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Synthesis of silver nanoparticles (AgNPs) of leaves extract of Rhynchoglossum notonianum wall. for enhancing its bioavailability and antibacterial activity

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***Abstract*---**Objective: Silver nanoparticles (AgNPs) derived from *Rhynchoglossum notonianum* Wall. leaf were the subject of this investigation to examine their synthesis, characterisation, and biological activity. Materials and Methods: Aqueous extracts of Rhynchoglossum notonianum Wall leaves and 0.1 M silver nitrate were used to make AgNPs. Particle size, Zeta potential, UV-Vis spectroscopy, and SEM were used to determine the AgNPs properties. Results: Rapid and homogeneous size and shape silver nanoparticles were synthesised utilising *Rhynchoglossum notonianum* Wall. extract. When methanolic leaf extracts of *Rhynchoglossum notonianum* Wall. were combined with an aqueous silver ion complex solution, the surface Plasmon vibrations were excited, resulting in the production of MERNAgNPs. UV-Visible spectroscopy was used to characterise the nanoparticles. The widening of the peak in the UV-visible spectrum suggested polydispersion. The silver nanoparticles in solution have a surface Plasmon band of 420nm. Aqueous silver nitrate solution is diffused throughout the reaction time, with no signs of agglomeration. The average particle size (z-average) was "132.6nm", the polydispersity index was "0.248", and the zeta values were -25.1mV with a 100% peak area. This implies a stable silver nanoparticle. The antibacterial activity of silver nanoparticles produced from MERN was greater than that of the MERN. MERNAgNPs boost oral bioavailability by improving the dissolving rate by lowering the particle size (132.6nm). Conclusion: AgNPs with high therapeutic potentials might be produced by optimising this green synthesis.

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Keywords---synthesis, silver nanoparticles, biological activity, rhynchoglossum notonianum.

Introduction

When anything has at least one dimension (or when it has components with at least one dimension) between 1 and 100 nm in length, it is termed nanoscale (or includes components with at least one dimension). The word "nanoparticle" refers to anything that is made up of nanoparticles [1]. Particle sizes below this level result in the production of materials with physical and chemical properties that vary dramatically from those of macroscale materials created from the same constituent [2].

Increasingly important are ecologically friendly synthesis methods, and the biosynthesis of nanoparticles has gained a significant lot of attention in recent years due to the increasing need for such procedures. In recent years, the biosynthetic approach, which makes use of plant extracts to synthesise metal nanoparticles, has attracted considerable interest as a simple and practical alternative to chemical and physical processes for the production of metal nanoparticles [3]. The food and health industries are becoming more dependent on nanotechnology for the development of their goods and manufacturing processes. Promising results and applications in the field of nanotechnology have already been achieved, including bioactive nanoencapsulation for drug delivery, biosensors for the detection and quantification of infections, and one-of-a-kind resources for the evaluation and development of better, safer, and more effective therapeutic formulations [4].

Plants, plant wastes, bacteria, and fungi have all been used to synthesise nanoparticles as templates for "green nanotechnology" in recent years, with plants, plant wastes, bacteria, and fungus being the most frequently used [5]. Plants, plant wastes, bacteria, and fungus have all been used to synthesise nanoparticles as templates for "green nanotechnology." As well as being sought after for their distinct optical, electrical and thermal properties, silver nanoparticles (AgNPs) are also being sought after for their ease of production and their ability to increase electrical conductivity, near-infrared absorption, and efficient charge separation, among other things [6].

In comparison to other biological sources, they are preferred due to their wide diversity of reducing metabolites and the volume of plant extracts available to them. Many potential pharmaceuticals, nutraceuticals, and food additives are generated from plant secondary products, which are found in abundance in nature. One of the most important subgroups of antioxidants are secondary antioxidant metabolites generated from plants, such as polyphenols. Researchers have linked the reduction characteristics of antioxidant metabolites to plant extracts that have a greater ability to synthesise nanoparticles with superior properties than other plant extracts [7]. Using plant sources for AgNP biosynthesis has a number of advantages, including cost-effectiveness and environmental friendliness, as well as the avoidance of high pressure and temperature, as well as the avoidance of hazardous chemicals and high energy consumption [8].

