

Synthesis Of Selenium Nanoparticles (Senps) Using *Silybum marianum* And *Aloe vera* For Biological Studies

Aqsa Iqbal

Faculty of Sciences the Superior University Lahore, Pakistan

Dr. Muhmmad Atif *

Faculty of Sciences the Superior University Lahore Pakistan

Email: muhammad.atif.fsd@superior.edu.pk

Ali Imran Mallhi

Faculty of Sciences, The Superior University Lahore, Pakistan

ABSTRACT

The development of environmentally benign nanomaterials has stimulated the interest in plant mediated synthesis routes in biomedical applications. In the present study, a green and chemically sustainable approach was determined for the phyto-assisted fabrication of selenium nanoparticles (SeNPs) using binary extracts of *Silybum marianum* and *Aloe vera*. The rationale of combining these botanical sources was to utilize the complementary phytochemical profiles (high in flavonoids, phenolic compounds, terpenoids and polysaccharides) for enhancing both the reduction efficiency and the colloidal stabilization. Sodium selenite (Na_2SeO_3) was used as the selenium precursor and its bioconversion to elemental selenium (Se_0) was done under mild aqueous conditions without the need for external toxic reducing agents. As preliminary evidence of the formation of SeNPs, a characteristic visual color transition was observed and later confirmed spectroscopically. Fourier transform infrared spectroscopy implied the active involvement of hydroxyl, carbonyl, and amide functional groups in nanoparticles reduction and surface capping, which proved the dual role of plant-derived biomolecules. Morphological and size-distribution analyses showed the formation of nanoscale and mostly spherical nanoparticles with satisfactory dispersion stability, which showed the efficiency of the binary extract system on inhibiting agglomeration. The biologically synthesized SeNPs showed a greater antimicrobial potential than crude extracts suggesting the synergistic interaction between oxidative stress mechanisms induced by selenium with membrane perturbation mediated by phytoconstituents. This study proposed an eco-friendly and scalable platform for binary phytochemical systems synthesis of biofunctionalized selenium nanostructures and highlighted the potential in the engineering field of stable nanotherapeutics. The findings add to the ongoing applications in green

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

nanobiotechnology providing a sustainable alternative to more conventional methods of chemical synthesis.

Keywords— Selenium nanoparticles (SeNPs), Green synthesis, Binary plant extract, Surface functionalization, Ultrasonic-assisted extraction (UAE), Antimicrobial activity, Nanobiotechnology.

Introduction

The worldwide escalation of antimicrobial resistance (AMR) is one of the greatest threats to modern healthcare systems [1–3] which critically reduces the therapeutic effectiveness of conventional antimicrobial agents and leads to higher treatment failure, longer hospitalization and higher mortality rates. The overuse and indiscriminate use of antibiotics in clinical medicine, veterinary medicine, agriculture and food production have increased the rate of emergence and spread of multidrug-resistant (MDR) microorganisms and promoted the horizontal transfer of resistance determinants between environmental reservoirs. Conventional antimicrobial discovery pipelines have failed to keep up with this fast-evolving resistance crisis resulting in an urgent need for alternative therapeutic strategies that work using different mechanisms of action with a reduced susceptibility to resistance development. In this context, the introduction of nanotechnology based antimicrobial platforms has been seen as a potential new generation approach that can avoid classical resistance mechanisms by multifunctional and physical antimicrobial mechanisms. Nanotechnology allows engineering of materials on the nanoscale with unique physicochemical, structural and biological properties [4–6] that are fundamentally different from the bulk counterpart. Among metal and metalloid-based nanomaterials, nanomaterials of selenium (SeNPs) have attracted more and more attention due to their inherent biological significance and multifunctional bioactivity. Selenium is an essential trace element [7–9] that is incorporated into selenoproteins that regulate the redox balance, antioxidant defense, immune modulation and cell signaling pathways. At the nanoscale, elemental selenium (Se₀) has been found to have better bioavailability, cellular interactions and lower systemic toxicity [10–12] than its ionic/inorganic forms. Mechanistically, SeNPs are antimicrobial through a variety of synergistic pathways; excessive ROS generation, microbial membrane integrity disruption, protein denaturation, DNA damage, metabolic interference and redox homeostasis perturbation are some of the antimicrobial mechanisms. The multimodal and non-specific nature of these mechanisms greatly decreases the chances of microbial resistance development and therefore SeNPs stand as promising antimicrobial nanotherapeutic agents.

