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# GREEN SYNTHESIS OF SILVER NANOPARTICLES VIA MEDICINAL PLANT EXTRACTS AND THEIR ANTIBACTERIAL ACTIVITIES

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## ABSTRACT

The green synthesis of silver nanoparticles (Ag NPs) were studied by bio reduction of silver nitrate solution using extractions of three commonly available medicinal plants found in Sri Lanka. Extractions were prepared using different plant parts obtained from *Stachytarphera indica* (leaves and stem), *Munronia pinnata* (leaves and stem) and *Rhipsalis baccifera* (stem). UV-Visible spectroscopy and Scanning Electron Microscopy (SEM) studies were carried out to confirm, characterize and analyze the physical parameters of produced NPs. The antimicrobial activity of the Ag NPs were evaluated against both gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram negative bacteria (*Escherichia coli*), using broth dilution (MIC) method. The UV-Visible spectroscopy results were discovered to be in the range of 425 nm - 460 nm indicating the formation of Ag NPs by all the plant extracts. SEM analysis confirmed the presence of spherical shaped NPs. The lowest particle diameter was observed for Ag NPs synthesized using *Stachytarphera indica* leaves extract (39.41 nm) and the highest was reported in *Stachytarphera indica* stem extract (82.08 nm). The synthesized Ag NPs proven to poses antibacterial activity against all tested pathogens and achieved Total Inhibition Concentration (MIC) value with in the range of 0.250 µg/mL- 0.750 µg/mL of Ag NPs concentration.

**KEYWORDS:** Silver nanoparticles, antimicrobial activity, green synthesis, plant extracts, antimicrobial activity, broth dilution method.

## INTRODUCTION

Nanotechnology is one of the topics discussed widely in the modern world with extensive range of applications, including in the fields of medicine, drug delivery and material sciences.[1,2,3] The differences in physicochemical properties of nanomaterials compared with the bulk materials, such as surface to volume ratio, size, distribution and morphology are the reason for their numerous biological, physical and pharmaceutical applications. These physiochemical properties are dependent upon their methods of production, which would in turn indicate different activities.[4,5]

Silver (Ag) is a naturally occurring transition metal element which is known to be one of the most universal antimicrobial substances throughout the history.[6,7] Before the use of modern antibiotics, silver has been used to cure bacterial infections in wound healing and also used as a food and water preservative.[7,8] Silver is normally a nontoxic substance, however in extreme cases, it can be toxic to mammalian cells.[9] The antimicrobial activity of silver has been attributed to the fact that it binds to bacterial DNA and RNA causing denaturing and thus inhibits bacterial replication.[10] Though the ionized silver is highly reactive and binds to tissue proteins, and brings structural changes in the bacterial cell wall and nuclear membrane, leading to cell distortion and death of the bacteria.[8,10]

There are a number of different processes in the production of metal nanoparticles.[1] Among those, the green synthesis of nanoparticles is becoming popular due to its relatively versatile nature. Green synthesis is the biological synthesis of NPs through the utilization of various plants parts, bacteria and fungus.[11,12] The secondary metabolite extracts collected from these biological sources contain numerous biomolecules such as enzymes, proteins, flavonoids, sterols, triterpenes, triterpenoids and saponins.[4,13] The antioxidant or reducing properties of these biomolecules are typically responsible for the reduction of metal compounds in to their respective nanoparticles and act as both reducing and capping agents during the process of synthesizing.[4,11,14] This “Bottom up” synthesizing of nanoparticles is widespread due to the yield of nanoparticles with uniform size, shape and distribution. Green synthesis has been proven to effectively cover chemical synthesis and precisely control the reaction to inhibit further particle growth. This method of bio synthesis of metal nanoparticles has been proposed as having significant advantages such as the usage of natural resources, cost-effectiveness, higher yields, rapidity, environmental friendliness and being able to acquire well defined and controlled size of nanoparticles.[5,12,15]

