



Green Synthesis and Characterization of SiO₂ Nanoparticles Using *Tridax Procumbens* Leaf Extract and Enhancing the Invitro Wound Healing Activity in L929 Fibroblast Cell Lines

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Abstract

This study aims to synthesize silica nanoparticles (SiO₂-NPs) using *Tridax procumbens* leaves. Previous studies have demonstrated the effectiveness of the leaf juice of *Tridax procumbens* in treating and healing fresh wounds. SiO₂-NPs have been found to enhance topical wound healing by promoting the migration of skin fibroblasts. Therefore, the current research focused on the green synthesis of SiO₂-NPs from *Tridax procumbens* leaves extract and evaluating their scratch wound healing activities. The synthesized SiO₂-NPs were characterized using various techniques like XRD, UV-visible spectrophotometry, FTIR, FESEM, EDX, Zeta potential and TG analysis. The characterization analysis confirmed that the particles were spherical, containing silicon (Si) and oxygen (O), exhibiting an average size of 44 nm, and experiencing a mass loss of 48.9%. The antioxidant activity of the synthesized SiO₂-NPs was evaluated through the DPPH assay, revealing an IC₅₀ value of 48.8 µg/mL. This indicates their capability to diminish free radical generation and alleviate oxidative stress. Moreover, the study aimed to explore the efficacy of these nanoparticles in wound healing activity. The in vitro scratch assay was employed to assess wound closure of L929 fibroblast cell lines. The results revealed that the synthesized SiO₂-NPs showed higher wound healing activity (74%) in L929 fibroblast cell lines than the control at 24 h, indicating the ability of the synthesized nanoparticles in enhancing wound closure processes. Overall, this study analyses the potential of plant-mediated synthesis of SiO₂-NPs for biomedical applications, particularly in wound healing therapies.

Keywords *Tridax procumbens* · SiO₂-NPs · XRD · EDX · Scratch wound healing · (DPPH) assay

Introduction

Nanotechnology is defined by its focus on investigating and progressing at the levels of atoms, molecules, or macromolecules. Nanotechnology represents an innovative strategy for advancing technology, specifically in the manipulation of materials at the nanoscale (1–100 nm). The synthesis of nanoparticles involves diverse techniques aimed at controlling their size and structure on the nano-scale [1]. Nanoscale materials find applications across various fields such as electronics, magnetism, pharmaceuticals, biomedicine, cosmetics, environmental remediation, and materials science. The promising future of nanotechnology has been attributed to the increase in worldwide expenditure in research and development [2].

In recent years, biocompatible nanomaterials obtained by green synthesis methods have attracted increasing attention

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due to their environmental friendliness, low toxicity, and high efficiency in regenerative medicine [3–6]. Particularly promising are nanoparticles modified with plant extracts, which demonstrate pronounced antioxidant, antibacterial, and wound-healing properties. For example, ZnO nanoparticles synthesized using *Calendula officinalis* extract showed 90% viability of L929 cells at a concentration of 10 µg/mL and accelerated wound closure to 69.1% compared to the control (64.8%) [7]. Gold nanoparticles obtained using *Limonia acidissima* demonstrated no cytotoxicity and active migration of L929 cells in a scratch test [8]. Composite nanofibers with *Syzygium cumini* extract provided accelerated wound healing in vivo in rats within 14 days, and in vitro confirmed antibacterial activity and compatibility with fibroblasts [9]. Silver nanoparticles synthesized using *Scutellaria barbata* showed no toxicity and significantly accelerated cell migration in the L929 model [10, 11].

Nanoparticles can be synthesized through chemical, physical, and biological processes. Silica nanoparticles (SiO₂-NPs) are used across multiple domains, including the healthcare sector, such as in sensor technology, drug delivery, optical imaging, and diagnosis. Their ease of synthesis, low toxicity, affordability, stability, and high compatibility make them ideal for various applications. These nanoparticles exhibit polymer-like behavior and can have specific molecule sizes [12]. SiO₂-NPs are synthesized using top-down and bottom-up methods like physical and chemical methods, such as sol-gel, ball milling, precipitation, photobleaching, chemical vapor condensation, and microemulsion techniques. However, these techniques have drawbacks such as increased chemical usage, high costs, elevated operating temperatures, and the generation of potentially toxic by-products during synthesis [13–15].

Nanoparticles could alternatively be synthesized from cost-effective and ecologically friendly biological processes, employing materials such as microbes, plants, seaweed, and agricultural wastes [16]. The biosynthesis of nanoparticles follows a bottom-up approach, primarily involving reduction/oxidation reactions [17]. Green synthesis methods have numerous benefits and have certain drawbacks associated with the previously mentioned physical and chemical approaches [18]. This approach is cost-effective, time-efficient, and environmentally sustainable [19]. The green synthesized SiO₂-NPs are used in various applications such as agriculture, cosmetics, medicine, paints, pharmaceuticals, and electrical applications. Green-synthesized SiO₂-NPs have a long and successful application history in medicine, particularly in tissue engineering, wound healing, and targeted drug delivery [20].

Tridax procumbens is a prevalent weed found in the rice fields of India. It is commonly referred to as ‘Common button’ or ‘Coat button’. *T. procumbens* is a widespread weed

across India [21]. It belongs to the Asteraceae family. Previous research has shown that the leaf juice of *T. procumbens* is effective in treating wounds. It may be used as a hair tonic as well as for curing new wounds and stopping bleeding. This plant possesses antimicrobial, wound-healing, anti-inflammatory, and immune-modulatory properties [21].

