

Bio-fabrication of bio-inspired silica nanomaterials from *Bryophyllum pinnatum* leaf for agricultural applications

M Sankareswaran

Muthayammal College of Arts and Science

Rajiv Periakaruppan (✉ rajivsmart15@gmail.com)

Karpagam Academy of Higher Education: Karpagam University

M. Sasivarnam

Karpagam Academy of Higher Education: Karpagam University

Jeyapragash Danaraj

Sathyabama Institute of Science and Technology: Sathyabama Institute of Science and Technology

Sugapriya Dhanasekaran

University of Bisha

Mosleh Mohammad Abomughaid

University of Bisha

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Abstract

Green chemistry approach was employed to synthesize Silica nanoparticles (SiNPs) using aqueous extract of *Bryophyllum pinnatum* leaf as capping agents. The physicochemical properties of formed nanoparticles were successfully completed through sophisticated characterization methods such as UV-Visible absorbance spectroscopy, Fourier Transform Infra-Red spectroscopy, X-Ray Diffraction, Scanning Electron Microscope, Energy Dispersive X-Ray, Zeta Potential Analysis and Thermo-Gravimetric Analysis. All the characterization results were indicating that the spherical morphology, amorphous nature with an average size of 24 nm of nanoparticles formed. FT-IR results highlighted the key bioactive compounds that could be responsible for capping and reduction to formation of SiNPs. As synthesized SiNPs shows excellent stability with negative zeta potential value - 32 mV. The biomolecules from *B. pinnatum* were successfully working for the formation of SiNPs with spherical shape. Moreover, to assess agricultural application of green synthesized SiNPs was carried out by seed germination assay on *Vigna radiata*. The seed germination assay confirms that the low concentration of SiNPs enhances the seed germination. Meanwhile, the higher concentration of the SiNPs inhibits the seed germination, shoot and root formation. SiNPs at optimum concentration could be used in the agriculture field as nano growth promoters.

Introduction

The field of nanoscience and nanotechnology has the potential for revolutionizing the methods to create the novel materials. It will give the significant impact on biological sciences and increase its values exponentially in the future (Kumar and Kumbhat, 2016; Hulla et al., 2015). These unique nanomaterials have wide-ranging physicochemical properties and are extensively used in food packaging industries, agricultural industries, biosensors, biomedical, textile and environmental applications (Hulla et al., 2015; Nguyen et al., 2018).

Among all the available nanoparticles, inorganic mesoporous SiNPs are the newcomers to the field, contributing with their unique physical and chemical properties and excellent properties (small size, strong surface energy, high scattered performance, and thermal resistance) (Ma et al., 2013; Wang et al., 2015). SiNPs have numerous applications, including drug delivery, biosensing, catalysis, bioimaging, and energy storage (Nandanwar et al., 2013; Kordasht et al., 2020). Silicon plays a vital role in increasing plant tolerance against pests and diseases. Mesoporous SiNPs were used as a solid adsorbent for the removal of lindane pesticides from aqueous solutions (El-Said et al., 2018). The synthesis, properties, and applications of SiNPs have become a quickly expanding field of research (Nandanwar et al., 2013). The SiNPs have been produced from agricultural wastes (Adebisi et al., 2019). Stober process is used for the preparation of monodispersed silica colloids 'white carbon black' by hydrolysis of alkyl silicates and silicic acid in alcoholic solutions using ammonia as catalyst (Ibrahim and Zikry et al., 2010).

Synthesis of metal/metal oxides nanomaterials, hybrid materials, and bioinspired materials using plants and microbes/other natural resources has received significant attention as a reliable, sustainable, cost-

effective and an eco-friendly technology in nanoscience alternative to chemical and physical methods (Singh et al., 2018; Parveen et al., 2016).