Despite their great therapeutic potential, the biomedical translation of selenium nanoparticles is still hampered by the challenges related to the reproducibility of synthesis, stability of the surface and biocompatibility optimization. Conventional physicochemical approaches to synthesis are often toxic (toxic reducing agents) and energy intensive, and require harsh reaction conditions as well, limiting biomedical applications as well as causing environmental concerns. While chemical reduction methods can be used to control the formation of nanoparticles, they often need a lot of purification to remove residual contaminants. Consequently, green approaches for

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

synthesis [13–18] have started to be considered as sustainable and environmentally friendly alternatives with plant-derived phytochemicals being used as reducing, capping and stabilizing agents in the fabrication of nanoparticles.

However, most of the current studies use single-plant extract systems that limit the chemical diversity of bioactive molecules that can be used for nanoparticles functionalization and surface modification. The concept of binary or combinatorial plant extracts mediated nanoparticle synthesis is still under explored and it is a serious gap in science. A dual-extract system may increase the reduction kinetics and nucleation efficiency, control the particle growth and create a more chemically diverse and functionally enriched surface corona. Such synergistic interactions of phytochemicals can potentially lead to improvement in colloidal stability, biological compatibility and antimicrobial performance compared to the conventional single extract approaches.

In the current study, a new synergy-based phytochemical approach was prepared by combining extracts of *Silybum marianum* and *Aloe vera* for green synthesis of selenium nanoparticles using sodium selenite (Na_2SeO_3) as a precursor of selenium. The innovation of this approach is based on the exploitation of complementary phytochemical compositions from two botanically distinct medicinal plants for improvement of reduction efficiency with simultaneous enrichment of the nanoparticle surface with multifunctional bioactive compounds. *Silybum marianum* contains flavonolignans, especially the silymarin complex, polyphenols, and antioxidant metabolites that have a good electron-donating capacity and binding affinity to the surface, which helps to efficiently reduce and stabilize in the formation of nanoparticles. In parallel, *Aloe vera* is a source of polysaccharides, anthraquinones, vitamins, amino acid and phenolic constituents with drawbacks for antimicrobial, immunomodulatory and wound healing. The combination of these extracts creates a redox-active reaction environment that promotes nucleation processes and functional surface modification by the capping of biomolecules. In aqueous plant-mediated systems, selenite ions are bio reduced through electron transfer from phytochemical functional groups, and lead to formation of elemental selenium nuclei followed by nucleation, particle growth and stabilization. The phytochemical corona formed on the surface of nanoparticles contributes to the better dispersion stability and provides other biological functionality in addition to the intrinsic properties of selenium. This biofunctionalization not only improves the antimicrobial performance, but also contributes to increase the surface reactivity and the potential biological targeting while ensuring environmental sustainability.

The current work presents several new contributions, such as development of a binary plant extract-mediated green synthesis platform for the synthesis of selenium nanoparticles, investigation of the synergistic phytochemical reduction mechanisms in comparison with the single plant conventional reduction systems, systematic physicochemical characterization to verify the formation of the structure and surface interactions, and evaluation of the antimicrobial efficacy against representative bacterial strains to determine the functional performance. Furthermore, the investigation of surface-functionalized SeNPs as multifunctional antimicrobial nano materials with improved bio interaction and possible therapeutic relevance are