Wound healing is an intricate process and involves multiple factors that lead to contraction, closure, and reconstruction of a functional barrier. This sequential process begins with hemostasis and inflammation, followed by the proliferation and migration of various cell types, facilitating remodeling, and ultimately resulting in tissue repair [18]. Interruptions in wound healing caused by infections lead to delays, emphasizing the need for managing and preventing infections, especially bacterial infections, to ensure rapid and effective healing. Many researchers have proposed evidence that supports the use of plant materials as topical antibacterial agents with wound-healing properties [22, 23]. In wound healing, antibacterial activity plays a critical role in preventing infections. Assessing antioxidant activities and wound scratch assays involves observing the impact of antioxidants on cell migration and wound healing. Skin fibroblasts exhibit morphological plasticity, and at every stage of tissue repair, which usually overlaps, skin fibroblasts are active [24].

To date, there are no data in the scientific literature on the synthesis of silicon oxide (SiO₂) nanoparticles using *Tridax procumbens* extract, as well as on their use to evaluate wound healing activity in vitro on the L929 fibroblast line, which emphasizes the novelty and relevance of this study. This investigation aimed to examine the wound healing capability of synthesized SiO₂-NPs using *Tridax procumbens* leaf extract, a remedy commonly used by traditional healers. The traditional usage of this substance was explained in a scientific analysis using antioxidant activity, antibacterial activity, and wound-healing activity.

Materials and Methods

Sample Collection and Plant Extract Preparation

The fresh and healthy leaves of the plant species *Tridax procumbens* were collected from the local region of Coimbatore. *T. procumbens* leaves were washed with tap water, air-dried for a few minutes, and then rinsed with double-distilled water. 5 g of the leaves were chopped into small pieces, finely ground with a mortar and pestle, and then added to 100 mL of deionized water. The entire mixture was boiled at 80 °C for 15 min and then cooled to room temperature. The aqueous extract was filtered by Whatman filter paper No.1 [16], and the purified extract was collected and stored at -4 °C for future investigations.

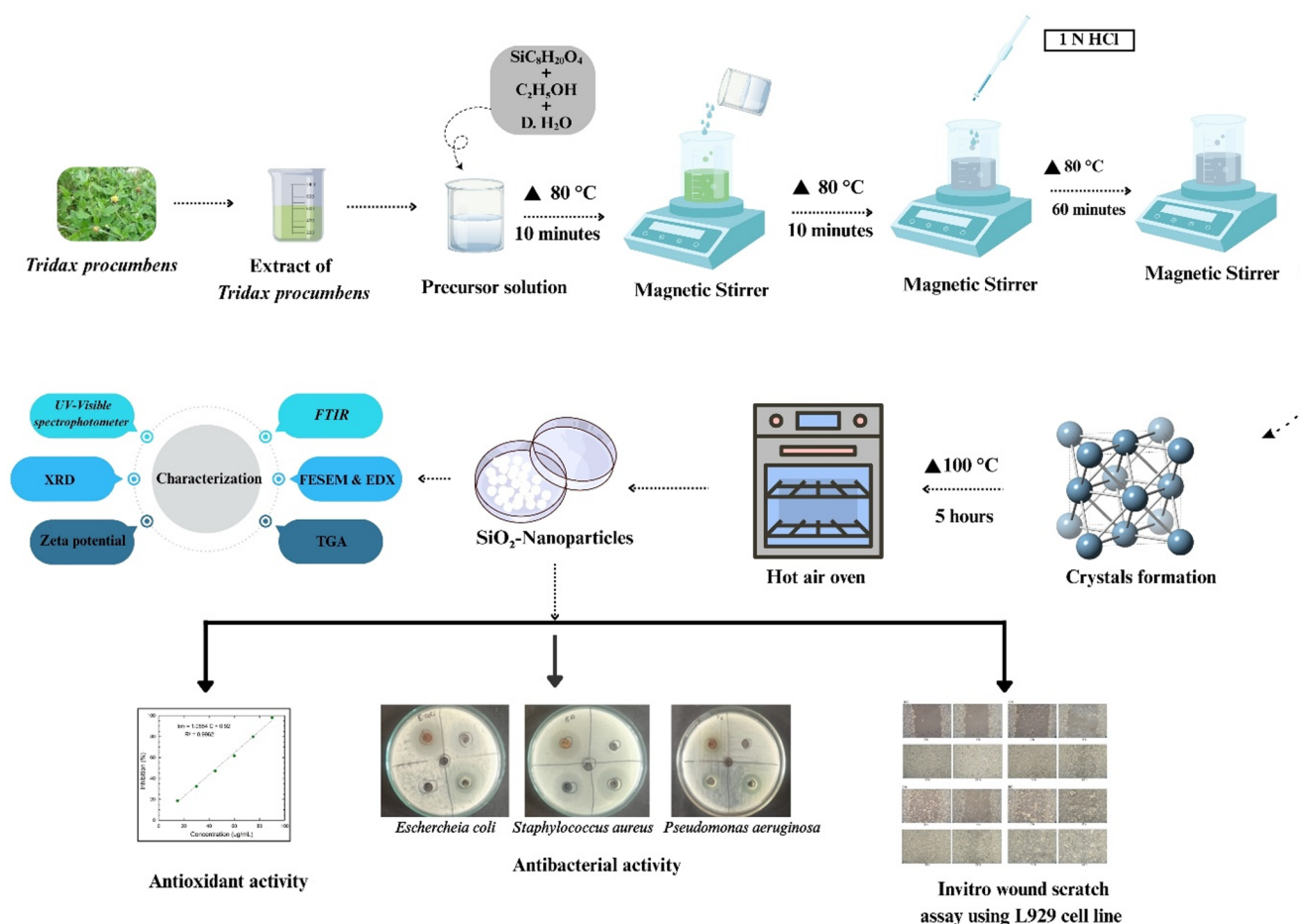


Fig. 1 Schematic diagram for the synthesis of SiO₂-NPs using *Tridax procumbens*

Green Synthesis and Characterization of *T. Procumbens* SiO₂-NPs

To synthesize SiO₂-NPs, 5 mL of TEOS (tetraethyl orthosilicate) was combined with 3 mL of distilled water and 7 mL of ethanol [25]. This solution was added to 10 mL of boiled *T. procumbens* aqueous extract, continuously stirred for the next 10 min at 80 °C, and 2 mL of concentrated 1 M HCl was gradually added while maintaining constant stirring; the precursor (TEOS) was then continuously stirred at 80 °C for 1 h (Fig. 1). Once crystal-like substances were formed, they were transferred and cooled to room temperature in a sterile Petri dish. It was kept in a hot air oven for 5 h at 100 °C and allowed to dry. Using a mortar and pestle, it was ground into a powder [26]. The resulting white powder was stored in a sterile bottle for subsequent analysis.