Preservatives, effective antibacterial and anticancer therapies, and biological sensors and detectors with low toxicity for use in both in vitro and in vivo applications are just a few of the numerous applications for inorganic metal nanoparticles that are now available (AgNPs). By the results of previous investigations, AgNPs have also been demonstrated to exhibit antioxidant, anti-inflammatory, anticoagulant, and larvicidal activities [9].

In today's world, medicinal plants that have been shown to be both safe and efficacious predominate in the pharmacopoeia. When it comes to the delivery of plant/herbal medicinal compounds as medicines, there are a variety of challenges to consider, including their low solubility, poor permeability, restricted bioavailability, and instability in the biological milieu [10]. In order to overcome the limitations of herbal remedies, nanoparticles may be connected or encapsulated to them in order to optimise their pharmacokinetics and hence significantly increase their overall efficacy. Pharmaceutical companies are concerned about nanoparticles because the capping layers given by medicinal plants enable them to manipulate the size and structure of nanoparticles, making them a special source of concern. In order to synthesise AgNPs, a range of medicinal and spice plants were used in the process [11].

Rhynchoglossum notonianum medicinal properties make it a valuable spice in a broad range of cuisines all over the world because of its medicinal properties. Each of these spices is regarded as a general treatment for a wide range of diseases, according to traditional medicine. Some of the benefits associated with these spices include antimicrobial, cancer-fighting, antioxidant, antidiabetic, antiemetic, blood pressure-lowering, hypoglycemic, hypolipidemic, and immunomodulatory effects. A range of antioxidants and alkaloids are found in these spices, according to phytochemical study, and these compounds may help to the reduction of silver to the nanoparticle form. Tannins, carotenoids, saponins, phenols, and flavonoids are some of the substances that may be found in plants [12].

During our continuous investigation into the medicinal, nutraceutical/economical, and environmental applications of these spices, we are investigating the antibacterial and antioxidant activities of *Rhynchoglossum notonianum* extract-derived nanoparticles.

Materials and Methods

Materials

The neighbourhood park provided the plants for this experiment. The specimens in the herbarium were used to identify and authenticate the plant. All of the chemicals employed in this investigation were of analytical quality, including the $AgNO_3$ (Sigma-Aldrich, United States). They were dried (60°C, 72 hrs) at 60°C, then ground up and kept in airtight containers at 4°C until required. Throughout the experiment, deionized water was utilised.

Synthesis of methanolic leaf extract of *rhynchoglossum notonianum* (Wall.) B.l. Burtt silver nanoparticles

Gathering the fallen foliage Running tap water was used to gather and clean the leaves of *Rhynchoglossum notonianum* (Wall.). Pulverizing and drying the leaves. The leaves were gathered and air dried in the shady areas. It was ground into a fine powder using a mixer. Using a No. 60 sieve, the powder was sifted and stored in an airtight container in a dry location.

Preparation of methanolic extracts of leaf of *Rhynchoglossum notonianum* (Wall.) B.L. Burtt

Defatting the dried powdered leaf of *Rhynchoglossum notonianum* by maceration with 1.5L petroleum ether (60-80 °C) yielded around 500g of dry powdered *Rhynchoglossum* leaf. To remove the solvent, the marc was filtered through a mesh screen, and then dried. The dried marc was soaked in 1.5 litres of methanol, and the extraction was carried out using triple maceration (72h process). Rota vapour was then used to evaporate the mixed filtrate, resulting in a solid mass.

Preparation of stock solution 2mg/20mL

To get a concentration of 2mg/mL, we used a methanolic extract and made a 20mL dilution. It was kept at a temperature of 4 °C for future use. (Fig. 1).

Aqueous solution containing 1mM silver nitrate ($AgNO_3$)

0.017g of silver nitrate was dissolved in 100mL of double distilled water and kept in an amber-colored container for future usage (Fig. 2).

Synthesis of methanolic leaf extracts of *Rhynchoglossum notonianum* (Wall.) B.L. Burtt silver nanoparticles (MERNAgNPs)

5mL of *Rhynchoglossum notonianum* (Wall.) B.L. Burtt's methanolic leaf extract was taken in a conical flask and put on a magnetic stirrer with a heated plate separately. Dropwise addition of 50 mL of 1 mM $AgNO_3$ solution was performed at 50-60 °C while stirring at 120 rpm. Periodically, the solution's colour changed. After 5 hours, the colour of the liquid changed from colourless to brown (Fig. 3). This showed that silver nanoparticles had formed. *Rhynchoglossum notonianum* (Wall) methanolic extract converted aqueous silver ions to very stable silver nanoparticles, as shown.

