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Green Synthesis of Silver Nanoparticles from Rhynchostylisretusa (L.) Blumeleaf extract under Sunlight

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ABSTRACT: ***A new green methods for the synthesis and stabilization of silver nanoparticles (AgNPs) using water extract of Rhynchostylisretusa(L.) Blume leaf with the help of Sun light as a source of energy has been developed. As obtained, the nanoparticles are characterized by FTIR, UV-visible (UV-Vis), X-ray diffraction (XRD) and SEM-EDX analysis.Thus, crystalline nature of AgNPs is confirmedby the prominent peaks in the XRD pattern.Distinct diffraction peaks in XRD spectrum is noticed at around 38^{O}, which are indexed by the (100) of the cubic face centered silver. FTIR spectrasuggest that the possible biomolecules are responsible for the efficient stabilization of the sample.The band between 3490-3500 cm^{-1}corresponds to O-H stretching,H-bonded alcohols and phenols. The peak found at around 1450-1500 cm-1 showed the bond stretch for N-H. The prepared nanoparticles had different antibacterial activity on both gram positive and gram negative bacteria.***

Keywords: Novel green synthesis, Silver nanoparticles, Rhynchostylisretusa(L.) Blume and antibacterial activity.

I. INTRODUCTION

Nano Particle synthesis may involve physical, chemical and biochemical approaches whichever the method use for synthesis, it provide a wide variety to create material, device and system with fundamentally new functions and properties [1]. Chemical and physical methods are most common for the synthesis of metal Nano particles but in this process use of toxic chemicals is very basic and not economically feasible one. Biochemical synthesis of metal process is most effective and economic methods over the chemical physical methods for the synthesis of nano particles [2]. For biochemical synthesis, different methods are available, synthesis of metal nano particle by using plant extracts and fruits extracts have a few advantages over the other methods viz. - a. Cell culture is not required, b. Non application of hurmful and toxic chemicals and c. Non release of any toxic byproduct. Synthesis of metal nano particle by using plant extracts has been already reported in several reports, such as Azadirachtaindica, aloevera, phyllanthusemblicaetc [3].

Here we have studied a novel approach for biochemical green synthesis of silver nanoparticle (AgNP) by using leaf extract (foxtail orchid leaf extract).

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Foxtail orchid is abundantly available in Assam of North East India and having variousmedicinal properties. It reduces the silver nitrate solution in presence of sunlight. After synthesis of silver nano particle antibacterial activities are tested on gram positive and gram negative bacteria- E.Coli, S.Aureus, Proteus Valgarisand Bacillus Subtilis.

II. MATERIALS& METHOD

A. Chemicals

Fresh leaves of Rhynchostylisretusa(L.)Blume collected from Golaghat Assam, India. All the chemicals from Hi Media Laboratories except nutrient agar, its form Marck. During the experiment Millipore water was used.

B. Leafextracts preparation for synthesis of silver nanoparticles.

Fresh leaves of Rhynchostylisretusa(L.) Blume (local name Foxtail orchid or kopouphool) were firstwashed thoroughly with water, and then by Millipore water, after this the leaves were cut to small pieces. 5g of these leaves was boiled in 100mL Millipore water for 10 minutes and filtered throughfilter paper (Whatmann No. 1). The filtrate, thus extracted, is transferred to an air-tight contained and stored in a refrigerator for future use in the synthesis of silvernanoparticles.

Fig1: Picture of Foxtail orchid or kopouphool

C. Silver nanoparticles-Preparation methodology

To achieve efficient silver nanoparticles, boiling time effect and effect of extract amount to be added to 0.3 Mm $AgNO_3$solution were varied and the best one was selected. AgNP was prepared in three ratios, Extract: $AgNO_3$ solution, these are 3:5, 3:10 and 3:15 and subjected to direct sunlight for 15 minutes. The best result was obtained in the 3:15 ratio.

D. Preparation of Antibacterial activity test well diffusion method.

The antibacterial activities of prepared AgNPs were tested by taking neomycin as the standard against both gran positive and gram negative bacteria by using agar well diffusion methods. Bacillus Subtilis&Staphylococcus Aureuswere selected as GramPositive Bacteria and Proteus Vulgaris and Escherichia Coli as gram negative bacteria for conduct of the test.