The green synthesized SiO₂-NPs were characterized using several techniques. The optical absorption spectrum was analyzed using a UV-Visible spectrophotometer (Shimadzu/206-26300-48 model, Avinashilingam Institute, Coimbatore). The functional groups of SiO₂-NPs

were determined using FTIR (Fourier-transform infrared spectroscopy) analysis (Shimadzu miracle 10 Model, Avinashilingam Institute, Coimbatore), recording wavelengths ranging from 4000 to 400 cm⁻¹. XRD analysis (X'Pert Pro Panalytical model, PSG Tech, Coimbatore) was used to determine the crystalline structure of SiO₂-NPs, and the average size was calculated using the Scherrer formula. FESEM (Field emission scanning electron microscopy) analysis provides information regarding the morphology of the SiO₂-NPs (TESCAN-MIRA3 XMU model, Sitra Coimbatore). The elemental composition of SiNPs was analyzed using an EDX (energy-dispersive X-ray) analyzer (TESCAN-MIRA3 XMU model, SITRA, Coimbatore). Zeta potential measurements were used to determine the surface charge of the SiO₂-NPs (Zetasizer Ver. 6.32 (Malvern Serial Number: MAL1037088) model, Karunya Institute, Coimbatore). A thermogravimetric analyzer (TGA) was used to analyze the mass loss of the SiO₂-NPs relating to the temperature (EXSTAR/6300 model, Avinashilingam Institute, Coimbatore). The stability of the synthesized SiO₂-NPs was determined by placing them in the TGA furnace at temperatures ranging from 30 to 925 °C [25].

Antibacterial Activity of *T. Procumbens* SiO₂-NPs

The agar well-diffusion method was used to determine the antibacterial potential of *T. procumbens*-mediated SiO₂-NPs against common human pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). These strains were obtained from the Department of Microbiology, PSG College of Arts & Science, Coimbatore. The collected bacterial samples were swabbed evenly on Mueller-Hinton Agar plates, and wells of a diameter of 5 mm were made. SiO₂-NPs in different concentrations (30 mg/mL) and tetracycline (1 mg/mL) were prepared, with 50 µL of SiO₂-NPs and 30 µL of tetracycline added to the wells, respectively [25]. *T. procumbens* extract, DMSO, and TEOS were also added to their designated wells. Zones of inhibition were observed after a 24-hour incubation period at 37 °C.

Antioxidant Activity of *T. Procumbens* SiO₂-NPs

The antioxidant potential of SiO₂-NPs synthesized from *T. procumbens* leaf extract was investigated using the DPPH assay [27]. This method assesses free radical scavenging activity by measuring the absorbance values of sample solutions at various concentrations. The absorbance at 520 nm was recorded using a microplate reader, with DMSO serving as the reference. The reaction mixture, varying concentrations of 15, 30, 45, 60, 75, and 90 µg/mL of green synthesized SiO₂-NPs were mixed with 1 mL of 0.1 mM DPPH solution [28]. Reduction is shown when the originally violet DPPH solution becomes yellow upon interaction with materials such as SiO₂-NPs that could possibly transfer hydrogen atoms [27]. The percentage of Radical Scavenging Activity (%RSA) was estimated and calculated using the following formula.

$$\%RSA = [(Abs_0 - Abs_1 / Abs_0) \cdot 100]$$

Abs0 - Absorption value of control; Abs1 - Absorption value of sample.

Wound Healing Activity

The L929 cells were cultured in 24-well plates at a density of $1 \cdot 10^6$ cells/ml and incubated until they attained about 80% consistency. At this phase, standard methods were followed to generate a tiny linear scratch in the completely covered cell layer using a cell scraper [29]. The cells were washed with 1X PBS buffer, and they were then exposed to various doses of SiO₂-NPs. Cell proliferation was measured at 0, 4, 18, 24, 48, and 72-hour intervals. An inverted phase contrast microscope (Radical Instruments, India) was used to image the migrated cells. The percentage of wound healing activity was calculated using the formula.

$$\% \text{ of wound healing activity} = [(Control - Treated) / Control] \cdot 100.$$

Results and Discussion

Characterization of Green Synthesized *T. Procumbens* SiO₂-NPs

The green-synthesized SiO₂-NPs' UV-visible absorption spectra were measured between the wavelengths of 200 and 800 nm, as illustrated in Fig. 2. The absorption peak of SiO₂-NPs was detected at 340 nm. The UV-vis spectrophotometer's report showed a wide peak that ranged from 300 to 370 nm, which was consistent with SiO₂-NPs absorption [26]. The peak at 340 nm suggests that the molecules interact strongly with light in the ultraviolet region. Electronic transformations inside the molecule, such as $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ transitions, might be the reason for this observation [30]. The bandgap of SiO₂-NPs is calculated using the Planck equation. The equation is expressed in terms of wavelength (λ) as $EBG = hc / \lambda_{max}$ where E is the energy, h is Planck's constant, and c is the speed of light. The *T. procumbens* mediated SiO₂ NPs with a bandgap of approximately 3.65 eV. The single peak in the UV-Vis spectrum indicates that the synthesized SiO₂ NPs were isomorphous. Kaabo et al. synthesized SiO₂ nanoparticles from *Penicillium oxalicum* in a similar manner. The combination's UV absorption spectra show an absorption band at 280 nm [31].

