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To cite this article: Pradeep Deshmukh, Yogesh Biradar, Dnyaneshwar Wankhede, Vikas Ragole, Sonaji Gayakwad & Kailas Kadam (30 Aug 2024): *Vachelia nilotica* seed-mediated green synthesis of sulfur nanoparticles (SNPs) and evaluation of anticancer & antifungal activities, Journal of Sulfur Chemistry, DOI: [10.1080/17415993.2024.2393391](https://doi.org/10.1080/17415993.2024.2393391)

To link to this article: <https://doi.org/10.1080/17415993.2024.2393391>



Published online: 30 Aug 2024.



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Vachelia nilotica seed-mediated green synthesis of sulfur nanoparticles (SNPs) and evaluation of anticancer & antifungal activities

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ABSTRACT

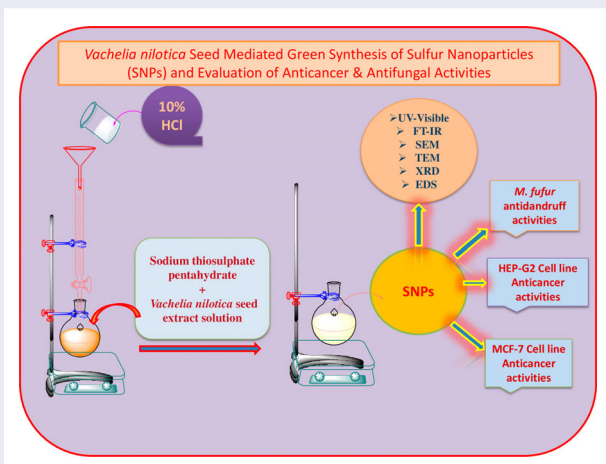
Sulfur nanoparticles (SNPs) have been successfully synthesized by applying *Vachelia nilotica* seed extract as a bioactive aqueous material by a disproportionation reaction of sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) with a 10% hydrochloric acid (HCl) at room temperature. The synthesized SNPs were characterized by UV–Visible, Fourier Transfer Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy-Dispersive X-ray Spectroscopy (EDS), and X-ray Diffraction (XRD) techniques. The usual size of SNPs was determined by an XRD study and calculated with the Debye–Scherrer formula. The SNPs are crystalline with a calculated average size of 45 nm. The SEM and TEM analyses revealed spherical shape and size around 55 nm of SNPs. The synthesized SNPs were screened for their antifungal activity against *M. furfur* species and for anticancer activities against (MCF-7, HepG2) cell lines. The obtained results suggested towards moderate to good antifungal and excellent anticancer activities.

ARTICLE HISTORY

Received 31 January 2024
Accepted 11 August 2024

KEYWORDS

Vachelia nilotica; sulfur nanoparticles (SNPs); anticancer; antifungal; *M. furfur*



1. Introduction

Nanoparticles are inorganic materials with specific characteristics such as size, distribution, chemical composition, and morphology containing structural dimension measuring 1–100 nanometers. Nanoparticles with greater surface area to volume ratio compared to that of bulk materials have gained enormous attention of researchers from various fields including chemistry, physics, material sciences, life sciences, medicinal fields, agriculture, biotechnology, food and safety, cosmetics, industrial area, and engineering. The high interest in nanoparticles is also because of their unique optical, magnetic, electronic, and catalytic properties with their distinctive feature of size and shape [1,2].

The application of various dyes in industrial application has created a major concern regarding environmental safety as these dyes are one of the main water pollutants and critical threats to environment and human health. Nanoparticles and nanocomposites are promising remedies for the degradation of these organic dyes from wastewaters as they can convert these toxic pollutants into non-toxic compounds under light. Thus photocatalytic degradation using nanomaterials is an important research area these days and many researchers have contributed [3–9].

Physical and chemical methods of nanoparticles synthesis have disadvantages such as high cost, environmentally hazardous chemicals, and non-availability for medical applications due to the presence of toxic capping agents which has prompted researchers to use green methods of synthesis [10,11]. The green methods emphasize on the synthesis of nanoparticles for maximum societal benefit, with minimal impact on the ecosystem [12–14]. In this regard, most of the researchers from academic and industry backgrounds have focused on applications of biological materials such as plants, marine algae, fungi, and bacteria for the green synthesis of nanoparticles [15–22]. In recent years, many researchers have synthesized metallic and non-metallic nanoparticles [23–30], metal oxide nanoparticles and nanocomposites [4–9,31–38] using green methods.