III. CHARACTERIZATION OF THE SYNTHESIZEDSILVER NANOPARTICLES

The Ag NPs obtained using silver nitrate and Rhynchostylisretusa(L.) Blume, leaves extract was characterized by using UV-VIS spectra,FTIR, XRD and SEM-EDX.UV-Visible spectrophotometer (SPECORD 50 PLUS) was used to monitor the reduction of pure Ag+ ions. Scanning electron microscopy (SEM) & EDX analysis of synthesized AgNPs was done using a **Make: Zeiss, Model:- Sigma 300.** XRD patterns were recorded through the use of Rigaku X-ray Diffractometer (Model: ULTIMA IV, Rigaku, Japan) with scanning rate of 3◦ per minute and 2θ value ranging from 5 to 100 using Cu K (= 1.54056 A) as the X-ray source and operate at a generator voltage of 40 KVA and current 40 mA, respectively. Furhter, IR Affinity, Shimadzu, Japan FTIR spectrophotometer mounted with a Shimadzu DRS-8000 DRIFT accessory andIR solution software with 4 cm-1 spectral resolution were used to record the Fourier Transform Infrared (FT-IR) Spectra.

IV. RESULTS AND DISCUSSION

A. UV-VIS Analysis

The prepared AgNP were observed under UV-Visspectrophotometer (SPECORD 50 PLUS) for itsmaximum absorbance and wavelength toconfirm the reduction of Silver nitrate[2]. UV spectra of AgNP were taken at different time interval are shown below:

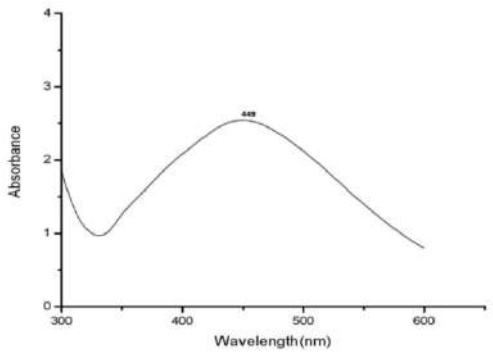

Fig 2: UV Spectra of Silvel Nano Particle

B. FT-IR(Fourier-Transform Infrared) Analysis

This analysis provides information about motion of the molecule in rotational and vibrational modes. Therefore, this technique is important forcharacterization and identification of the synthesized particles.

The band, between 3490-3500cm^{1}corresponds to O-H stretching H-bonded alcohols and phenols. The peak foundaround 1450-1500 cm-1 showed the bond stretch for N-H [6].

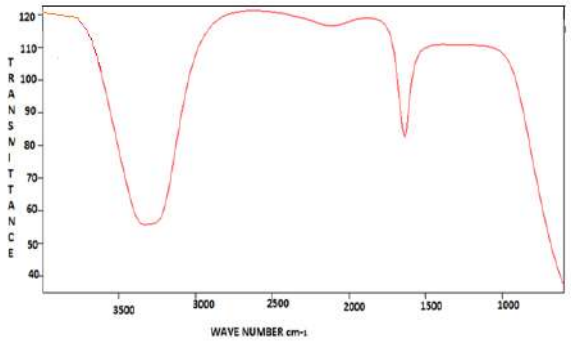

Fig 3: IR spectra of Silver Nano particle

C. XRD ANALYSIS:

Distinct diffraction peaks were observed at around 38^{O}during theXRD spectrum analysis, indexed by the (100) of the cubic face centered silver. The sharp Bragg peaks might have occurred due to presence of capping agent that has helped to stabilize the nanoparticles. Further, the intense Bragg reflections suggest that strong X-ray scattering centers in the crystalline phase and this could also be a result of presence of capping agents. In this regard, due toredispersion and centrifugation of the pellet in Millipore water as a process of purification after synthesis of nanoparticles,independent crystallization of the capping agents is ruled. This also suggests that the crystallization of the bio-organic phase occurs on the surface of the silver nanoparticles or vice versa. Usually, a broader peak in the XRD patterns of solids is attributed to particle size effect, where, it signify smaller particle size and reflect the effects due to experimental conditions on the nucleation and growth of the crystal nuclei.[7]

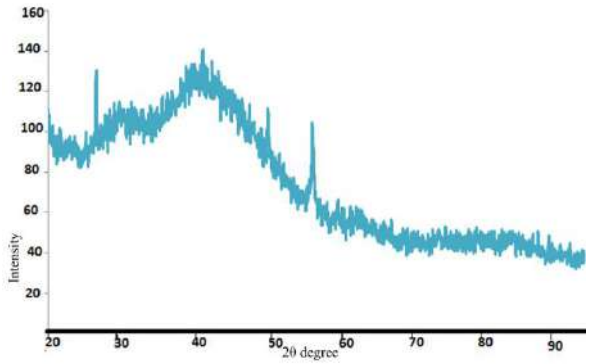

Fig 4: XRD spectra of Silver Nano Particle

D. SEM Analysis:

.The SEM image of silver nanoparticals synthesized is shown below. The AgNPs are monodispersed with star & cuboids' shaped. The average size of the particles is between 160-180 nm. Some Ag NPs showed size of approximately below 100 nm. A few agglomerated particles are also observed due to the spectral shift [8].