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

discussed. Future research directions should be on mechanistic molecular levels investigations to unravel phytochemical and selenium interaction pathways during nucleation and growth processes, advanced surface analytical techniques to characterize specific biomolecular binding patterns, extended and antimicrobial evaluation against clinically relevant multidrug resistant and biofilm forming pathogens. Comprehensive in vitro cytotoxicity testing as well as in vivo biocompatibility assessments will be necessary methods to translate into validation for use. Additionally, nano-drug conjugation strategies allowing to load antibiotics or bioactive compounds onto the surfaces of SeNPs may bring in synergistic antimicrobial effects as well as reduce the therapeutic dosages. The development of target delivery systems and smart-responsive selenium nanoplates is another promising direction of development and precision antimicrobial therapy. Scaling up of the green synthesis protocol while ensuring the reproducibility of the batches and physicochemical stability will also be crucial for the industrial and biomedical implementation. Overall, this study presents a sustainable, bioinspired, and function-wise optimized selenium-based nanosystem, and the binary plant extract strategy is a scalable strategy to support the design of novel antimicrobial nanomaterials to counter the emerging problem of the global burden of antimicrobial resistance

2. EXPERIMENTAL

2.1 Materials

1. **Selenium sources** 0.5 g **Sodium selenite** (Na_2SeO_3 , analytical grade) used as the selenium
2. Local collection of fresh leaves of *Silybum marianum* and fresh leaves of *Aloe vera* were collected and authenticated.
3. **Materials** Ethanol (96%, analytical grade), distilled and deionized water.
4. Use **some natural materials** like cotton, straw, twigs and even leaves.
5. Nutrient agar and MacConkey agar media, used for conducting antimicrobial test.

2.2 Instrumentation

1. **Ultrasonic bath (Skymen, China):** Ultrasonic bath (Skymen, China): For dispersion, to prevent the aggregation of nanoparticles and for improving the homogenization of the plant extracts and the nanoparticles in suspension.
2. **A hot plate with magnetic stirrer** (Dawn analytical) was used to ensure controlled heating and continuous agitation of the phytochemical extraction and in the reaction for the green synthesis.
3. **An analytical balance** (OHAUS, USA) was used for the accurate weighing of plant materials, chemicals and reagents for experimental accuracy and reproducibility.
4. **A centrifuge** (DAWN-04, China) was used in separation, purification and collection of plant extracts and synthesis selenium nanoparticles respectively after the reaction process.
5. UV-Visible spectroscopy [22–24] (Mitash, China) was employed to monitor the formation of nanoparticles by measurement of the changes in optical absorption

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

related to the synthesis of selenium nanoparticles.

6. Fourier Transform Infrared (FTIR) [25–27] spectroscopy (Shimadzu, Japan) was used for identification of functional groups in reduction and stabilization of nanoparticles, and for phytochemical interaction with the surface of nanoparticles.
7. **Characterization of phytochemicals:** After extraction, the samples were analyzed and characterized using High- Performance Liquid Chromatography (HPLC) (Shimadzu, Japan) with a UV detector.
8. **Drying of plant materials and synthesized liquor of nanoparticles:** A drying oven was used to dry plant materials and synthesized nanoparticles under controlled temperature.
9. **pH control and monitoring:** pH meter (Benchtop model, China
10. Scanning Electron Microscopy [28–30] (SEM) (JSM-5910, JEOL Japan) was outsourced and used to check the surface morphology and the structural features of the synthesized nanoparticles.
11. **Determination of the Particle size:** Zetasizer (Malvern, UK) was outsourced and used for determination of the particle size distribution with Dynamic Light Scattering (DLS) analysis and for zeta potential determination for the determination of colloidal stability.

