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RESEARCH

Production and Characterization of *Azadirachta indica*-Mediated SiO_2 Nanoparticles and an Evaluation of Their Antioxidant and Antimicrobial Activities

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Abstract
Azadirachta indica leaf extract was used in the current study to create silicon dioxide (SiO_2) nanoparticles securely. The unique aspect of this research is to utilize the biomass of *A. indica* as a capping, reducing, and stabilizing agent to produceSiO_2 nanoparticles with potential antioxidant activity. The size, functional groups, shape, purity, and stability of the as-produced nanoparticles were analyzed using spectroscopic and microscopic assessments. In addition, the antioxidant and antimicrobial activity were determined by DPPH assay and agar well diffusion method. As-synthesized SiO_2 nanoparticles were spherical, extremely stable, and amorphous nanoparticles with an average size of 22 nm. FT-IR analysis reveals that the occurrence of Si–O confirms the formation of SiO_2 nanoparticles. The purity of silicon dioxide nanoparticles wa sinvestigated by EDX analysis through the presence of Si and O. Thermal stability was determined and investigated by TGA analysis. DPPH analysis and Agar well diffusion assay confirmed that *A. indica*-mediated SiO_2 nanoparticles show good antioxidant and antimicrobial activity. The end product of biogenic SiO_2 nanoparticles may be useful for the formulation of new drugs.

Keywords *Azadirachta indica* · SiO_2 nanoparticles · FTIR · TGA · Antioxidant · Antimicrobial activity

1 Introduction

The term "nanotechnology" applies to atomic, molecular, and macromolecular scale research and technology development that enables the controlled manipulation and study of structures and devices with length scales between one and one hundred nanometers [1]. A potential tool for sustainable agriculture is nanobiotechnology. However, some nanoparticles with special physiochemical properties naturally promote crop improvement and resilience to stress rather than serve as nanocarriers [2]. Because of the potential to use chemical innovation to concurrently achieve environmental and economic objectives, green chemistry is a relatively new field that aims to work at the molecular level to achieve sustainability [3].

According to the World Health Organization, "good health" is a condition of physical and emotional well-being that is unaffected by any illness or disease [4]. This state was specifically described in ancient Sanskrit as "Nimba," which subsequently became Neem [5]. Neem has become popular in modern medicine due to its widespread use in Ayurveda, Unani, and

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Table 1 Phyto-constituents analysis of aqueous extract of *A. indica*

S.no	Phyto-constituents	Aqueous extract
1	Tannins	Present
2	Glycosides	Present
3	Saponins	Present
4	Reducing sugar	Present
5	Steroids	Absent
6	Alkaloids	Absent
7	Flavonoids	Absent

Homoeopathic systems of medicine. Neem (*A. indica*) produces a wide range of chemically varied and structurally complicated biologically active compounds. From various neem plant sections, more than 140 different compounds have been discovered [6]. In the past 50 years, significant advancements have been made in the study of neem's biological activity and its potential uses as a medicine, in addition to the chemistry of its components. Herbalists in Southeast Asia have used the plant *A. indica* for millennia to shrink tumors, and research suggests that prostate cancer cells (PC-3) are killed by an ethanolic extract of neem [7]. It is now regarded as a valuable source of distinctive natural products for the creation of both industrial goods and medicines to treat a variety of diseases [8]. In addition to its effectiveness as a medicine, neem has already demonstrated its promise as a source of naturally occurring pesticides, insecticides, and agrochemicals [9].

Pansambal et al. [10] reported that the green synthesis approach offers eco-friendly, low-toxic, less expensive, biocompatible, and efficient nanomaterials. No toxic wastes and by-products are produced during the synthesis, which doesn't require purification processes [11]

T. absoluta infection is significantly reduced by the use of silica nanoparticles [12]. Using silica nanoparticles, novel classes of fluorescent labels are created. As it has a multifunctional potential it is used in various applications such as Barcoding tags, DNA and microarray detection, single bacterium detection, and cancer cell imaging [13]. It has been reported that mesoporous silica nanoparticles created through hydrothermal treatment are stable for long in a variety of environments at both room temperature and body temperature. Highly regulated mesoporous silica nanoparticles exhibit substantially improved biocompatibility and reduced macrophage uptake when compared to bare mesoporous silica nanoparticles, making them suitable for in vivo hidden drug delivery functions [14].Though it is used in drug delivery researchers have proved it as a hepatotoxicant based on its chemical modification, size, and structures [15]. Silica nanoparticles in agriculture conform to the literature pertinent to the use of nanoparticles as soil-improving agents, pesticides, genetic and drug transfer agents, fertilizers, tools for soil analysis, and herbicides [16].