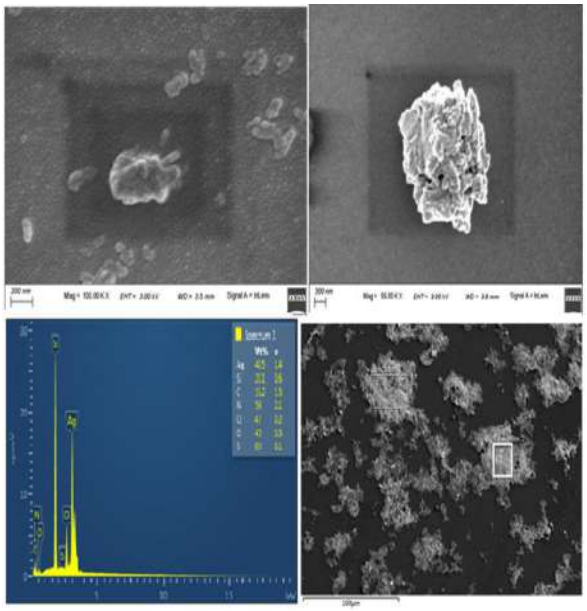

Fig 5: SEM-EDX Analysis of Silver nano particle

E. Antimicrobial activities:

In the study presented by Wen-Ru Li et al.,[9] stated that silver nano particle can act against both Positive and Negative Gram species of bacteria. The result of our analysis also states the same- the synthesized nano particles are effective against both Positive and Negative Gram species of bacteria. Further, we have observed that the prepared silver nanoparticle acted more efficiently than that of standard neomycin.

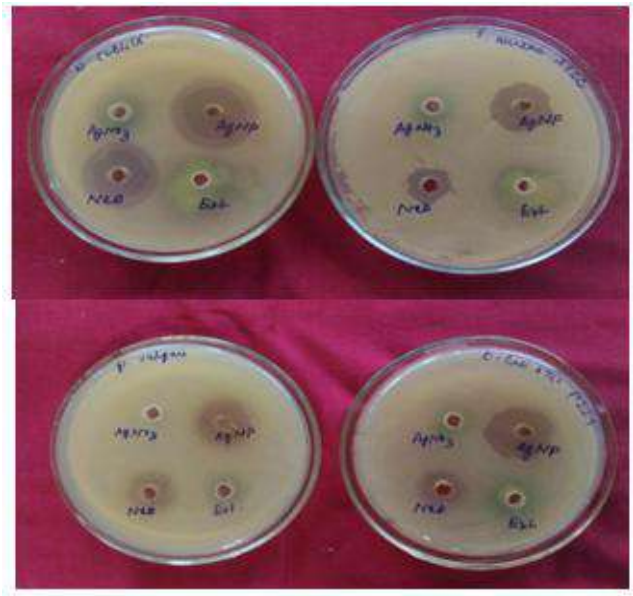

Fig6: The pictures showing the zone of inhibition for antibacterial activity for AgNP, $Ag(NO)_3$, Leaf Extract and standard neomycin.

The following table showing the result of antibacterial activity for AgNP, $Ag(NO)^3$, Leaf Extract and standard neomycin.

Table 1:- Showing zone of inhibitions found in Bacillus subtilis, Proteus vulgaris, Staphylococcus aureus and Escherichia coli

Name of the bacteria	gram positive		gram negative	
	Bacillus subtilis MTCC 441	Staphylococcus aureus MTCC 96	Proteus vulgaris MTCC 426	Escherichia coli MTCC 2582
AgNP	21.6 mm	19.3 mm	18.02 mm	22.4 mm
Ag(NO)3	no result	no result	no result	no result
Extract	no result	no result	no result	no result
Neomycin	21.2 mm	14 mm	14.3 mm	16 mm

V. CONCLUSION

The above studies are an environment friendly synthesis method of biological silver nanoparticle and can be potentially used in many applications. Benefits of new green synthesis over conventionally used methods include non-hazardous byproduct products, less processing efforts, stable material and material can be easily reproduced etc. Further, it has been observed that the prepared silver nano particles are highly reactive against bacteria of Gram positive and Negative species. Therefore, in the days to come, this method can be applied for development of different pharmaceutical products related to some bacterial diseases.

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