Characterization of Silver Nanoparticles in Bryophytes: Insights from LC-MS and Enzyme Activity Analysis

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***Abstract*—The present investigation is focused on bryophytes association for the characterization of silver nanoparticles, LC-MS and enzyme activity of the liverwort *Targionia hypophylla* L. and two mosses as *Trachypodopsis blanda* (Mitt.) Fleisch.and *Stereophyllum anceps (Bosch* and Lac.) Broth. From the Khambataki ghat Khandala and Wai, Dist.-Satara of Maharashtra, India. Nanotechnology is useful to solving different environmental and health problems of human beings. Which is also useful for chemical fertilizers in agriculture aspects. Nanoparticles are useful in increasing the growth and production of crops. Synthesis of nanoparticles change in color was observed from pale yellow to finally reddish brown. The peak is between 400nm to 440nm was acquired on a UV Visible Spectrophotometer. which established silver nanoparticles.LC- MS is an analytical chemistry technique that combines liquid chromatography and mass spectroscopy.**

Which is useful for the analysis of metabolites like Phenol. In *S. anceps* phenol is present named carvacrol. In *Targionia hypophylla is a* tricyclic glycopeptide antibiotic named Vancomycin. Study of enzymes like Amylase and Nitrate reductase. Amylase is a group of enzymes that reduce starch into simple sugar. There are valuable industrial enzymes, a major resource of biologically active compounds in bryophytes. Another enzyme is nitrate reductase is the first enzyme of the nitrogen reduction pathway in plants. The production of ammonia plays a crucial role in nitrogen-fixing symbiosis, particularly in bryophytes.

HIGHLIGHTS

- **The article provides a comprehensive overview of the biologically active compounds present in bryophytes.**
- **Elucidate the therapeutic potential of bryophytes as antibiotics, antimicrobials, antioxidants, and anti-inflammatory agents.**
- **The article presents a detailed account of the characterization of silver nanoparticles, LC-MS, and enzymes.**

***Index Terms*—Enzymes, Liverwort, Mosses, Silver nanoparticles- LC-MS.**

I. INTRODUCTION

Nanotechnology is a field of nanoscience that deals with the size of nanoparticles approximately 1100 nm play an important role in drug delivery. Nanotechnology is a green method. Synthesis of nanoparticles and their uses eco-friendly. The Synthesis of silver, zinc and gold, platinum and copper. Use of green synthesis process is used as a medical field ant microbiological agent and anticancer Richard Feynman, a physicist and professor at the California Institute of Technology, was presented in his historical address as the guy who invented nanotechnology and wrote "There Is Plenty of Room at the Bottom" on December 29,

1959. Professor Norio Taniguchi of Tokyo Science University is credited with coining "nanotechnology" (Vimala and Sathish, 2017). Nanotechnology in the 21st century is the most important field in nanoscience and novel technologies. Currently, nanotechnology has achieved remarkable success at the research level. Nanoscience serves as the foundation of nanotechnology (Vimala, 2018). The biosynthesis of silver nanoparticles is a discovery of bryophytes (Khamgal *et al.,* 2021).

Metal oxides such as nano titanium dioxide (nano-TiO_2) and zinc oxide (ZnO) are commonly used for nanomaterials in various applications. Bryophytes, especially mosses, are used to monitor water and air pollution (Motyka *et.al.,* 2016). However, the use of

bryophytes for synthesizing nanoparticles is still limited. In the last decade, there has been a growing interest in using nanoparticles in bryophytes to study current phenomena (*Srivastava et.al.,* 2021).

Silver nanoparticles were produced using higher plants such as angiosperms, gymnosperms, and cryptogamic plants like *Riccia* and mosses. For this study, *Stereophyllum anceps*, *Targionia hypophylla*, and *Trachypodopsis blanda* were chosen to synthesize silver nanoparticles. *Stereophyllum anceps* belongs to the family *Stereophyllum* and is a major group of bryophyte mosses and liverworts. It has a prostrate, irregularly branched main stem that is 8.0-10cm long and glossy in appearance, with crowded leaves. *Targionia hypophylla* is a genus of liverworts in the order Marchantiales. Its thallus is green with dichotomous branching of 5.10 nm, attached to soil with an entire margin, and purple air pores present. *Trachypodopsis blanda*, belonging to the family Meteoriaceae, has a shiny, dirty green, yellow or golden-brown appearance, with mostly elongated secondary stems. The study of bryophytes was conducted using LC-MS.

II. MATERIAL AND METHOD

Collection of plant material: Thallus or leafy parts of *Stereophyllum anceps (Bosch and* Lac.) Broth. *Targionia hypophylla L.*, *Trachypodopsis blanda* (Mitt.) Fleisch) are collected and washed thoroughly under tap water and then with distilled water.

Preparation of plant extracts: The leaves (Thallus) under shade dried for 5 days were blended and produced into fine coarse powder.1gm of powder is mixed with 10 ml of 80% ethanol and boiled for 15 min. When the extract is filtered through filter paper. Residue is removed and pure filtrate is obtained.