The green synthesis of SiO_2 nanoparticles from the leaf extract of *A. indica* (neem) and the characterization of as-synthesized SiO_2 nanoparticles using various microscopic and spectroscopic methods are the subjects of the current study. Additionally, the antioxidant capacity of SiO_2 nanoparticles was performed.

2 Materials and Reagents

All the materials chemicals, glassware, reagents, and solvents of 99% of purity used in the research, and all chemicals and reagents were obtained from Sigma-Aldrich.

2.1 Sample Collection

In the middle of March 2023, fresh and healthy leaves of the *A. indica* were collected from in and around PSG College of Arts & Science, Civil Aerodrome, Coimbatore, Tamilnadu, India (11.0328° N, 77.0349° E).

2.2 Preparations of Aqueous Extract Using *A. indica* Leaves and Analysis of Phytoconstituents

The collected leaves were washed three times in flowing tap water and then with distilled water. 10 g of cleaned leaves were ground using a mortar and pestle and 100 mL of distilled water [17]. The extract was boiled for 15 min in a water bath under 75ºC. Later, the extract was then filtered using Whatman No. 1 filter paper, and the purified extract was stored at 4 °C for

Table 2 Comparative study of green synthesized SiO_2 nanoparticles and other methods

S.NO	Parameters	Green synthesis of SiO_2 nanoparticles	Other methods SiO_2 nanoparticles
1	Protocols/Steps/Procedures	Easy	Complex
2	Usage of toxic agents	Low	High
3	Quantity of chemical consumption	Less	High
4	Toxicity level	Low	High
5	Yield of the end product	High	Moderate
6	Biological activity	High	Low
7	Time consumption	Quick process	Time taking process

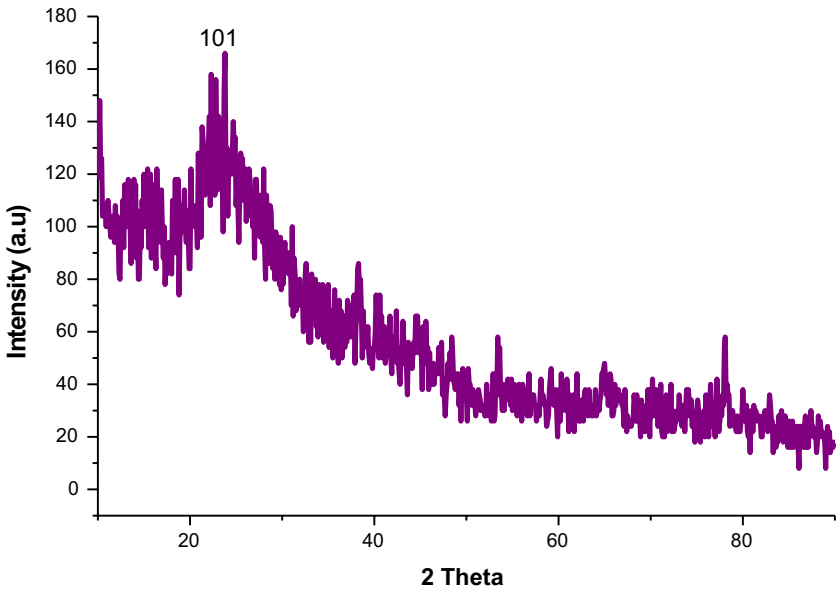


Fig. 1 XRD analysis of *A. indica*-mediated SiO_2 nanoparticles

further analysis [18].The phytoconstituents such as tannins, glycosides, saponins, reducing sugar, steroids, alkaloids, and flavonoids were assessed using standard protocols [19].