Bryophyllum pinnatum (Fig. 1) is commonly known as *Pattharcaṭṭa*. It belongs to Crassulaceae family. The herb contains a wide range of valuable chemicals that could be responsible for its various pharmacological effects (Latif et al., 2019). It is mainly used as a natural anti-inflammatory agent (Fernandes et al., 2019). It is used in ethnomedicinal practices to treat kidney stones, jaundice, skin diseases, hypertension, and urinary problems (Oufir et al., 2015; Yadav et al., 2016). The leaves of *B. pinnatum* possess both sedative and muscle relaxant properties (Von Manitiuset al., 2019)

This study focused on the green synthesis of SiNPs using extract of

B. pinnatum by sustainable approach, simple, cheap, and environmentally friendly manner. In addition, the current research aimed to investigate the effect of SiNPs on seed germination of *Vigna radiata* and its development of root and shoot after the germination.

Materials And Methods

The analytical grade chemicals and reagents were used in the experimental studies. Tetra Ethyl Ortho Silicate (TEOS), Hydrochloric acid, Ethanol and Double distilled water (DDW) were obtained for this investigation. All the glasswares were soaked in acids and washed with distilled water.

PREPARATION OF PLANT EXTRACT AND PHYTOCHEMICAL SCREENING

Fresh and healthy leaves were collected from villages of south Coimbatore (District), Tamilnadu, India, on January 2021. The leaves were cleaned with running tap water to remove debris. The aqueous extract was made by mixing and macerating 10 grams of fresh *B. pinnatum* leaves with 200 ml of double-distilled water. The mixture was boiled for 20 minutes at 50°C. The extract was cooled down and filtered by Whatman filter paper, and the extract was stored at 4 °C for further analysis. The phytochemicals of *B. pinnatum* were screened by the methods of Harborne (1998).

SYNTHESIS OF SiNPS

Tetra Ethyl Ortho Silicate (TEOS) is a precursor for the production of SiNPs. 20 mL of plant extract was mixed with 12 mL of precursor solution and allowed continuous stirring at 50 to 65°C for 10 min. Then, 1 M HCl was mixed on the above mixture to reduce the impurities and attain the highly purified silica nanomaterials. After 20 min, jelly-like precipitation was formed. Next, the precipitation was dried in a hot air oven. Finally, the white color power was obtained and stored in a sterile air tight container for further analysis.

CHARACTERIZATION OF SYNTHESIZED SiNPs

Various techniques were used to characterize the synthesized biogenic SiNPs. The optical property of biogenic SiNPs was determined by the UV-Visible Double beam spectrophotometer. The nature of biogenic SiNPs was determined by the X-ray diffractometer. The atomic percentage of elements present in the biogenic silica nanomaterials was determined by EDX. The surface morphology of SiNPs was assessed by the SEM analysis. The functional group of SiNPs and aqueous plant extract were identified by the FT-IR spectrometer. The charge of SiNPs was determined using a Malvern particle size analyzer. Thermal stability of synthesized SiNPs was analyzed by Mettler-Toledo TGA.

AGRICULTURAL APPLICATIONS

SEED GERMINATION ANALYSIS

Seeds of *Vigna radiata* were used for this investigation. Four different concentrations (25, 50, 75, and 100 µg/mL) of SiNPs were prepared using distilled water. The seeds were surface sterilized using distilled water. Meantime, the sterile Petri plates were taken, and cotton was placed on them. Then, the 15 seeds were placed on respective plates. A 15 mL of biogenic SiNPs at various concentrations was poured on seeds in corresponding plates. Positive control (distilled water) was maintained. Next, the plates with seeds were incubated in the dark condition at 37°C for five days. After incubation, the germinated seeds were counted, and the root and shoot length were measured. Five replications were used for this analysis.

Results And Discussion

PHYTOCHEMICAL ANALYSIS

Table 1 determined the phytochemicals analysis of *B. pinnatum* (aqueous extract). The steroids, alkaloids, flavonoids, tannins, saponins, and reducing sugar were found in *B. pinnatum* leaf extract. Terpenoids and Glycosides were absent in the extract. Kavita et al., (2013) reported the medicinally active phytoconstituents like tannins, alkaloids, terpenoids, steroids, and saponins in the leaves of *Phyllanthus fraternus*.