Separation of silver nanoparticles

In a centrifuge, the silver nanoparticles were separated after 15 minutes of 10,000 rpm centrifugation. The supernatant liquid was resuspended in sterile double-distilled water. The procedure was repeated three times in order to remove any biomolecules that were not properly coordinated. The supernatant liquid was discarded after the necessary reaction time, and the pellets were collected and kept for future use at 4° C [13].

Lyophilization

Once the pellet was formed, it was then freeze-dried to increase the stability of the silver nanoparticles [14]. Cryoprotective agent is used to lyophilize the just-made MERNAgNPs before they may be used (mannitol). After that, it was immediately chilled to -50 °C for two hours before being dried in two stages: first at 1.03 mbar, then at 0.001 mbar. The generated MERNAgNPs were lyophilized and kept at 4 C until they were needed again (Fig. 4). For the purpose of characterising synthesised MERNAgNPs, the analytical parameters like particle size, zeta potential, UV-Visible spectal and morphological studies using SEM were employed:

Determination of "Particle size and Zeta potential

Particle size distribution, polydispersity index, and zeta potential of MERNAgNPs were all assessed using a zeta size analyzer (Nano ZS 90, Malvern Instruments Ltd.,UK). Water was added to freeze-dried powders and the water content was adjusted to 15 before the scattering intensity was measured. Table 1 and Fig. 5 and 6 summarise the findings.

UV-Visible spectroscopy

UV-Visible spectroscopy was used to examine the synthesis and completion of silver nanoparticles. Periodic aliquots of the solution were taken and the UV-Visible spectra of these aliquots were recorded as the reaction progressed in the region of 200-600nm, with a resolution of 1nm, to monitor the bioreduction of Ag+ ions in the solution. The void was filled up with distilled water [16]. It's possible to see the final findings in Fig. 7.

Morphological studies of synthesized MERNAgNPs by using Scanning Electron Microscopy (SEM)

When it came to the MERNAgNPs' structure, the SEM was used to examine them (Hitachi X650, Tokyo, Japan). The sample's surface was captured in high-resolution photos using SEM. Using the same concept as an optical microscope, a scanning electron microscope measures electrons dispersed from the sample instead of photons. It is possible to shorten the wavelength of electrons by applying an electric voltage. The SEM was able to magnify pictures up to 200.000 times as a result of this. Nanoparticle shape and size may also be determined via high-resolution images of the surface, making the tool very helpful. SEM grid was coated with a thin film of a sample, which was dried under a mercury lamp for five minutes after a very little quantity of the sample was dropped on the grid. Any excess solution was removed using a blotting paper. Afterwards, secondary electron sputtering at 20 kV was used to record the SEM images [17]. The Fig. 8 shows the outcomes of the study.

Antibacterial activity of *Rhynchoglossum notonianum* (Wall.) and Silver Nanoparticles

By using an agar disc diffusion technique, silver nanoparticles made from *Rhynchoglossum notonianum* flower extract were examined for antibacterial efficacy against test organisms. Gram positive and Gram-negative organisms (*Staphylococcus aureus* and *Bacillus subtilis*) were both employed in this investigation. An antimicrobial agent's susceptibility to a specific test organism was determined using the ZoI (zone of inhibition) method, which is fast and affordable. Vernier callipers were used to measure the area of inhibition. Antibacterial activity was significantly reduced after 24 hours of incubation with the leaf extract of *Rhynchoglossum notonianum* (Wall), coupled with conventional chloramphenicol, in the presence of AgNPs from *Rhynchoglossum notonianum* (Wall). Comparing the bactericidal activity of various $AgNO_3$ was done using the zone of inhibition (ZoI). Antibacterial activity of AgNPs from *Rhynchoglossum notonianum* (Wall) B.L. Burtt leaf extract against the test organisms *E. coli* was found to be around 3.28 mm wide. AgNPs from *Rhynchoglossum notonianum* B.L. Burtt flower demonstrated 2.30mm and 2.11mm ZoI against S. aureusa and *Bacillus subtilis*, respectively, in a similar way (Table 2 and Plate 9) [18].