The use of seed powder aqueous extract of various plants for the synthesis of metallic nanoparticles such as silver [39,40], gold [41,42], copper [43], magnesium [44], and so on is well documented, but no such report is available in case of SNPs synthesis. Thus, in present investigation, we report the synthesis of SNPs using the seed powder aqueous extract of the *Vachelia nilotica*, a plant with large number of medicinal properties [45]. The *Vachelia nilotica* seed powder aqueous extract contains various phytochemicals that actively participate as reducing and capping agent to generate and stabilize the SNPs by converting the S^{2-} to S^0 state [46]. The shape, size, and crystalline nature of synthesized 'green' SNPs are defined by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy-Dispersive X-ray Spectroscopy (EDS), and X-ray Diffraction (XRD) analytical methods. The biomolecules attached to the SNPs on the surface are responsible for their reduction as well as stabilization and are identified by UV–visible and FT-IR techniques. The synthesized SNPs were tested for their antifungal and anticancer activities and observed to exhibit good antifungal and excellent anticancer activities.

2. Results and discussion

2.1. UV–Visible spectroscopy

The UV–Visible absorption spectral studies were carried out to confirm the formation of SNPs using *Vachelia nilotica* seed extract solution (Figure 1a). After completion of

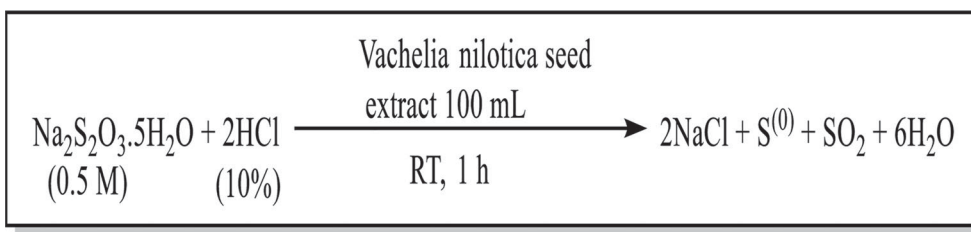


Figure 1. (a) UV–Visible spectrum of *Vachelia nilotica* aqueous seed extract solution and (b) UV–Visible spectrum of synthesized SNPs.

reaction, the samples were subjected to record absorption peaks for the SNPs (Figure 1b). The UV–Visible spectrum recorded in the range of 200–800 nm for *Vachelia nilotica* seed extract solution exhibited peaks at 207.11 and 261.46 nm indicating the presence of different phytochemicals like fatty acids, terpenoids, alkaloids, and phenolic compounds. The sample solution exhibited absorption peak at 264.24 nm indicating the presence of SNPs. Reports indicating the presence of SNPs in the range of 250–300 nm with maximum absorbance at 264 nm wavelength [49] are available.

2.2. Fourier Transform Infrared (FT-IR) analysis

FT-IR data readings are performed to find out the possible biomolecules accountable for the reduction, capping, and efficacious stabilization of SNPs. FT-IR spectrum of *Vachelia nilotica* aqueous seeds extract solution is illustrated in (Figure 2a). It was observed that the groups responsible for the formation of the SNPs were $-\text{OH}$ (3321 cm^{-1}), $-\text{C}=\text{O}$ (1365 cm^{-1}), and $\text{C}-\text{O}-\text{C}$ (1042 cm^{-1}). These functional groups act as dispersing, capping, and stabilizing agents for SNPs during synthesis.

The analysis of FT-IR spectrum of SNPs (Figure 2b) clearly indicated the presence of peak at 465 cm^{-1} due to SNPs. These results show that the reaction took place and nanoparticles were formed. The spectrum indicated a new chemistry linkage on the surface of the SNPs and is an enough evidence in favor of binding of *Vachelia nilotica* aqueous seeds extract to SNPs acting as a stabilizer and dispersing agent to prevent agglomeration of SNPs [50].