2.3 Synthesis of SiO_2 Nanoparticles

For the production of SiO_2 nanoparticles, Tetraethyl orthosilicate was used as a precursor. 10 mL of Ethanol, 5 mL of distilled water, and 20 mL of *A. indica* aqueous extract were mixed with 12 mL of Tetraethyl orthosilicate. The mixture was kept in a magnetic stirrer at 70ºC for 10 min. 1 ml of hydrochloric acid was added carefully. Finally, white color precipitation was obtained. Then it was dried using a hot air oven to completely remove the moisture and the resulting powder was collected and stored.

2.4 Characterization

The bio-reduction and formation of SiO_2 nanoparticles were analyzed by using various spectroscopic methods. Fourier Transmission Infra-Red analysis (FTIR) was used to identify whether bio-molecules were present in the purified SiO_2 nanoparticles (Thermo scientific Nicolet 380 FT-IR Spectrometer) [20]. X-ray diffraction (XRD) analysis was used to determine the SiO_2 nanoparticles' size and nature. The facts were evaluated with the help of Origin software (version 9.0) [21]. By using field emission scanning electron microscopy (FESEM) with energy dispersive x-ray spectroscopy (EDX) the morphology and purity of the SiO_2 nanoparticles were evaluated. Thermogravimetric analysis (TGA) was used to examine the thermal stability and volatile compound content of the synthesized SiO_2 nanoparticles [13].

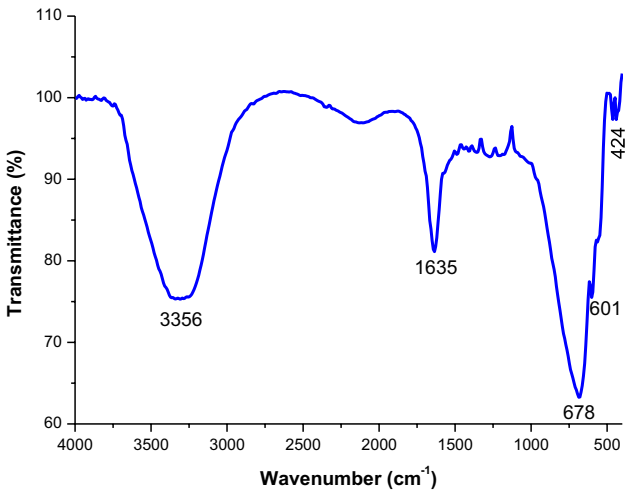


Fig. 2 FT-IR analysis *A. indica* leaf extract

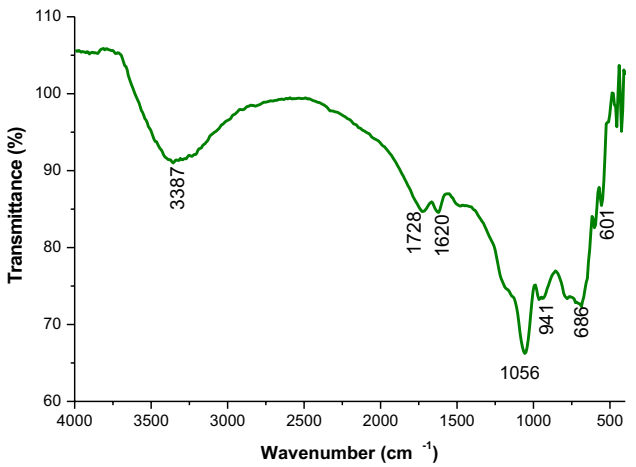


Fig. 3 FT-IR analysis *A. indica*-mediated SiO_2 nanoparticles

2.5 Antioxidant Activity

For the antioxidant assay, various concentrations of SiO_2 nanoparticles were prepared (50 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml). A test for DPPH (2,2-Diphenyl-1-picrylhydrazyl) and scavenging assay was used. Three replications were employed [22]. The formula that is used to measure the free radical inhibition is:

$$\% = (\mathbf{A}_0 - \mathbf{A}_1)/\mathbf{A}_0\mathbf{X}100$$

where, % = Scavenging activity, A_0 = Absorbance of the control, and A_1 = Absorbance of the sample.