2.3 Plant Material Preparation

Fresh, healthy and morphologically intact specimens of the edible plants *Silybum marianum* and *Aloe vera* were collected from geographically defined local regions and authenticated by a qualified botanist. Voucher specimens were put in the institutional herbarium for future reference for ensuring taxonomic verification and reproducibility. Only disease-free and mature plant materials were used in order to reduce variability of phytochemicals and increase bioactive compound yield. The samples collected were extensively washed under running tap water and then rinsed with distilled water to remove the adhering soil particles, microbial contaminants and surface impurities. For *Silybum marianum*, the cleaned plant material was shade-dried under controlled laboratory condition (temperature: about 25-30 deg C; relative humidity controlled) until they reached constant weight condition to minimize moisture content and prevent any enzymatic degradation of phytochemicals. The dried material was pulverized into fine powder with the help of a sterilized mechanical grinder to increase its surface area and thus the solvent penetration during extraction. The powdered material was passed through a mesh sieve to get uniform particle size distribution for consistency of extraction. In the case of *Aloe Vera*, fresh leaves returning was processed in a delicate way in order to get the gel matrix of the leaves inside, but first, it had to be separated from the outer green rind. The gel was homogenized to achieve a homogeneous suspension and also filtered to remove the fibrous material. The prepared plant materials were stored in sterile and air tight containers at four degree Celsius to extract to maintain phytochemical stability. This standardized preparation protocol was responsible for maximum preservation of reducing agents, flavonolignans, polysaccharides, phenolics and antioxidant metabolites responsible for the efficient bio-reduction and stabilization of the selenium nanoparticles.

2.4 Application of Ultrasound Assisted Extraction of Bioactive Phytochemicals

Ultrasound-assisted extraction [19–21] (UAE) was used as a controlled and efficiency-enhanced application for the extraction of phytochemicals from the powder of *Silybum marianum* and homogenized Aloe vera gel for further use as the reducing and stabilizing agents in the binary green synthesis of selenium nanoparticles. A accurately weighed amount of plant powder or gel (e.g. X g) was dispersed in distilled water at a certain solid: **solvent ratio** (e.g. 1: 10 or optimized ratio w/v) in a closed extraction vessel. Suspension was ultrasonically treated that is done in ultrasonic bath under controlled frequency and controlled power conditions. Acoustic cavitation induced by ultrasonic irradiation could create a localized high pressure and high temperature micro environment which favors the disruption of plant cell walls and increases mass transfer of intracellular bioactive compounds to the solvent. These mechanical and physicochemical phenomena gave important improvement for extraction efficiency of polyphenols, flavonolignans, polysaccharides, vitamins and antioxidant molecules. The extraction process was carried out for a set optimized period of time while keeping the temperature controlled to avoid thermal degradation of heat-sensitive phytochemicals. Temperature was constantly monitored to make sure it was kept in a biologically safe range during the sonication process. After ultrasonication is completed, the extracts were cooled to room temperature and filtered using No. 1 filter paper of Whatman to remove solid residues. The filtrates were centrifuged at 5000 rpm for 10 minutes in order to obtain clear supernatant free of particulate matter. The purified extracts were then kept at 4°C for further characterization and utilization in the synthesis of nanoparticles. To obtain reproducibility and reliability of the experimental results, the extraction efficiency can be evaluated by the phytochemical quantification methods such as total phenolic content (TPC) and total flavonoid content (TFC), which can verify the availability of active reducing agents in the formation of selenium nanoparticles. The optimized ultrasound-assisted extraction procedure provided for the enhanced yield, reproducibility, shorter extraction time, and maximum recovery of bioactive compounds required for controlled nucleation, growth, and stabilization of selenium nanoparticles in the binary plant-mediated system.

2.5 HPLC Analysis

A study was done using the technique of High-performance Liquid Chromatography (HPLC) for characterizing and quantifying the phytochemical constituents that can be found in optimized extracts of *Silybum marianum* and Aloe vera. The main aim of this analysis was to verify the existence and relative concentration of bioactive compounds responsible for the reduction of sodium selenite and stabilization of selenium nanoparticles in the binary extract system. Prior to analysis, filtered extracts were further purified by membrane filtration (0.45 µm), and diluted appropriately with a solvent of high purity for quantum chemistry analysis (HPLC grade) so that compatibility with the chromatographic system is ensured. HPLC profiling allowed to identify phenolic compounds, flavonolignans, flavonoids, and antioxidant metabolites involved in the electron transfer and surface functionalization during the synthesis of nanoparticles. Compound identification was done by comparing the retention times and the peak areas to authenticated analytical standards. Quantification was done using calibration curves using standard solutions. The obtained chromatographic profile

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

provided the experimental validation of reducing and capping potential of the binary plant extracts and supported their functional role in the fabrication of selenium nanoparticles.