The FT-IR (Fourier-Transform Infrared Spectroscopy) characterization is used to identify molecules and their respective functional groups present in both the synthesized SiO₂-NPs and *T. procumbens* extract. Figure 3

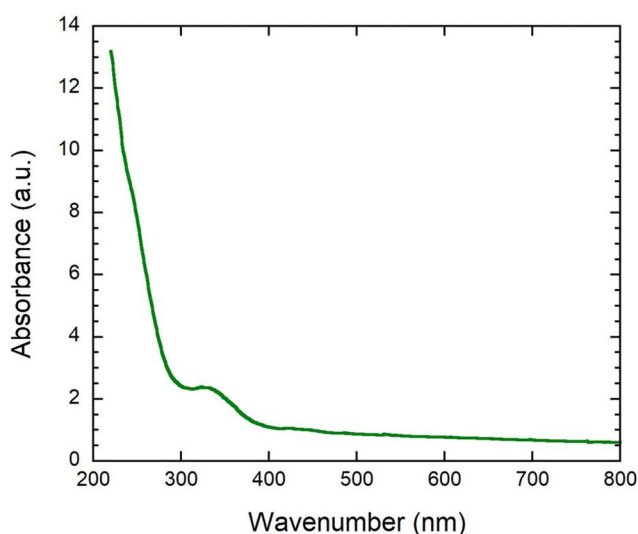


Fig. 2 UV-vis analysis of SiO₂-NPs synthesized using *Tridax procumbens* leaves extract

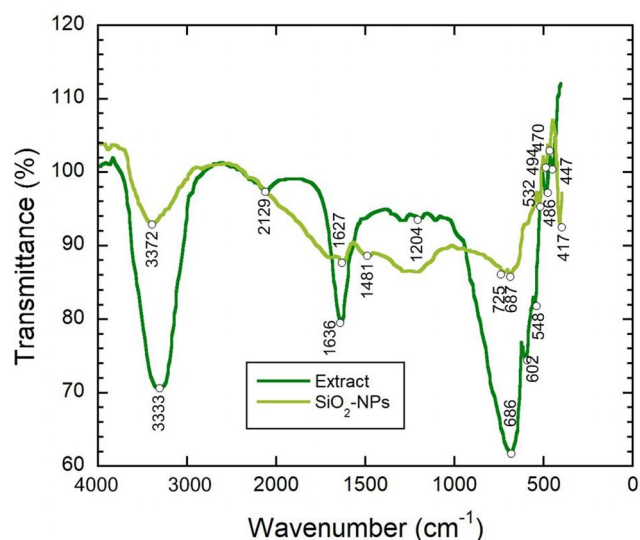


Fig. 3 FTIR analysis of *Tridax procumbens* leaves extract and SiO₂-NPs synthesized using *Tridax procumbens* leaves extract

shows the FT-IR spectral results of *T. procumbens* extract, showing different peaks observed at 3332.99 cm⁻¹ (O-H bond - hydroxyl groups), 2129.41 cm⁻¹ (C ≡ N - Nitrile functional group), 1635.64 cm⁻¹ (C = O bond - carbonyl groups), 1203.58 cm⁻¹ (C-O bond - carboxylic groups), and 686.66 cm⁻¹ (C-H bond - alkyl groups), similar to the results observed by Rao et al. [32]. The FTIR spectrum of the SiO₂-NPs (Fig. 3) showed that the different peaks were observed at 3371.57, 1627.92, 1481.33, 725.23, and 532.35 cm⁻¹ corresponding to O-H bond - hydroxyl groups, C = O bond - carbonyl groups, C-H bond - alkene groups, benzene groups, and metal-oxygen bonds. This appearance of new peaks indicates modification of the surface of nanoparticles by functional groups. Sankareswaran et al., [33] determined that the FT-IR analysis of *Phyllanthus emblica*-mediated SiO₂-NPs has hydroxyl, amide, and carboxyl groups. Similar results were reported by Periakaruppan et al. [26]. These peaks indicate potential surface functional groups associated with SiO₂-NPs.

The presence of hydroxyl (–OH) and carbonyl (C=O) groups on the surface of the synthesized SiO₂ nanoparticles has a key impact on their stability and biological activity. Hydroxyl groups contribute to the formation of a negative charge, as evidenced by a ζ-potential value of –9.99 mV (see below), ensuring electrostatic repulsion and stability of the colloidal system. Carbonyl and hydroxyl groups also participate in antioxidant activity by stabilizing free radicals, as evidenced by an IC₅₀ value of 48.8 µg/mL in the DPPH test (see below). Furthermore, they facilitate interaction with cell membranes, improving fibroblast adhesion and migration, as reflected by a 74% wound closure rate on the L929 line after 24 h. Thus, the functional groups formed

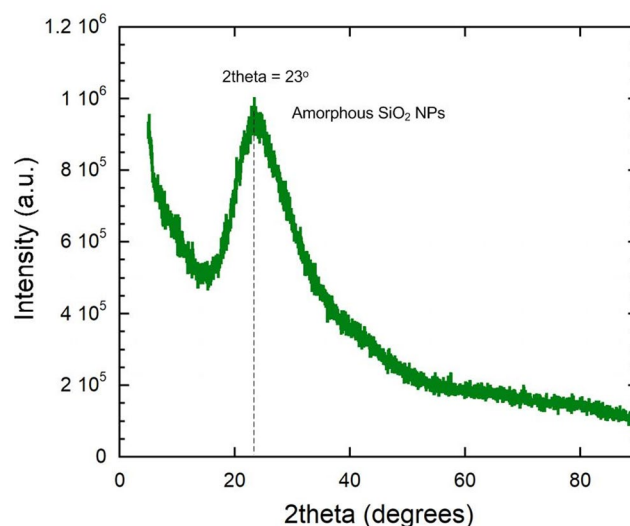


Fig. 4 XRD analysis of SiO₂-NPs synthesized using *Tridax procumbens*

Table 1 Percentage of elemental composition of SiO₂-NPs synthesized using *Tridax procumbens* by EDX analysis

Element	Weight%	Atomic%
O K	58.89	71.55
Si K	41.11	28.46
Totals	100.00	

during green synthesis not only stabilize the nanoparticles but also enhance their therapeutic potential.