2.6 Analysis of Antibacterial and Antifungal Properties

Antibacterial and antifungal properties of *A. indica*-mediated SiO_2 nanoparticles were studied by the Well diffusion method. Different concentrations of *A. indica*-mediated SiO_2 nanoparticles such as 10 µg/mL, 20 µg/mL, and 30 µg/mL were employed. Tetracycline and Amphotericin B were used as a positive control. Muller Hinton Agar plates were prepared and 100 µl of microbes (*Escherichia coli* and *Aspergillus niger*) were swabbed. Later, the wells were created and various concentrations of *A. indica*-mediated SiO_2 nanoparticles were loaded. The plates were incubated and the zone of inhibitions was measured according to the protocol [3].

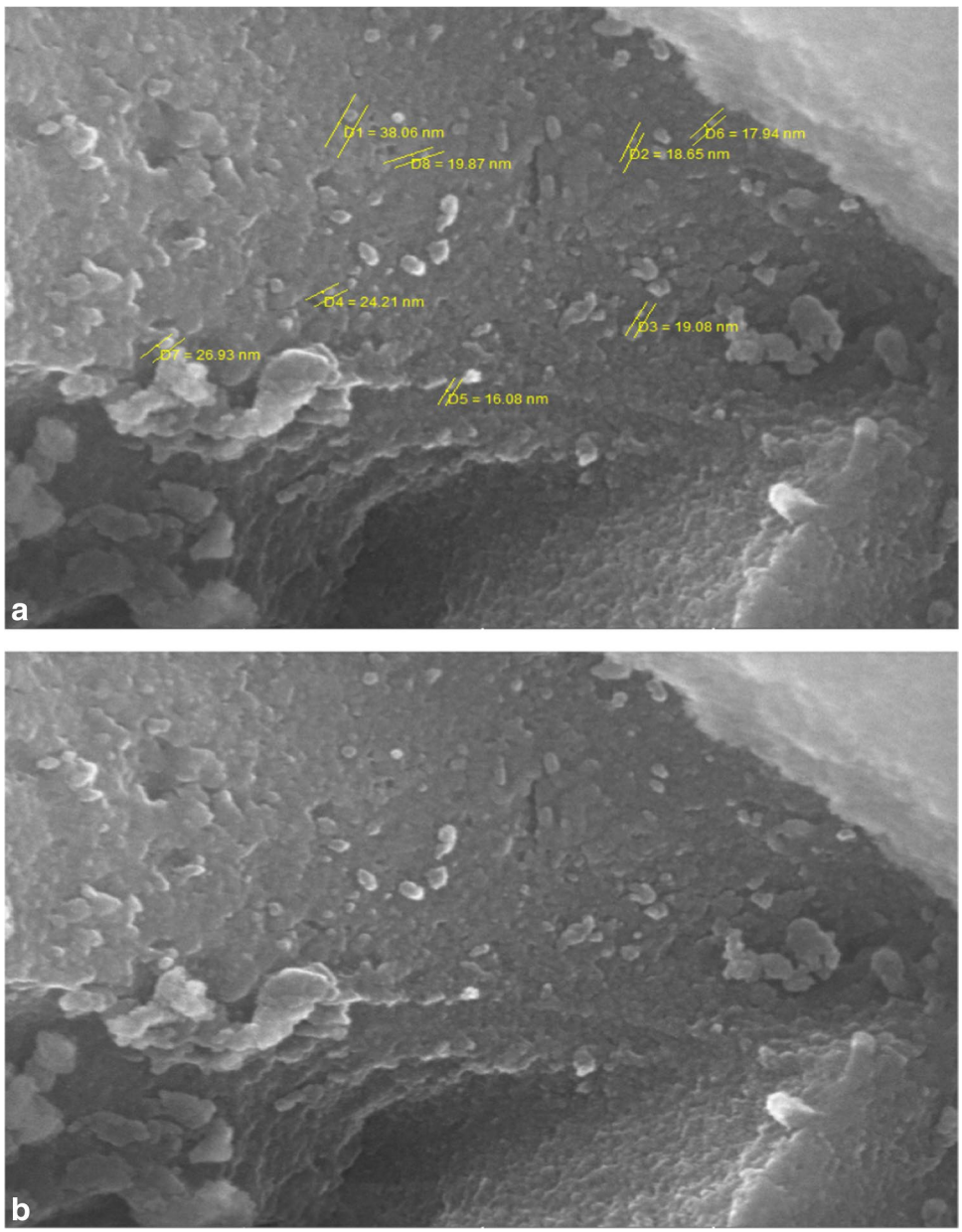


Fig. 4 **a** & **b** FESEM analysis *A. indica*-mediated SiO_2 nanoparticles

3 Results and Discussion

3.1 Analysis of Phyto-Constituents in *A. indica*

Table 1 shows the presence of phyto-constituents such as tannins, glycosides, saponins, and reducing sugar in the aqueous leaf of *A. indica.* Similar results were reported by Itelima et al. [23] and they confirm the presence of alkaloids, flavonoids, steroids, reducing sugar, glycosides, saponins, and tannins.

3.2 A Mechanism for the Synthesis of SiO_2 Nanoparticles

The synthesis of SiO_2 nanoparticles was followed by hydrolysis and condensation of tetra-ethyl-ortho-silicate in the presence of Hydrochloric acid as a catalyst [24]. The phyto-constituents in *A. indica* act as reducing, coupling, and capping agents during the synthesis to form the SiO_2 nanoparticles. The reducing agents of phyto-constituents from *A. indica* were controlling the size and shape of the SiO_2 nanoparticles. Table 2 determines the comparative analysis of green synthesized SiO_2 nanoparticles and chemical methods.