2.6 Chromatographic conditions are given, including eluent rates, flow rates

Phytochemical separation was performed on a reverse phase column of an HP liquid chromatography instrument combined with an ultraviolet and visible light detector and C18 analytical column under controlled temperature conditions. The mobile phase was solvent A (water with formic or acetic acid 0.1%) and solvent B (methanol, acetonitrile (HPLC grade)). A gradient elution program was used in order to perform efficient separation of compounds with different polarities and obtain better peak resolution.

Some of the important chromatographic parameters were:

- Flow rate: 0.8–1.0 mL/min
- Injection volume: 10–20 μ L
- Column temperature: 25–35°C

To detect and measure the sample dispensed in a pipette by the flow analyzer by registering the following parameters:

1. Total run time Optimized to ensure complete chromatographic separation
2. Calibration curves were made using external standards to ensure linearity, accuracy and reproducibility.
3. Data acquisition and processing was done using validated software for the chromatography.

These controlled analytical conditions were used for precise profiling of bioactive molecules directly involved in the bioreduction and stabilization mechanism of the Se nanoparticles.

2.7 Green synthesis of the Selenium Nanoparticles

Selenium nanoparticles were prepared by a green reduction method using the sodium selenite as the selenium precursor and the optimized binary extract of *Silybum marianum* and *Aloe vera* as reducing and stabilizing agents. An aqueous solution of sodium selenite Na_2SeO_3 was made at a predetermined optimized concentration with constant magnetic stirring. Reaction reagents such as pH, temperature, precursor concentration and extract volume were optimized systematically in order to control nucleation kinetics and particle growth. The binary plant extract was dropwise added in selenium precursor solution under constant stirring condition to ensure homogeneous mixing for uniform reaction condition. The reduction of Se^{4+} ions to elemental selenium (Se^0) was evidenced by a gradual change in color from colorless to reddish-brown, which was an indication of the formation of nanoparticles. The reaction was allowed to proceed over a defined optimized length of time to ensure that complete reduction and stabilization occurred. During the synthesis, phytochemicals that were present in the binary extract worked in synergy by serving as:

- Electron donors which favour reduction of selenite ions
- Capping agents to prevent the particle aggregation
- Colloidal dispersion and surface functions & Agent

Upon the completion of synthesis, the resulting nanoparticles were collected by centrifugation at 10,000 - 15,000 rpm for 15-20 min. The precipitated nanoparticles were washed several times using distilled water and ethanol to remove the residual ions and unbound phytochemical components. The purified nanoparticles were dried under controlled conditions and kept in air tight containers which were further subjected to physicochemical characterization and antimicrobial evaluation. This environmentally sustainable approach of synthesis allowed fabricating surface-functionalized selenium nanoparticles with an enhanced stability, raised biological compatibility, and controlled structural characteristics.

2.8 Preparation of Nanosuspension and Stabilizer Screening

The purified selenium nanoparticles prepared by the green synthesis method were subsequently prepared as an aqueous nanosuspension in order to enhance the dispersibility, physicochemical stability and suitable biological evaluation. For nanosuspension preparation, a well weighed amount of dried selenium nanoparticles was dispersed in distilled water under continuous magnetic stirring. To prevent the aggregation of the nanoparticles and to increase colloidal stability, various types of biocompatible stabilizers (e.g. polymers and/or surfactants that provide a steric or electrostatic stabilization effect) were tested in a systematic manner. Each stabilizer was introduced separately in separate nanoparticle dispersions at determined concentrations under controlled stirring and/or ultrasonication in order to achieve homogeneous dispersion. The formulations were allowed to equilibrate under standardized conditions in order to determine stability performance. Stabilizer screening was conducted by testing significant physicochemical parameters which include: particle size and size distribution, polydispersity index (PDI), Zeta potential, sedimentation behavior, homogeneity and phase separation of the visual and short and intermediate term stability of storage. The best stabilizer was chosen on the basis of a minimal aggregation, a narrow particle size distribution, high absolute zeta potential values and long-term dispersion stability. The optimized nanosuspension formulation was stored under controlled conditions in the laboratory and was subjected to further physicochemical characterization so that reproducibility and stability of the formulation could be confirmed.

