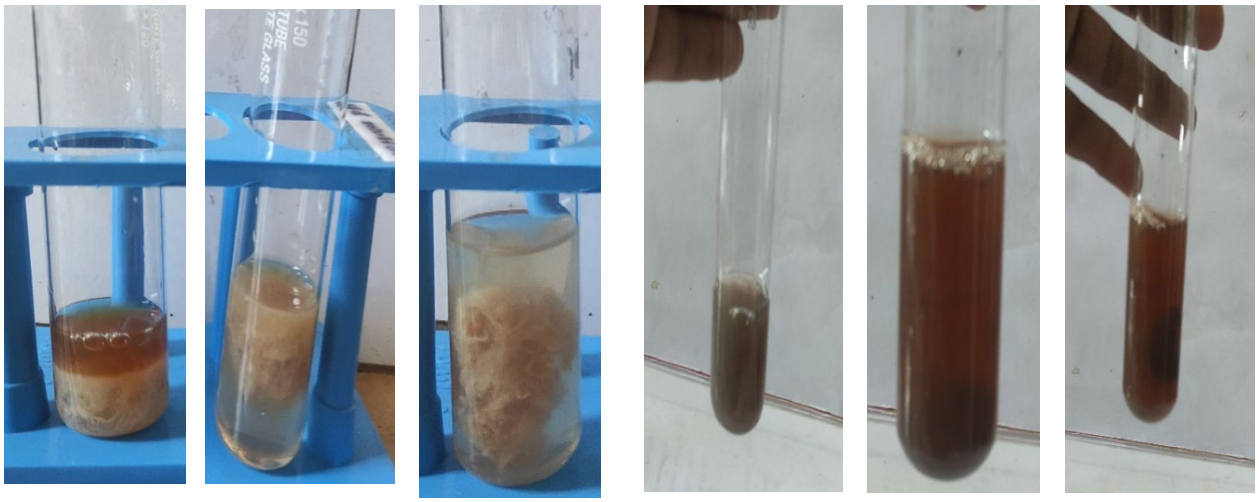
Characterization of Synthesized Nanoparticles

UV–vis spectrometry analysis was carried out using CT-2600 UV-vis Spectrometers (BioTek©) with a resolution of 1 nm between 200 and 700 nm. The resulting AgNps or FeONPs pellets were re-suspended in deionized water and used for characterization. SU6600 Scanning Electrone Micreoscope (HITACHI) was used for the morphology, size and analysis of the particle distribution of iron oxide nanoparticles, and microscopic structure was observed at 50.00 KV and 100 KV under multiple (KX) magnifications.

Results and Discussion

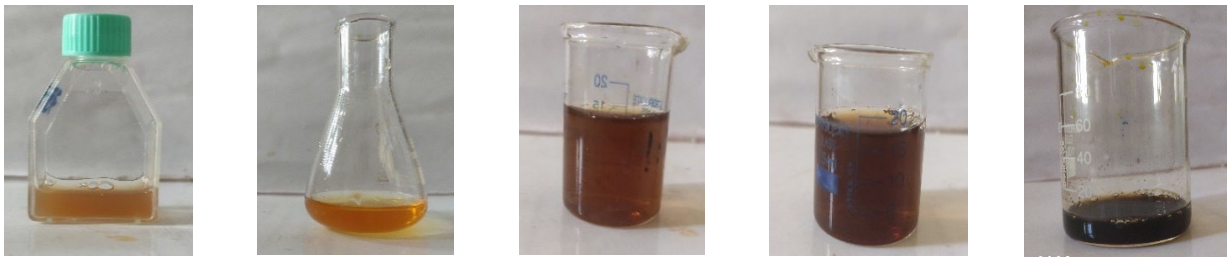
Preliminary Test

Bio-reduction process was first confirmed by changing of colour of reaction mixture. Within 30 min colourless reaction mixture was gradually changed to pale brown colure and within next 24 hrs it was turn to dark brown (Figure 2 and 3).



A B C D E F

Figure 2. AgNPs Synthesised Using *M. quadrifolia* Leaf Extract.
(A); Colour of the Reaction Mixture 1:1 Ratio, (B); Colour of the Reaction Mixture 1:2 Ratio, (C); Colour of the Reaction Mixture 1:3 ratio, (D); Colour of the Reaction Mixture after 24 hrs 1:1 Ratio, (E); Colour of the Reaction Mixture after 24 hrs 1:2 Ratio, (F), Colour of the Reaction Mixture after 24 hrs 1:3 Ratio



a b c d e

Figure 3. FeONPs Synthesised Using *M. quadrifolia* Leaf Extract.
(a); *M. quadrifolia* Leaf Extract, (b); Colour of Ferrous Nitrate (c); 2:1 Leaf Extract: Salt (After 24 hrs), (d); 1:1 Leaf Extract Salt(After 24 hrs), (e); 1:2 Leaf Extract: Salt (After 24 hrs).