The X-ray diffraction (XRD) analysis determined the crystal-like nature of synthesized SiO₂-NPs using *T. procumbens* leaves extract. The broad absorption peak was found at 2θ = 24° in the XRD spectrum (Fig. 4). Similarly, Sharmiladevi et al., [34] reported that the SiO₂-NPs synthesized have a 2θ value range of 30–80°. Rahimzadeh et al. [35] investigated the *Rhus coriaria* mediated SiO₂-NPs that showed a broad XRD spectrum at 2θ = 23°.

The Energy-dispersive X-ray (EDX) analysis ascertained the purity and elemental composition of the green synthesized SiO₂-NPs. The EDX spectra determined the atomic percentages of 28.46% for silica and 71.55% for oxygen, along with corresponding weight percentages of 58.89% for silica and 41.11% for oxygen (Table 1), as illustrated in Fig. 5. This analysis confirmed the presence of silicon (Si) and oxygen (O) in the SiO₂-NPs. Periakaruppan et al. [16] demonstrated that the SiO₂-NPs synthesized using *E. thymifolia* show the atomic percentage of silica at 42.02% and oxygen at 58.98%. Marousek et al. [36] stated that the SiO₂-NPs synthesized from coir pith showed a weight% of 25.58% for silica, 41.58% for oxygen, 12.12% for Na, and 20.72% for Cl.

The Field Emission Scanning Electron Microscope (FESEM) analyzed the size and shape of SiO₂-NPs

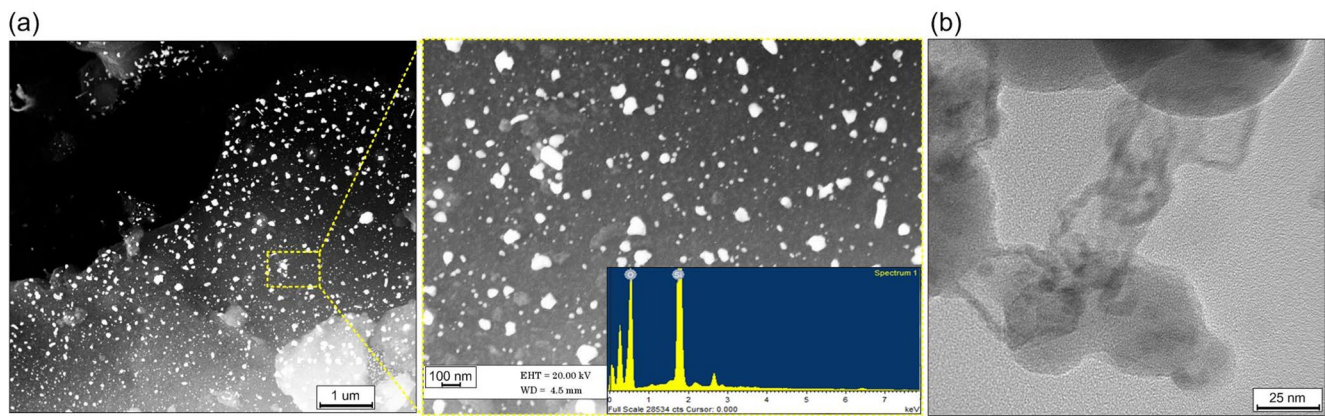


Fig. 5 SEM-EDX (a) and TEM (b) analysis of SiO₂-NPs synthesized using *Tridax procumbens*

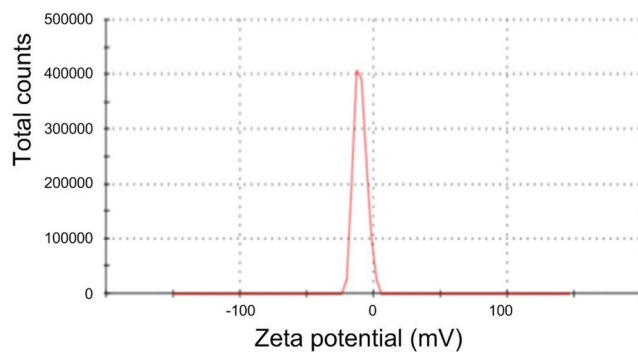


Fig. 6 Zeta potential analysis of SiO₂-NPs synthesized using *Tridax procumbens*

synthesized using *T. procumbens* leaf extract. The findings revealed an average SiO₂-NPs size of 44 nm, and they have a spherical shape (Fig. 5). The obtained SiO₂ nanoparticles exhibited spherical morphology and an average size of about 44 nm, which was consistent with the results presented by Patil et al. [37], where similar nanoparticles synthesized using rice (*Oryza sativa* L.) husk also exhibited spherical shape and uniform distribution. Similar morphological characteristics were reported by Kaabo et al. [31] and Rahimzadeh et al. [35], where biogenic SiO₂-NPs prepared using fungi and plant extracts exhibited similar shape and stability. This confirms that the green synthesis method using *Tridax procumbens* provides reproducible morphology comparable to other green approaches and can be applicable for the scalable production of biocompatible nanomaterials.