In this study, bio synthesis of Ag NPs was conducted using the extracts of three plant varieties with medicinal properties which are widely available in Sri Lanka. Various parts of each plant were used to evaluate their NP synthesizing potential. *Munronia pinnata* (Binkohomba) is a small plant (Figure 1), about 15 cm in height containing no branches. It has white colored flowers with pleasant smell and parts of this plant is used in treatment for fever, skin disorders, diarrhea and swellings.[16] *Stachytarphera indica* (Balunakuta) is a plant about 30 -90 cm high (Figure 1) that contains dark blue colored flowers and is used in indigenous Ayurveda medical system for the treatment of intestinal worms, venereal diseases, ulcers, dropsy, and stomach ailments. Coactions made from the plant is used against cataract and open wounds.[17,18] Plant *Rhipsalis baccifera* (Nawahandi) is habited in the wet zone on trees as a bush like epiphyte which belongs to the cactus family (Figure 1).[20] It has fibrous roots and cylindrical mushy stem which divides in to two stems and grows. *Rhipsalis baccifera* is a plant known to have various uses in Ayurveda medical system such as curing toothache, scorpion stings, snake bites and wounds.

**Figure 1: Plants used in the study (a) *Munronia pinnata* (https://apeosupela.blogspot.com) (b) *Stachytarphera indica,* (https://www.flickr.com/) (c) *Rhipsalis baccifera*, (https://commons.wikimedia.org/wiki)**

The objective of this study was to evaluate the potential of using *Stachytarphera indica, Munronia pinnata* and *Rhipsalis baccifera* plant extracts as a way of synthesizing Ag NPs and to investigate physical properties and antibacterial properties of synthesized NPs.

## MATERIALS AND METHODS

### Collection of Plant Materials and Plant Extract Preparation

Specimens of plant materials, *Stachytarphera indica, Munronia pinnata* and *Rhipsalis baccifera,* were collected and authenticated by the National Herbarium, Peradeniya. Collected plant materials were thoroughly washed with deionized water to remove any dirt and dust. Subsequently, cleaned plant materials were shade dried in open air.[4] Extraction

was carried out by placing 1 g of each plant material in 100 mL of deionized water and then heated at 70 °C for an hour. Then, the solid plant materials were filtered and the final volume was brought up to 100 mL by adding deionized water. The extractions were stored at 4°C until further use.

**Synthesis and Collection of Silver Nanoparticles**

A 50.00 mL volume of *Stachytarphera indica* (leaves), *Munronia pinnata* (leaves) and *Rhipsalis baccifera* (stem) extract was separately mixed with 2.5 mmol $dm^{-3}$ $AgNO_3$ solution, in 1:5 plant extract to $AgNO_3$ solution ratio. The *Stachytarphera indica* (stem), *Munronia pinnata* (stem) plant extracts were also used with same proportions except the concentration of the used $AgNO_3$ solution was 1.0 mmol $dm^{-3}$. The mixtures were kept under sunlight for 180 minutes[20,21] until the colour change occurred from initial pale yellow to intense red colour (Figure 2). Then, they were stored in a dark cupboard for another 24 hours, after covering the flasks with aluminum foil. The Ag NPs were collected by centrifuging these reacted mixtures for 15 minutes, at 4500 rpm. Deposited NPs at the bottom of the centrifuge tubes were rinsed with deionized water until the supernatant was clear in colour. The precipitated NPs were collected and oven dried at 40 °C to obtain a dry product.

**Characterization of the Synthesized Silver Nanoparticles**

The presence of Ag NPs was confirmed by performing UV-Visible spectroscopy and further characterization was carried out using Scanning Electron Microscopy (SEM).[6,22] UV-Visible analysis was performed by using spectrometer (Hitachi, U-2910 model) with in the range of 300 - 600 nm. The Scanning Electron microscope (Zeiss, EVO|LS15 model) was used to analyze the particle size and appearance.[6]

**Antibacterial Activity of the Silver Nanoparticles**

Broth Dilution method[23] was used to determine the antibacterial activity of the synthesized NPs.[24] The bacterial strains *Staphylococcus aureus*, *Bacillus subtitles*, and *Escherichia coli*[25] were obtained from the Medical Research Institute (MRI), Sri Lanka.

The preparation of Bacteria Inoculum: At the start of every anti-bacterial assay, a small portion of bacteria collected with a sterilized loop were diluted with deionized water to match the turbidity of the 0.5 McFarland Standard, and used as the bacteria inoculum.

Preparation of Ag Stock Solutions from NPs: A stock solution with a concentration of 1 mg/mL was prepared using isolated Ag NPs. The stock solution was made by weighing followed by homogenizing by vortexing with deionized water. Subsequently, a diluted series was prepared with a concentration from 0.125 µg/mL - 0.750 µg/mL from this stock solution by adding deionized water.