CHARACTERIZATION OF SiNPs

UV-Visible absorbance spectroscopy is broadly being utilized as a technique to determine the optical properties of nanosized particles. The result obtained from the analysis of UV-Visible spectroscopy of the sample is presented in Figure 2. The SiNPs formation was confirmed by the peak occurrence in the range between 230 to 300 nm in UV spectra because of the Surface Plasmon Resonance nature of silica nanoparticles in the reaction medium. The optical feature is similar to earlier report (Djangang et al., 2015) and indicated to Si-O-Si bond approving the existence of silica nanoparticles. Babu et al., (2018) were analysis the optical properties of *Cynodon dactylon* assisted silica nanoparticles using the UV-Visible spectroscopy and obtained the peak at wavelength at 350 nm in UV spectra.

The nature of biogenic SiNPs was evaluated from the X-Ray Diffraction pattern. The most substantial peaks at 2theta values of 22.01 correspond to the amorphous nature of SiNPs and are shown in Figure

3. The average size of the SiNPs was calculated by Debye-Scherrer's formula. The size of the *B. pinnatum* leaf extract mediated SiNPs is 24 nm. Mohd et al., (2017) carried out the XRD analysis to find out the crystalline nature and size of the sugarcane bagasse mediated nanomaterials. The XRD was used to confirm the formation of amorphous silica nanoparticles from palm kernel shell ash by the modified sol-gel extraction method (Imoisili et al., 2020).

The elemental composition and purity of *B. pinnatum* leaf extract mediated SiNPs were determined by the EDAX analysis. Figure 4 shows the EDAX spectrum for phyto-genic mediated SiNPs. The atomic percentages of carbon (21.68%), oxygen (50.95%), and silica (27.37%) were present in green synthesized SiNPs. The carbon was derived from plant extract. The SiNPs were synthesized by Dubey et al., (2021), and they assessed the level of its composition. They reported the presence of silica and oxygen with no other impurities.

The shape or morphology of the synthesized SiNPs was determined by the SEM analysis. The microscopic images of *B. pinnatum* leaf extract mediated SiNPs are presented in Figure 5 and clearly show the distribution and spherical shape of the SiNPs. The synthesized silica nanoparticles were spherical in shape and agglomerated because of existence of biomolecules from *B. pinnatum* leaf extract. Adebisi et al., (2020) reported the production of biogenic SiNPs using maize stalk and determined its morphology using the SEM analysis. It is worthy to note that the outcome of SEM analysis is compared to Zamani et al., (2020), and spherical shaped silica nanoparticles have been produced using the extract of *Saccharomyces cerevisiae* confirmed by SEM analysis.

FT-IR spectra of plant extract and SiNPs are shown in Figures 6 *a* and *b*. The peaks such as 3348, 2970, 2885, 1921, 1658, 1442, 1087, 879, 671, and 555 cm^{-1} are in Figure 4 *a* and correspond to the hydroxyl, amide, and carboxyl functional groups. The spectrum of SiNPs shows the peaks such as 2978, 2893, 1589, 1396, 1143, 1072, 956, 678, and 555 cm^{-1} . Agreeing to result of FT-IR (Figure 4a) it was detected that the chemical bond of silicon and oxygen in the biogenic silica nanoparticles. The peaks of 1072, 956, 678, and 555 cm^{-1} are referring to the Si-O-Si bonds. The intense peaks of 2978, 2893, 1589, 1396, 1143 cm^{-1} were corresponding to C-H stretching, hydroxyl, amide, and carboxyl functional groups which were derived from *B. pinnatum* leaf extract. FT-IR study was determined the functional groups of capping, reducing, and stabilizing agents from *B. pinnatum* leaf extract to form the nanomaterials. Anuar et al., (2020) were found the functional groups or chemical groups, namely Si-O-Si, CH_2 , -OH, and Si-OH form the FT-IR spectrum of coconut husk ash mediated silica nanomaterials.

