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Green synthesis and antimicrobial studies of silver nanoparticles from some *ex-situ* conserved Bryophytes in coastal sites of Raigad district of Maharashtra, India

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Abstract
This research will use aqueous extracts of *Targionia indica* Kash., *Cyathodium tuberosum* Kash., and *Anthoceros erectus* Kash., to synthesize and classify silver nanoparticles. These bryophytes were grown ex-situ in the lab and further research was conducted. The current study focuses on the green synthesis of silver nanoparticles and their antimicrobial properties. In the Raigad district, bryophytes were collected from the Revdanda and Kashid coastal site vegetation. The appearance of a reddish brown colour shift suggests the development of AgNps. UV-Vis was used to classify these AgNps. The green synthesis extracts of valued bryophyte species display antimicrobial activity, i.e. zone of inhibition against bacterial strains such as *E. coli*, *Pseudmonas aeroginosa*, and *Staphylococcus aureus*, as well as fungal strains such as *Rhizopus* spp. and *Alternaria* spp.

Keywords: bryophytes, AgNps, *Ex-situ*, UV-Vis, antimicrobial activity

Introduction
Bryophytes are primitive, nonvascular land plants with a basic thalloid plant body organization. The advanced community of bryophytes mosses held a special role between lower cryptogams and vascular cryptogams. Bryophytes are non-vascular land plants with a cosmopolitan nature and the second largest primitive group. According to Crum (2001) [12], there are about 20,000 to 25,000 species worldwide. 1980 (Barbara & Crandall Stotler) Sathish *et al.* (2013) [29] reported 482 genera and 2486 species of bryophytes in India. There are 128 species of mosses in Maharashtra's Western Ghats, belonging to 11 orders, 26 families, and 59 genera Magdum *et al.* (2017) [25]. According to Vanderpoorten and Engels, bryophytes have many applications such as pollution indicator, ornamental purpose, drug extraction, natural pesticides, fungicides, pharmaceutical industry, horticulture, fuel, antibiotic activities, and antimicrobial activities (2003).
Nanotechnology is the most promising field in nanoscience, and it is a critical factor in the advancement of new technologies in the twenty-first century. Nanotechnology is concerned with the production and application of nanomaterials for the desired object. According to Parashar *et al.* (2009) [43], there are numerous methods used to produce nanoparticle synthesis, including physical, chemical, biological, microwave assisted radiation assisted, electrochemical, and non-chemical methods.
In comparison to other approaches, the biological approach is more environmentally friendly and does not contain any toxic chemicals. The green synthesis approach is superior to the chemical and physical methods (Kowshik *et al.*, 2003) [19, 27]. Silver, gold, platinum, zinc, and copper were used in the synthesis of metal nanoparticles (Caroling *et al.*, 2013) [15]. Ashton and Callaghan (2009) [14] Ex-situ conservation basically translates to "off-site" conservation. It is the method of bringing an endangered species to life. Ex-situ material is a Set of experimental methods that can be evaluated both in the lab and in the field. Ex-situ conservation of bryophytes includes many steps that are all equally important: material selection, propagation, storage (including cryopreservation), and reintroduction.
The current research is being conducted to investigate the antibacterial activity of silver nanoparticles synthesized from bryophytes *Targionia indica* Kash, *Cyathodium tuberosum* Kash, and Anthoceros erectus Kash. The study's aim is to evaluate antibacterial activity and establish the zone of inhibition on a specific bacterial strain. The antibacterial activity of the extract is calculated using the agar disc diffusion process. The antibacterial activity of bryophyte extract was tested against three bacteria: *E. coli*, *Pseudmonas aeroginosa* and *Staphylococcus aureus*. The antifungal activity of silver nanoparticles synthesized from bryophyte extracts on some fungal strains included *Rhizopus* spp., *Alternaria* spp. Biosynthesis is a novel method of producing nanoparticles from biological sources. It is gaining popularity due to its low-cost, environmentally sustainable, and large-scale production capabilities. Nanomaterial's are the atomic and molecular building blocks of matter (0.2 nm).Nanoparticles are a form of nanomaterial that can be amorphous or crystalline, and their surfaces can serve as carriers for liquid droplets or gases (Buzea *et al*., 2007). Since ancient times, nanoparticles have been used in pottery and medicine. Since the last decade, the most thoroughly researched nanoparticles have been those made of noble metals such as silver, gold, and platinum (Duran *et al.*, 2005; Ankamwar, 2010; Deng *et al.*, 2009) [30, 5, 34].

Materials and Methods
Collection and extraction of plant material
The plant material was collected from Revdanda and Kashid beach nearby costal vegetation in Raigad District of

Maharashtra during the period of September 2020. The collected samples were packed separately in sterilized polythene bags and noted with their locality, date of collection, time and brought to laboratory for further analysis. The bryophytes were identified by Gametophytic Plant body and Sporophytic plant body by using available standard literature.

