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Research Article

# PHYTOCHEMICAL SCREENING AND SYNTHESIS OF SILVER NANOPARTICLES USING LEAF EXTRACT OF *RHYNCHOTECHUM ELLIPTICUM*

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**ABSTRACT**

Objective: This study aims to investigate the hexane and ethanol extracts of the leaves of *Rhynchotechum ellipticum* for the presence of various phytochemicals and to synthesize silver nanoparticles using the ethanol extract of *Rhynchotechum ellipticum* leaves.

Methods: The presence of various phytochemicals viz. polyphenols, alkaloids, terpenoids, flavonoids, carbohydrates and steroids were investigated by following standard biochemical methods. The synthesized silver nanoparticles (SNPs) were characterized by using UV-Vis spectroscopy, FTIR, XRD and SEM.

Results: The extracts contain various phytochemicals. The synthesized nanoparticles were found to be spherical in shape with average size in the range of 0.51 to 0.73 μm.

Conclusion: The results revealed that the ethanol extract of *Rhynchotechum ellipticum* leaves is a very good bioreductant for the synthesis of silver nanoparticles.

**Keywords**: Phytochemicals; Bioreductant; Stabilizing; Silver nanoparticles

## INTRODUCTION

Natural products such as plant extracts provide unlimited opportunities for new drug discoveries because of unmatched availability of chemical diversity, either as pure compounds or as standardized extracts [1], and recent evidences from the pharmaceutical companies shows that it still represents an extremely valuable source for the production of valuable chemical entities that can be used for the treatment of some complex diseases [2].These medicinal plants can be rich in phenolic compounds, alkaloids, diterpenoid, steroid and other compounds which inhibit the development of various microorganisms [3].Besides these, phytochemicals in the plant extracts can act as reducing and capping agent in the reduction of metal ions to metal nanoparticles [4] and thus have found widespread use in the biosynthesis of metal nanoparticles which can be used in drug delivery [5], tissue/tumor imaging [6], biosensing [7], catalysis [8] and surface-enhanced Raman scattering-based sensors [9]. Recently the biosynthesis of metal nanoparticles using plant extracts has received considerable attention as a suitable alternative to existing chemical procedures and physical methods [10, 11].It is also very cost effective [12, 13] and thereby it can be used as an economic and valuable alternative for the large scale production of metal nanoparticles. Many researchers have reported the biosynthesis of metal nanoparticles by using various plant extracts [14, 15].

Considering the vast potentiality of plants as a source for drugs and its usefulness in the biosynthesis of metal nanoparticles, a systematic investigation was undertaken to screen a local flora, *Rhynchotechum ellipticum* for the presence of various phytochemicals. *Rhynchotechum ellipticum* is an erect undershrub and bears rose purple flowers. Leaf extract alleviates cough in children. Further, the ethanol extract of *Rhynchotechum ellipticum* leaf extract was used for the biosynthesis of silver nanoparticles.

## MATERIALS AND METHODS

### Chemicals

All reagents used in the study were of analytical grade. Silver nitrate ($AgNO_3$) was obtained from Sigma Aldrich.

### Collection and processing of plant samples

Fresh leaves of *Rhynchotechum ellipticum* were collected in the month of January 2013 from Tinsukia district of Assam, India. The collected leaves were washed and then air dried in the shade and crushed to obtain fine powder. 3.12 gm of *Rhynchotechum ellipticum* was extracted successively with hexane and then ethanol in a 500 ml round bottomed (R.B) flask separately. The extraction was done for 2 days. The extracts were concentrated under reduced pressure with the help of a Rotary Evaporator and preserved at 5°C in air tight bottles until required.

### Preliminary investigation for the presence of phytochemicals

Hexane and ethanol extracts of the leaves of *Rhynchotechum ellipticum* were investigated for the presence of phytochemicals viz. polyphenols, alkaloids, terpenoids, flavonoids, carbohydrates, steroids and steroids by following standard biochemical methods [16].

### Biosynthesis of silver nanoparticles from the ethanol extract of *Rhynchotechum ellipticum*

2 ml of the ethanol extract was added into 30 ml of aqueous solution of 1 mM silver nitrate for reduction of $Ag^+$ ions and stirred at room temperature for 5 hours.

### UV-Vis spectra analysis

The reduction of pure $Ag^+$ ion was monitored by measuring the UV-Visible spectrum of the reaction mixture after diluting a small aliquot of the sample with distilled water after regular interval of time. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-1700 (Shimadzu).

### FTIR analysis of dried biomass after bioreduction

To remove any free biomass residue, the residual solution was centrifuged at 5000 rpm for 15 min and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR.

### XRD analysis

X-ray diffraction (XRD) measurement was carried out by Rigaku X-ray diffractometer (Model: ULTIMA IV, Rigaku, Japan) with CuKα X-ray source (λ = 1.54056 Å) at a generator voltage 40 kV, a generator current 40 mA with the scanning rate 2° $min^{-1}$.