2.3. X-ray Diffraction analysis

The XRD pattern of synthesized SNPs by *Vachelia nilotica* aqueous seed extract illustrated in Figure 3 exhibited sharp peaks indicating bigger crystals. The 2θ peaks observed at 21.02° , 22.13° , 25.77° , 26.02° , 27.87° , 28.71° , 31.49° , 34.28° , 37.07° are attributed to the crystal planes of sulfur at (113), (222), (133), (026), (311), (206), (313), (044), and (244) respectively [51,52]. The formation of SNPs also involves sodium chloride formation as a side product and the extra peaks at 42.92° , 47.80° , and 51.03° can be attributed to the crystal planes of sodium chloride at (404), (319), and (515) respectively. The results indicated that SNPs are well crystalline and the position and relative intensity of the diffraction peaks match well with the standard monoclinic phase sulfur diffraction pattern [JCPDSN-34-094]. The crystal planes corresponding to the peaks are indicated in Figure 3.

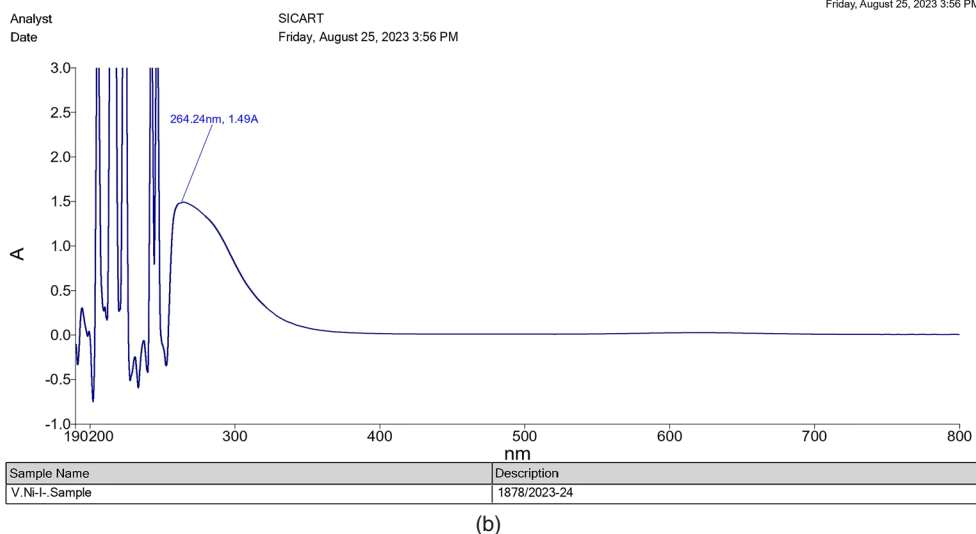
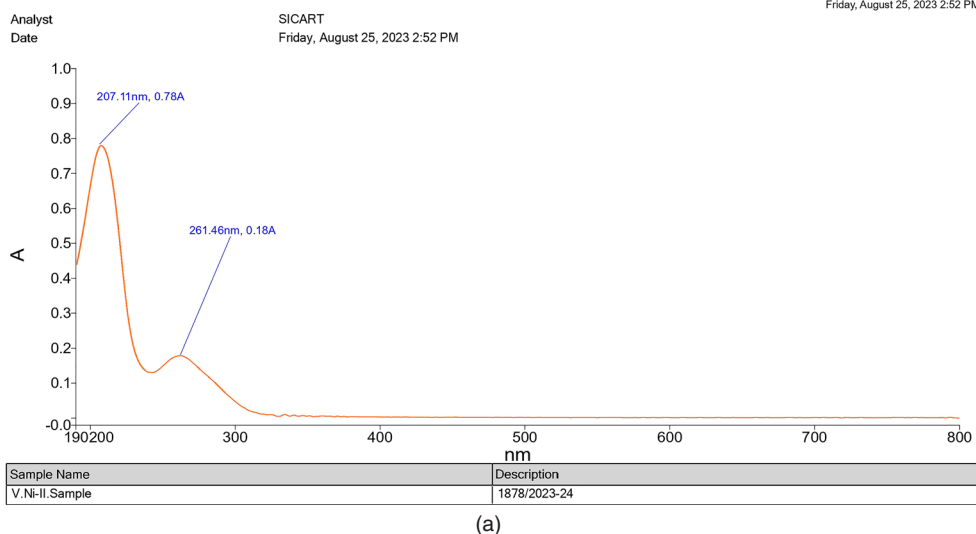
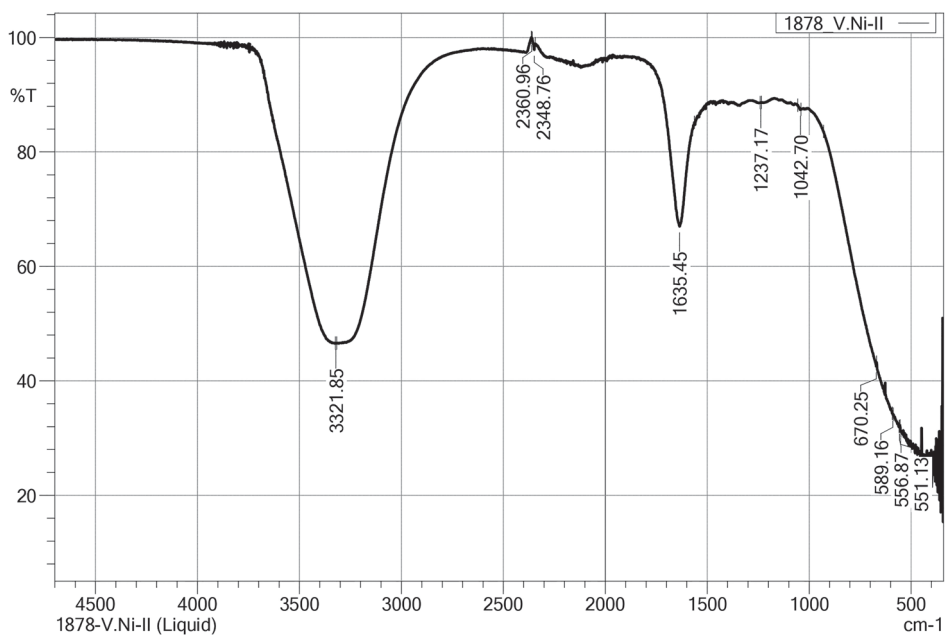