Zeta potential analysis is a traditional method to determine the stability of the nanomaterials. The SiNPs from *B. pinnatum* show negative charges and the zeta potential value of -32.4 mV (Figure 7). The high value of zeta potential refers to the stability of the suspension due to the increased force of electrostatic repulsion between the particles. The low zeta potential value indicates the aggregation of the nanomaterials (Wang et al., 2010). The SiNPs with antibacterial properties were produced by Joni et al., (2020). In addition, they reported the zeta potential value of -24.69 mV for the SiNPs.

Thermal stability of synthesized SiNPs was performed to determine the weight loss of green synthesized SiNPs at different temperatures (the range between 30 to 1000°C) (Figure 8 a & b). It was obtained that, mass of the SiNPs decreased in two stages on different temperature range of 30°C - 800°C. At first stage, 53.8% of mass loss was acquired at a temperature of 250°C because of evaporation of water molecules on surface of silica nanoparticles. In the second stage, about 7.6% of mass loss was obtained at the temperature ranges from 300 – 800°C due to the vaporization of the remaining residue of the phyto-compounds. The mass reduction of bio-synthesized silica nanomaterials was assessed by the exothermic peak in the DTA curve. The similar results were reported by Maroušek et al., (2022) and Sankareswaran et al., (2022).

AGRICULTURAL APPLICATIONS

SEED GERMINATION ASSAY

Different concentrations (5, 10, 15, and 20 µg/mL) of SiNPs were used in the present investigation. The maximum level (100%) of seed germination was achieved on 5 µg/mL SiNPs treated treatment, and meanwhile, the minimum level (40%) of seed germination was observed on 20 µg/mL of SiNPs treated treatment. The increased shoot length of 4.3 cm was observed on the treatment of 5 µg/mL SiNPs, and decreased shoot length of 1.8 cm was achieved with a concentration of 20 µg/mL of SiNPs. The highest root length (1.0 cm) was observed on T2 treatment (5 µg/mL of SiNPs), and the lowest root length (0.2 cm) was recorded on T4 treatment (20 µg/mL of SiNPs) (Table 2). Results showed that SiNPs have a significant effect on seed germination, the length of the shoot, and the root. Roohizadeh et al., (2015) observed that SiNPs improved the seed germination on *Vicia faba*.

Conclusion

This investigation has been performed to produce highly stable silica nanoparticles in a simple, bio-based, less toxic and environmental friendly to study its effects on enhanced seed germination and growth of shoot and root formation. The aqueous extract of *B. pinnatum* leaf has been used as capping and reducing agents for green synthesis of SiNPs. The phyto-constituents namely steroids, alkaloids, flavonoids, tannins, saponins, and glycosides were present in leaf extract. Bio-synthesized silica nanomaterials were characterized by various techniques. Spherical and amorphous nature biogenic SiNPs were produced with an average size of 24 nm. The green synthesized SiNPs enhanced seed germination, shoot, and root formation at low concentration levels. So, SiNPs could be used to improve seed germination and crop production in agriculture areas.

Declarations

DECLARATION OF INTERESTS

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval – Not applicable

Consent to participate – Not applicable

Consent for publication – Not applicable

Availability of data and materials – Not applicable

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

SM: Conceptualization

PR: Supervision, Funding acquisition and Project administration

MS: Investigation

JD: Methodology

SD: Data Curation and Writing- Original draft preparation

MMA: Data Curation and Writing- Original draft preparation

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Please include the sub-sections below of Compliance with Ethical Standards section. – Not applicable

Disclosure of potential conflicts of interest – Not applicable

Research involving Human Participants and/or Animals – Not applicable

Informed consent – Not applicable

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Tables

Table 1. Screening of phytochemicals in *B. pinnatum* extract.

