

Bombax Ceiba Flower Extract Induced Surface Groups on Se Nanoparticles to Enhance Antibacterial Activity and Urea Detection

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Abstract

Biosynthesis of metallic-nanomaterials has emerged as a non-toxic and economical approach to their applications in diverse fields especially in biomedical sciences. Herein, this study first time reporting the use of *Bombax ceiba* flower extract for synthesis of selenium nanoparticles (SeNPs). Initially, SeNPs were confirmed by turning the color of reaction mixtures from light yellow to red-brick. Scanning electron microscope (SEM) and Transmission electron microscopy (TEM) images showed spherical shaped nanoparticles with smooth surface, size ranges between 30–150 nm. Dynamic light scattering (DLS) showed 100–150 nm for the distribution of particle size. X-ray diffraction (XRD) analysis revealed SeNPs crystallinity and confirmed by matching with selenium JCPD card No. 06-362. Energy-dispersive X-ray (EDX) spectra showed presence of pure Se peaks that corroborate the conversion of selenium ions into its elemental form by bio-reduction. Fourier-transform infrared spectroscopy (FTIR) spectra demonstrated that involvement of -OH, C-H, C = C, and C = O functional groups for SeNPs formation. Raman Spectra peaks at 250 cm^{-1} represent asymmetric trigonal selenium (t-Se). Ultraviolet-visible spectrophotometer (UV-Vis) peaks at 296 and 306 nm which is an indication of surface plasmon resonance (SPR). Moreover, maximum antibacterial activity of SeNPs were observed against *Staphylococcus aureus*- a gram positive bacteria that possess zone of inhibition (ZOI) 20 mm and *Klebsiella pneumonia* and *Pseudomonas aeruginosa*-gram negative bacterias with ZOI 28 mm, respectively, at concentration 100 $\mu\text{g}/\text{ml}$. In addition, the surface functionalities induced through extract components adhere over Se binds with urea and give its detection up to 1mM in milk sample. Conclusively, synthesized SeNPs may act as a potent potential antibacterial pharmaceutical candidate.

1. Introduction

The plant extracts has been a promising and ecofriendly approach to biosynthesis of metallic nanoparticles (MNPs) as compared to conventional physicochemical synthesis methods for last decade [1]. These conventional methods involves toxic, costly and non-biodegradable chemical and also yielding of hazardous by-products. The plant extracts consist of phytochemicals that involves in nanoparticles synthesis and forming of layers on their surfaces; are known as natural reducing and oxidizing agents. [2, 3]. Thus, phytochemicals coating enhances nanoparticles life span in in vivo by preventing the aggregation of nanoparticles as compared to conventionally methods' synthesized[4]. Therefore, plant extract mediated biosynthesis of MNPs are used in different fields safely especially in biomedical sciences[5].

In plant, there are present a wide range of phytochemicals such as phenols, proteins, enzymes, carbohydrates and secondary metabolites. Secondary metabolites have different forms of functional groups on their surfaces including carbonyl, hydroxyl, and amine [6]. These functional groups are involved in formation of metallic nanoparticles by reducing their ions, especially hydroxyl group. Therefore; MNPs are considered stable and biocompatible in in vivo. [7]. The applications of metallic nanoparticles including copper, silver and gold depends upon their type and nature relating to their uses in biomedical sciences for treating various diseases such as bacterial, viral and cancer. Moreover, these kinds of MNPs have been widely prepared using plant extracts of various plants species [5, 8]. However, these metals still have limitations in biomedical sciences like possessing higher cost, increased toxicity and inertness[9].