Preparation of Test Samples for Antibacterial Assay: A test sample contained 25 µL of the freshly prepared bacteria inoculum, 100 µL of the Ag NP solution from the prepared series and 5.0 mL of the broth solution as the medium. The test control contained 5.0 mL of broth solution and 100 µL of deionized water and 25 µL of bacteria inoculum. The test samples were kept at room temperature for 18 hours and the absorbance was measured at 600 nm. The Percentage Inhibition was calculated according to the equation shown below, where Ac is the absorbance given by the test controls and As is the absorbance given by the test samples.

Percentage Inhibition (%) = (Ac-As / Ac) 100%

All the preparations and media used in the antibacterial assay were either autoclaved or cleaned with 80% Ethanol to meet the necessary aseptic conditions.

*each test was triplicated and an average value was taken to determine the calculation.

## RESULTS AND DISCUSSION

### Synthesis and Collection of Silver Nanoparticles

Trials were executed by mixing a series of concentrations of $AgNO_3$ (0.5 – 5.0 mmol $dm^{-3}$) with different volumes of each plant extract to determine the optimum concentration for the production of Ag NPs (Figure 2). These photo-reacted mixtures were kept in darkness for another 24 hours before analyzing by the UV-Visible spectrometer to confirm the presence of NPs through the observation of the characteristic absorption band at the 425 - 450 nm wave length (Table 1).

**Table 1: Maximum wave length of synthesized Ag NPs mediated via each plant extract**.

| Type of silver nanoparticles | Wavelength of the peak (nm) |
|---|---|
| *Stachytarphera indica* leaves | 444±2 |
| *Stachytarphera indica* stem | 444±3 |
| *Rhipsalis baccifera* stem | 445±2 |
| *Munronia pinnata* leaves | 440±3 |
| *Munronia pinnata* stem | 425±5 |

The optimum concentration of $AgNO_3$ for the production of NPs were categorized as 1.0 mmol $dm^{-3}$ for *Stachytarphera indica* (stem) and *Munronia pinnata* (stem) extracts whereas, a 2.5 mmol $dm^{-3}$ $AgNO_3$ solution was used for *Stachytarphera indica* (leaves), *Munronia pinnata* (leaves) and *Rhipsalis baccifera* (stem) extracts. However, it was detected that, all the plant extracts used in this study were capable of producing Ag NPs, even at a lower $AgNO_3$ concentration, and with less volume of plant extract. This was confirmed by the observation of the characteristic color change and by UV-Visible analysis. Nevertheless, collection of NPs were only possible with the 1.0 and 2.5 mmol $dm^{-3}$ $AgNO_3$ with 1:5 volume of silver solution to plant extract ratio, thus it was maintained as the optimum concentration in this study.

The NPs were collected separately and were labeled according to their plant extracts that was used to synthesis them. Ag NPs produced from *Stachytarphera indica* leaves and stem were labeled as SI/L and SI/S. Ag NPs synthesized using *Munronia pinnata* leaves and stem were named as MP/L and MP/S respectively where, NPs collected from *Rhipsalis baccifera* stem extracts were labeled as RB/S (Figure 2).


**Figure 2: (a) A trial Concentration series prepared to determine the ability to synthesize Ag NPs from MP/L extract and the colour change illustrated when nanoparticles are formed. (b) Mixture of the plant extracts and $AgNO_3$ solutions exposed to the sun light for the initiation of the reaction.**

UV-Visible spectra generated after 24-hour reaction time (with 50 mL of plant extract and different concentrations of $AgNO_3$ varying from 0.5 to 5.0 mmol $dm^{-3}$) are depicted below (Figure 3). In most cases, maximum wave length was observed in close proximity to 450 nm range whereas, some instance, with higher concentration of $AgNO_3$ a shift to longer wavelength of the maximum absorption was observed. Figure 3 shows the UV-Visible spectra of SI/L, SI/S, MP/L, MP/S and RB/S as recorded by the spectrometer, for all the five

samples. Here, λ max closer to 450 nm level was selected as the optimum concentration of silver nitrate.