Results

Synthesis of methanolic leaf extract of *rhynchoglossum notonianum* (wall.) B.l. Burtt silver nanoparticles

A substance having overall dimensions in the nanoscale is known as a nanoparticle. Contrast agents in medical imaging and carriers for gene transfer into particular cells are only two of the many uses for these materials that have arisen in recent years. The features of nanoparticles, such as “chemical reactivity, energy absorption, and biological mobility”, are distinct from those of bulk materials because of their small size.

There are a wide range of medicinal uses for metallic nanoparticles, which have been employed in a variety of ways. For the creation of metallic nanoparticles on a massive scale, plants, among other biological agents, offer a safe and advantageous method. Aside from these advantages, the synthesis pathway is more environmentally friendly than that of bacteria, algae, and fungus.

Preparation of methanolic leaf extracts of *Rhynchoglossum notonianum* (Wall.) B.L. Burtt

Defatting the dried powdered leaf of *Rhynchoglossum notonianum* by maceration with 1.5L petroleum ether (60-80 °C) yielded around 500g of dry powdered *Rhynchoglossum* leaf. To remove the solvent, the marc was filtered through a mesh screen, and then dried. The dried marc was soaked in 1.5 litres of methanol, and the extraction was carried out using triple maceration (72h process). Rota vapour was then used to evaporate the mixed filtrate, resulting in a solid mass.

Preparation of stock solution 2mg/20mL

To get a concentration of 2mg/mL, we used a methanolic extract and made a 20mL dilution. It was kept at a temperature of 4 °C for future use (Fig. 1).

Fig. 1: Stock solution MERN of 2mg/20mL

Preparation of aqueous solution containing 1mM silver nitrate ($AgNO_3$)

Double-distilled water was used to dissolve an exact weight of 0.017g silver nitrate, and the solution was kept in an amber-colored container until it was needed (Fig. 8.5).

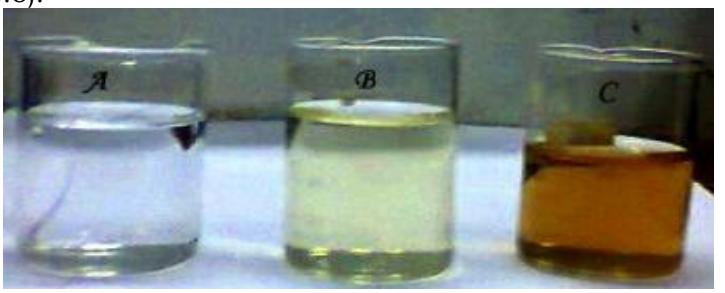

Fig. 2: A. Aqueous solution of 1Mm $AgNO_3$; B. Aqueous solution of 1Mm $AgNO_3$ with MERN after 0 min; C. Aqueous solution of 1Mm $AgNO_3$ with MERN afrer 5 hr.

Silver nanoparticles synthesized from *Rhynchoglossum notonianum* (Wall) leaf extracts in methanol by B.L. Burtt (MERNAgNPs)

As a result of the introduction of the substrate, there was a considerable colour change in the plant extract. Originally, the plant extract had no discernible colour. After the silver salt was added, the colour changed to brown. After the 5-hour period had elapsed, there was no visible change in the colouring of the specimen. Indication that the reaction was complete occurred when the silver nitrate solution grew darker, indicating that the reaction had been finished It was discovered that the methanolic leaf extract of *Rhynchoglossum notonianum* (Wall.) B.L. Burtt was capable of reducing silver ions into silver nanoparticles, as shown by the change in colour from colourless to brown in Figs. 3 and 4. This was owing

to the activation of surface Plasmon vibration in silver nanoparticles, which was responsible for the phenomenon.

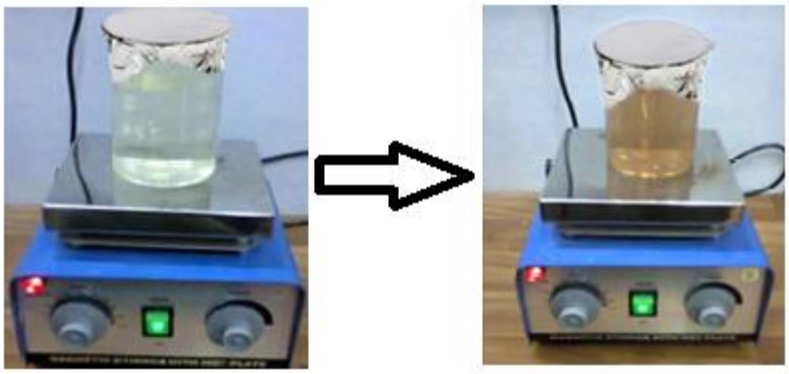

Fig. 3: After 5 hours, the colour of the medium has changed from colourless to brown