Selenium (Se) is a one of inexpensive, safe essential trace dietary mineral and widely used as antibacterial. It has broad functions in biological system such as for maintenance of health and growth[10]. It is commercially available in different form of products like Selisseo® in food for chicks and sealants as antibacterial agent [11]. Moreover, preparation of nanoparticles from selenium holds prominent position due to unique physicochemical properties. Selenium nanoformulations also have advantages over its bulk form due to low dose, low toxicity, maximum bioavailability and better reactivity[9]. Therefore, selenium nanoparticles have been biosynthesized using different plant extracts, microorganism, and enzymes. These nanoparticles showed various biological activities such as antimicrobial, anti-inflammatory and anticancer. Vetrivel et al prepared SeNPs using extract of *Ceropegia bulbosa* Roxb and evaluated their activities against *Bacillus subtilis* and *Escherichia coli* and MDA-MB-231 breast cancer cells [12]. Ghalia et al biogenically synthesized SeNPs of citrus fruits and showed antibacterial activities against *E. coli*, *M. luteus*, *B. subtilis* and *K. pneumoniae*[1]. Mechanistically, SeNPs attaches with bacterial surfaces, as it has different characteristics including biological activities and good adsorptive with functional groups of proteins namely C-O, C-N, NH and COO- that are present on it, where it releases the selenium ions into bacterial cell. These ions causes oxidative stresses and inhibit the protein synthesis or DNA mutations and kill the bacteria[13].

Bombax ceiba is a wild Asian deciduous tree belonging to the family Bombacaceae[14]. The whole plant parts including flowers are used as folk medicine for treatment of various ailments such as healing wound, gastrointestinal and expectorant due to possessing of wide range of phytochemicals[15]. Its flower also showed strong different pharmacological activities such as antimicrobial, anti-inflammatory, and antioxidant. These characteristics are due to having number of phytochemicals like flavonoids, terpenoid, saponins, carbohydrates, tannins and glycosides which add active surface moieties over metallic nanoparticles[16]. Moreover, researches shown that these phytoconstituents are potential candidates for the stabilization and reduction of metal ions into nano size[4]. Recently, AgNPs biosynthesis have been reported using extracts of *Bombax Ceiba* leaf, bark, and flower and their activities were observed against bacterias[17]. But its flower for the synthesis of SeNPs has not been reported yet. Present study is the first-time reporting for using of *Bombax ceiba* flower extract as bioreducing and stabilizing agent for synthesis of SeNPs and characterized these SeNPs. The sodium selenite is used as a precursor. The obtained SeNPs antibacterial activity was evaluated against six different strains of clinical bacterial strains along with urea detection in milk sample.

2. Material And Methods

2.1 Synthesis of Selenium nanoparticles

Bombax ceiba flower extract was managed by conventional methanolic extraction method. Selenium nanoparticles were synthesized by using 25ml flower extract and sodium selenite (2.8g). The mixture heated (60°C) with continuous stirring (150 RPM) until solution color turned into brick red from light yellow. The obtained precipitates washed with distilled water and dried overnight incubation at 60°C. The final powder stored for further analysis.

2.2 Antibacterial assay

Antibacterial activity of obtained SeNPs were assessed using well diffusion method. A normal saline solution (10–15 ml) with 3–5 well separated colonies of bacterial suspension density 0.5 McFarland standard were used. Only 0.1 ml out of total amount (1ml) was taken and placed on the Solid plate, a hole was also made in solid agar having a diameter of 5 mm. Then a small volume of 10 µL obtained from a solution of synthesized SeNPs added into wells and kept for 24 hours as incubation period at 37°C. The antibacterial activity of SeNPs was compared to size and diameter of the zone around the mentioned holes. Different concentrations of SeNPs, 20–100 µg/mL were also tested using similar procedure.

