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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *RHYNCHOSIA HEYNEI* PLANT LEAF EXTRACT: CHARACTERIZATION, ANTIMICROBIAL, ANTIOXIDANT, AND BIOCATALYTIC PROPERTIES.

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ABSTRACT

Objective: To study the green synthesis of silver Nanoparticles (AgNps) from plant leaf of *Rhynchosia heynei*, for the characterization of AgNPs by UV, zeta potential, FTIR and SEM techniques and to study the antioxidant, antimicrobial, and biocatalytic properties. **Methods**: the *Rhynchosia heynei* leaf extract was used for the green synthesis of AgNPs. The antioxidant, antibacterial, antifungal activity, biocatalytic activity and characterisation of Nanoparticles with UV- Vis Spectrophotometer, Zeta potential, FTIR, and SEM. **Results:** the plant leaf of *Rhynchosia heynei* has shown good antioxidant activity, also shown good antibacterial activity against Gram positive bacteria (*Bacillus substilis)* and Gram negative bacteria(*Pseudomonas auregenosa*)**.** The leaf also has good antifungal activity against *Aspergillus niger.* The leaf also shows good bio catalytic activity of Brilliant green 16% and Bromothymol 18%. The UV absorbance is at 450nm. The size and shape of AgNps (30-120nm) were confirmed by scanning electron microscopy (SEM) studies**. Conclusions**: The synthesized AgNps shows very good antibacterial antifungal and antioxidant properties.

KEYWORDS: *Rhynehosia heynei*, Silver Nanoparticles, Characterization. Antioxidant, Antimicrobial and Bio-catalytic properties.

1. INTRODUCTION

Present days Nanotechnology has come to the limelight because it is promising to face the challenges. Nanotechnology has a wide range of applications; they are optical receptors[1] bioactive materials[2] electrical batteries[3] sensors[4] and biomedical fields for instance; antioxidant, antimicrobial activity[5] gene delivery, and catalyst.[6] The different inorganic elements are used in Nanotechnology such as copper (Cu) Silver (Ag) Gold (Au) Palladium (Pd) etc, in these elements silver has excellent activity.[7] The silver Nanoparticles have a broad spectrum of infections.[8] The silver Nanoparticles have good antimicrobial activity, against bacterial strains like *Bacillus subtilis*, *Pseudomonas aeruginosa*, and fungi, *Aspergillus niger*.[9] The Nanoparticles disperse into Cations and Anions; the Ag^+ Cation binds to electron donor groups, which leads to the loss of their function.[10]

The Nanoparticles inhibit the growth of Microbes, due to the large surface area of AgNPs. It attaches the Microbes on the surface and makes a hole in the membrane as a result; the intracellular materials come out.[11] The Nanoparticles inactivate the proteins and DNA by binding the minerals sulphur and phosphorous, which are present in the Biomolecules.[12] Whenever the reduction reaction occurs with the Thiol group of enzymes and proteins, the ions were formed. These ions inactivate the respiratory system.[13] The free radicals were origin whenever primary metabolism proceeds, these radicals were neutralized by the antioxidant or antiradical elements.[14] The secondary metabolites such as phenols act as antioxidants and neutralize the free radicals. If the free radicals were left without neutralization, it leads to an aging process and pathogens will attack.[15]

The dyes are of two types, they are natural and synthetic dyes. These dyes are mainly generated from the textile industry as well as pharmaceutical, paper, plastic, and tanneries, these dyes are toxic.[16] The water ecosystem was disturbed by the release of the dyes, whichever comes from industries into rivers, lakes, ponds etc.[17] The plant *Rhynchosia heynei* habitat is under a shrub, which grows up to 1.5m tall. The branches are greying down. The vernacular name is *adavivulava*. It occurs in scrub jungles & dry deciduous forests. The essential oils from this plant have medicinal properties. This plant has antimicrobial activity against *Klebsiella pneumonia*, which causes lung infections, and antifungal property against *Candida albicans*, which appear in diabetes patients in foot infections.[18] The main aim is to green synthesize the silver Nanoparticles from the leaf of

Rhynchosia heynei. Characterization, and to study their antimicrobial, antioxidant, and bio catalytic properties.