3.3 Characterization of *A. indica*-Mediated SiO_2 Nanoparticles

3.3.1 X-Ray Diffraction Analysis

Figure 1 displays the x-ray diffraction spectra of *A. indica*-mediated SiO_2 nanoparticles. XRD spectra show a wide band with a reflection at $2\theta = 101°$ peak. It confirms the formation of amorphous SiO_2 nanoparticles that are free from impurities [25].The core area of the composite particles was identified by X-Ray Diffraction patterns as amorphous SiO_2 nanoparticles [26–28].

3.3.2 FTIR Spectroscopy Analysis

The vibration frequency bands at 3356 cm^{-1}(Amide groups of proteins and enzymes), 1635 cm^{-1} (Diketones), 678 cm^{-1} (C–Cl), 601 cm^{-1} (C-I), and 424 cm^{-1} (Halogen compounds) were found in FTIR spectra of the *A. indica* leaf extract (Fig. 2) [29]. Figure 3 displays asymmetric and symmetric stretching peaks at 1620 and 1056 cm^{-1}, respectively, whereas the amorphous stretching and bending were seen at 3387 and 1728 cm^{-1}, respectively. There were maxima for the asymmetric extending at 941 cm^{-1}, 686 cm^{-1}, and 601 cm^{-1}, which is very similar to a previous report [30].

3.3.3 FESEM Analysis

Micrographs of *A. indica*-mediated SiO_2 nanoparticles from field emission scanning electron microscopy are shown in Figs. 4a and b. The greater magnification of FESEM images clearly shows the spherical and clumped morphologies of SiO_2 nanoparticles. Strong hydrogen bond interactions in the precipitate during biological synthesis may have led to these clumped formations. The typical size of the nanoparticles in the SiO_2 sample was estimated to be 22.15 nm [31]. So, the size distribution of SiO_2 nanoparticles and the FESEM findings are well congruent [32].