3. Results And Discussion

The size and morphology of biosynthesized SeNPs were characterized using SEM and TEM images, as indicated in Fig. 1 (a-c). SEM images in Fig. 1a showing SeNPs spherical shapes with variable sizes in the ranges of 30–150 nm. TEM images in Fig. 1 (c) clearly depicting smooth surface and sizes with 30 nm. The biomolecules present in *Bombax Ceiba* are involved for bioreduction of selenite and controlling the sizes. Further, these biomolecules prevent agglomeration due to forming of capping on nanoparticles and facilitate for in vivo stability. In Fig. 1 (d) & (e), EDX and XRD spectra are representing existence of elemental selenium and confirming its crystallinity, respectively. EDX spectra showed different characteristics peaks in Fig. (d) These SeNPs confirmed the bioreduction of selenium ion into elemental form of selenium. XRD spectra is described in Fig. 1 (e). Diffraction peaks appear at 28.76, 47.6 and 56.33 are indexed as (101), (111) and (112) planes, matched with selenium trigonal phase that has lattice constant $a = 4.362 \text{ \AA}$ and $c = 4.958 \text{ \AA}$, with agreement to Joint Committee on Powder Diffraction Standards (JCPDS) card No. 6-362 of t-SeNPs[18]. As amorphous crystalline characteristics are advantageous in biological systems, due to better solubility, subsequent adsorption and bioavailability in in vivo[19]. Further, size distribution measurements of nanoparticles done using DLS technique as shown in Fig. 1(f). the obtained single scan values are at 100–150 nm for particle size distribution, considered more positive value in suspension representing lesser agglomeration. FTIR analysis confirmed presence of different functional groups on synthesized SeNPs and shown in Fig. 2 (a). A broad absorption peak of stretching of –OH at 3416 cm^{-1} , representing the presence of aromatic rings (alcoholic and phenolic compounds). According to published studies, this peak must be followed by peaks between $800 - 600 \text{ cm}^{-1}$, in our results there is peak at 620 cm^{-1} . Therefore, our results correspond to published studies and confirms the presence of –OH containing aromatic rings. Two sharp peaks at 2911 and 2923 cm^{-1} , indicating C-H stretch of alkynes (ether-methoxy-OCH₃ groups). Peaks at 1637 and 1617 cm^{-1} , representing C = C stretch for alkenes, C = O stretch of ester group, these confirm the appearance of amid I band. Peaks at 1384 cm^{-1} indicating the bending of C-H asymmetric in CH₂ and CH₃ groups, this confirm appearance of

amide II band. The plane of C-H bending in superposition showing intense band at 1070 cm^{-1} , this is also characteristics features for vibrational stretching of Se-O, it's confirmed by literature [20]. FTIR results confirm that SeNPs were biosynthesized and coated with phytochemicals of Bombax ceiba flower, that have different functional groups. Moreover, the following peaks at 1070.31 cm^{-1} , 620.97 cm^{-1} , and 478.26 cm^{-1} confirms the vibrational stretching and bending relating to Se-O.

Raman spectroscopy determined the synthesized SeNPs vibrational characteristics for crystalline or amorphous nature as shown in Fig. 2(c) resonance peak analyzed that whether nanoparticles are monoclinic or trigonal. It was observed a resonance peak centered at 250 cm^{-1} , that is attributed to A1 symmetric stretching mode of amorphous selenium for tri-gonal selenium. Zhang et al observed the similar results for the monoclinic selenium (m-Se) as well as for trigonal Se (t-Se) [21]. The reaction mixture's yellow color turned into red ruby shown the biosynthesis of SeNPs by reducing with flower extract. Further, confirmed using UV-Vis absorption spectrum peaks at 296 nm and 306 nm (Fig.), formations of these peaks are due to Surface Plasmon Resonance (SPR). These peaks are also due to presence of different phytochemicals in Bombax ceiba flower extract, showing biosynthesized SeNPs possess capping layers of phytochemicals. Our results are corroborated previous studies that synthesis of SeNPs with different plant species extracts[13].

3.1 Antibacterial Activity

Biosynthesized Selenium Nanoparticles (SeNPs) antibacterial activities were observed using four different levels of SeNPs concentrations such as 20, 40, 80 and 100 $\mu\text{g/ml}$, against six clinical strains of bacteria. The antibacterial activity was indicated by increased diameters of zones of inhibition from 5 to 28 mm in photographs shown in Fig. 3 (a-f) and Table 1. Table 1 shows the each concentration's inhibitory zones. The appearance of clear inhibition zones around the wells, showed maximum sensitivity of SeNPs with zone of inhibition ($20 \pm 0.3\text{ mm}$) and ($18 \pm 0.4\text{ mm}$) against *S. aruras* and *S. Pneumonia* respectively, while *K. pneumonia* ($27 \pm 0.3\text{ mm}$), *P. aeruginosa* ($5 \pm 0.2\text{ mm}$), *Acinetobacter* ($29 \pm 0.3\text{ mm}$), *Escherichia coli* ($20 \pm 0.5\text{ mm}$).