2.9 UV-Visible Spectroscopy

UV-Visible spectroscopy [22–24] was used to determine the successful formation of selenium nanoparticles as well as the optical properties and colloidal stability of the prepared nanosuspension. Spectral measurements were carried out with the help of a calibrated UV-Visible spectrophotometer. The spectrum of absorbance measurements of the prepared nanoparticles was measured in a suitable wavelength range to detect the characteristic optical behaviour of nanoscale selenium. The existence of a defined absorption band confirmed the formation of nanoparticles while the change in absorbance intensity gave an insight into their concentration and dispersion uniformity. For the nanosuspension formulation, UV-Visible spectra were collected at known time intervals to follow the temporal stability and detect the occurrence of aggregation, precipitation, or shift in spectra which could be an indication of physicochemical instability. All the measurements were performed with distilled water or the respective solvent as a blank reference to ensure the accuracy and reproducibility of the

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

measurements.

2.10 Fourier Transform Infrared (FTIR) [25–27]

Spectroscopy Fourier Transform Infrared (FTIR) [25–27] Spectroscopy illustration Fourier Transform Spectroscopy Spectroscopy represents a crucial method employed to analyze chemical compositions via spectrophotometry. 2.10 Fourier Transform Infrared (FTIR) [25–27] Spectroscopy Fourier Transform Infrared (FTIR) [25–27] Fourier Transform Infrared (FTIR) [25–27] spectroscopy was used to study surface chemistry, functional group participation and molecular interaction related to the synthesized selenium nanoparticles and stabilized nanosuspension. Dried samples of the nanoparticles and representative formulation components were investigated in the spectral range from roughly 4000 to 400 cm^{-1} in an FTIR Spectrometer. The analysis was aiming at the identification of characteristic bands of absorption for functional groups such as:

1. Hydroxyl ($-\text{OH}$)
2. Carbonyl ($\text{C}=\text{O}$)
3. Carboxyl ($-\text{COOH}$)
4. Amino ($-\text{NH}$)
5. Phenolic groups

2.11 Scanning electron microscope

The surface morphology and the structural characteristics of the green synthesized selenium nanoparticles were analyzed by a Scanning Electron Microscopy [28–30] (SEM). Dried samples of nanoparticles were attached to aluminum stubs using conductive adhesive carbon tape. The samples were sputter coated with a conductive thin layer of gold, in order not to have charging effects during the imaging. Micrographs were made in a vacuum under high vacuum at optimum accelerating voltage.

SEM analysis has been undertaken to:

1. Determine the morphology of the particles
2. Assess surface topology
3. Investigate the tendency of aggregation
4. Approximate range of particle sizes Estimate in dry state

Morphological evaluation provided visual evidence of formation of nanoscale particles and evaluation of the structural uniformity as a consequence of reduction and stabilization by phytochemical-mediated binary plants extract system.

2.12 Antimicrobial Activity

The antimicrobial effect of the green synthesized selenium nanoparticles was investigated against selected bacterial strains to know the potential of the nanoparticles in biology. Fresh cultures of the bacteria were prepared and adjusted to the turbidity of 0.5 McFarland standard to assure uniformity of concentration of the inoculum.

The antibacterial assay was performed using aseptic condition using agar well diffusion method [36–38]. Generous inoculation of the sterile nutrient agar plates using the sterile cotton swabs with the bacterial suspension was done. Definitely diameter wells were aseptically punched out into the agar medium.