2. MATERIALS AND METHODS

2. 1 Collection of plant material

The plant selected for the study was collected from the Talakona forest, Chittoor District, Andhra Pradesh, India. The plant was identified and confirmed as *Rhynchosia heynei* with the help of flora.[19]

2.2. Preparation of Silver Nanoparticle

For the green synthesis of silver Nanoparticles, *Rhynchosia heynei* leaf was taken and cleaned under tap water. Crushed the leaves with the mortar and pestle and added distilled water step by step and filter the sample then 0.1mM of silver nitrate was added to the sample, formation of the brown colour from green is an indication of AgNps formation in the sample.

2.3. Antioxidant activity of AgNps

For the antioxidant activity different concentrations (100, 200, 300, 400, 500uL) AgNps were prepared and ascorbic acid was used as standard and DPPH was used as control. Later 0.1 mM of DPPH solution was added to the test tubes and was incubated for 30minutes later formation of colour change was measured at 517 nm in the spectrophotometer.[20]

Percentage (%) of inhibition = (A control- A sample/ A control) x 100

A sample = absorbance of sample

A control = absorbance of DPPH

2.4. 2, 2, Diphenyl-1-Picrylhydrazyl scavenging activity

To measure the scavenging activity of silver Nanoparticles, the DPPH solution (0.004%. in methanol) The AgNps solution with different concentrations was mixed with 1ml of DPPH solution and kept for incubation in a dark place for 15mins. After incubation, the decolouration of the test samples of was compared with the standard (Ascorbic acid).[21]

2.5 Antimicrobial activity

2.5.1 Antibacterial activity of AgNps

The antibacterial activity of AgNps was tested against both gram-positive and gram-negative bacterial strains like *Bacillus substilis* and *Pseudomonas aeruginosa.* The nutrient agar media was prepared. The antibiotic Azithromycin was added to the media as a control and autoclaved along with the petri plates.[5] Later 20mL the media was transferred to sterilized plates and left for solidification. After solidification, the young bacterial cultures were streaked on the media. The discs were prepared by puncturing the whatmann No1 filter paper, these discs were dipped into the colloidal Nanoparticles suspension (10µl) and the discs were placed on the media. The petri plates were kept in the incubator at 37^0C for 24 hrs. After incubation, formation of a zone of inhibition surrounding the strips was measured with the scale.[22]

2.5.2 Antifungal activity of AgNPs

The fungal culture used for the experiment is *Aspergillus niger*. For this the PDA medium was prepared; the antibiotic streptomycin was added to control. After solidification of medium the *A. Niger* spore suspension was poured on the medium (0.1ml) and spread with a sterile L glass rod, the Nanoparticles suspension was poured in the wells (100µl) in the three wells separately and one well was maintained as control. The plates were incubated at room temperature for 5-7 days. After the incubation, formation of a zone of inhibition surrounding of the wells was measured.[23]

2.6 Biocatalytic activity (Dye decolouration) of AgNPs

The bio catalytic activity of silver Nanoparticles on synthetic dyes like Bromothymol and Brilliant green was studied. For this 100ppm stock solution was prepared. The two test tubes were taken one for Bromothymol and one for Brilliant green dye, 100uL of AgNPs was added in each test tube 10ml of dye i.e. 1ml of stock 9ml of distilled water and kept for incubation for different incubation periods. The decolourization of dye was observed for every 30mins, after that, the readings were taken in a spectrophotometer calibration curve was 670nm.[24]

The calculation of Brilliant green dye decolourization

Dye decolouration = Initial concentration - final concentration / initial concentration x 100

The calculation of Bromothymol

Dye decolouration = Initial concentration - final concentration / initial concentration x 100.

3. RESULTS AND DISCUSSION

3.1 Collection of plant material

The plant selected for the study was collected from the Talakona forest, Chittoor District, Andhra Pradesh, India. The plant was identified and confirmed as *Rhynchosia heynei* with the help of flora.[20]

Fig.1. *Rhynchosia heynei* plant.

3.2 Green syntheses of silver Nanoparticles

For the synthesis of silver Nanoparticles, *Rhynchosia heynei* plant leaf (5g) was used. After mixing the leaf extract with silver nitrate (0.1mM) solution, Nanoparticles began to form. The formation of AgNPs was confirmed by the observable changes in the solution colour from pale green to brown (shown *in Fig. 2.).*

Fig.2 A. Plant leaf extract without $AgNo_3$.

