

Optimization & Characterization of Silver Nanoparticles from *Rhizophora mucronata* Mangrove bark extract

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Abstract: The major goal of nanotechnology is to produce nano materials with controlled properties like, size and shape which can serve the human being, marine species and ecosystem through diverse applications. *Rhizophora mucronata* Mangrove bark extract supported the synthesis of highly effective and substantial AgNPs. The synthesis of AgNPs from 1.0 mL of *Rhizophora mucronata* extract was optimized at different Physiological and biological condition at a concentration of pH 7.0 and 1.0 mM of AgNO₃ at 37°C. The synthesized AgNPs were characterized by ZETA potential and Atomic force Microscopy (AFM). The Zeta potential value was -20.3 mV wherein the AFM envisage the 3D structure of the nanoparticles ranging from ~78 to ~99 nm. The results confirm, consistent and high degree of stable nanoparticles of *Rhizophora mucronata* Mangrove bark extract could be used as immunostimulants, which will expand the possibilities of further research on disease controlling trial on marine animals.

Key words: Mangrove bark extract, ZETA potential, Atomic force Microscopy

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I. Introduction

Nanoparticles of noble metals are being used widely nowadays because of their unique applications in the field of textile, medicine, energy saving, environment, cosmetics, electronics, and many more [1]. Various techniques, such as chemical, physical, and mechanical techniques, have been developed to prepare metal nanoparticles, as these methods are costly, toxic, and non-ecofriendly. A green synthesis of nanoparticles with the help of biological sources like plant and microorganisms is carried out because they are less toxic to human and environment [2]. Although such particles can be synthesized by physical, chemical and biological methods in the past few years, among them biological method has gained more importance [3]. The green synthesis or biological production of nanosized particles with specific functions is attracting an increasing amount of interest in bio-nanotechnology [4].

Mangrove plants are rich in natural antioxidants and also contain bioactive compounds which are active against many pathogenic bacteria [5]. Most of the studies related to the synthesis of nanoparticles using mangrove and mangrove associate plants are very limited. It is proven and well known that mangrove plants have different characteristics from those of terrestrial including the Nanoparticles size and morphology [6-7]. The present study dealt with the extracellular synthesis of AgNPs using *Rhizophora mucronata* mangrove plant belongs to the Rhizophoraceae family, followed by its characterization and optimization for rapid AgNPs synthesis.

II. Materials and Methods

2.1 Collection of *Rhizophora mucronata* bark

The bark of the mangrove plant *Rhizophora mucronata* was collected from Pichavaram mangrove forest located in the higher land of Vellar estuarine complex, Tamilnadu, India. The Collected samples underwent three step cleaning process starting from sea water, tap water and at last with distilled water to eliminate all the contaminants before shade dried at room temperature for 2 weeks.

2.2 Preparations of *Rhizophora mucronata* bark extract

The air-dried bark extract was well grounded to coarse powder using a blender. A sample of 5.0 g of the dried bark was taken and mixed with 100 ml of distilled water. This mixture was kept at 55°C for 15 minutes in a water bath, cooled to room temperature and filtered with Whatman No 1 filter paper. This aqueous bark extract was refrigerated and used as base sample for further experiments.

2.3 Biosynthesis of Silver nanoparticles

Silver nitrate was purchased from HiMedia Laboratories (Chennai, India). A total of 1 ml of the *R. mucronata* bark aqueous extract was added to 9 ml of 1 mM silver nitrate solution in a 15 ml test tube. The aqueous bark extracts of *R. mucronata* and AgNO₃ solution were used as control. After the desired reaction period, the solution containing the AgNPs was centrifuged at 10,000 rpm for 15 minutes. The pellet was collected and dispersed in distilled water, eliminating any interactive biotic molecules. The reaction was performed in dark condition at room temperature for 24 h in a static condition and recorded for any reactions changing in colour. The synthesized AgNPs were analyzed under UV–vis spectrophotometer from the peaks range of 300 nm to 700 nm. In addition, the *R. mucronata* bark extract solution was visualized and recorded for any precipitation or accumulation of nanoparticles. AgNPs synthesized with *R. mucronata* bark showed good stability and better separation of the AgNPs and was used for characterization and optimization studies.