The collected mosses were shade dried and made it into powder. 10gm of powder taken in 250 ml Erlenmeyer flask along with 100 ml of distilled water and boiled the mixture for 30 min. The extract was filtered through the Whitman No.1 filter paper and stored in the refrigerator for further use.

Synthesis of silver nanoparticles

To synthesize the silver nanoparticles 10ml of plant extract were mixed with 90ml of aqueous 1mM AgNo3 solution in a 250 ml Erlenmeyer flask. The flask was exposed to sun light for an hour and stored in the refrigerator for further use. Then the synthesized sample was used for characterization and antimicrobial activity.

Characterization of synthesized silver nanoparticles

Characterization of synthesized silver nanoparticles by using UV- Vis spectra analysis-The reduction of metallic Ag+ ion was monitored by measuring the UV- Vis spectrum after overnight of reaction. Spectrum of reaction mixture was taken on a wavelength from 200 nm to 800 nm on UV-Vis spectrophotometer (Systronics Double beam spectrophotometer 2202).

Preparation of media for microbial growth

Preparation of nutrient agar plate: The extracts were screened for antimicrobial activity through the disc diffusion assay method according to National Committee for Clinical Laboratory Standards (NCCLS, 1999; 2000 and Santra *et al.*1999). Antimicrobial activity was assayed using standard well diffusion method against human pathogenic bacteria *Escherichia coli*, *Pseudmonas aeroginosa* and *Staphylococcus aureus*. For antimicrobial test the sterile discs dip in the solution containing nanoparticles then put on the Nutrient agar plates and for control test sterile discs dip in distilled water then put on the Nutrient agar plates. Petri plates are incubated in BOD chamber at 370c overnight for 24 hours. The next day, zone of inhibition in the bacterial mat was measured.

Potato Dextrose Agar (PDA) is used for the cultivation of fungi (Uthayasooriyan *et al.*, 2016). The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophytes. Many standard procedures use a specified amount of sterile tartaric acid (10%) to lower the pH of this medium to 3.5 +/- 0.1, inhibiting bacterial growth. To prepare potato infusion, boil 200 g sliced, unpeeled potatoes in 1 liter distilled water for 30 min. Filter through cheesecloth, saving effluent, which is potato infusion. Mix with Dextrose, Agar and Water and boil to dissolve. Final pH maintained at 5.6 ± 0.2. Plugged the flasks with cotton plug and sterilize at 121 0C, 15 lbs. pressure for 15 minutes in an autoclave. (Aneja, 2003) [4].

Antifungal activity was assayed using standard well diffusion method against plant pathogenic fungi Rhizopus species and Alternaria species. For antifungal test the sterile discs dip in the solution containing nanoparticles then put on the Potato Dextrose Agar plates and for control test sterile discs dip in penicillin then put on the Potato Dextrose Agar plates. Petri plates are incubated in incubation chamber at 370c overnight for 24 hours. The next day, zone of inhibition was measured.

Photograph

Field photographs

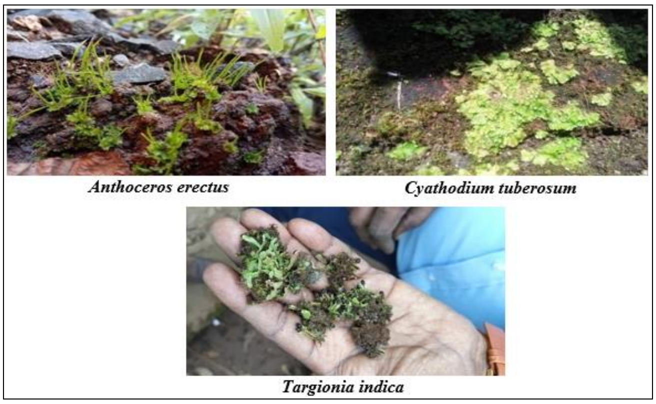

Fig 1

Ex-sit conservation

Fig 2

***Ex-situ* Conservation of bryophytes**

Result and Discussion

Synthesis and characterization of AgNps is done by visible color change after the substrate was provided to the plant extract. The synthesis of nanoparticles is confirmed by change in colour from plae green to reddish brown. The presence of silver nanoparticles was confirmed by obtaining a spectrum in visible range of 200nm to 600nm.