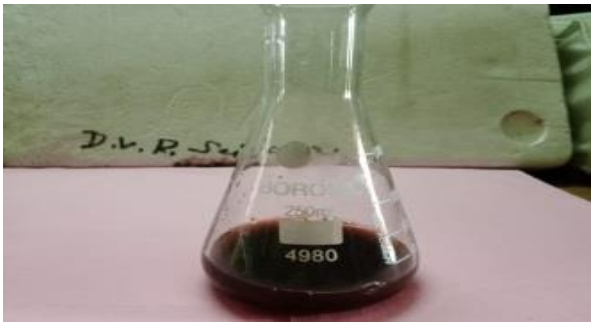


Fig.2 B. Plant leaf extract with $AgNo_3$.

3.3. UV-Visible spectroscopy studies

The confirmation of developed AgNPs was also done by spectral analysis. The absorption of synthesized AgNPs from leaf extracts of was observed at 425nm, 423nm, 410nm, and 400nm as shown in below Fig. 3. Due to surface Plasmon resonance (SPR).The optical property of AgnNps was determined by a UV-Visible spectrophotometer. A surface Plasmon resonance shows a characteristic peak at a range of 300- 700nm, it conforms to the synthesis of $Agno_3$. *R.hyenei* shows the absorbance at 450nm.

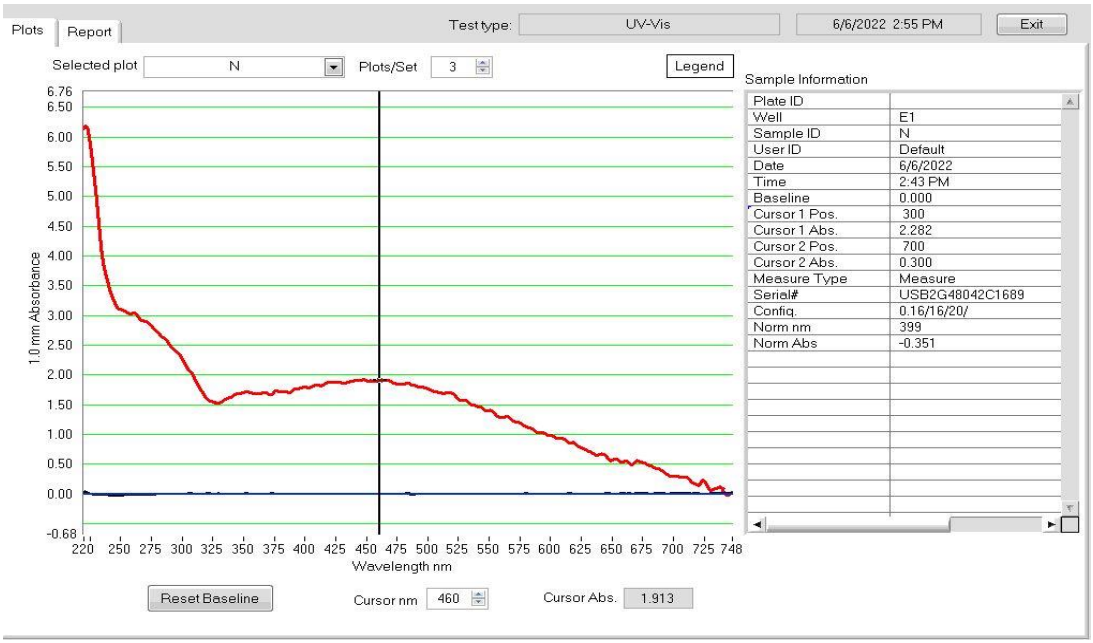


Fig 3: UV spectrum of AgNps.

3.4 Nanoparticle size analysis

The particle analyzer is the scientific tool, which measures, and depicts particle size distribution for a synthesized $AgNo_3$. The particle size of the AgNPS obtained is detected by the intensity and laser diffraction. Here, we observed a larger particle size. The size of synthesized Nanoparticles ranges from 30to50nm. The small-size synthesized Nanoparticle has good antimicrobial activity. Because these particles work when they contact the bacterial cell wall. It shows the Nanoparticle bacterial interaction. Nanoparticles can penetrate microbial membranes, interact with the metabolic proposes and cause membrane damage.