2.5 Optimization of phytosynthesis of AgNPs

2.5.1 Effect of different pH

1.0 ml of *R. mucronata* bark aqueous extract 9.0 mL of 1 mM AgNO₃ was added and maintained at different pH (4, 5, 6, 7, 8, 9 and 10) at 37°C. In addition to this, the absorbance spectrum of solution was calculated between 300 and 700 nm using HITACHI U-2900 spectrophotometer after 24 hours. The pH 7.0 indicated high synthesis and good durability of Silver nanoparticles and hence this pH level was maintained for further studies.

2.5.2 Effect of different concentrations of aqueous *R. mucronata* bark

The *R. mucronata* bark aqueous extract was taken in the variance of 0.5 mL, 1 mL, 1.5 mL, 2 mL, 2.5 mL and 3 mL were added with 1.0 mM AgNO₃ (pH 7.0) to make 10 mL of solution which was kept under 37°C for 24 hrs. From the absorbance calculated through Spectrophotometer out of all variance, the 1.0 mL bark extract added with 1.0 mM was consistent and hold up maximum biosynthesis of nanoparticles. Hence all the further studies on optimization and characterization will be carried out with this solution.

2.5.3 Effect of different temperature

Multiple samples prepared in the combination of 1.0 ml of *R. mucronata* bark aqueous extract added to 1 mM AgNO₃ (pH 7.0) and incubated at different temperature such as 22°C, 27°C, 32°C, 37°C, 42°C, 47°C and 52°C for 24 h. The absorbance of the resulting solutions was calculated thoroughly with Spectrophotometer. The sample incubated at 37°C temperature showed good synthesis of nanoparticles and stability among all other samples. Hence 37°C was standardized for further studies.

2.5.4 Stability of Phyto synthesized silver nanoparticles

The sample of *R. mucronata* bark extract Phyto synthesized AgNPs was kept in dark at room temperature for a period of 2 months. The stability of the sample was recorded on different time intervals starting from 0 hrs, 12 hrs, 24 hrs, 5th day, 15th day, 1 month, and 2 months.

2.6 Characterization of AgNPs

2.6.1 Zeta potential measurement

The *R. Mucronata* bark extract containing Silver nanoparticle sample was vortexed and transferred into 1.0 mL of zeta potential cuvette ((DTS1060, Malvern). The electrophoretic potency of the sample was measured by Zeta sizer Nano ZS (Malvern Instruments Ltd, Malvern, UK) and analyzed by applying the Henry equation to get the results for interpretation.

2.6.2 Atomic Force Microscopy (AFM)

The exterior structure and size of optimized Silver nanoparticles were considered by Atomic Force Microscope (AFM, XE-70 Parksystem, German) below standard atmospheric state. The working condition of AFM was determined with $F_0 = 241$ kHz—high-resonant-frequency, 41 N/m—silicon probes force constants, and 2 Hz scan speeds. The working conditions were adjusted and fixed with AFM image software.

III. Results and Discussion

3.1 Optimization of the phytosynthesis of silver nanoparticles

3.1.1 Effect of different Ph

The reaction of phytosynthesis of nanoparticles at pH 4, 5, and 6 was moderate. The Silver nanoparticles exhibited light brown colour at the end of 24 h. At neutral pH 7 the reactions begin instantly after the addition of silver nitrate in the *R. Mucronata* bark extract. The pale-yellow solution become dark brown for AgNPs. The colour formation was quick at pH 9 and pH 10 but agglomeration was observed after the inclusion of

AgNO₃. At pH8 the pale yellow colour was reformed to dark brown for AgNPs but they were not stable after 4 days. It exposed agglomeration of nanoparticles. The stability was high at the pH 7 when compared with other pH 4,5,6 and 10. (Fig. 1). This observation was in consonance with the prior studies via [8]. [9] reported that there was a slow rate of origination and aggregation of AgNPs at acidic pH wherein, [10] observed that at basic pH there was a possibility of Ag⁺ precipitating as AgOH. They also found that the optimum condition for the preparation of AgNPs using *Terminalia chebula* was at pH 7.0. Similar results were also observed by [11] which has reported as pH 7.0 was optimum for the formation of AgNPs using *Acalypha indica* leaf extract.