Figure 2. (a) The FT-IR spectrum of *Vachelia nilotica* aqueous seed extract solution and (b) the FT-IR spectrum of synthesized SNPs.

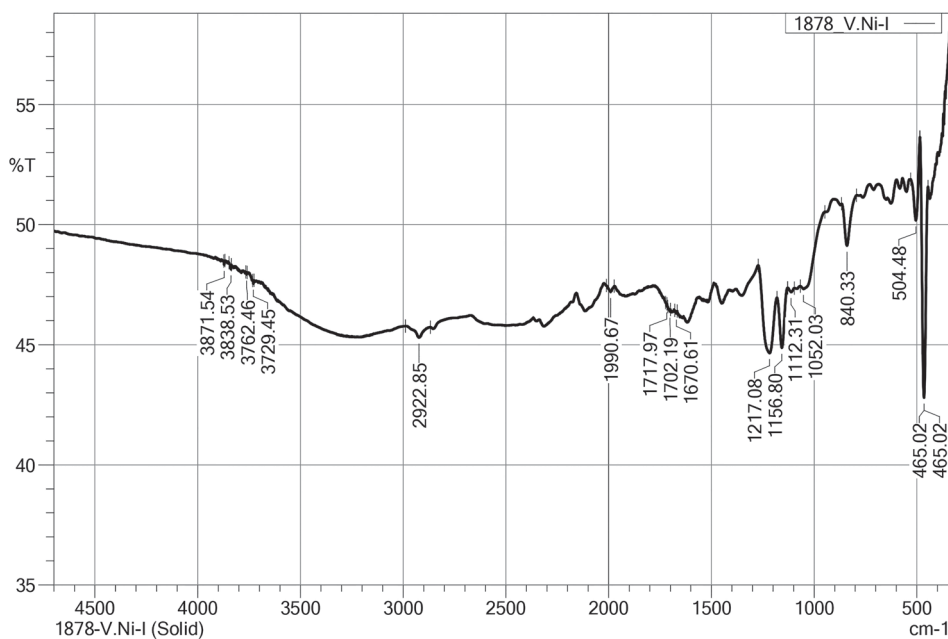
The particle size of the synthesized SNPs was calculated using the Debye–Scherrer formula [8,9,53,54], $D = K\lambda/(\beta\cos\theta)$, where D = the nanoparticles crystalline size, K = Scherrer constant (0.90), λ = wavelength 1.54 \AA , β = full width at half maximum (FWHM), and θ = half diffraction angle or Bragg angle or peak position (radians). The crystallite size was estimated to be 45 nm using the diffraction peak associated with crystal plane (222).