The trend of enhancement of inhibition in zone was noted directly to increase in SeNPs concentration as shown in Fig. 3(i) at different concentrations from 20–100 $\mu\text{g/ml}$. Our results corresponded to SeNPs prepared using citrus fruit extract, higher zone of inhibition (ZOI) were observed in comparable inhibition zone [1]. These SeNPs may have potential candidates for pharmaceutical industries Moreover, the ethanolic extract of Bombax ceiba bark extract showed inhibitory effect against the most resistant type of strain Staphylococcus typhi and the most sensitive one the Staphylococcus aureus with zone of inhibition 10–18 mm in diameter) [22].

Table 1
SeNPs Showed Zone of inhibition (mm)

Concentration of SeNPs	Replicates	Gram-Positive Bacteria		Gram-Negative Bacteria			
		<i>Staphylococcus aureus</i>	<i>Streptococcus pneumonia</i>	<i>Klebsiella pneumonia</i>	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter</i>	<i>Escherichia coli</i>
20 µg	R ₁	14.00	11.50	14.00	13.50	13.50	13.00
	R ₂	13.00	13.50	15.00	16.00	14.00	12.50
	R ₃	12.00	14.00	16.00	15.50	14.50	10.50
	Mean ± SD	13.00 ± 0.816	13.00 ± 1.08	15.00 ± 0.81	15.00 ± 1.08	14.00 ± 0.70	12.00 ± 1.08
40 µg	R ₁	13.50	13.50	19.00	20.00	14.00	18.00
	R ₂	14.00	16.00	20.00	18.50	15.00	16.00
	R ₃	14.50	15.50	21.00	21.50	16.00	14.00
	Mean ± SD	14.00 ± 0.407	15.00 ± 1.08	20.00 ± 0.81	20.00 ± 1.22	15.00 ± 0.81	16.00 ± 1.63
80 µg	R ₁	15.00	15.50	21.00	17.00	14.50	17.50
	R ₂	14.00	17.00	22.00	25.00	16.00	18.00
	R ₃	16.00	18.50	23.00	24.00	17.50	18.50
	Mean ± SD	15.00 ± 0.816	17.00 ± 1.22	22.00 ± 0.81	22.00 ± 3.55	16.00 ± 1.22	18.00 ± 0.40
100 µg	R ₁	19.50	17.00	27.00	26.50	19.50	19.50
	R ₂	19.00	18.00	28.00	29.50	19.00	20.00
	R ₃	21.50	19.00	29.00	28.00	21.50	20.50
	Mean ± SD	20.00 ± 1.47	18.00 ± 0.81	28.00 ± 0.81	28.00 ± 1.22	20.00 ± 1.47	20.00 ± 0.40

3.2 Urea Detection in Milk sample

The as synthesized Se NPs with induced surface groups are then treated with milk sample which is treated before use to remove protein and fats as discussed in (Fig S1 and S2). The skimmed milk is adulterated with urea in 10, 20, 30, 40mM to form standard solution (Fig. 4a). pH of sample solution was adjusted to 8.0 which facilitated complex formation (Fig S3). To check activity of Se NPs the adulterated sample 3ml is treated with 1m of 1mM of Se NPs and warmed the mixture up to 40°C which turns the mixture to slight orange color which indicated the complex formation. The surface groups i.e. -COOH may bind with -NH₂ group to form a metal complex with λ_{max} at 625nm (Fig. 4b). Peak shift from λ_{max} 274nm (urea) to 625nm is resultant from metal-urea complex indicated by color change. The same process was repeated with treated milk with no adulterant added which declared same result which proves the activity of surface modified Se NPs to detect urea in sample solution (Fig S4).

Conclusion

Present study first time reporting the using of *Bombax ceiba* flower extract for biosynthesis of selenium nanoparticles (SeNPs). SeNPs sizes between 100–150 nm and spherical morphological shapes were confirmed using SEM, TEM and DLS. EDX and XRD spectra are showed presence of elemental selenium and confirmed its crystallinity. Further, FTIR spectra also revealed the different types of functional groups (-OH, C-H, C = C, C = O) on SeNPs that are involved for bioreducing the selenite into nanoparticles and stabilizing them. Moreover, SeNPs revealed strong antibacterial activities against gram-positive and gram-negative bacteria.