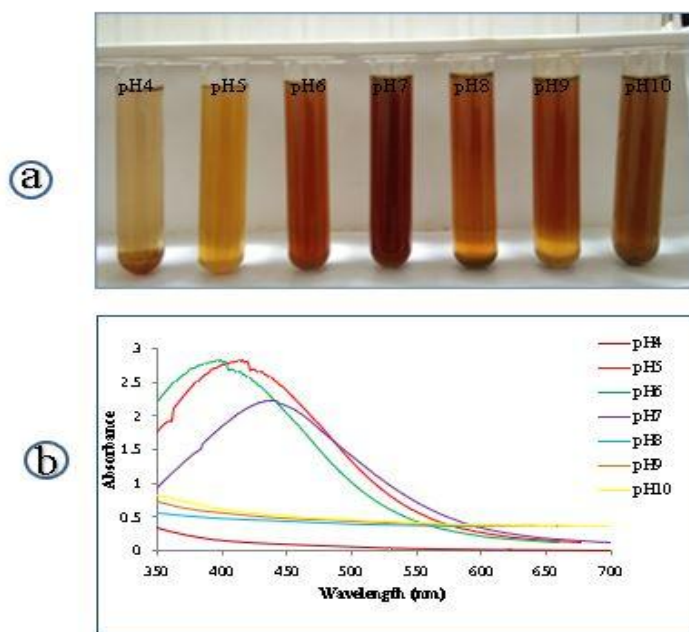


Fig.1. Effect of different pH on synthesis of AgNPs from *R. Mucronata* bark extract (a) Photographs showing change in colour of AgNPs at different pH (b) UV–vis spectrum of AgNPs at different pH at 24 h.

3.1.2 Effect of different concentration of silver nitrate

Among the different concentration of AgNO₃ examined at pH 7 the concentration at 0.5 mM did not turn to dark brown for Silver nanoparticles. Although the AgNO₃ of 1.5, 2.0, 2.5, and 3 mM resulted in agglomeration of nanoparticles and thus resulting a shift in peak was recorded, the 1.0 mM of AgNO₃ supported hasty development of colour change from pale yellow to dark brown at 430 nm and showed high stability (Fig.2). These results were accepted, when compared with the earlier investigations made by Mock et al. [12][11]. Increasing various concentrations of AgNO₃ raised the agglomeration of Ag⁺ to AgNPs [13].

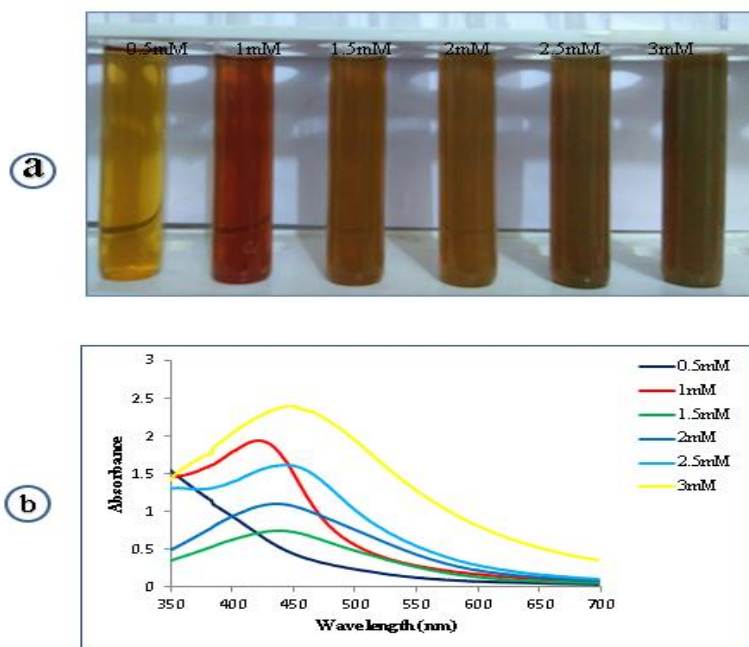


Fig:2. Effect of different concentrations of AgNO_3 on synthesis of AgNPs from *R. Mucronata* bark extract (a) Photographs showing change in colour of AgNPs at different concentrations of AgNO_3 (b) UV–vis spectrum of AgNPs at different concentrations of AgNO_3 at 24 h.

3.1.3. Effect of different concentrations of aqueous bark extract of *R. Mucronata*

Different quantities of the *R. Mucronata* bark extracts were used for the synthesis of Silver nanoparticles. Increase in bark extract volumes increased in the colour intensity from golden brown to dark brown (Fig. 3) at 430 nm for AgNPs. The different peaks corresponding to 1.0 mL–3.0 mL added deliberately caused a steady shifting of the peaks up to 450 nm. However, 1.0 mL of bark extracts maintained high stability than the rest.