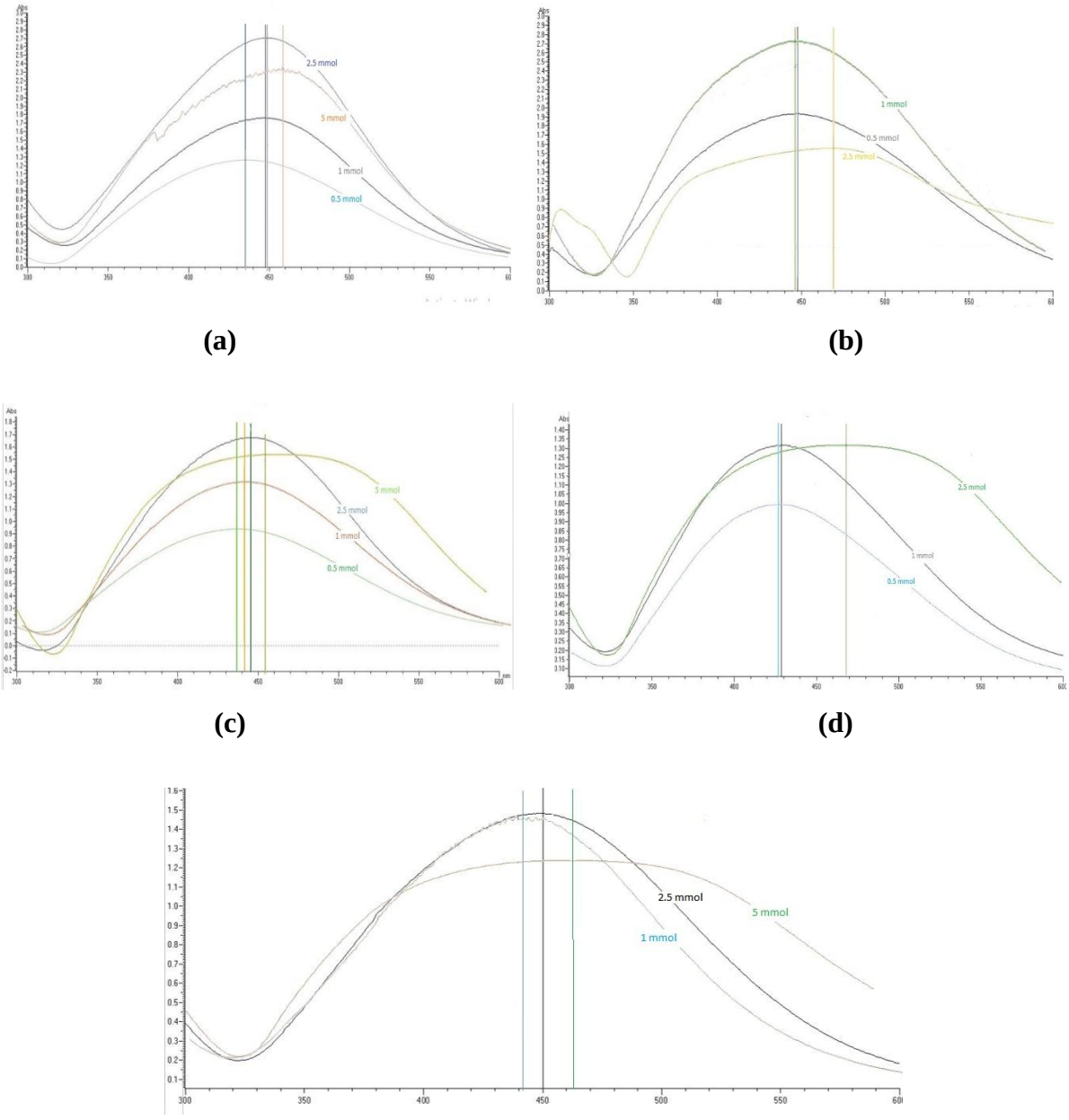


**Figure 3: The absorbance vs wavelength plots generated for various concentrations of $AgNO_3$ reacted with 50 mL plant extracts of (a) SI/L (b) SI/S (c) MP/L (d) MP/S and (e) RB/S.**

**Characterization of the Synthesized Silver Nanoparticles**

The figure shown below present the selected images obtained from the SEM analysis of the synthesized Ag NPs from the plant extracts (Figure 4). The NPs were deposited on carbon

strips and observed at different magnitudes in varying areas increasing from micrometer to nanometer scale. The synthesized NPs were observed to be in spherical shape with particle diameters varying from 39.41 – 82.08 nm. Most of the NPs appeared to have define spherical shape, at the same time low aggregation was also observed in some samples. The lowest particle diameter sizes were observed in Ag NPs synthesized from SI/L within the range of 39.41-52.82 nm and the highest particle size was reported in NPs produced by the SI/S extract 70.31-82.08 nm. NPs collected from RB/S, MP/L and MP/S extracts had particle diameter ranges of 45.48 - 56.35 nm, 52.16 - 64.52 nm and 55.97 - 76.42 nm respectively.