Maximum zone of inhibition (ZOI) 20 mm against gram-positive bacteria (*Staphylococcus aureus*) and 28 mm against gram-negative bacteria (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) were observed. Also, the surface functionalized NPs give urea detection up to 1mM from raw milk sample which implies its uses in food sciences to check adulterant contamination in food samples. However, further investigations are required to confirm the efficacy of these SeNPs as potential candidates for pharmaceutical industries.

Declarations

Author Contribution.

Muhammad Waqas: data curation, methodology, writing – original draft, visualization writing – review and editing; **Sidra Aslam:** methodology, formal analysis; **Misbah Akram:** data curation, formal analysis; **Adnan Khaliq:** methodology; **Samreen Ahsan:** data curation, formal analysis; **Atif Liaqat:** formal analysis, resources, bioinformatics; **Misbah Mirza:** data curation, formal analysis; **Muhammad Safdar:** data curation, validation, writing – review and editing; **Waseem Akhtar Qureshi:** conceptualization, writing – review and editing; supervision, validation, visualization writing – review and editing.

Data Availability

This article is written with the data created or analyzed during this study.

Declarations Ethics Approval and Consent to Participate

The results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration (from you or one of your Contributing Authors) by another publisher.

Consent for Publication

Not applicable.

Conflict of Interest

No, I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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Not applicable