Lyophilization

The silver nanoparticles in the pellet were then freeze-dried to ensure their long-term viability. MERNAgNPs that have recently been manufactured are first lyophilized using a cryoprotective agent (mannitol). Two hours of cooling and two stages of drying later, it was finally dried at 0.001 mbar before being sent back to the freezer. It was decided to lyophilize the MERNAgNPs and store them at 4 °C until they were required again (Fig. 4).

Fig. 4: Synthesized MERNAgNPs

Determination of Particle size and Zeta potential

There are a number of variables that are controlled by the zeta potential of silver nanoparticles, including particle size distribution, saturation solubility, and dissolving velocity, which are all influenced by the potential.

Particle size measurements

The mean particle diameter and polydispersity indices of solutions were measured using photon correlation spectroscopy immediately upon synthesis (PCS). For the first time, the granulometric distribution and volume of colloidal silver nanoparticles have been reported. The average particle size (z-average) is 132.6 nm. According to particle size analysis, there were nanoparticles with polydispersity indices (PDIs) between 0.224 and 0.643. Table 1 and Figure 5 demonstrate this.

Table 1: The Polydispersity Index (PDI) and Mean Particle Diameter (MPD) of Biosynthesised MERNAgNPs

"Parameter	Value	Peak No	Peak Size (d.nm)	Peak Intensity %	Peak Width (d.nm)
Z-Average (d.nm)	132.6	Peak 1	141.8	100.0	102.5
PDI	0.248	Peak 2	0.000	0.000	0.000
Intercept	0.643	Peak 3	0.000	0.000	0.000"

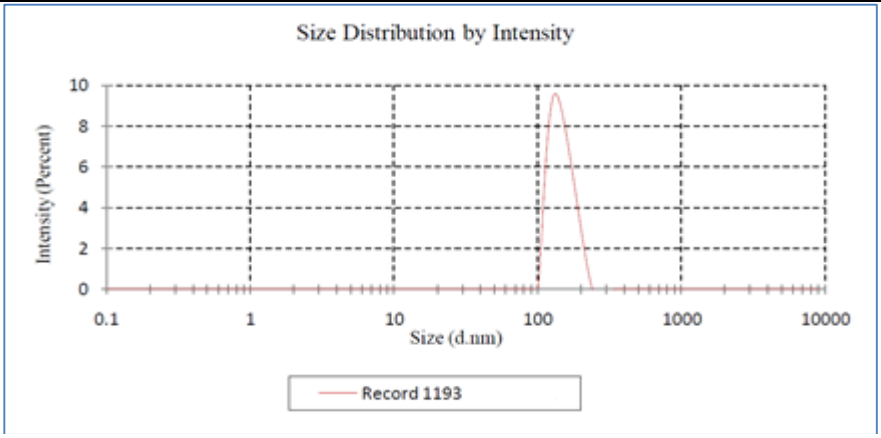

Fig. 5: Particle size distribution of biosynthesised MERNAgNPs as a percentage of intensity

Zeta Potential measurement

Nanoparticle surface potential was measured using a Zeta Potential technique. Determine the zeta potential of silver nanoparticles in aqueous solution to determine how stable they are. The zeta potential of stable silver nanoparticles must be at least 30 mV. It was found that the zeta potential of the nanoparticles was -25.1 mV with a peak intensity of 100%, according to the data. The stabilising effects of these values may lead nanoparticles with narrow size distribution indices to do so (Fig. 6).