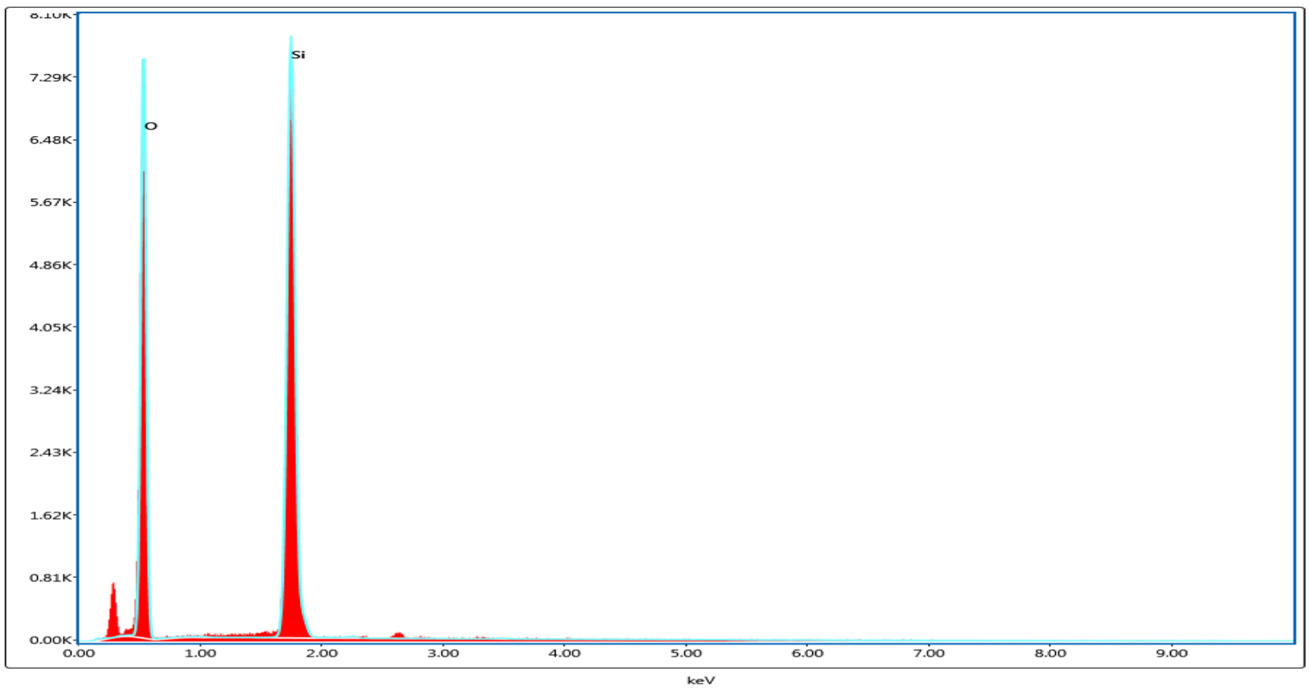
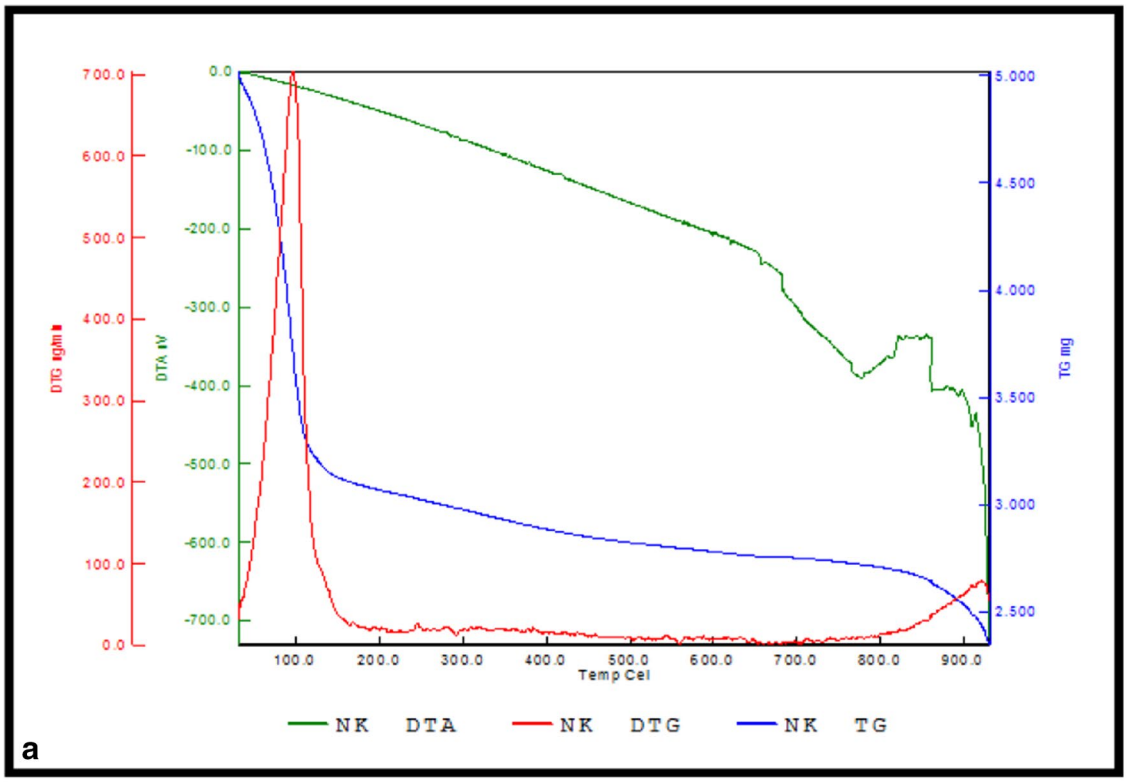