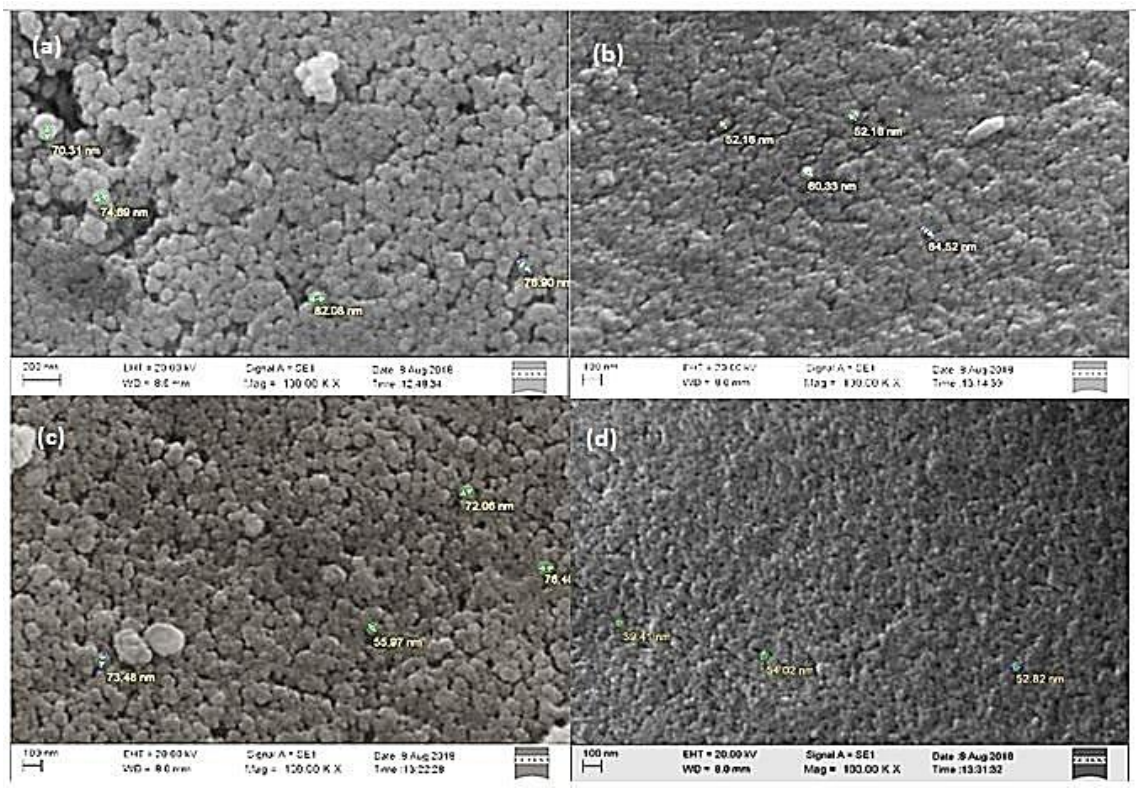


**Figure 4: SEM images of Ag NPs prepared with (a) SI/S extract, (b) MP/L extract, (c) MP/S extract and (d) SI/L extract.**

**Antibacterial Properties of the Synthesized Ag NPs**

All the NPs synthesized from the plant extracts, except by the extractions of SI/S demonstrated antibacterial activity against the three tested pathogens in the Ag nanoparticle concentration of 0.250 - 0.750 µg/mL levels. The Minimum Inhibitory Concentration (MIC) value was defined as the lowest concentration of Ag NPs concentration that will inhibit the growth of the microorganism used in the assay after the incubation time period. The Inhibition Percentage calculated for the test samples were plotted against the NP

concentration series that was used and a graph was plotted to observe the inhibition patterns (Figure 5).