2.4. Scanning Electron Microscopy analysis

Scanning Electron Microscopy or SEM is useful in determining topography of materials and provides detailed surface data of solid samples. In present investigation, the SEM



(a)



(b)

Figure 3. The XRD pattern of synthesized SNPs.

images indicated that synthesized SNPs are almost spherical in shape and were of uniform size (Figure 4) [55]. Figure 4 indicated that the SNPs obtained using aqueous seed extract solution are having uniform particle size, spherical, and having tendency to prevent agglomeration. This is enough evidence to emphasize the role of aqueous seed extract

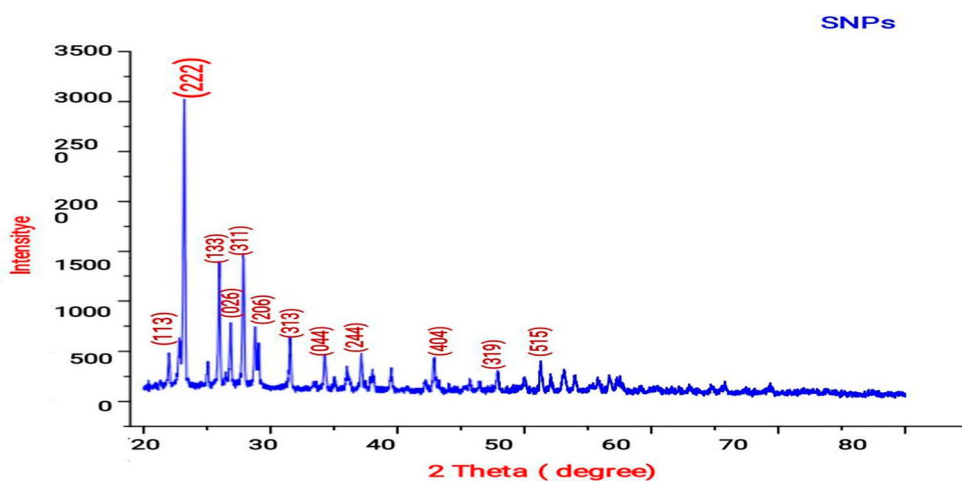


Figure 4. The SEM images of SNPs.

Table 1. The elemental analysis of SNPs.

S. no.	Test parameter	Name of the instrument	Results (%)
1	CHNS (%)	CHNS/O Analyzer, Elementar, Unicube	Carbon 1.37 Hydrogen 4.125 Nitrogen 0.13 Sulphur 5.091

solution of *Vachelia nilotica* in the reduction of sulfur and in the formation and stabilization of SNPs [56].

2.5. Transmission Electron Microscopy analysis

Transmission Electron Microscopy or TEM technique is used preferentially in case of nanoparticles to measure particle size, size distribution, and morphology. It is having better resolution than SEM. In present investigation, the TEM images represented that the SNPs are spread homogeneously and the size of the SNPs observed to be 5–100 nm (Figure 5) [24,57].

2.6. Energy-Dispersive X-ray Spectroscopy analysis

EDS is an analytical technique used for chemical characterization or elemental analysis of SNPs which provided information on the chemical composition, phase identification, and required purity of the biosynthesized SNPs [57]. The obtained results are represented in Table 1.