Preparation of plant extract for Synthesis of silver Nanoparticle: 5µM aqueous solution of silver nitrate ($AgNo_3$) was prepared and used for the synthesis of silver nanoparticles 10 ml of extract was mixed with 1ml (aq.) solution of 5µM silver nitrate for reduction into Ag^+ ions and incubate overnight at room temperature in dark. Analysis of UV-visible spectroscopy, the bio reduction of pure silver nanoparticles is monitored using UV-visible spectroscopy at regular intervals during reduction sample was taken and centrifuged at 12000 rpm. The supernatant was scanned by UV 300-400nm.

Preparation of methanolic extract for LC-MS: The thallus or leafy parts of the plant are collected, washed thoroughly under tap water, and then rinsed with distilled water. The dried plant material is powdered. The plant material is then extracted by soaking it in methanol at room temperature. A 1 mg/mL solution is prepared from the dry extract obtained after filtration and evaporation.

The enzyme activity of Bryophytes

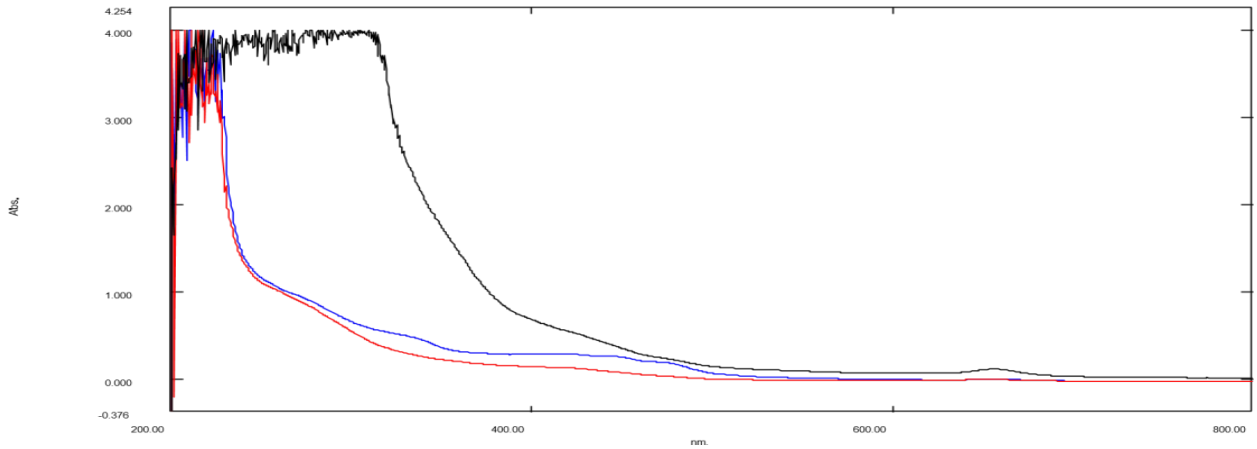
Nitrate reductase: The in vivo activity of the enzyme is determined by the Jaworski method (1967).

Amylase: Peter and Bernfeld (1955) developed a method for determining amylase activity using a spectroscopic technique.

Fig. Shown Synthesis of Silver Nanoparticles

Fig.1Before synthesis of AgNo3

fig.2After synthesis AgNo3



T1 T2 S1

Graphic representation shown was Spectral peak at variable concentration Synthesis of silver nanoparticles in T1 (T. hypophylla), T2 (T. blanda), T3 (S. anceps)