### Scanning Electron Microscopy (SEM) Analysis

The morphology and size of the synthesized silver nanoparticles were identified by using Scanning Electron Microscope (SEM)

(Model 6390 LV JEOL Asia PTE Ltd., Singapore/JEOL JSM). Thin films of the sample were prepared by dropping a very small amount of the sample on glass plates and then allowed to dry at room temperature.

## RESULTS AND DISCUSSION

The results of the qualitative screening of the phytochemical components such as polyphenols, flavonoids, alkaloids, terpenoids, carbohydrate and steroids in the hexane and ethanol extracts of the plant species are shown in table 1. The ethanol extracts of the leaves of *Rhynchotechum ellipticum* revealed the presence of polyphenols, flavonoids, alkaloids, terpenoids and carbohydrate and absence of steroids. Hexane extract of *Rhynchotechum ellipticum* leaf showed the presence of polyphenols, alkaloids and terpenoids and absence of other tested phytoconstituents.

### Characterization of the Ag nanoparticles

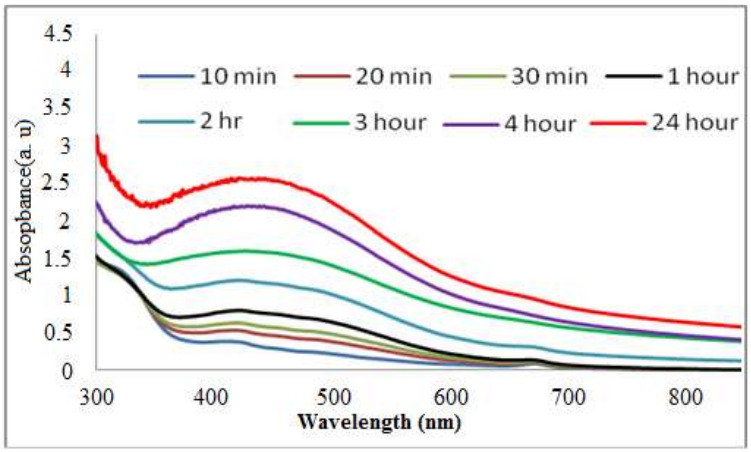
### UV-Vis study

The addition of *Rhynchotechum ellipticum* leaf extract to 1mM silver nitrate solution led to the appearance of a yellow brown colour solution after 10 minutes indicating the formation of Ag nanoparticles. Further UV- Vis spectral analysis showed surface plasmon resonance (SPR) band at 459 nm, a typical of silver nanoparticles. Fig. 1A shows the UV-Vis spectra of silver nanoparticles synthesized by using *Rhynchotechum ellipticum* leaf extract. It was observed that the intensity of absorption peaks increased as a function of time without any shift in the peak position.

**Table 1: Results of phytochemical screening of hexane and ethanol extracts of *Rhynchotechum ellipticum***

| Constituents | *Rhychontechum ellipticum* | |
|---|---|---|
| | Hexane | Ethanol |
| Polyphenols | + | + |
| Flavonoids | - | + |
| Alkaloids | + | + |
| Terpenoids | + | + |
| Carbohydrates | - | + |
| Steroids | - | - |

(+) indicates presence of constituents and (–) indicates absence of constituents.



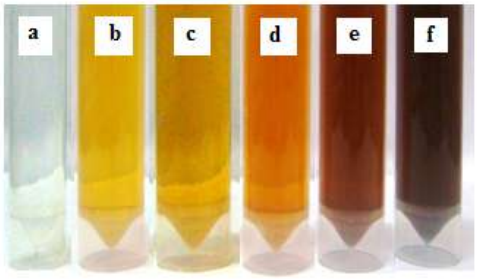
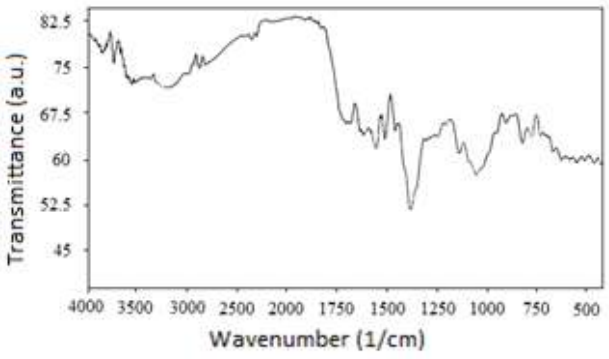




**Fig. 1A** **Fig. 1B**

**Fig. 1A: UV-Vis spectra recorded as a function of time of reaction of $10^{-3}$ M aqueous solution of $AgNO_3$ with *Rhynchotechum ellipticum* leaf extract; Fig. 1B: Formation of Ag nanoparticles. a is the solution of $AgNO_3$, b, c, d, e, f are the solution of $AgNO_3$ after addition of plant extract after time 10 min, 20 min, 30 min, 1 h, 4 h respectively.**