Predetermined concentrations of nanoparticles suspension of selenium were added to

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

the wells. Control groups included:

1. **Negative control** (sterile distilled water, formulation medium)
2. **Positive control** (normal antibiotic).

Plant extract only (of course the extract effect must be differentiated from the nanoparticle effect if any)

The plates were incubated at 37 degrees C for 18- 24 hours. After incubation, the zones of inhibition were measured in millimeters after the use of a calibrated scale.

All the experiments were done in triplicates and results expressed as mean + standard deviation.

The performance of antibacterial was tested on the basis of:

1. Presence or absence of the inhibition zones
2. Diameter of Inhibition by magnitude
3. Concentration dependent pattern of response

Interpretation of antimicrobial activity [31–35] was done with caution without trying to over ascribe mechanism. Observed activity correlated with the size and surface characteristics in nano-scale and potential interactions of selenium nanoparticle and bacterial cell membrane.

3.RESULTS AND DISCUSSION

3.1 Optimization of the Ultrasonic-Assisted Extraction

Extraction temperature had a significant effect on the yield of the binary extract obtained from **Silybum marianum** and **Aloe vera** under ultrasonic assisted extraction condition. The extract yield showed progressive increase in the temperature range of 30 to 50 degC and maximum at 50 degC (Table 3.1), which showed higher cavitation efficiency and better mass transfer at moderately higher temperatures. However, the decline in yield was seen at a temperature above 50 degC, indicating that there is a possibility of thermal degradation of heat-sensitive phytoconstituents. These results show that thermal controlled conditions are important in maintaining bioactive compounds that are responsible in reducing and stabilizing selenium nanoparticles.

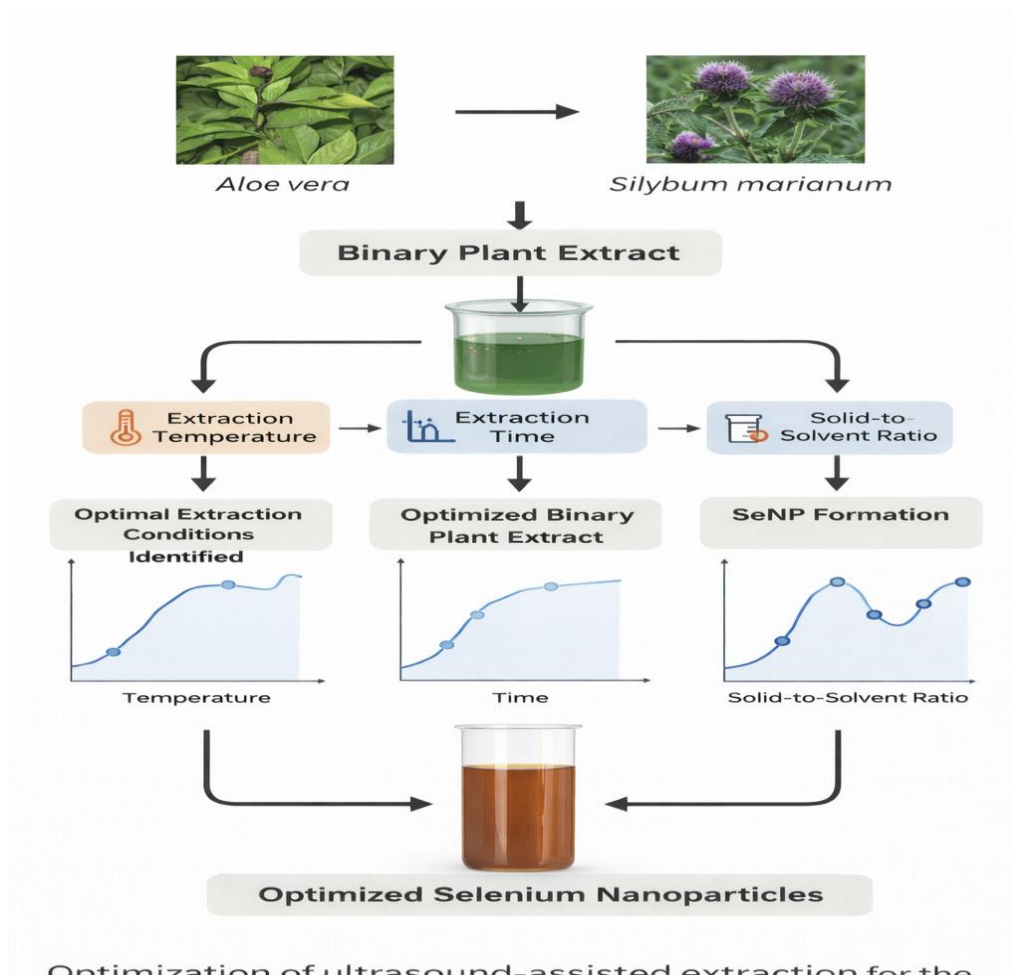
Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>