The absorption spectra were recorded on UV–Visible Spectrophotometer 300-650 nm wavelength and under 25^{0}C temperature condition. Figure 4 shows the UV-vis absorption spectrum of the *M. quadrifolia* leaf extract. Figure 5 shows UV spectrum of synthesized AgNPs. Ali *et al.* (2015) reported that surface Plasmon resonance peaks in the range of 435–445 nm using *Artemisia absinthium* leaf extract. Furthermore, reported that narrow peak was observed when both plant extraction and chemical used in 1:1 (v/v) ratio. Rane *et al.* (2014) reported that silver nanoparticle synthesized as a broad peak from 402- 420 nm using *A. moschatus* leaf extract. Figure 5 depicts the different surfaces resonance peak in between wavelength of 430-435 nm. Most pronounced increase in the peak is visible in the 1:3 ratio. Further, much narrow peak shows under the 70% of plant extraction mixed with the Silver Nitrate. Reaction mixture gets rapidly darker and in the preliminary test highlighted colour changed visualized in the 1:3 ratio comparative to other two. According to the readings of UV-Vis spectrometer optimum quantity of NPs was formed in the 1:3 ratio in the *M. quadrifolia* leaf extraction.

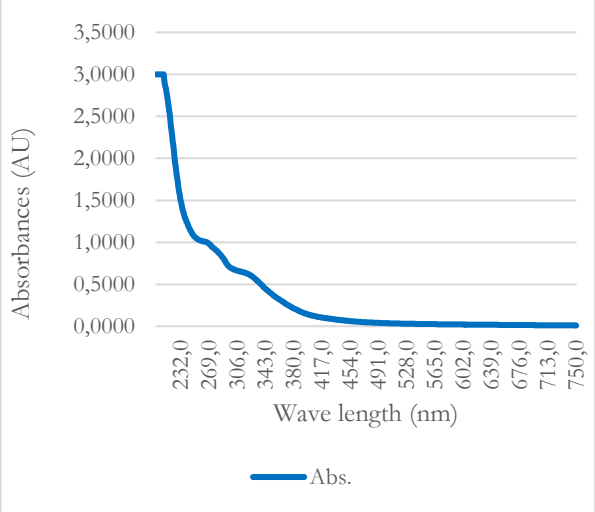


Figure 4. UV-Vis Spectrum of *M. quadrifolia* Leaf Extract

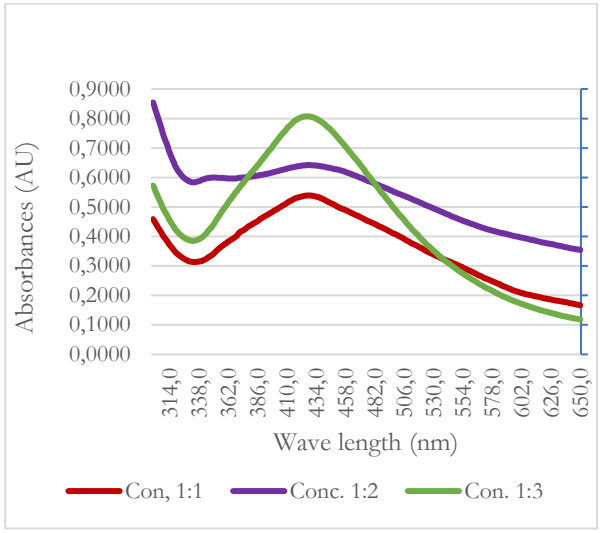


Figure 5. UV-Spectroscopy Data 300-650 nm, Synthesizing of AgNPs under Dissferent $AgNo_3$ Concentrations

Ferrous Oxide Nanoparticle (FeONPs)

Shape and average particle sizes of FeONPs synthesis using *M. quadrifolia* was determined by suing the SEM image analysis (Figures 6 and 7).