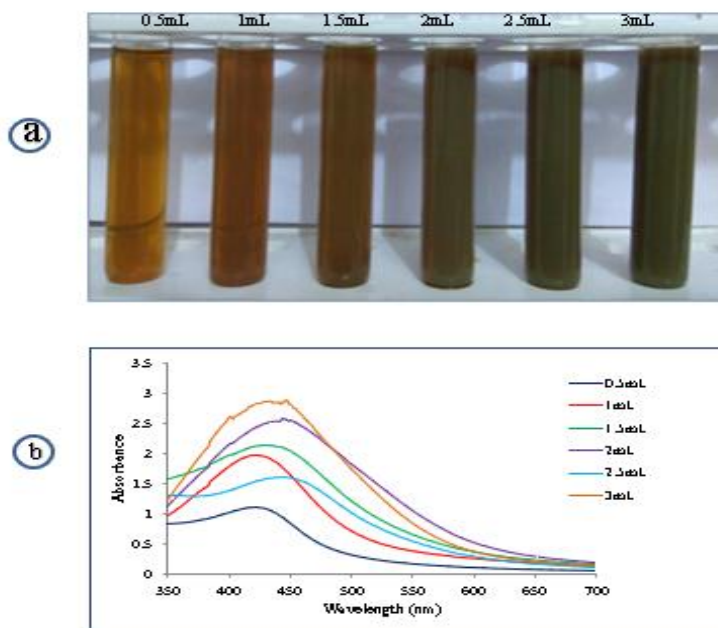


Fig:3. Effect of different concentrations of *R. Mucronata* bark extract on the synthesis of AgNPs (a) Photographs showing change in colour of AgNPs at different concentrations of *R. Mucronata* bark extract (b) UV–vis spectrum of AgNPs different concentration of *R. Mucronata* bark extract at 24 h.

3.1.4 Effect of different temperatures

The AgNPs of the *R. Mucronata* bark extracts photosynthesized at different temperatures starting 22°C, 27°C, 32°C 37°C, 42°C, 47°C and 52°C exposed that, increase in temperature (Fig. 4) improved the formation of AgNPs. though, the AgNPs obtained at 47°C–52°C shows agglomeration of nanoparticles, the formation of AgNPs observed between the temperature 32°C–42°C was noticeable color change from pale yellow to dark brown. Furthermore, the peak of 430nm formed by AgNPs at 37°C was sharp. Since 37°C maintained the formation of nanoparticles, this temperature is standardized for further researches. In an earlier study, [14] described that the 37°C favored the synthesis of Silver nanoparticles. [15] stated that among the different temperatures (20°C, 40°C, 60°C, 80°C and 100°C) tested for the synthesis of AgNPs and AuNPs using *Chenopodium album* leaf extract, 40°C maintained the formation of nanoparticles with high solidity.

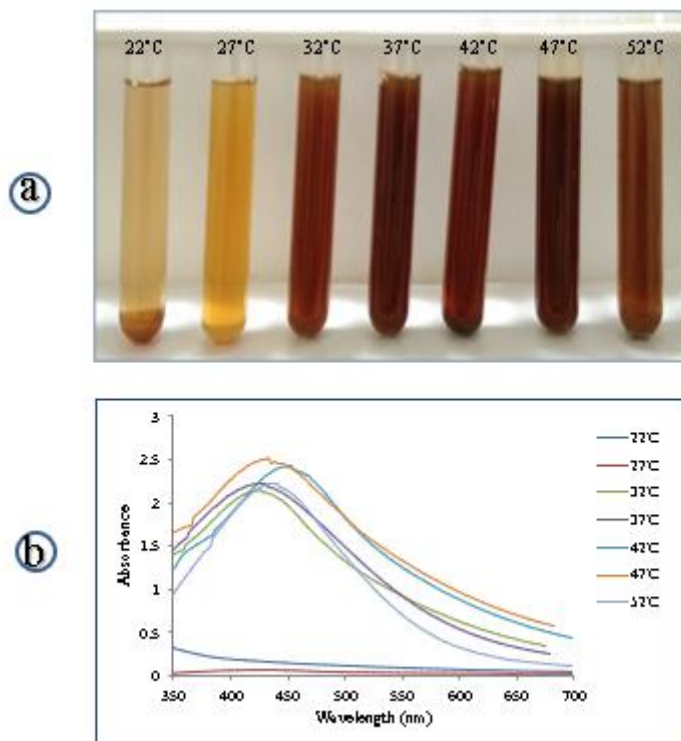


Fig:4. Effect of different temperatures on the synthesis of AgNPs from *R. Mucronata* bark extract (a) Photographs showing change in colour of AgNPs at different temperatures (b) UV–vis spectrum of AgNPs different temperatures at 24 h.