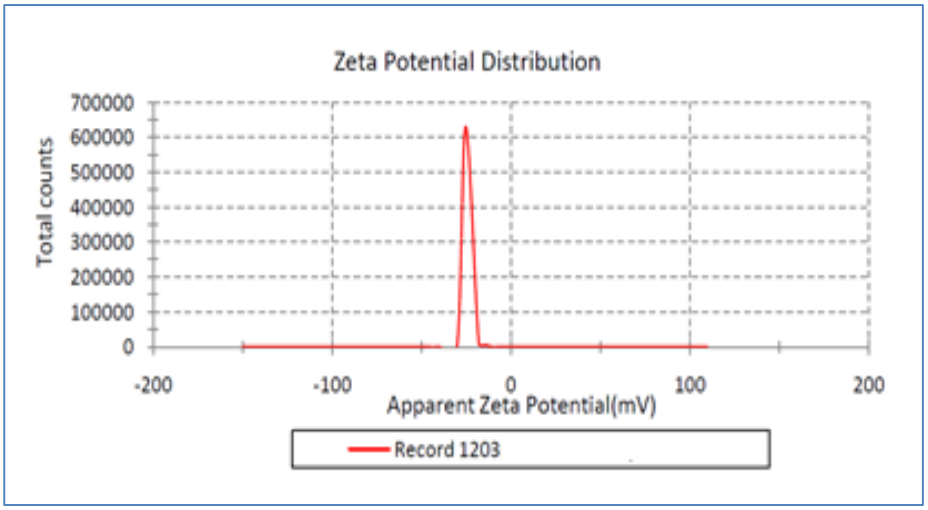

Fig. 6: Zeta potential distribution of bio-synthesized MERNAgNPs

UV-Visible Spectroscopy

To begin the process of characterizing the silver nanoparticles, a UV-Vis spectrometer was used. UV-Vis spectrum of the reaction medium was evaluated to verify that pure Ag+ was reduced after the sample was diluted in distilled water. The UV-Vis spectrophotometer Model 1800 from Shimadzu was used to analyse the spectra between 200 and 600 nm. The colloidal solution's UV-Vis Spectra show a significant absorption peak at 420 nm, which may be due to the reduction of silver ions in the solution, as seen in Fig. 7. Silver nanoparticles exhibited a typical surface Plasmon resonance peak.

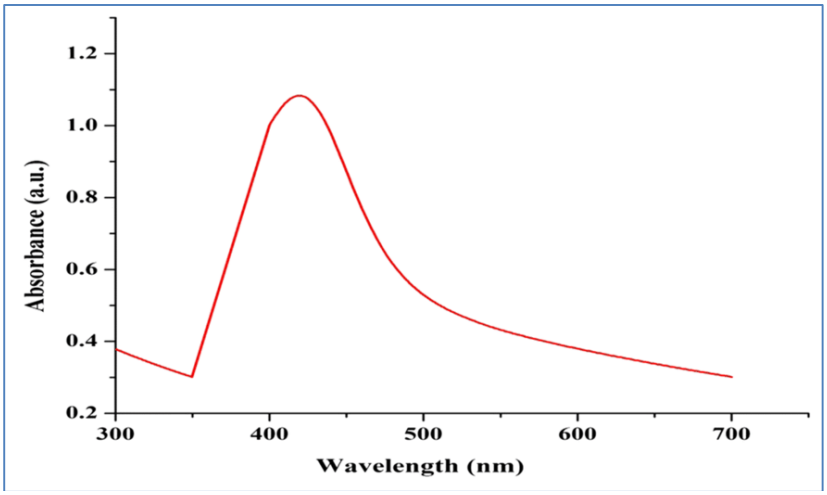

Fig. 7: UV-Vis spectra of MERNAgNPs

Surface Plasmon resonance

It is a collective oscillation of the electrons in solids and liquids induced by light. Surface electrons vibrating against positive nuclei form a resonance condition when light photons match their inherent frequency. The term "localised surface plasmon resonance" refers to SPR in nanometer-sized objects. SPR may be used to evaluate the adsorption of metal nanoparticles on flat metal surfaces (often gold and silver). On this basis, there are a number of colour biosensors and chip sensors in use today.

Silver nanoparticle morphological investigations were conducted using Scanning Electron Microscopy (SEM)

Silver nanoparticles were examined using a scanning electron microscope (SEM) for shape and size distribution. The silver nanoparticles seemed to be spherical and evenly distributed on the cells' surfaces, with an average diameter of roughly 100nm (Fig. 8).