3. Biological activity

3.1. Antidandruff

The results of the antidandruff activities of the SNPs against *M. furfur* species were determined by the Agar well diffusion method. Samples showed moderate to good inhibition

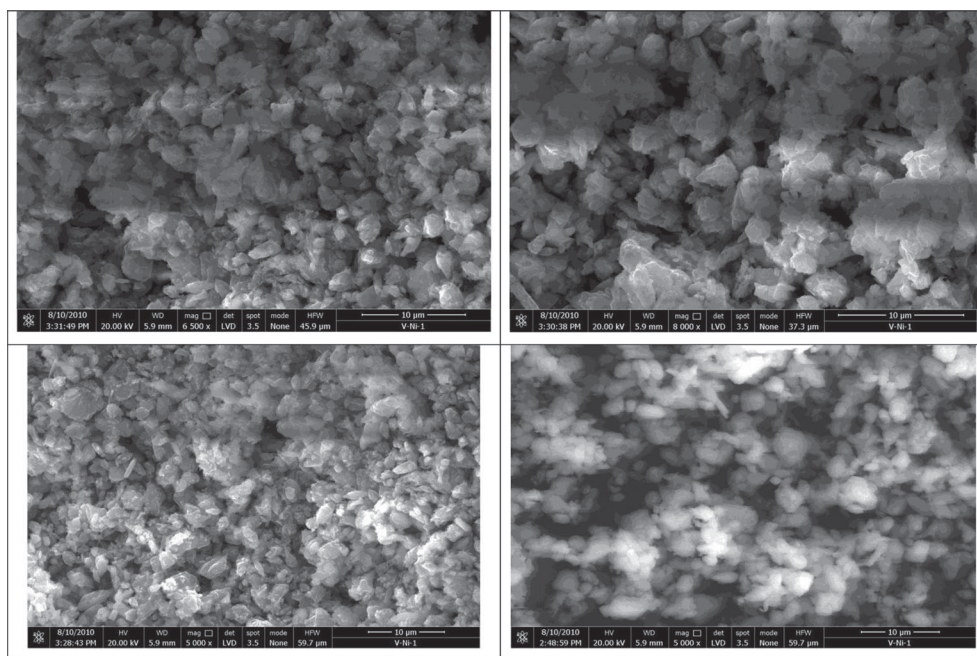


Figure 5. The TEM images of SNPs.

Table 2. Antidandruff activities of the SNPs against *M. furfur* species.

S. no.	Sample	Concentration	Zone of inhibition
1.	Control	–	–
2.	Standard	1 mg/mL	20
3.	SNPs	5 mg/mL	10
		10 mg/mL	14

to the growth of fungal strain *M. Furfur* (Figure 6) [58,59] in comparison to standard. Table 2 represents the results obtained for antidandruff activity. The inhibition was directly proportional with increasing concentration of SNPs.

3.2. Anticancer

The synthesized SNPs exhibited moderate inhibition of cancer cells against *MCF-7* (Breast Cancer Cell Line) and *Hep-G2* (Liver Cancer Cell Line) when compared to standard drug 5-Fluorouracil (5-FU). The synthesized SNPs, however, exhibited lower IC_{50} values compared to that of standard drug 5-FU. This indicated lower concentration required for the killing of 50 percent of cancer cells, thus indicating potential of synthesized SNPs as anti-cancer agent in comparison to standard 5-FU [60,61]. Tables 3 and 4 represent effects of synthesized SNPs against *MCF-7* and *Hep-G2* cell lines.

Previous studies have established that the depletion of copper in the cells by chelating agents is a promising strategy to improve cancer therapy and SNPs are able to detain copper [62,63]. Thus SNPs are able to inhibit the growth of cancer cells via inactivation of the MEK/ERK pathway followed by deceleration of mitosis in cancer cells [64,65]. In