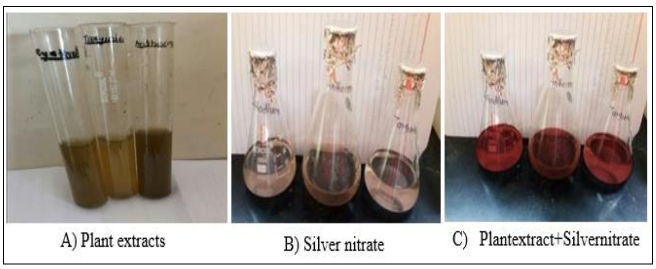

Fig 3: A) plant extract (pale yellow)B) Silver Nitrate C) plant extract + silver nitrate color change (Reddish brown)

The present study shows antimicrobial activity of silver nanoparticles from*Targioniaindica* Kash.,*Cyathodium* *tuberosum* Kash., and *Anthoceros erectus* Kash. Against three strains of laboratory pathogens include *Escherichia coli, Pseudomonas aeroginosa*and *Staphylococcus aureus.* The zone of inhibition measured is summarized in table no 1. The antimicrobial activity of *A. erectus* shows small zone of inhibition to *E. coli* and *C. tuberosum* large zone of inhibition to *P. aeruginosa.*

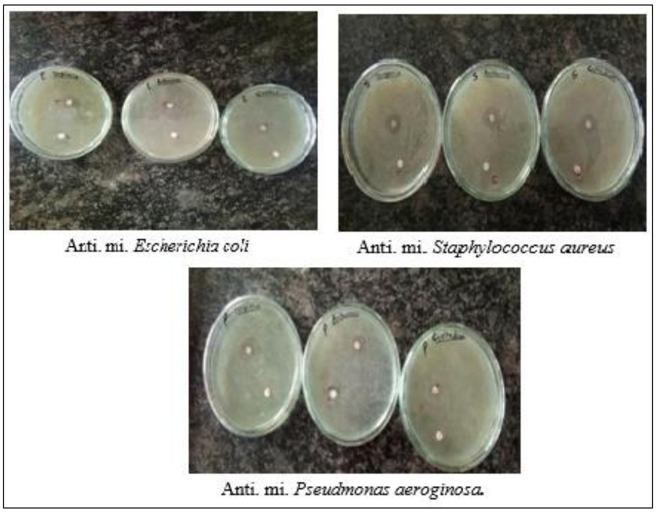

Fig 4: Petri plates showing Antimicrobial activity (Zone of inhibition)

Antimicrobial activity

Table 1: Antimicrobial activity

Bryophytes	Bacteria	Zone of inhibition(mm)
Targionia indica Kash.	*Escherichia coli.*	16
	Pseudmonasaeroginosa.	23
	Staphylococcus aureus.	11
Cyathodium tuberosum Kash.	*Escherichia coli.*	11
	Pseudmonasaeroginosa.	26
	Staphylococcus aureus.	13
Anthoceros erectus Kash.	*Escherichia coli.*	08
	Pseudmonasaeroginosa.	14
	Staphylococcus aureus.	14

The present study also shows antifungal activity of silver nanoparticles from *Targionia indica*Kash., *Cyathodium tuberosum* Kash., and *Anthoceros erectus* Kash. Against two strains of laboratory pathogens includes *Rhizopus* spp and *Alternaria* spp. The zone of inhibition measured is summarized in table no 2. Zone of inhibition of *Cyathodium tuberosum*is smaller against *Rhizopus* spp and larger against *Alternaria* spp.

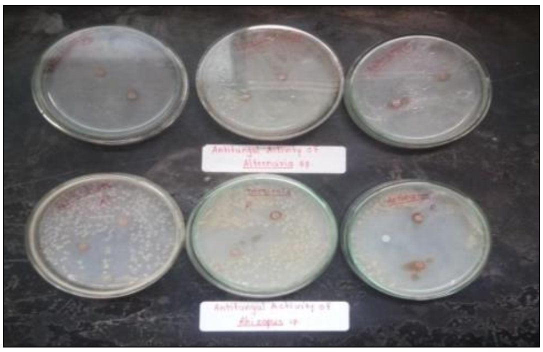

Fig 5: Anti. Fungal activity against *Rhizopus* sp. and *Alternaria* sp.

Anti-fungal activity

Table 2: Antifungal activity

Bryophytes	Fungi	Zone of inhibition (mm)
Targionia indica Kash.	*Rhizopus* spp.	8
	Alternaria spp.	11
Cyathodium tuberosum Kash.	*Rhizopus* spp.	6
	Alternaria spp.	13
Anthoceros erectus Kash.	*Rhizopus* spp.	12
	Alternaria spp.	8

Conclusion

Plant-mediated metallic nanoparticle synthesis has had a significant impact in the field of bio nanotechnology, but work on green synthesis by bryophytes has been restricted. As a result, the current study focuses on the synthesis and characterization of silver nanoparticles derived from moss (bryophyte). Bryophytes are discovered to be a novel source of silver nanoparticle biosynthesis. The extraction and synthesis of nanoparticles is a simple process due to the simple organization of thallus. The nanoparticles exhibit antimicrobial activity and are extremely harmful to microorganisms. Silver nanoparticles have inhibitory and microbial effects, which expands their use as antimicrobial agents. The nanoparticles have antifungal activity and are used on a large scale to prevent fungal infections, especially in the pharmaceutical industry.

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