Fig. 5 EDX analysis *A. indica*-mediated SiO_2 nanoparticles



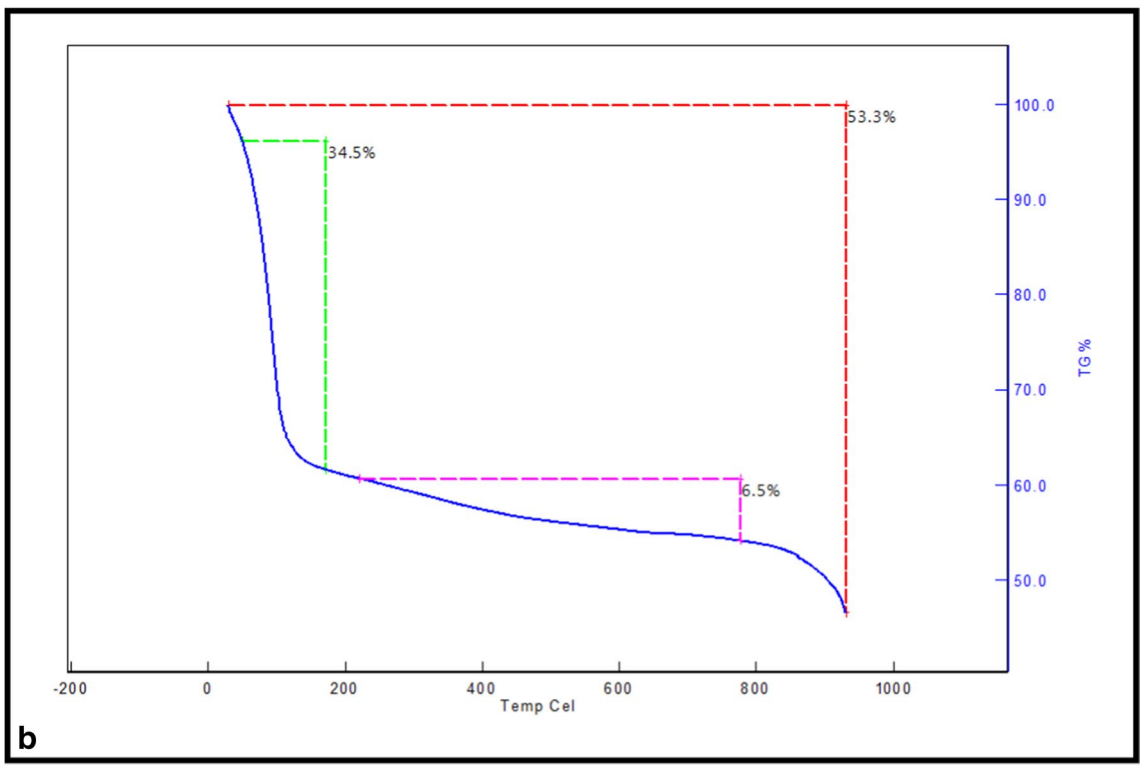


Fig. 6 **a** & **b** TGA analysis of *A. indica*-mediated SiO_2 nanoparticles

Table 3 Antioxidant (DPPH) assay for *A. indica*-mediated SiO_2 Nanoparticles

S.NO	Concentration (µg/ml)	% Inhibition	IC_{50} value
1	50	48.36	617.4923
2	250	52.46	
3	500	59.02	
4	750	63.93	

3.3.4 EDX analysis

According to Fig. 5, it is evident that green synthesized *A. indica*-mediated SiO_2 nanoparticles have silica and oxide. EDX profile displays the purity of SiO_2 nanoparticles and shows the peaks of Si and O. It has also been found that the elemental composition of silica was high. No impurity was found in as-synthesized SiO_2 nanoparticles. Periakaruppan et al. [33] produced biogenic silica nanoparticles via green chemistry approach and confirm their purity with the help of EDX analysis.