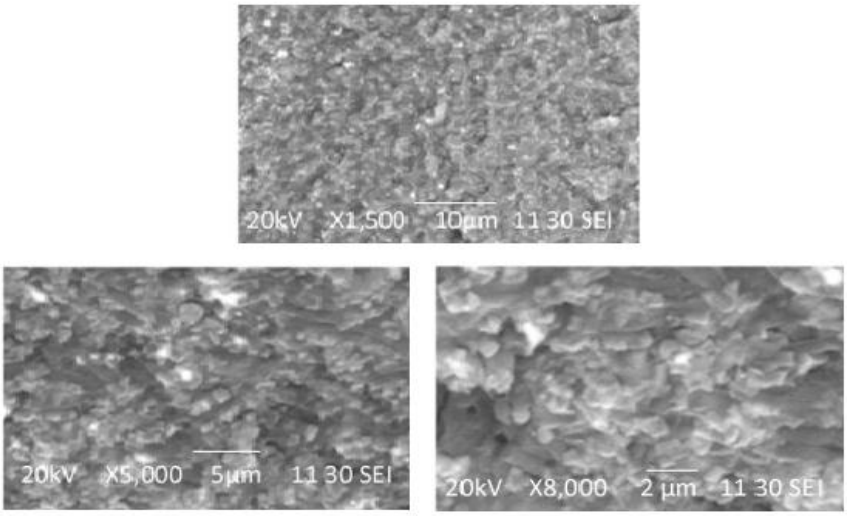

Fig. 8: SEM images of MERNAgNPs

Rhynchoglossum notonianum (Wall.) B.L. Burtt and Silver Nanoparticles having antibacterial properties

The antibacterial efficacy of silver nanoparticles generated from *Rhynchoglossum notonianum* leaves extract was studied using an agar disc diffusion technique. *Staph aureus* and *Bacillus subtilis*, two Gram-positive and Gram-negative species, were used in this study. The zone of inhibition (ZoI) approach was used to measure the susceptibility of an antimicrobial agent to a particular test organism. Using vernier callipers, the area of inhibition was measured. After 24 hours of incubation, AgNPs from *Rhynchoglossum notonianum* (Wall.) B.L. Burtt leaf extract significantly inhibited *Rhynchoglossum notonianum* (Wall.) B.L. Burtt leaves extract and standard chloramphenicol. The zone of inhibition was used to compare the bactericidal activity of different $AgNO_3$ (ZoI). *Rhynchoglossum*

notonianum B.L. Burtt leaf extract was discovered to have antibacterial activity against E. coli with an antibacterial activity of 3.28 millimetres wide. For S. aureus and Bacillus subtilis, *Rhynchoglossum notonianum* B.L. Burtt leaf AgNPs showed 2.30mm and 2.11mm ZoI identical to those of S. aureus and Bacillus subtilis (Table 2 and Fig. 9).

Table 2: AgNPs, $AgNO_3$, and *Rhynchoglossum notonianum* (Wall.) B.L. Burtt leaf extract have antibacterial action

"Samples	Doses	Escherichia coli (mm)	Staphylococcus aureus (mm)	Bacillus subtilis (mm)
$AgNO_3$	30µl/ml	1.60±0.12	1.78±0.14	1.70±0.16
Rhynchoglossum notonianum	30µl/ml	1.08±0.08	0.84±0.06	0.77±0.04
AgNPs	30µl/ml	3.30±0.23	2.32±0.18	2.13±0.16
Standard (Chloramphenicol)	30µl/ml	6.31±0.45	5.80±0.41	5.73±0.40"

Triplicate data were summarised as the mean standard deviation (SD).