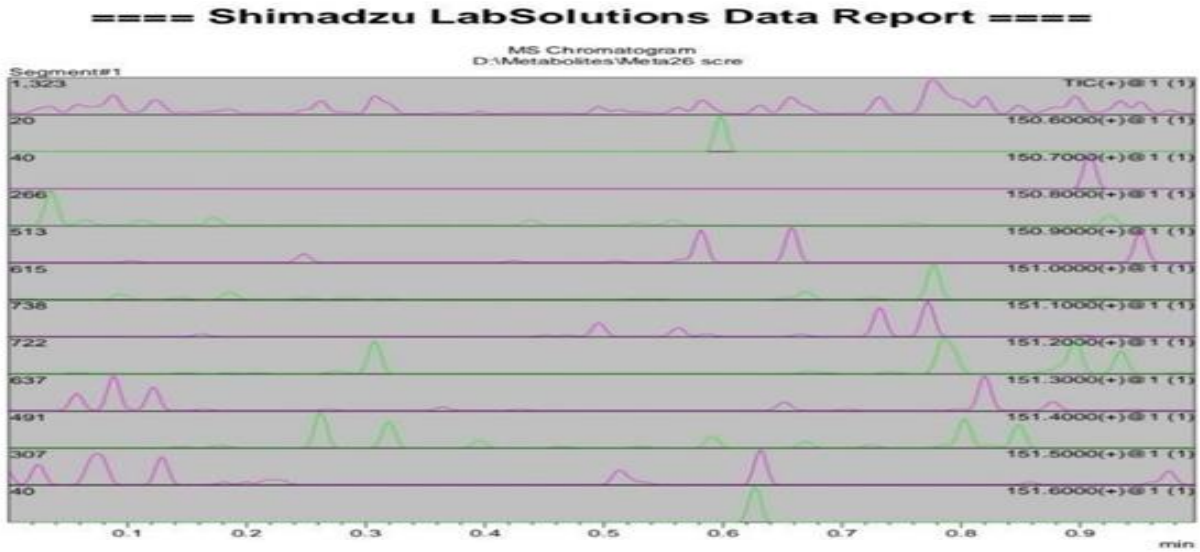


Fig.3.LC-MS chromatogram of methanolic extract Targionia *hypophylla*

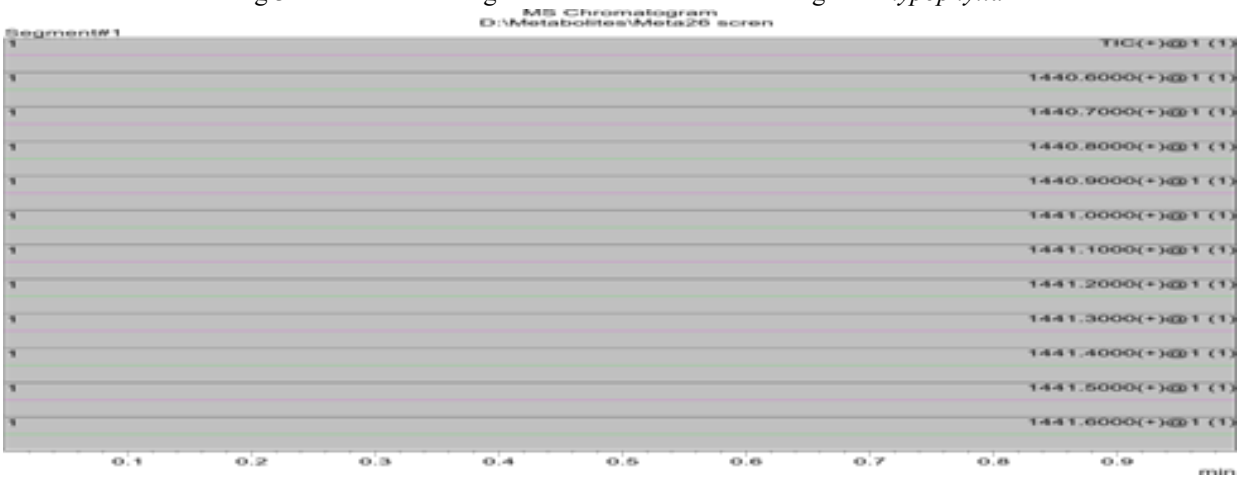


Fig.4 LC-MS Chromatogram of methanolic extract of Stereophyllum anceps Enzyme composition and LC-MS components

Name of species	Name of enzymes		LC-MS component's
	Nitrate Reductase	Amylase	
Targionia hypophylla	0.0872 Δ O.D.hr^{1}fresh tissue	-	Vancomycin
Stereophyllum anceps	0.352 Δ O. D. hr. $^{-1}$ fresh tissue	2.105mg maltose	Carvacrol

III. RESULT AND DISCUSSION

LC-MS (Liquid chromatography, Mass Spectroscopy), to trace the visible chemical compound Liquid chromatography, Mass spectroscopy analysis of methanolic extract was done. The compound obtained from LC-MS in *S. anceps* extract shows the presence of Carvacrol to detect the LC-MS spectrum. Carvacrol is a phenolic monoterpene useful as an antimicrobial, antitumor, and antioxidant. Due to the presence of oil bodies, liverworts are characterized by the widest range of aromas among bryophytes. The sources of odors are volatile mono- and sesquiterpenes and terpenoids (Michał D *et.al.,*2022). The Tannin was higher in *S. anceps* (Wadavkar *et.al.,*2017)

Targionia hypophylla extract shows the presence of Vancomycin to detect the LC-MS spectrum. Vancomycin is the tricyclic glycopeptide antibiotic originally derived from the organism *Streptococcus orientalis* useful to treat and prevent various bacterial infections, for complicated skin infections. The enzyme activity of *T. hypophylla, S anceps* is showing the nitrate reductase activity is 0.0872 Δ O.D.hr $^{-1}$fresh tissue in S. anceps 0.352 Δ O. D. hr. $^{-1}$ fresh tissue *T. hypophylla* The Amylase activity shows *S. anceps* the result as 2.105mg maltose 5 min incubation. Nanoparticles show a gradual decrease of 400-800 nm.

IV. CONCLUSION

The LC-MS of Methanolic extract of *S. anceps* and *Targionia hypophylla* the presence of carvacrol and vancomycin These are new active compounds for possible application in antibacterial, antitumor, antioxidants, and anti-inflammatory and also useful as an antibiotic and to treatment of skin disease. Future studies on character elucidation of compounds will be remarkable insights for drug designing and benefit of mankind.

The enzyme activity of Nitrate reductase in *T. hypophylla* is greater than*, in S anceps* The Amylase activity is higher in *S. anceps.*

The nanoparticles show the species of in T1 *(T. hypophylla*), T2*(Trachypodopsis blanda*), T3(*S. anceps*) gradual decrease 400to 800nm

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