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Figures

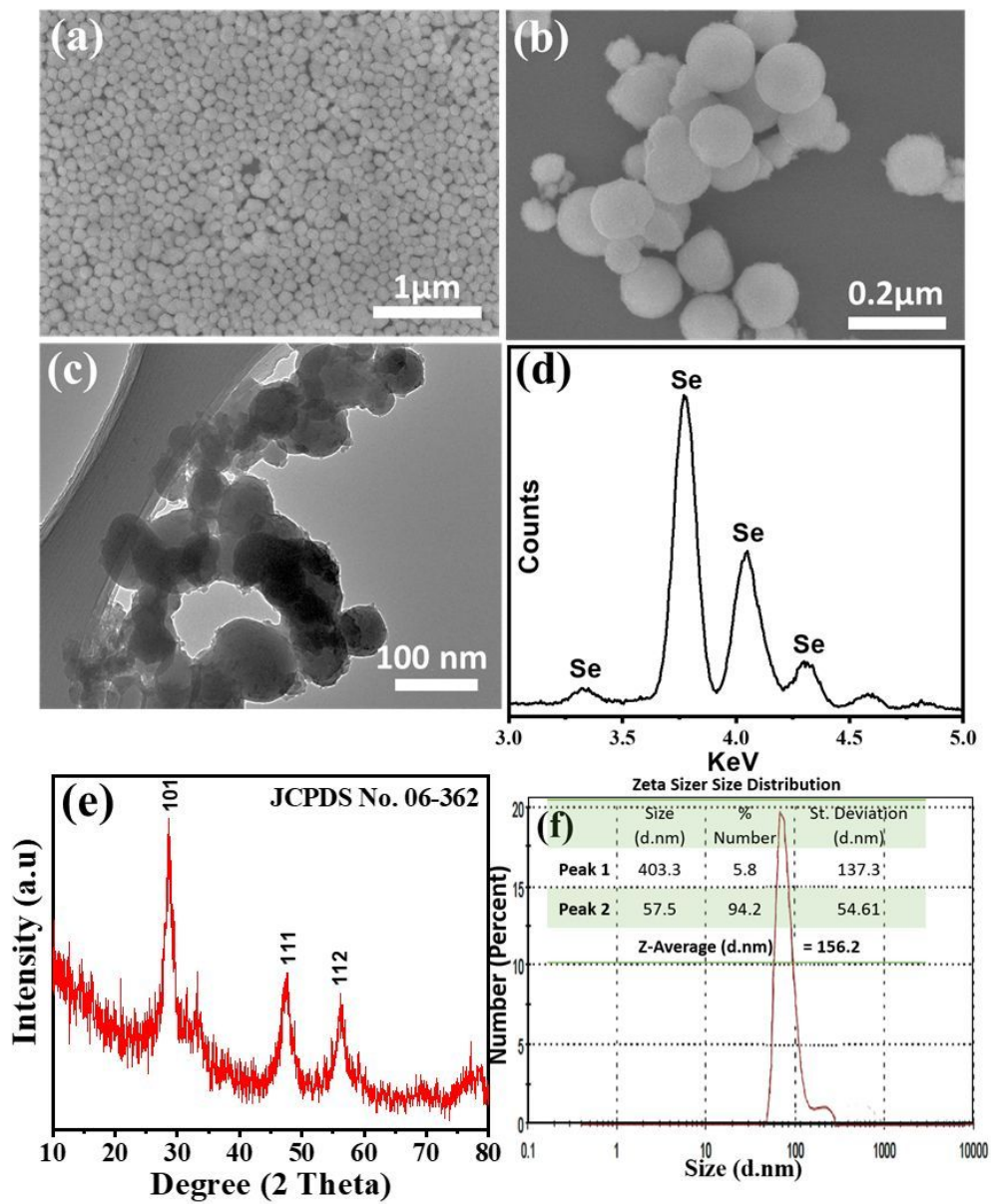


Figure 1

(a)-(c) SEM images of bio-reduced Se NPs (d) EDX spectra of Se NPs (e) XRD spectra of Se NPs powder (f) Size distribution of Se NPs through DLS spectra