3.1.5 Stability of photosynthesized silver nanoparticles

The stability of photosynthesized AgNPs were revealed that there was no alteration in the peak even after 2 months of incubation under dark room at 37°. However, there was a shift in the peak from 432 nm on the 1st month and 435 nm on the 2nd month (Fig. 5). The reaction time for the formation of AgNPs was found substantially lower than the earlier reports [16].

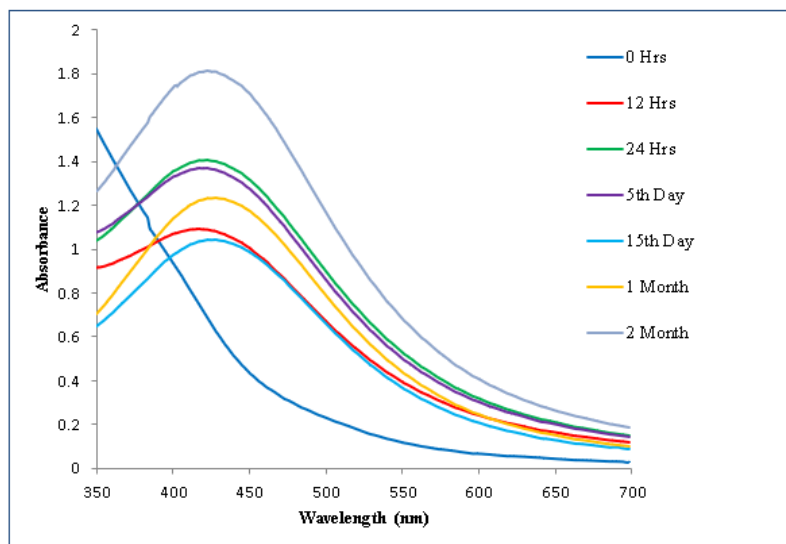


Fig:5. Stability on AgNPs synthesized from *R. mucronata* bark extract at different duration: UV–vis spectrum of AgNPs stability at different durations.

3.2 Characterization of AgNPs

3.2.1 Zeta potential analysis

This Zeta potential analysis of an electrophoretic mobility gives the charges which has an impact on the particle distribution [17]. Here, using water as dispersant the zeta potential is found to be -20.3 mV for AgNPs (Fig.6) [18] recorded that the zeta potential of AgNPs was -18.2 mV and -18.9 mV synthesized from *Hydrastis canadensis* and *Thuja occidentalis*, respectively. As per the instrumental function of the intensity of negative charge indicates that the AgNPs are found stable [19].