Zeta potential analysis assessed the exterior surface charge and consistency of synthesized SiO₂-NPs using *T. procumbens* leaf extract. The zeta potential differs due to the concentration, pH, and temperature of the nanoparticles. The obtained zeta potential value is -9.99 mV indicates that these SiO₂-NPs possess a negative charge, as shown in Fig. 6. In previous research [38], it was found that SiO₂-NPs

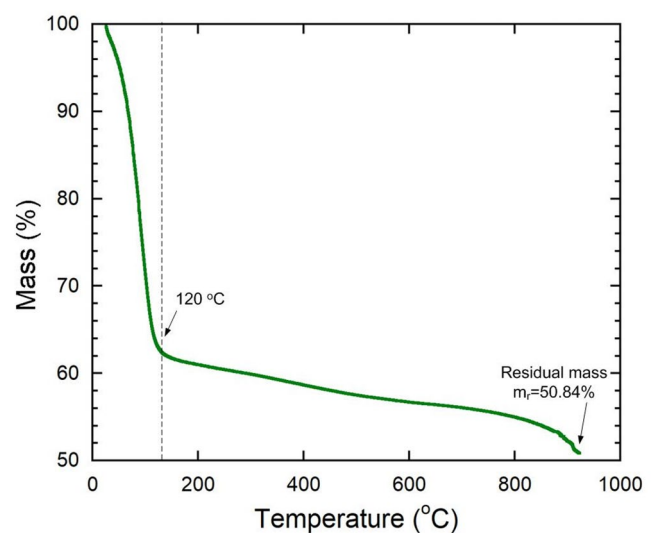


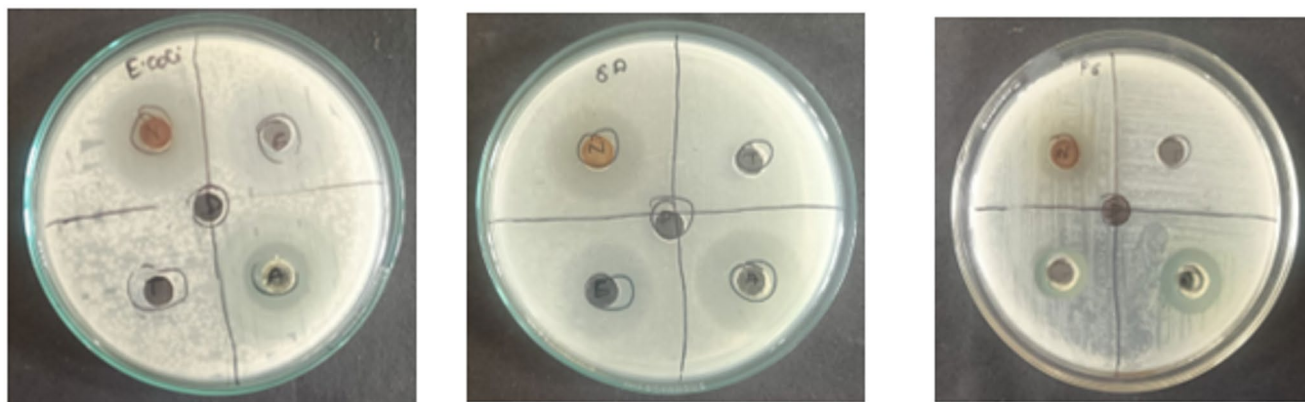
Fig. 7 TGA analysis of SiO₂-NPs synthesized using *Tridax procumbens*

have a negative zeta potential, such as -10 mV. Therefore, the negative zeta potential observed in the synthesized nanoparticles from *T. procumbens* confirms their stability and suitability for further applications.

A thermogravimetric analyzer (TG) was employed to measure the thermal stability and mass degradation of SiO₂-NPs synthesized by means of *T. procumbens* leaf extract. The mass loss of the SiO₂-NPs was monitored across a temperature spectrum from 30 to 925 °C (Fig. 7). It was noted that the SiO₂-NPs synthesized using *Tridax procumbens* reduced the mass loss by 48.9% at elevated temperatures. This results from the removal of functional groups that were present on the synthesized nanoparticles. Ganesan et al. [25] demonstrated that the *H. floresia*-mediated SiO₂-NPs synthesized have a weight loss of around 44% that was reduced at 30–925 °C. Sachan et al. [39] reported that the SiO₂-NPs synthesized from the dried leaf extract of *Saccharum ravan-nae*, *Saccharum officinarum*, and *Oryza sativa* showed 21%

Table 2 Antibacterial activity for the *Tridax procumbens*-mediated SiO₂-NPs by agar well diffusion method

Name of the bacteria	Zone of inhibition (mm) in diameter				
	Positive control Tetracycline (30 µL/mL)	SiO ₂ -NPs (50 µL/ mL)	<i>T. procumbens</i> (75 µL/mL)	Precursor solution (50 µL/mL)	Negative control DMSO (30 µL/mL)
<i>Escherichia coli</i>	32±0.2	25±0.1	19±0.1	No zone formation	No zone formation
<i>Staphylococcus aureus</i>	28±0.1	22±0.1	17±0.2	No zone formation	No zone formation
<i>Pseudomonas aeruginosa</i>	20±0.1	18±0.2	15±0.1	No zone formation	No zone formation

**Fig. 8** Antibacterial potential of SiO₂-NPs synthesized using *Tridax procumbens*: (a) *Escherichia coli*; (b) *Staphylococcus aureus*; (c) *Pseudomonas aeruginosa*

of weight loss over a wide range of temperatures around 100–1100 °C.