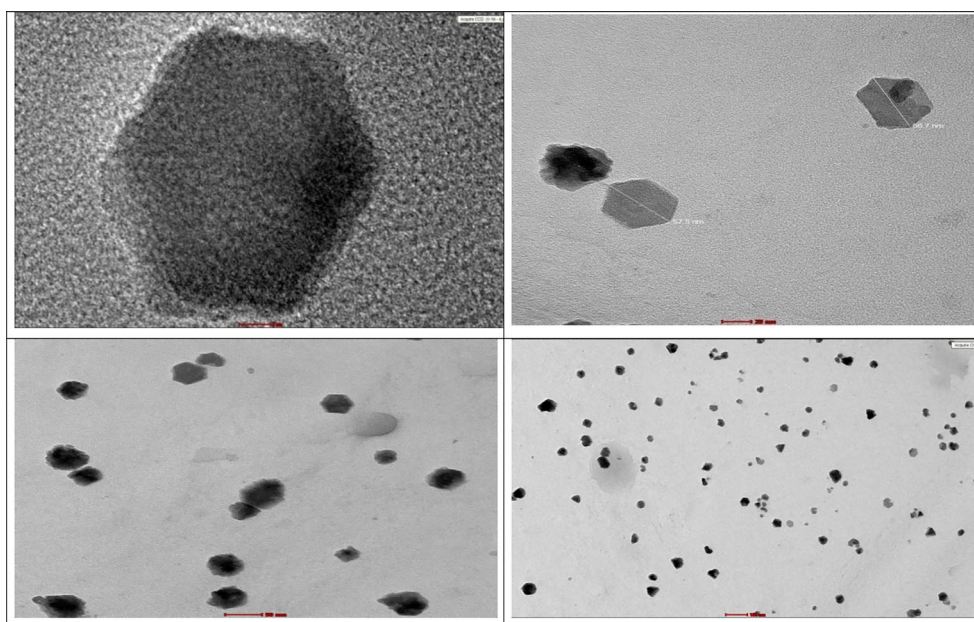


Figure 6. The antidandruff analysis of SNPs.

Table 3. Effects of SNPs against *MCF-7* (Breast cancer cell line) by MTT assay.

S. No.	Sample	Concentration (µg/mL)	OD	Mean	Percentage inhibition (%)	IC ₅₀
1.	Control DMSO (0.2% in PBS)		1.1101.1211.113	1.114		
2.	Standard 5FU	10	0.3550.3410.352	0.349	68.67	40.81
		40	0.2840.2740.261	0.273	75.49	
		100	0.1950.1810.191	0.189	83.03	
3.	SNPs	10	0.5400.5560.542	0.546	50.98	34.64
		40	0.4100.4120.411	0.411	63.10	
		100	0.3550.3570.347	0.353	68.31	

Table 4. Effects of SNPs against *Hep-G2* (Liver cancer cell line) by MTT assay.

S. No.	Sample	Concentration (µg/mL)	OD	Mean	Percentage inhibition (%)	IC ₅₀
1.	Control DMSO (0.2% in PBS)		0.9850.9760.973	0.978		
2.	Standard 5FU	10	0.3550.3460.341	0.347	64.51	43.16
		40	0.2860.2860.272	0.281	71.26	
		100	0.1920.1830.175	0.183	81.28	
3.	SNPs	10	0.4890.4810.467	0.479	51.02	35.03
		40	0.3830.3730.370	0.375	61.65	
		100	0.3380.3240.319	0.327	66.56	

present investigation, the anticancer activity effect of SNPs can be dedicated to similar reason.

4. Conclusion

A facile, without surfactant, green route for synthesizing sulfur nanoparticles (SNPs) from sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) in the presence of *Vachelia nilotica* aqueous seed extract solution was developed. The synthesized nanoparticles were characterized by UV-visible, FT-IR, SEM, TEM, EDS and powder XRD techniques. The results indicated a spherical shape of SNPs with an average size diameter of 55 nm. The SNPs exhibited lower IC_{50} values compared to that of standard 5-Fluorouracil (5-FU) and are thus potential anticancer agents against *MCF-7* (Breast Cancer) & *Hep-G2* (Liver cancer) cell lines.

5. Experimental

5.1. Materials

Vachelia nilotica plant seeds were procured at the Swami Ramanand Teerth Marathwada University Nanded, Maharashtra State, India. Sodium thiosulfate pentahydrate (AR) ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 99.5%), 10% hydrochloric acid (AR), and rectified spirit (AR) were purchased from S. D. Fine Chemicals Pvt. Ltd. India. All chemicals were used as received.