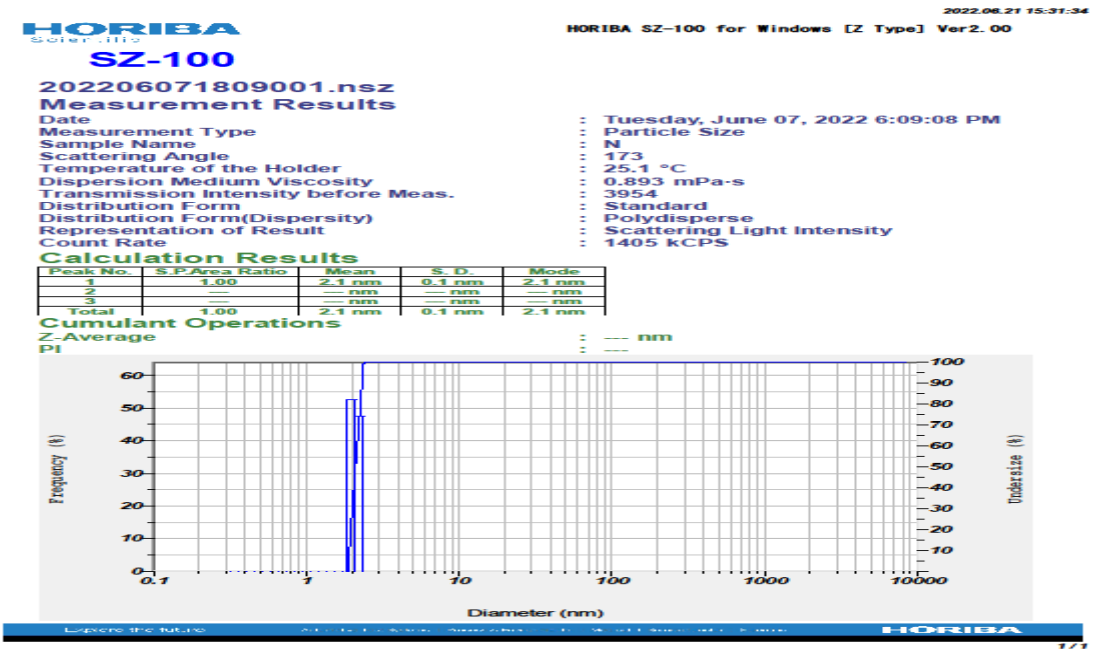


Fig 4: Particle Analyzer.

3.5 SEM studies

The size of the silver Nanoparticles from *Rhynchosia heynei* leaf observed under Scanning electron microscopy shown in (Fig.5) from the results, the AgNPs ranges from 30 to 120nm.

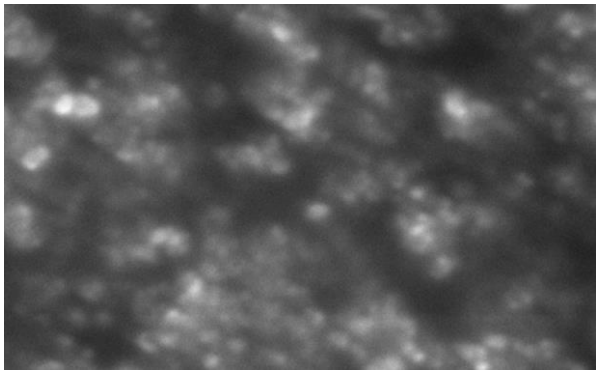


Fig.5: SEM image of silver Nanoparticles.

3.6. Antibacterial activity of AgNPs

The antibacterial activity of the AgNps was confirmed by measuring the formation of zone of inhibition. The antimicrobial activity of *Rhynchosia* heynei leaf extract was studied against, Gram-positive (*Bacillus substilis*) and Gram-negative (*Pseudomonas auregenosa*) bacteria.[25] The inhibition zone of bacteria *Pseudomonas auregenosa* was measured as 3cm 3/2 = 1.5cm. The inhibition zone of bacteria *Bacillus substilis* as 2 cm 2/2 =1 cm. The zone of inhibition of standard antibiotic for bacteria *Pseudomonas auregenosa* is 4 cm 4/2 = 2 cm. The zone of inhibition of standard antibiotic for bacteria *Bacillus subtilis* is 3 cm 3/2 = 1.5. The concentration of AgNPs is 100µl/well.

Table 1: Antibacterial activity of Nanoparticles on *Rhynchosia heynei* leaf extract.