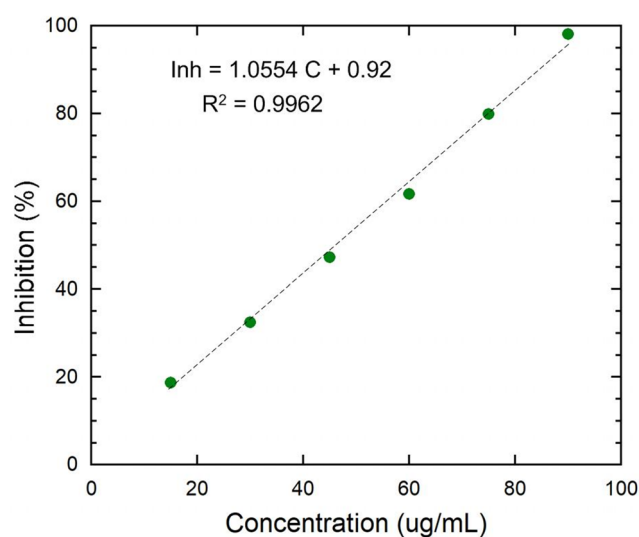
Biological Activities of Green Synthesized *T. Procumbens* SiO₂-NPs

Antibacterial Activity

The study demonstrated the antibacterial activity of biosynthesized SiO₂-NPs derived from *T. procumbens* leaf extract against the bacterial strains of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [25]. The zone of inhibition diameter was compared along with positive control (tetracycline), negative control (DMSO), and TEOS solutions (Table 2). The results of the antibacterial tests indicated that SiO₂-NPs exhibited the maximal zone of inhibition against *Escherichia coli*, as illustrated in Fig. 8a, *Escherichia coli* exhibited a greater zone of inhibition compared to *Staphylococcus aureus* (Fig. 8b) and *Pseudomonas aeruginosa* (Fig. 8c). Overall, these findings highlight the antibacterial potential of *T. procumbens*-mediated SiO₂-NPs.

Antioxidant Activity

The antioxidant activity of biosynthesized SiO₂-NPs from the *Tridax procumbens* leaf extract was done using the DPPH assay. Antioxidant activity is the capacity of a

**Fig. 9** Antioxidant activity of SiO₂-NPs synthesized using *Tridax procumbens*

substance to neutralize free radicals, which can inflict damage on cells and tissues in the body [27]. Antioxidants function by donating electrons to unstable free radicals, thereby stabilizing them and preventing them from inducing oxidative stress and damage. The results on antioxidants for Radical Scavenging Activity (%RSA) were analyzed (Fig. 9). In this study, the IC₅₀ values obtained from the DPPH assay serve as a measure of the antioxidant activity of SiO₂-NPs, indicating the concentration required to inhibit 50% of the

DPPH radicals [27] To determine the IC₅₀ value, we compared it with a standard antioxidant molecule, ascorbic acid [40]. The IC₅₀ value for our SiO₂-NPs sample was measured as 48.8 µg/mL. The maximum antioxidant activity was found to be at 90 µg/mL of SiO₂-NPs, and the lowest activity was found at 15 µg/mL of SiO₂-NPs. The maximum antioxidant activity was due to the interaction of SiO₂-NPs with the free radicals, thereby reducing the oxidative stress. As the concentration of SiO₂-NPs increased, the inhibition of free radicals was greater. Therefore, the bioactive compound from *T. procumbens* present on the surface of the nanoparticles increases the antioxidant activity. Antioxidants play a significant role in wound healing by reducing oxidative stress, inflammation, and tissue damage, thereby promoting faster wound closure [40].

Invitro Wound Scratch Assay Using L929 Cell Line

The in vitro scratch assay, or wound healing assay, has been used for studying cell migration dynamics [41]. In this method L929 cell lines were used to analyze the therapeutic potential of green synthesized SiO₂-NPs in wound healing activity [28]. Microscopic analysis provides insight into the dynamics of cell migration and wound closure, facilitating the development of sustainable and effective interventions for tissue regeneration and healing. Interpretation involves determining the percentage of wound closure at specific intervals of time (0, 4, 18, and 24 h) in L929 cell lines (Table 3). In the beginning, a scratch or wound is introduced to the cell monolayer, triggering cell migration and proliferation near the scratch edge. After 4 h, early responses to the wound become superficial, with cells starting to migrate into the scratched area (Fig. 10a and b). The considerable progress in wound closure is typically observed as cell migration and proliferation continue around 18 h. Finally, by 24 h, substantial closure of the wound scratch is estimated, with the majority of the scratch area covered by migrated cells (Fig. 10c and d). The outcomes of the SiO₂-NPs sample synthesis demonstrate a substantial 74% increase in wound

healing activity within L929 cells after only 24 h, exceeding the expected healing rate of the control group. This highlights the potential of the synthesized nanoparticles to significantly enhance wound closure progressions [29]. Besides, the control group exhibited normal wound healing, emphasizing the specificity of the observed effect, suggesting the essential role of the synthesized nanoparticles in increasing the healing process. These results provide plausible alternatives for the utilization of SiO₂-NPs as effective agents for stimulating wound healing in numerous biological applications.

Table 4 shows that the characteristics of modified SiO₂ particles obtained by us are comparable with published analogues. As can be seen from the comparative analysis, the modified SiO₂ particles obtained in this work demonstrate significantly lower cytotoxicity compared to a number of industrial and energy nanomaterials, which confirms their biocompatibility and potential safety for biomedical applications.

Conclusion

The present study demonstrated the successful green synthesis of silica nanoparticles (SiO₂-NPs) using *Tridax procumbens* leaf extract, which is an eco-friendly and sustainable approach to prepare functional nanomaterials. Comprehensive characterization, including UV-Vis, FTIR, XRD, EDX, FESEM, and TG analysis, confirmed the presence of surface functional groups, spherical shape, crystallinity, high purity, and thermal stability of the particles with an average particle size of about 44 nm. The synthesized SiO₂-NPs exhibited significant antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as antioxidant potential with an IC₅₀ value of 48.8 µg/mL. In the scratch test model on the L929 fibroblast line, the nanoparticles contributed to accelerated wound closure 74% in 24 h, which is comparable to the control and indicates their biological activity. It was shown for the first time that SiO₂-NPs obtained using *T. procumbens* simultaneously possess antibacterial, antioxidant, and wound-healing properties that are superior to standard drugs. The absence of similar publications confirms the novelty of the approach, and the results obtained open up prospects for the use of such nanoparticles in the biomedical industry in particular, for the development of new treatments for skin lesions and infections.