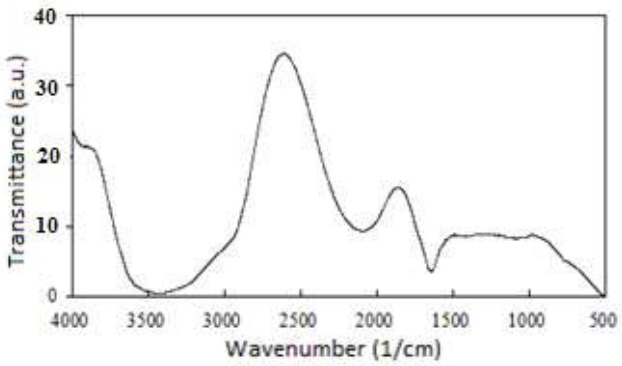




**Fig. 2A** **Fig. 2B**

**Fig. 2A: FTIR spectra of Ag nanoparticles synthesized by reduction of $Ag^+$ ion by *Rhynchotechum ellipticum* leaf extract; Fig. 2B: FTIR spectra of ethanol extract of *Rhynchotechum ellipticum***

### FTIR spectroscopy

The FTIR analysis was carried out to identify the possible biomolecules responsible for the reduction of $Ag^+$ ions and capping of the bioreduced nanoparticles synthesized by the ethanol leaf extract of *Rhynchotechum ellipticum*. FTIR spectra of water soluble plant extract before reduction of $Ag^+$ions (Fig. 2B) showed FTIR peak at 3344 $cm^{-1}$(strong peak for O-H vibration). 1932 $cm^{-1}$, 1440 $cm^{-1}$ and Ag nanoparticle formed by reduction of $Ag^+$ ions (Fig. 2A) using *Rhynchotechum ellipticum* leaf extract showed peak at 3248 $cm^{-1}$(weak peak for O-H vibration), 1704

$cm^{-1}$, 1682 $cm^{-1}$, 1514 $cm^{-1}$, 1381 $cm^{-1}$, 1139 $cm^{-1}$. The strong peak 3344 $cm^{-1}$ is due to presence of phenolic group and for water present in the sticky part of the ethanol extract of the plant material. This result suggested the presence of flavonoids adsorbed on the surface of metal nanoparticles.

**Powder X-Ray diffraction studies**

The biosynthesized silver nanoparticle by employing *Rhynchotechum ellipticum* leaf extract was further demonstrated and confirmed by the characteristic peaks observed in XRD analysis. The diffraction peak of silver nanoparticle at 2θ values 38.10°, 44.16°, 64.52°, 77.45° are assigned to plane (111), (200), (220) and (311) of lattice plane of face centered cubic(fcc) for Ag nanoparticle respectively. The corresponding 'd' spacing value of Ag nanoparticles were 2.34, 2.04, 1.41 and 1.21 respectively (Fig. 3). The high intensity diffraction peak was observed at 38.18°, corresponding to the crystalline Ag. It is confirmed that the nanoparticles were composed of pure crystalline Ag.

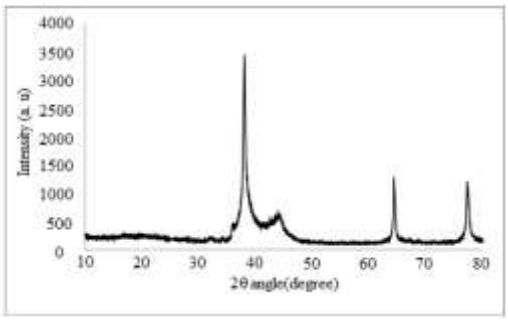



**Fig. 3: XRD pattern of Ag nanoparticles synthesized by treating *Rhynchotechum ellipticum* leaf extract with $AgNO_3$ solution**

**SEM Analysis**

The SEM image of silver nanoparticles synthesized by using *Rhynchotechum ellipticum* leaf extract is shown in Fig. 4 which shows distinct and clear image of synthesized silver nanoparticles having spherical shapes in the average size in the range between 0.51 to 0.73 µm. This image further indicates that the silver nanoparticles are not aggregated i.e monodisperse in nature.

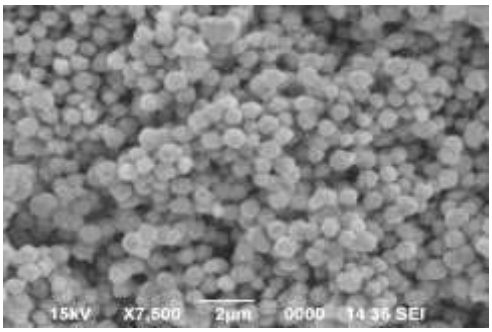



**Fig. 4: SEM image of the silver nanoparticles synthesized by using *Rhynchotechum ellipticum* leaf extract.**

**CONCLUSION**

It can be concluded that the hexane and ethanol extracts of the leaves of *Rhynchotechum ellipticum* contains various phytochemicals. Further the ethanol extract of *Rhynchotechum ellipticum* leaf is capable of producing stable spherical silver nanoparticles which indicates its potential in the production of other valuable nanostructures in the future.

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