Bacterial species	100 µl	Standard Antibiotic
Bacillus substilis	1	1.5
Pseudomonas auregenosa	1.5	2

Note; Zone of inhibition represented in cm diameter for mean values.

The Antibacterial activity of AgNps against Gram-positive *Bacillus substilis* and Gram-negative *Pseudomonas auregenosa* bacteria. The zone of inhibition was calculated with the help of Metric scale.[26] The *Psedomonas auregenosa* species has good and potential antibacterial activity than the *Bacillus substilis* species. The plant extract showed antimicrobial activity at different ranges. The maximum zone of inhibition was observed in *pseudomonas auregenosa* species followed by *Bacillus substillis*. Similar reports made from AgNps by *Actinomycetes* species exhibited good antibacterial activity.[27]

3.7. Antifungal Activity

The antifungal activity of the Silver Nanoparticle was studied by measuring the zone of inhibition. The size of the zone of inhibition is a measure of the effectiveness of the compound. The antifungal activity of *Rhynchosia heynei* leaf extract was studied against the fungi *Aspergillus niger*. The inhibition zone measured is 1.7

cm.1.7/2 = 0.85 cm. The zone of inhibition of standard antibiotic for *Aspergillus niger* is 2.5 cm 2.5/2 = 1.25 cm. The concentration is 100µl of Nanoparticles.

Table 2: Antifungal activities of Nanoparticles on *Rhynchosia heynei* leaf extract.

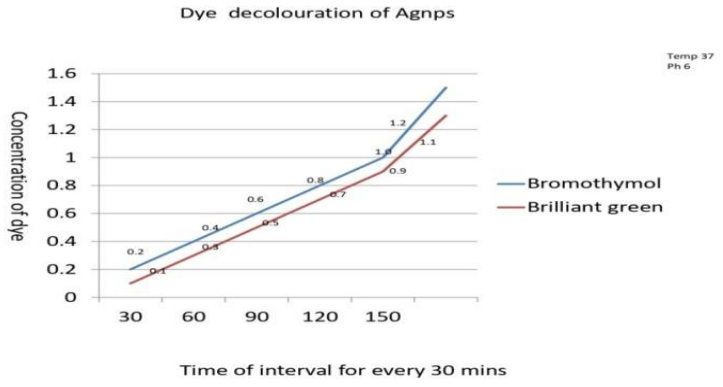
Fungi	100µl	Standard Antibiotic
Aspergillus niger	0.85	1.25

Note; Zone of inhibition was represented in cm diameter for mean values.

The antifungal activity of AgNps on *Aspergillus niger*. The maximum zone of inhibition was observed. Similarly AgNPs from *A. niger* exhibited good antifungal efficacy.[28]

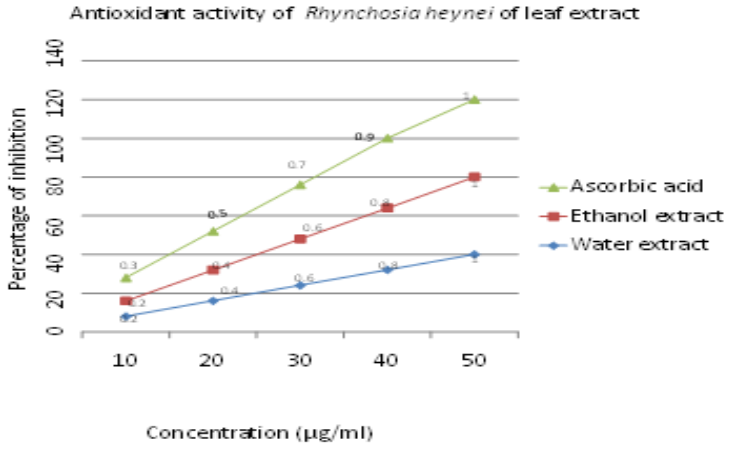
3.8. Dye decolouration studies

The catalytic property of silver Nanoparicles on Bromothymol and Brilliant green dyes were studied. While increasing the concentrations of AgNps the dye decolouration also increased, maximum dye decolouration was observed Brilliant green where as Bromothymol showed less decolouration.[29] The dye decolouration was observed by measuring in UV-Spectrophotometer. The dye decolouration of Brilliant green is 61%. The dye decolouration of Bromothymol is 18%. The dye decolouration has good activity by the AgNps solution. Dyes are two types; they are organic and synthetic dyes. Synthetic dyes are widely using now a days, which were recognised as a potential carcinogenic, these dyes pollute the water. So, waste water treatment should be required.[30] Now a day's waste water containing the dyes also treated with a variety of Nanoparticles.[31]



Graph 1: Dye decolourization studies.