5.2. Preparation of seed extract

Vachelia nilotica seeds were washed thoroughly with tap water and then with deionized water until all the unwanted visible dirt particles were removed. Subsequently, the seeds were dried and powdered through mortar and pestle. The fine powder measured about 20 g poured into 100 mL deionized water in a 200 mL Erlenmeyer flask. The mixture was boiled at 75–80°C for 2 h and filtered using Whatmann No.1 filter paper to remove solid particles. Then the extract was centrifuged through centrifugation at 5000 rpm for 15 min to remove heavy bioactive materials. The filtrate was stored under low temperature for further experimental work.

5.3. Phytochemical screening of *Vachelia nilotica* seeds extract

A simple classification system divided phytochemicals into three chemically distinct groups. They are the phenolics, terpenes, N, and S containing compounds. All determinations were done in triplicates [47,48]. In present investigation, the phytochemical screening results indicated the presence of alkaloids, phenolic compounds, terpenoids, and fatty acids in the extract solution.

5.4. Synthesis of sulfur nanoparticles (SNPs)

All the glasswares were cleaned using an aqua regia solution (1:3 nitric acid and hydrochloric acid mixture) to remove the potential nucleation sites on the surface of the glassware before the synthesis.

Sodium thiosulfate pentahydrate [12.406 g/mol (0.5 M)] was dissolved in 100 mL aqueous brick-red seed extract directly and made a homogeneous mixture. After 5 min of

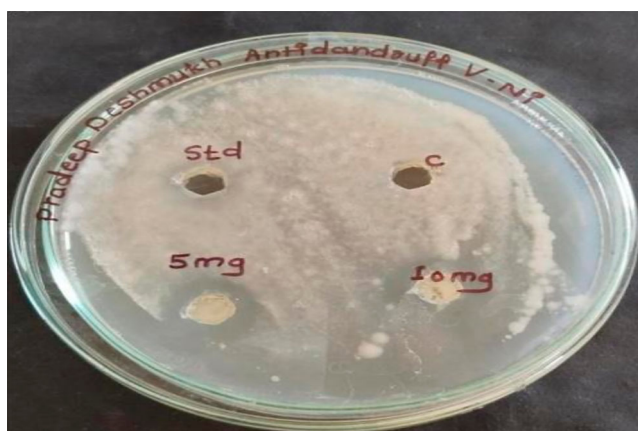


Figure 7. General reaction for synthesis of SNPs.

continuous stirring, 50 mL of 10% hydrochloric acid was added to the homogeneous reaction mixture. During reaction the brick-red colored solution changed to Whitish-yellow precipitate. The whitish-yellow precipitate thus formed was collected and then washed with deionized water and transferred to centrifugation process at 5000 rpm for 30 min under ambient temperature. The supernatant was discarded and the precipitate was repeatedly washed with distilled water and absolute ethanol to get rid of any biological material. The product was finally dried in a vacuum oven at 50°C for 30 min. The general reaction representing formation of SNPs is represented in Figure 7.

5.5. Characterization of sulfur nanoparticles

The response of sulfur nanoparticles to UV range of electromagnetic radiations was determined through recording UV–VIS spectrum (Perkin Elmer; Lambda 1050+ instrument). The FT-IR spectrum recorded (Shimadzu, IR SPIRI instrument) was used to examine synthesized SNPs. The morphology and size distribution of SNPs were determined using SEM (FEI-Nova Nano SEM 450 instrument) and TEM (TALOS F 200I FEG TEM). The crystalline structure of SNPs was characterized by XRD (Rigaku MiniFlex-II instrument) with Cu- K_{α} ($\lambda = 1.542 \text{ \AA}$) radiation. Purity and chemical composition of the fabricated nanomaterials thus obtained were analyzed with Energy-Dispersive X-ray Spectroscopy (EDS/EDAX) using CHNS/O Analyzer, Elementary, Unicube instrument.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Authors Pradeep Deshmukh and Yogesh Biradar acknowledge the financial support of Chhatrapati Shahu Maharaj Research, Training and Human Development Institute (SARTHI) Junior Research Fellowship (JRF), Pune, Maharashtra State, India, to carry out this research work.

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