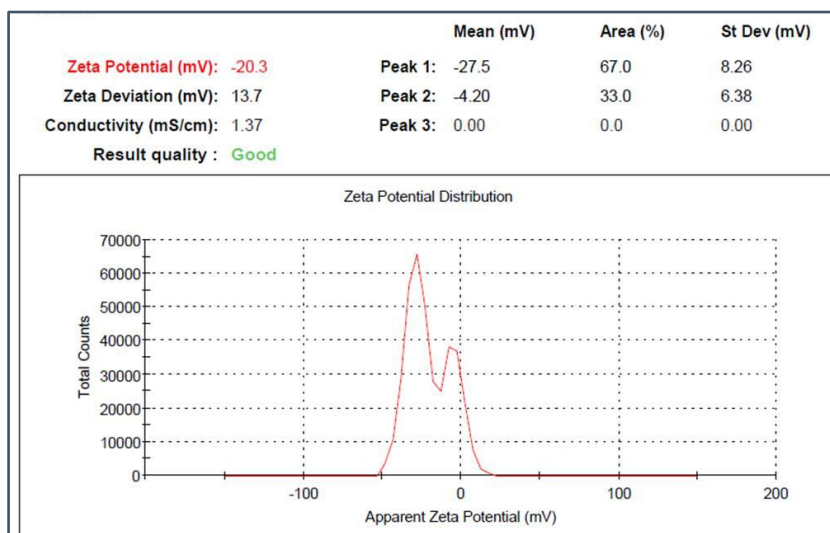


Fig: 6. Zeta Potential measurement analysis of silver nanoparticles

3.2.2 Atomic Force Microscopy (AFM) analysis

AFM studies revealed that the external morphology of *R. mucronata* bark extracts silver nanoparticles shown in (Fig.7) is impressive. The sizes of the particles are ranging from ~ 70 – ~ 90 nm and the respective 3D images (AFM) are displayed in (Fig.8) shows the Silver nanoparticles are spherical in shape with a smooth surface and also without any depths and clefts. The spherical shape of Silver nanoparticles was noticed in the existing efforts [20]. The AgNPs were in the size of 28 nm in *Ocimum tenuiflorum*, 26.5 nm in *Syzygium cumini* and 65 nm in *Camellia sinensis*. [21].

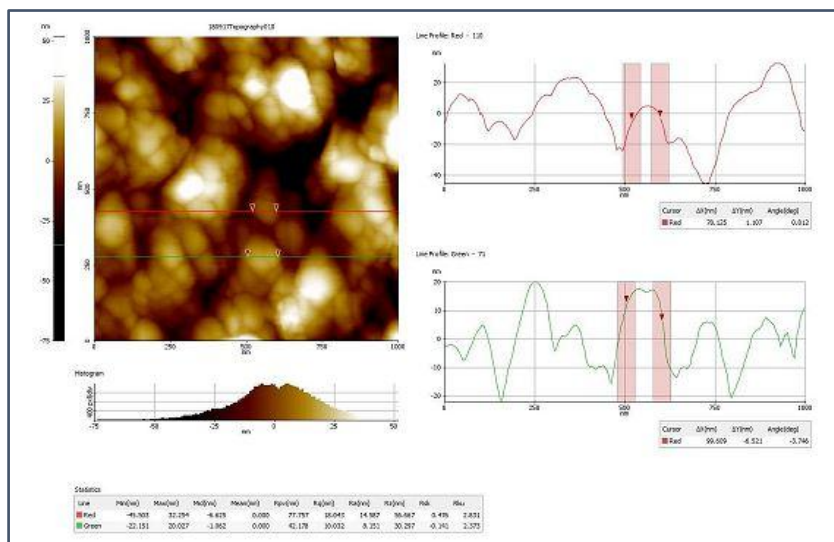


Fig: 7. AFM analysis of optimized, photosynthesized AgNPs

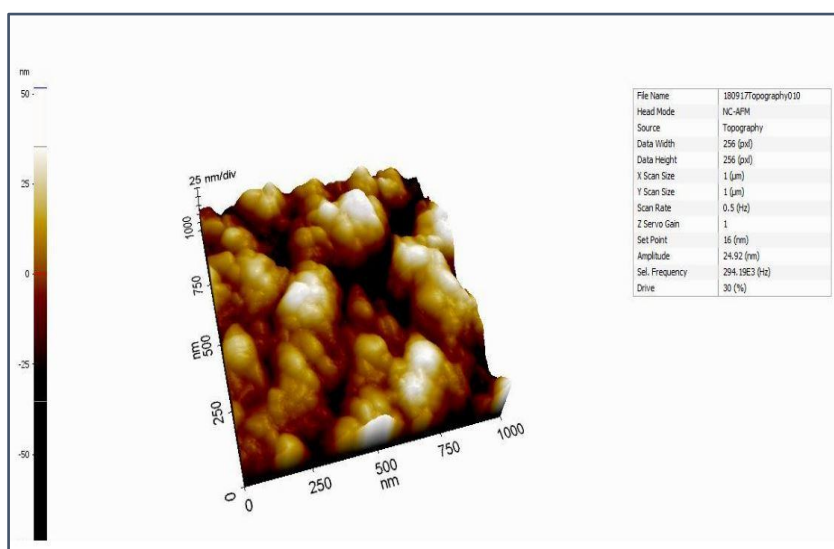


Fig: 8. AFM analysis of optimized and photosynthesized AgNPs

IV. Conclusion

The biocompatible AgNPs were effectively optimized and characterized from *R. mucronata* bark extract and found to be constant and stable for up to 2 months. The 3D structure and size of nanoparticles formation from *R. Mucronata* bark extract shows good consistency and solidity. All the optimization and characterization results exposed, the biologically synthesized AgNPs could be useful in the medical field for their antimicrobial function and would be an effective immunostimulant for the marine animals which is to be proved in future.

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