The graph shows the dye decolouration of AgNps. The dyes were used for decolouration, they were Bromothymol and Brilliant green and the decolouration was noted every 30 minutes of incubation at temperature 37°C and PH 6. The decolouration of Bromothymol blue dye by the AgNps declined at every 30 minutes the decolouration declined from 1.4 to 0.2 mg/ml. Similarly, Brilliant green from 1.45 mg/ml to 0.2 mg/ml respectively.



Graph 2: Antioxidant activity of *Rhynchosia heynei* of leaf extract.

Percentage (%) of inhibition = (A control – A sample/ A control x 100)
A Sample = absorbance of sample
A control = absorbance of DPPH

The Antioxidant activity was confirmed by the appearance of blue colour as a positive result. The antioxidant of *R. heynei* was estimated by (2, 2, Diphenyl 1-1-Picrylhydrazyl scavenging activity)**.** Free radicals which were originated from metabolism were neutralized by the antioxidant. If these free radicals were not neutralized, diseases will occur for instance; cardiovascular disease, cancer, diabetes etc.[32] The graph shows the antioxidant compared with the standard. The Ascorbic acid was taken as standard.

The 10 µg/ml of ascorbic acid of 0.3 concentration has 20% of inhibition, 20 µg/ml of 0.5 concentration has 40% of inhibition, 30 µg/ml of 0.7 concentration has 60% of inhibition, 40 µg/ml of 0.9 concentration has 80% of inhibition, 50 µg/ml has 1.0 concentration has 100%.

The ethanol extract of 10 µg/ml of 0.2 concentration has 20% of inhibition, 20 µg/ml of 0.4 concentration has 40% of inhibition, 30µg/ml of 0.6 concentration has 60% of inhibition, 40µg/ml of 0.8 concentration has 80% of inhibition, 50µg/ml of 1.0 concentration has 100% of inhibition.

The water extract 10 µl/ml of 0.2 concentration has 10% of inhibition, 20 µl/ml of 0.4 concentration has 20% of inhibition, 30 µl/ml of 0.6 concentration has 30% of inhibition, 40 µl/ml of 0.8 concentration and 50 µl/ml of 1.0 concentration has 40% and 60% of inhibition. The ethanol extract has higher concentration than water extract.

3.9 Zeta potential studies

The electrostatic repulsive force between the Nanoparticles depends on the charge present on the particle's surface. The negative zeta potential value confirms the repulsion using the particles; thereby increasing the stability of the formulation and preventing the Nanoparticles from agglomeration in the medium, leading the long stability. The zeta potential of the AGNPS of *Rhynchosia heynei* of leaf extract is -0.2 mv. It was concluded that the AgNPs synthesized with *Rhynchosia heynei* were moderately stable.