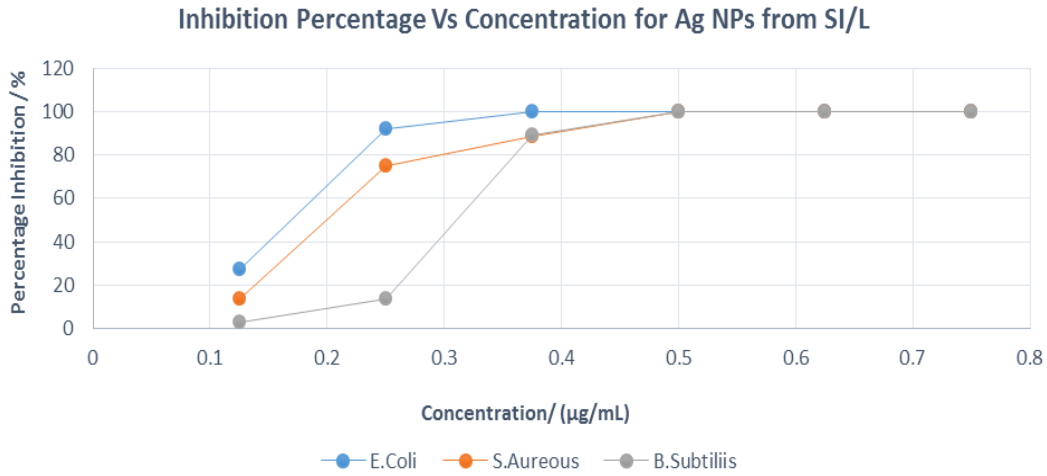


**Figure 5: The Inhibition Percentage calculated for the test samples plotted against the NP concentration series for Ag NPs synthesized from SI/L.**

Ag NPs synthesized from RB/S extract demonstrated the lowest MIC value of 0.250 µg/mL for all the bacteria species used. It was observed that the MIC value of the SI/S for *Escherichia coli* and *Staphylococcus aureus* exceeded the rest of the concentration series and thus, it was included in the data table in that manner and no consideration were given for results analysis. The lowest MIC value for *Escherichia coli* was 0.250 µg/mL which had been observed for both MP/L and RB/S, while the highest MIC values had been observed for *Staphylococcus aureus* and *Bacillus subtitles* was 0.500 µg/mL (in both SI/L and MP/S) and 0.750 µg/mL (MP/L) respectively. The lowest MIC for both *Staphylococcus aureus* and *Bacillus subtitles* was given by the NPs collected from RB/S at 0.250 µg/mL concentration (Table 2). The data indicates that Ag NPs possess antibacterial activities against both gram negative and gram positive bacteria used in micro gram levels. This has been documented in the previous studies as well.

**Table 2- summery on results of the anti-bacterial assay.**

| Bacteria Sp. | Minimum Inhibitory Concentrations ($\mu g/mL$) of Ag NPs extracted from | | | | |
|---|---|---|---|---|---|
| | SI/L | SI/S | MP/L | MP/S | RB/S |
| *Escherichia coli* | 0.375 | >0.750 | 0.250 | 0.375 | 0.250 |
| *Staphylococcus aureus* | 0.500 | >0.750 | 0.375 | 0.500 | 0.250 |
| *Bacillus subtitles* | 0.500 | 0.500 | 0.750 | 0.625 | 0.250 |

## 4. CONCLUSION

The extractions used from the widely found plants *Stachytarphera indica*, *Munronia pinnata* and *Rhipsalis baccifera* were able to produce Ag NPs collectible at 1.0 mmol $dm^{-3}$ and 2.5 mmol $dm^{-3}$ $AgNO_3$ concentrations for *Stachytarphera indica* (leaves), *Munronia pinnata* (leaves) and *Rhipsalis baccifera* (stem) extracts respectively in 1:5 ratios..

The SEM analysis yielded that the synthesized NPs were to be in spherical shape with 39.41 – 82.08 nm of particle diameter, and in some samples, the aggregation was less than the others. It was concluded that this aggregation occurred in the centrifuging and oven drying steps.

With the exception for NPs collected from Stachytarphera indica stem extract, all others samples exhibited antibacterial activities against the used pathogens in 0.250 - 0.750 µg/mL range of Ag NPs concentration levels.

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