S.No	Qualitative test	Colour Obtained	Aqueous extract (<i>B. pinnatum</i>)
1.	Steroids	Blue green	Presence
2.	Alkaloids	Orange to Red	Presence
3.	Flavonoids	Yellow	Presence
4.	Terpenoids	Red, Pink or Violet	Absence
5.	Tannins	White precipitate	Presence
6.	Saponins	Foam formation	Presence
7.	Glycosides	Blue or Green	Absence
8.	Reducing sugar	Yellow to Orange	Presence

Table 2. Analysis of seed germination, root and shoot length on Silica nanoparticles treated *Vigna radiata*.

S.No	Concentrations	% of seed germination	Shoot length (cm)	Root length (cm)
1	Control – Distilled water	100%	4.0 ± 0.1	1.0 ± 0.2
2	T1 – 5 µg/mL	100%	4.3 ± 0.1	1.5 ± 0.1
3	T2 - 10 µg/mL	70%	3.4 ± 0.2	0.8 ± 0.1
4	T3 - 15 µg/mL	50%	2.0 ± 0.3	0.5 ± 0.1
5	T4 - 20 µg/mL	40%	1.8 ± 0.1	0.2 ± 0.1

Figures

Figure 1

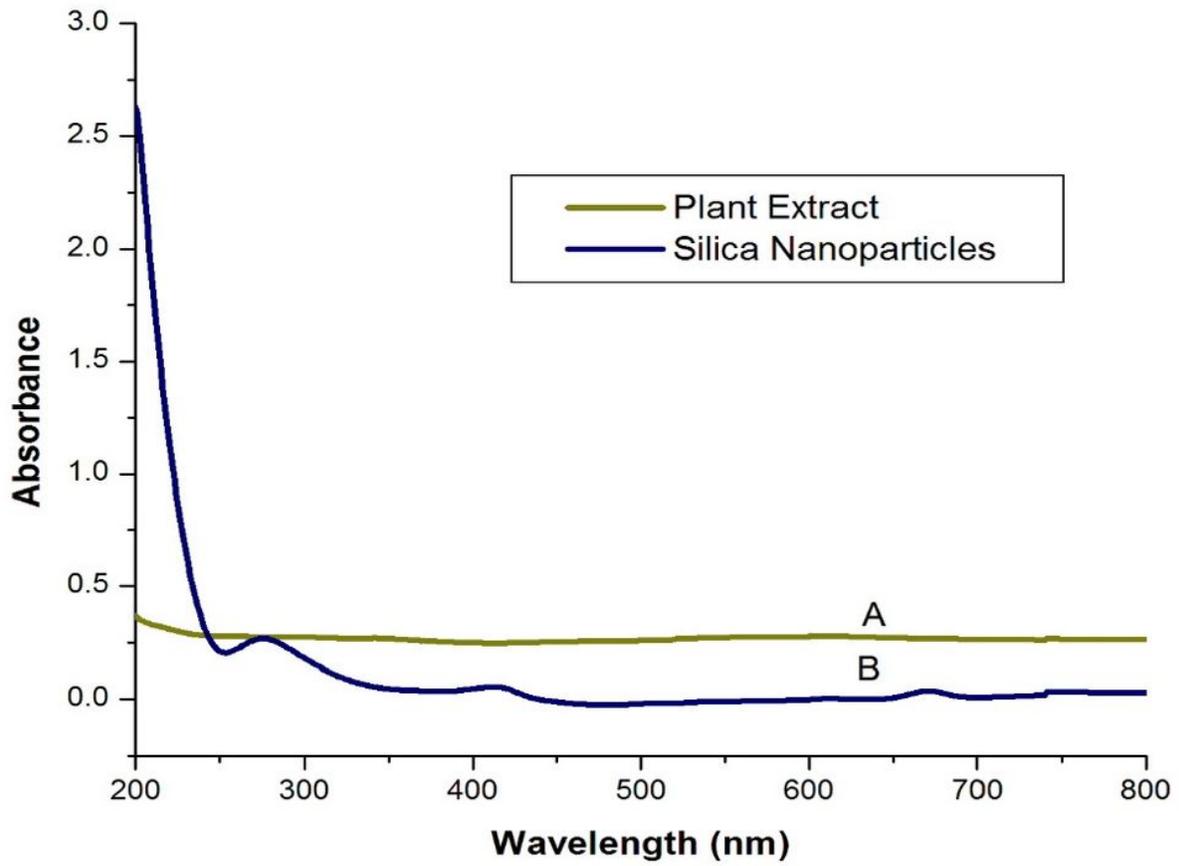


Figure 2

UV spectra of a) plant extract and b) Silica nanoparticles

Figure 3

XRD spectrum of Silica nanoparticles

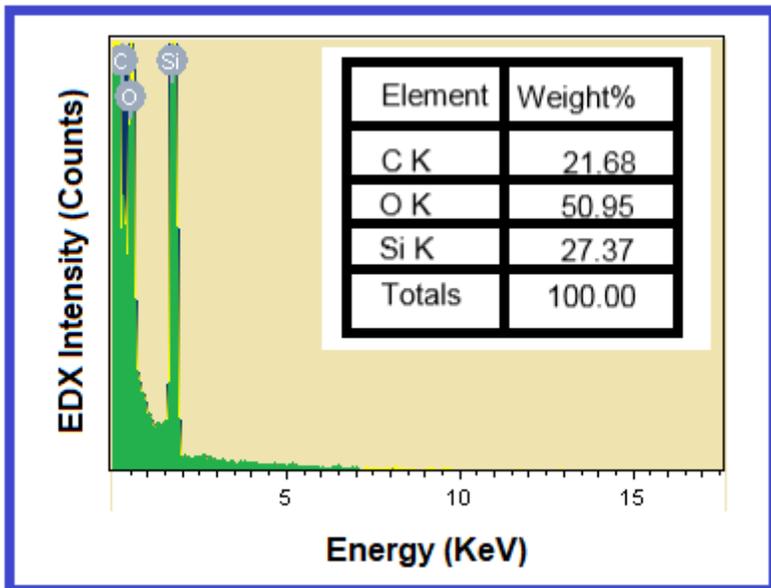


Figure 4

EDX spectrum of Silica nanoparticles

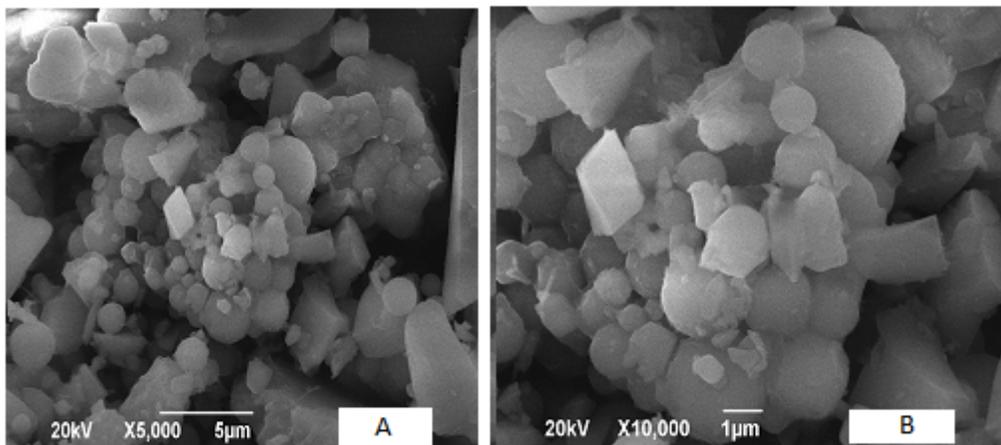


Figure 5

SEM images of Silica nanoparticles

Figure 6

FT-IR spectrum of a) Silica nanoparticles and b) plant extract

Figure 7

Zeta potential analysis of Silica nanoparticles

Figure 8

a. TGA/DTG/DTA analysis of Silica nanoparticles

b. TG analysis of Silica nanoparticles