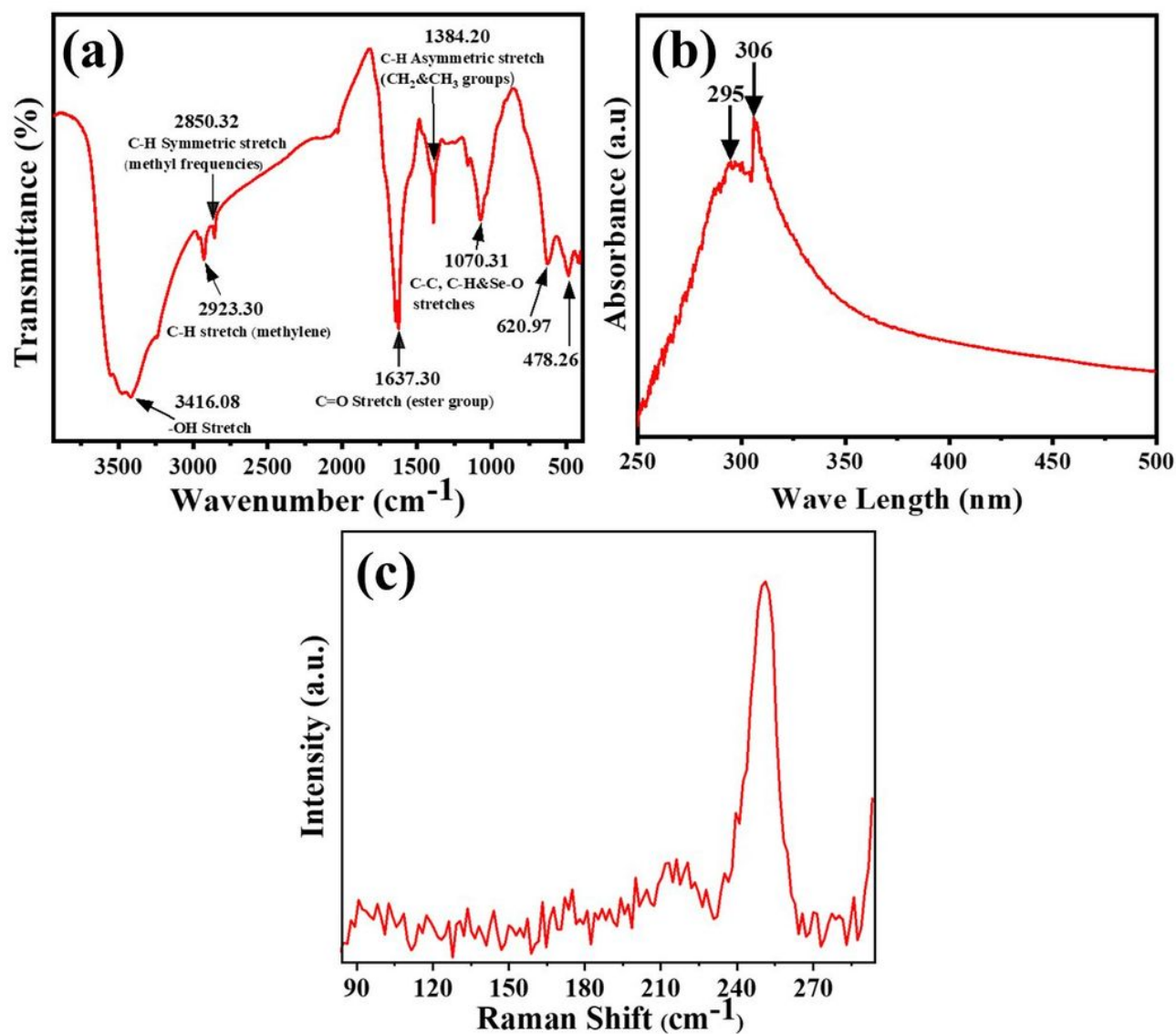


Figure 2

SeNPs synthesized through Bombax ceiba extracts (a) FT-IR spectra (b) UV-Vis spectra and (c) Raman shift spectra.