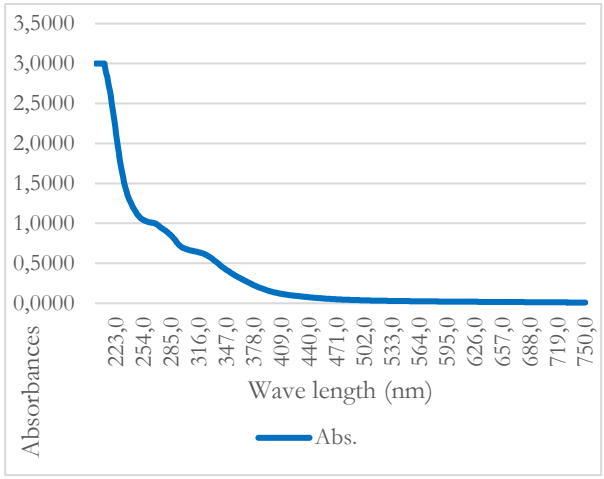


Figure 6. UV-Visible Spectroscopy of *M. quadrifolia* Leaf Extract

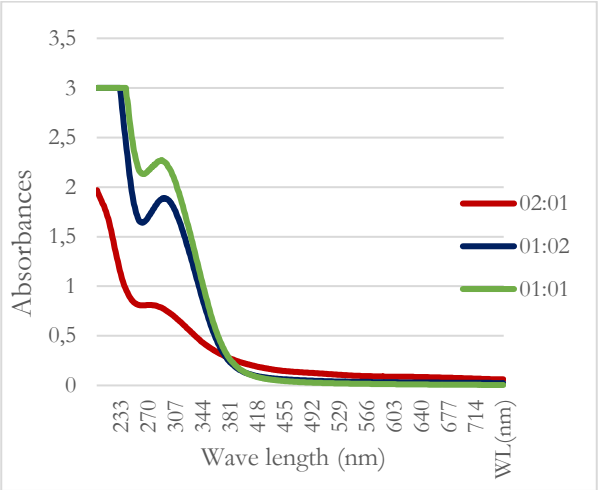


Figure 7. UV-Visible Spectra Image Showing Absorption Peaks

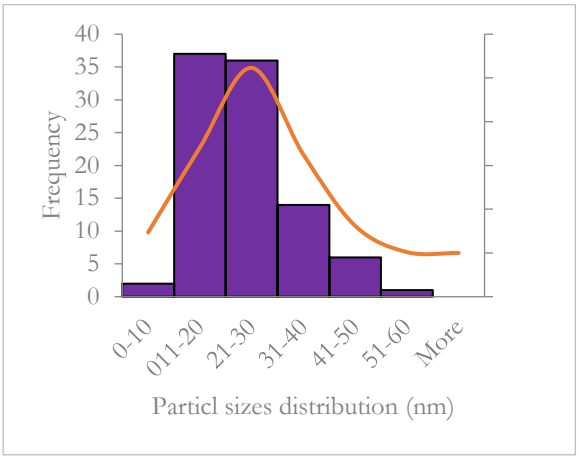


Figure 9. Particle Sizes Distribution of SNPs of *M. quadrifolia* Leaf Extract

SEM Analysis

Silver Nanoparticles (AgNPs)

SEM images of AgNPs are shown in the Figure 8. The surface morphology of AgNPs show a unique shape with uniform size distribution. Average particle sizes were calculated as 25.19 nm in sizes. Mani et al., (2015) reported the similar results during the synthesis of silver nanoparticle using the *Pedalium murex* leaf extract. Figure 9 shows, particle sizes distribution of AgNPs of *M. quadrifolia* leaf extract

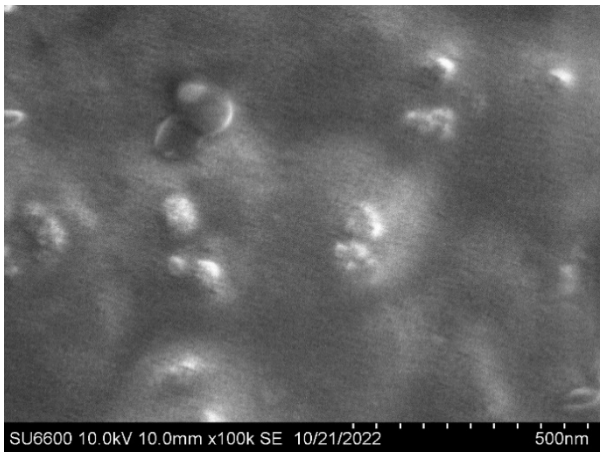


Figure 8. SEM Images of SNPs of *M. quadrifolia* Leaf Extract

According to Beer-Lambert law, concentration of a solution is directly proportional to its absorption of light. The proportion of the light absorbed will depend on how many molecules it interacts with light beam. According to law, the linear relationship between the concentration and the absorbance of the solution, which enables the concentration of a solution to be calculated by measuring its absorbance (Rahman 2016). There are number of reports about several factors affect the nanoparticle yield, Such as concentration of $AgNO_3$, plant extract, solvent type as well as capping and stabilizing agent, OH group. Meanwhile, Krishna et al., 2015 reported that by increasing reaction time can increase formation of nanoparticles.