Table 3 Wound scratch assay performed at the specific time intervals for green synthesized SiO₂-NPs

Time Duration (h)	Wound scratch assay performed at the specified time periods				
Concentration (μg/μL)	Wound area (μm)	0 h	4 h	18 h	24 h
25	2137	0	11	55	67
50	1789	0	13	55	70
75	1957	0	19	68	73
100	1961	0	19	70	74

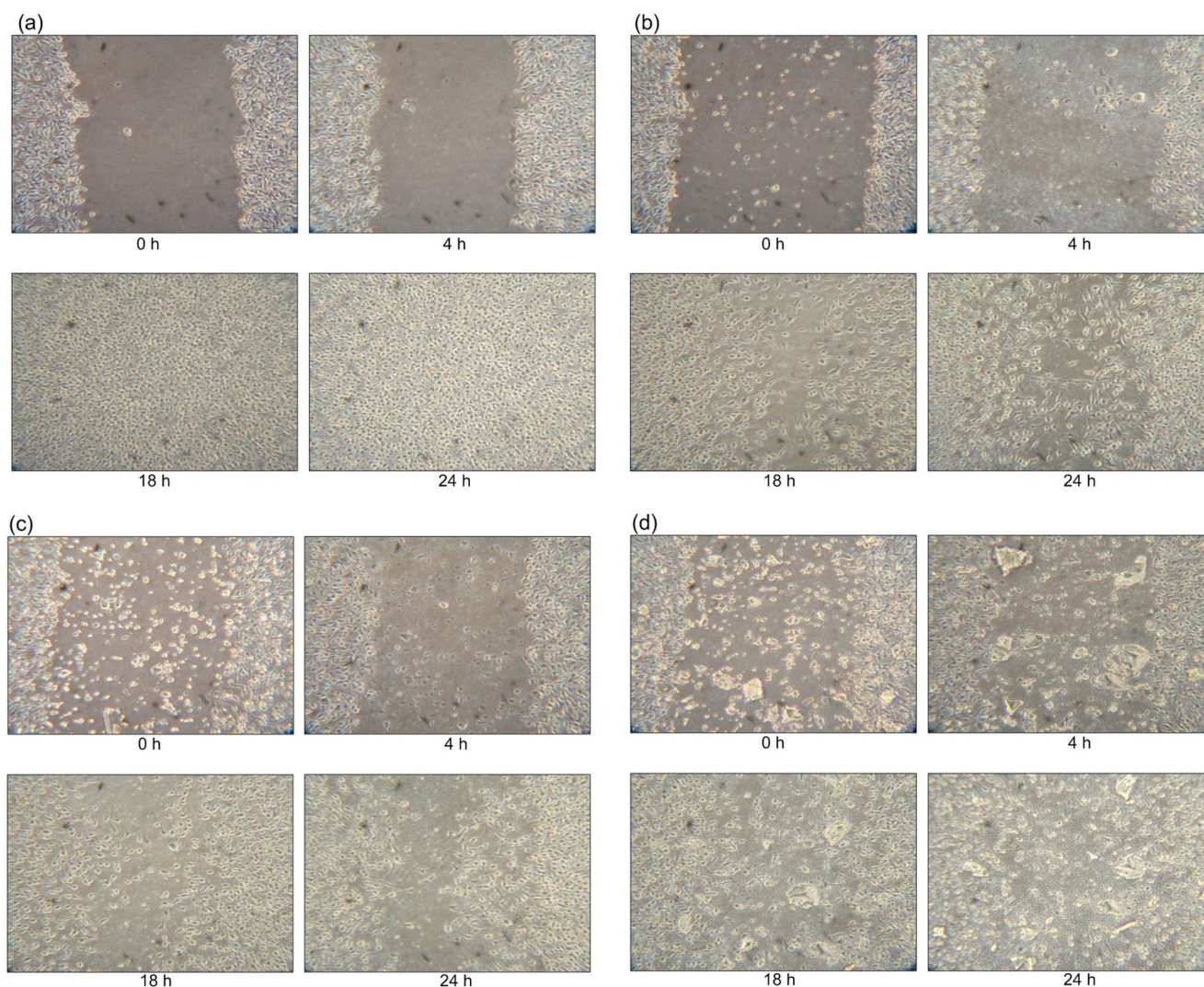


Fig. 10 In vitro wound scratch assay for control (a) and different concentration SiO₂-NPs synthesized using *Tridax procumbens* (25 (b), 75 (c) and 100 μL (d))

Table 4 Comparison of obtained SiO₂-NPs with published analogs

Material / Modification	Concentration (ug/mL)	L929 Viability (%)	Evaluation method	References
SiO ₂ with plant functional groups (our data)	100	>90%	MTT	This work
SiO ₂ (20–30 nm), unmodified	100	~ 70%	MTT	[42]
Graphene oxide	100	~ 60%	MTT	[43]
Nanoclay	100	~ 75%	MTT	[43]
Nano-TATB (energy material)	100	< 50%	LDH, SOD	[44]

Author Contributions Vanathi Palanimuthu: investigation, writing-original draft, project administration; Nishanthi Rajendran: formal analysis, validation, data curation; Rajiv Periakaruppan: Conceptualization, supervision, writing-review and editing; Valentin Romanovski: formal analysis, validation, visualization, data curation, investigation, writing-review and editing. Priyanka Govindaraju: formal analysis, validation, data curation; Mani Natarajan: formal analysis, validation, data curation.

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Data Availability All data, models, and code generated or used during the study appear in the submitted article.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interest with any previous work.

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