3.3.5 TGA Analysis

Thermogravimetric analysis (TGA) is an effective method to examine the thermal decomposition behavior of nanomaterials. The TGA curve in Figs. 6a and b determines that thermal transition takes place in two different temperature ranges (30–150 and 200- 750 °C). The first weight loss was 34.5%, which corresponded to the evaporation of water molecules and moisture content at 30 to 150 °C. In the second zone the loss of weight was 6.5% due to the loss of bio-molecules in *A. indica*-mediated SiO_2 nanoparticles and it was proved by the FT-IR analysis. A few researchers attributed weight loss in biologically-synthesized SiO_2 nanoparticles to the presence of water molecules, which was confirmed by the TGA analysis [34]. Sharma et al. [35] produced the *A. indica*-based thin film and determined its thermal stability by thermogravimetric analysis.

3.4 Antioxidant Analysis by DPPH Assay

Antioxidants are chemicals that can stop oxidation processes in their tracks. Free radicals can contribute to a number of pathological situations in cellular macromolecules. The DPPH technique is employed to evaluate the level of antioxidant activity [36]. The DPPH technique uses a nitrogen radical molecule called 2,2-diphenyl-1-picrihydrazyl. The hydrogen capture process by DPPH from the antioxidant itself forms the basis of this technique. By using the IC_{50} value (617 µg/ml), antioxidant activity can be measured (Table 3). The effective concentration of the *A. indica*-mediated SiO_2 nanoparticles that are needed to eliminate 50% of all free radicals is known as the IC_{50} value. It is very similar to the findings of Khorrami et al. [37].

3.5 Analysis of Antibacterial and Antifungal Properties

A. indica-mediated SiO_2 nanoparticles have good antimicrobial properties as displayed in Table 4. The growth of *Escherichia coli* and *Aspergillus niger* were suppressed by various concentrations of *A. indica*-mediated SiO_2 nanoparticles. Cell walls of microbes can be injured by the penetration of nanoparticles, which leads to cause the inhibition of microbial growth and death [38]. Similar results have also been reported by Otari et al., [38] who have proved the antimicrobial properties of the Ag–$GSiO_2$ nanomaterials against *Staphylococcus aureus* and *Escherichia coli*.

4 Conclusion

A. indica leaf extract was used in the present study to synthesize SiO_2 nanoparticles in such a way that it has been proved to be simple, environmental-friendly, cost-effective, and low-toxic. All the microscopic and spectroscopic analyses reveal that as-synthesized SiO_2 nanoparticles were both amorphous and spherical in nature with an average size of 22 nm. *A. indica*-mediated SiO_2 nanoparticles show good antioxidant and antimicrobial activity, which was confirmed by DPPH assay and well diffusion method. It may therefore be useful for finding new drugs that may be of use to improve human health too.

Table 4 Antibacterial and antifungal properties of *A. indica*-mediated SiO_2 Nanoparticles

S.NO	Name of the organisms	Zone of inhibition in diameter (mm)			
		10 µg/mL	20 µg/mL	30 µg/mL	Positive control (10 µg/mL)
1	*Escherichia coli*	8.1 ± 1.0	9.2 ± 1.0	10.2 ± 1.0	10 ± 1.0
2	*Aspergillus niger*	7.5 ± 0.5	8.0 ± 1.0	9.2 ± 0.2	12 ± 0.2

Authors' Contributions Mr. Naveen Kanna Duraisamy: Investigation.
Dr. Rajiv Periakaruppan: Conceptualization, Supervision, Original draft and Project administration.
Dr. Salwan Ali Abed: Data Curation.
Dr. Noura Al-Dayan: Data Curation.
Dr. Sugapriya Dhanasekaran: Data Curation.
Dr. Saad Hamad Abdullah Aldhayan: Data Curation.

Data Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Research involving Human Participants and/or Animals Not applicable.

Informed consent Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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.

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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