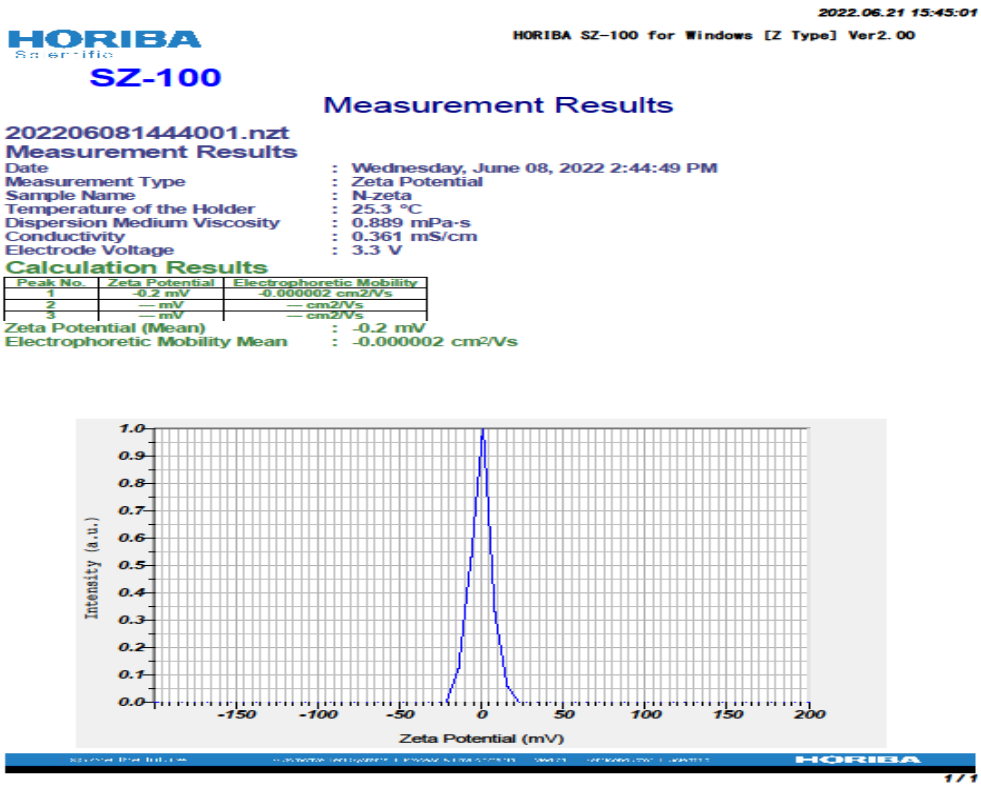


Fig 6: Zeta potential of AgNps.

3.10 FTIR studies

The FTIR is a technique which is used as the emission or absorption of solid, liquid, or gas. Which measure the intensity of range of wavelengths at a time.

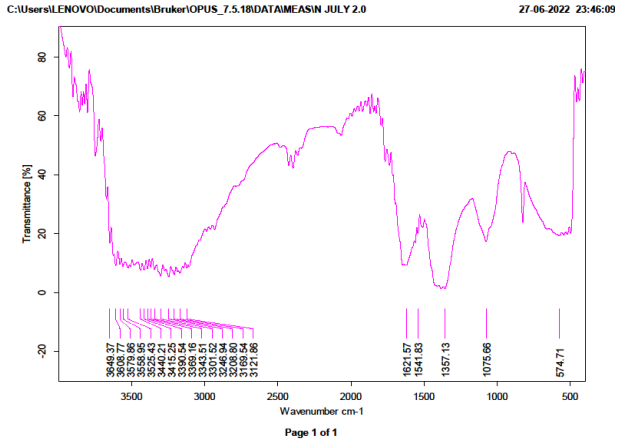


Fig 7: FTIR Spectrum of AgNps.

When comparing the results with another plant for instance; *Tecoma*[33] plant leaf has good antioxidant, and antimicrobial activity. The *Bacillus substilis* is a Gram-positive *eubacterium.* It forms spores; it is a non-pathogenic bacterium.[34] It appears in the gastrointestinal tract of Humans.[35] The *Pseudomonas auregenosa* is an aerobic rod bacterium that belongs to the *Pseudomonadaceae* family. It is a Gram–negative bacterium. It causes diseases for both plants and animals. It is believed to be a few true pathogens of plant pathogens. In animals, it causes urinary tract, and respiratory tract, infections, as well as it cause infection of soft tissue and dermis.[36] The fungi *Aspergillus niger* is a filamentous fungus, it is an ascomycete fungus. It is ubiquitous in the environment and it is an opportunistic fungus. Mainly it is used for the synthesis of citric acid and in the biotechnology industry. It is used as an experimental tool in the research area.[37] In this study we report that the silver Nanoparticles synthesized from *Rhynchosia heynei* plant leaf extracts could be used as an excellent antibacterial, antifungal and biocatalytic agent for modern medicine and bioremediation sectors.

4. CONCLUSION

In this work, silver nanoparticles were synthesized by using Rhynchosia heynei leaf extract and characterized. The antioxidant, antimicrobial, dye decolorization properties of AgNPs were studied. The silver Nanoparticles were characterized by UV-Visible Spectrophotometer, FTIR, and Zeta potential, The Nanoparticle size was characterized. The *Rhynehosia heynei* leaf has potent antioxidant and biocatalytic activity. The plant leaf extract showed good antibacterial activity against both gram-positive and gram-negative bacteria and also antifungal efficacy on *Aspergillus niger*.

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