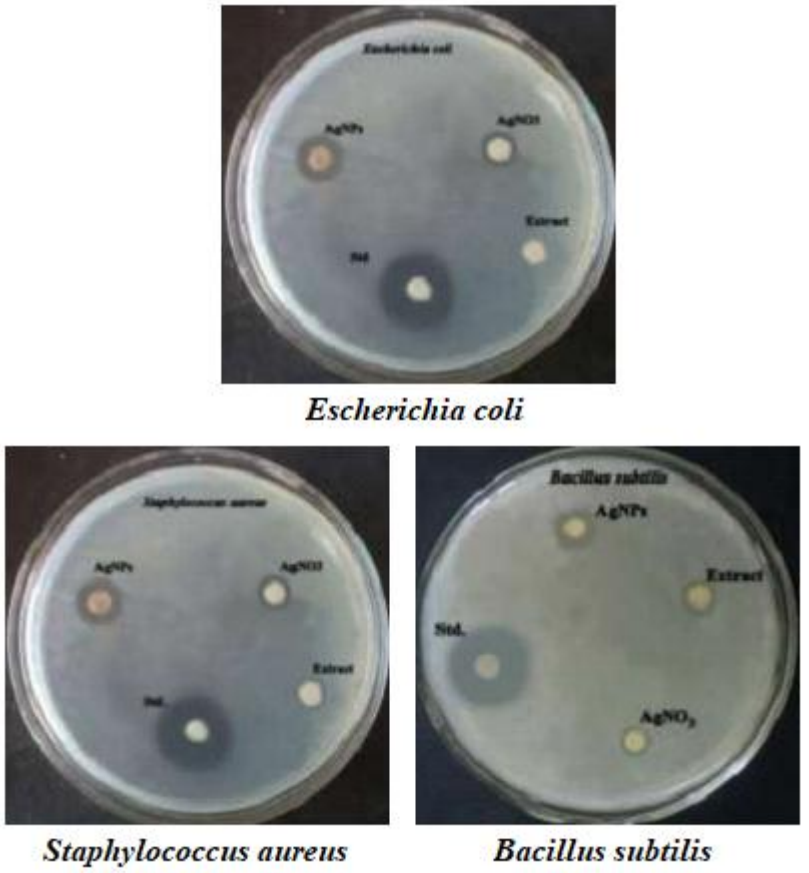

Fig. 9: AgNPs, $AgNO_3$, and *Rhynchoglossum notonianum* (Wall.) B.L. Burtt leaf extract have antibacterial action

Discussion

Rhynchoglossum notonianum Wall. silver nanoparticles are mostly synthesised using a green approach. herbal medicines were previously overlooked as innovative formulations due to a lack of scientific explanation and difficulty extracting and identifying particular medicinal components in complicated polyherbal systems that were difficult to standardise and extract. While traditional herbal medicine cannot be used in new drug delivery systems like "nanoparticles, microemulsions, matrix systems, solid dispersion liposomes and solid lipid nanoparticles", modern phytopharmaceutical research can meet the scientific needs (such as pharmacokinetics, mechanism of action and site of action determination and the precise dose required).

Rhynchoglossum notonianum Wall. extract was used in the biological production of silver nanoparticles, and the results were impressive in terms of particle size and shape uniformity. *Rhynchoglossum notonianum* Wall's methanolic leaf extracts were brown in colour when they were combined with an aqueous solution of silver ion complex, indicating that MERNAgNPs had formed.

Ultraviolet spectroscopy, which has shown to be a very helpful approach for the investigation of nanoparticles, was used to characterise the nanoparticles. The widening of the peak in the UV-Visible spectrum revealed that the particles were poly distributed.. Silver nanoparticles in solution retain a surface Plasmon band of around 420 nm. Particles remain distributed in the aqueous solution of silver nitrate throughout the reaction time, with no indication of agglomeration. Zeta values were determined to be -25.1 mV with the peak region of 100% intensity, and the average particle size (z-average) was found to be 132.6 nanometers. This demonstrates that the silver nanoparticle generated is stable in this environment. The silver nanoparticles generated had a spherical shape and were roughly 100nm in size, as seen by SEM pictures. The cells' surfaces were found to have silver nanoparticles scattered in a consistent pattern by SEM. Silver nanoparticle production using green chemistry provides several benefits, such as ease of scaling up, economic feasibility, and so on.

The biological assessment compares the antibacterial activity of MERNAgNPs with MERN. The antibacterial activity of silver nanoparticles produced from MERN was greater than that of MERN. Due to a decrease in particle size (132.6nm), MERNAgNPs boost oral bioavailability by enhancing the dissolving rate.

Conclusion

Silver nanoparticle synthesis utilising *Rhynchoglossum notonianum* Wall. leaves seems to be more successful than MERN in terms of creating antibacterial silver nanoparticles, according to the results of the study. In the biomedical and nanotechnology industries, the medicinal plant *Rhynchoglossum notonianum* Wall. will be employed to create a value-added product that will be sold to customers. The antibacterial activity of silver nanoparticles produced from MERN was greater than that of MERN. Due to a decrease in particle size (132.6nm), MERNAgNPs boost oral bioavailability by enhancing the dissolving rate.

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