Reduction of Ag2+ iron is due to the presence of phytochemicals in *Marsilea* leaf extraction. The ability of tannins, saponins, flavonoids, steroids, terpenoids, alkaloids contained in *Marseillea* leaf extract to convert Ag2+ in to zero is demonstrated by surface plasmon resonance. According to the Ali et al., 2015, long wavelength in the peak indicates the higher practical sizes while short or narrow wavelength indicated the small size particles. Relevant to the observation in Figure 9, narrow peak indicated the small sized nanoparticles were formed. Furthermore, secondary metabolites also responsible for determining the sizes of the practical. Content of secondary metabolites in the extraction performs in different way in the

reduction process and affect the growth and nucleation of the practical. Size of the particle is highly effect on its active nature and stability (Ali et al., 2015). Due to the nontoxic nature, green synthesise of AgNPs are currently widely applied for anti-microbial, anti-insecticide, and cytotoxicity activities. Through this study it was observed that *M. quadrifolia* plant has a strong ability to reduce Ag iron in to its zero form and stabilize AgNPs.

Ferrous Oxide Nanoparticles (FeONPs)

Figure 10 shows SEM images of ferric oxide nanoparticles, it shows the structural features of FeO nanoparticles and Figure 11 shows the average practical sizes and distribution of the synthesized FeONPs. The synthesized FeONPs are cubic shaped and approximately average particle size distributed around 30- 39 nm. Results were consistent with previous reports. According to Abid et al. (2020), 27.91 to 40.94 nm size particles were observed. According to Razack et al., (2020), size and the shape of particle depended based on the concentration and nature of the plant extract.

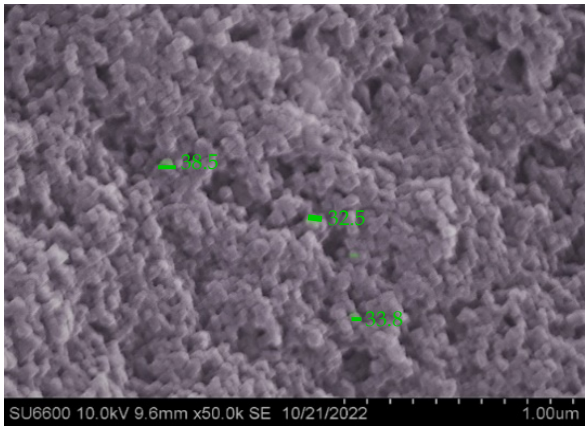


Figure 10. SEM Images from Ferric Oxide Nanoparticles

Natural iron oxide exists in number of different ways; magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), and hematite (α-Fe_2O_3). According to their phase, chemical composition and properties also differ each other (Syarifah *et al.,* 2018). The phase colour changed as; in iron oxide magnetite has a black colour, maghemite has light brown colour, and hematite has red colours.

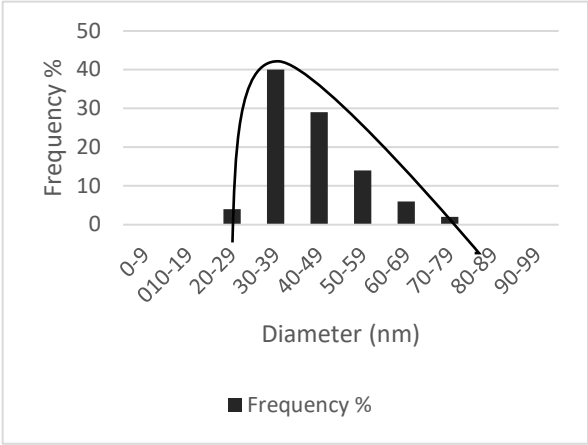


Figure 11. Frequency of Particle Size Distribution of FeONPs

Conclusion

M. diplotricha mediated AgNPs 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 AgNPs. The temperature range 60-80°C optimize synthesis of AgNPs comparative to the room temperature.

Biosynthesis of FeONPs will be a promising area in nanotechnology. As biosynthesis lacks usage of toxic chemicals and excessive energy consumption, it could be an eco-friendly sustainable method. Physical and chemical methods have shown several drawbacks. *M. quadrifolia* leaf extract has the potential to act as a stabilizing agent and the best concentration ratio of salt and *M. quadrifolia* leaf extract is 1:1 with a temperature range 60-80°C optimize the synthesis of FeONPs.

Bio-synthesised AgNPs and FeONPs 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 AgNPs and FeONPs production. The present study is useful to optimize protocols for biological synthesis of Ag and FeO NPs using other plants.

Acknowledgement

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Conflict of Interests

No conflict of interest.

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