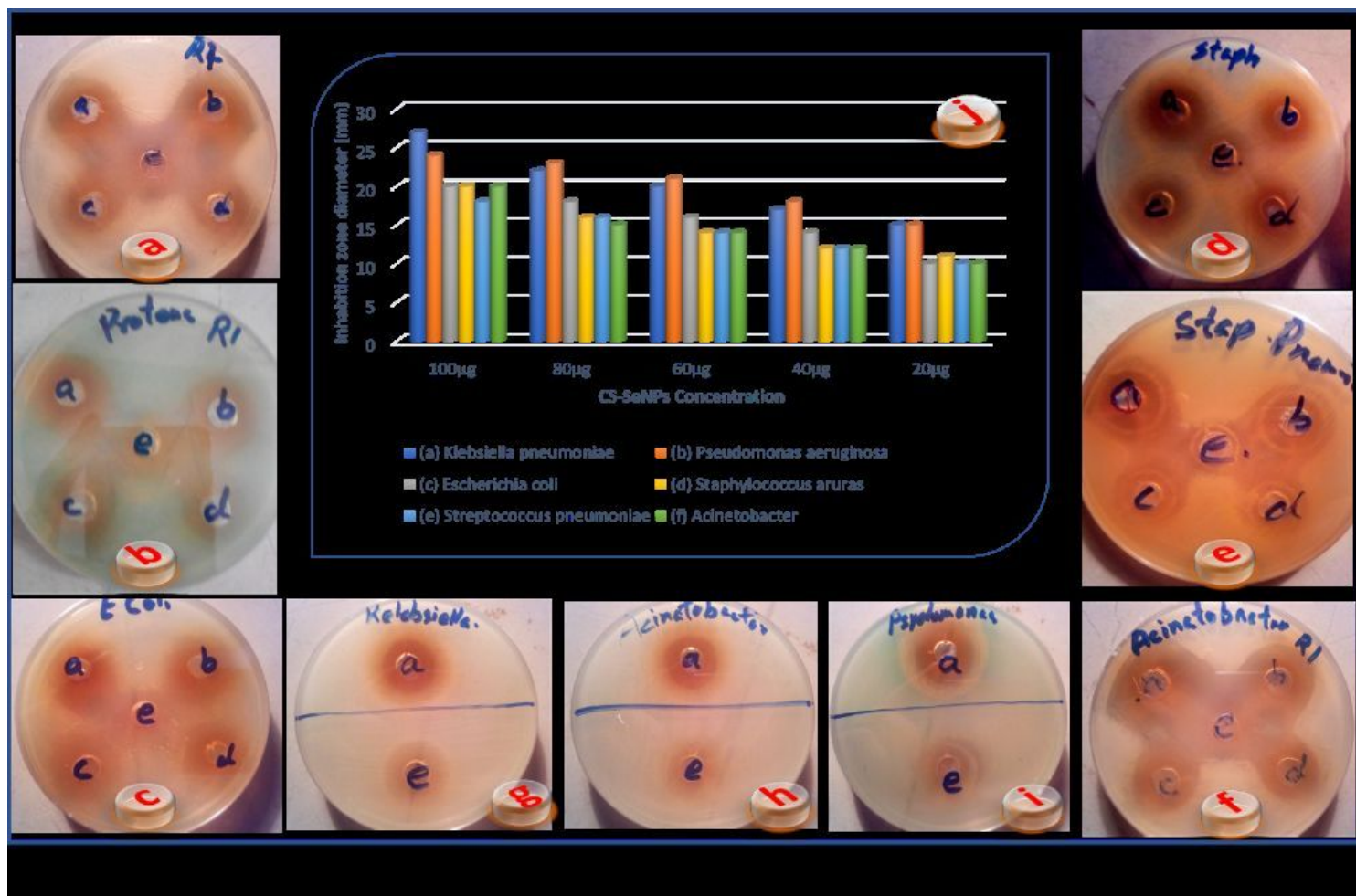


Figure 3

Antibacterial activity of SeNPs synthesized using *Bombax ceiba* flower extract towards (a) *K. pneumoniae* (b) *P. aeruginosa* (c) *E. coli* (d) *S. aureus* (e) *S. pneumoniae* (f) *Acinetobacter* (g) Photograph of concentration effect on zone of inhibition by (a) (h) (f) and (i) (b). Concentration effect in bar graph is given in (j).

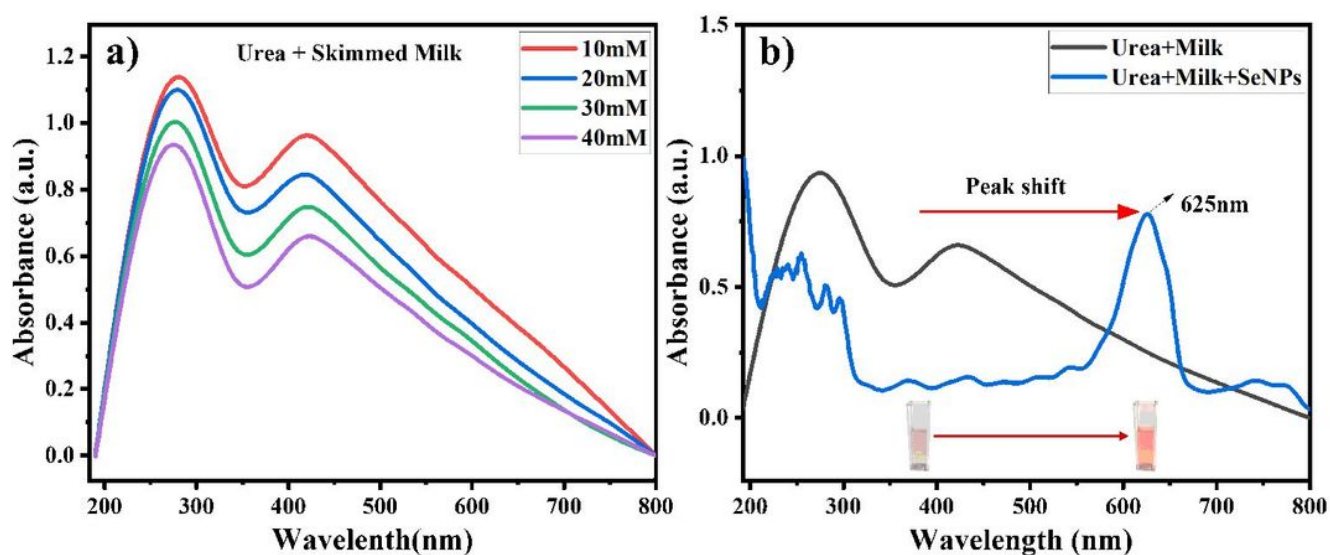


Figure 4

SeNPs urea detection activity (a) UV-Vis spectra of adulterated milk sample (treated) (b) UV-Vis spectra of added SeNPs indicating peak shift after complex formation

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