Optimization of ultrasound-assisted extraction for the
Figure 3.1 Graphical presentation of Formulation Development

Extraction Temperature (C)	Yield (%w/w)	Total Phenolic content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g)	Observation
30	8.12 ± 0.24	41.6 ± 1.2	26.4±0.9	Incomplete extraction
40	11.34 ±0.31	58.7± 1.6	3.48±1.1	Improved solubilization
50	14.21 ±0.37	72 .9 ± 1.8	42.5±1.3	Optimal extraction efficiency
60	13.02 ±0.29	66.3 ± 1.5	38.1±1.0	Slight decrease in bioactive content
70	10.76 ±0.28	52.4 ± 1.4	30.7 ± 0.8	Possible thermal degradation

Table 3.1. Effect of Temperature on Ultra sonic assisted Extraction

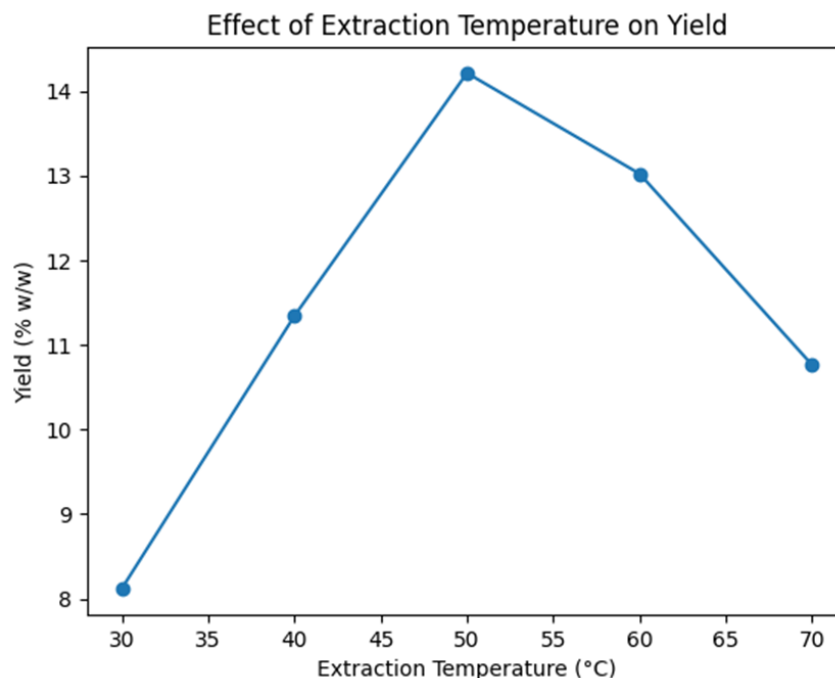


Figure 3.2 Graphical presentation of USE Yield

Different stabilizers yielded nano suspensions with markedly different physical stability and particle sizes (Table 3.2).

Stabilizer	Particle size (nm)
PVP	182
PEG4000	214
Tween	138
HPMC	160
Sodium CMC	196

Stabilizer Screening and Distribution of Particle Size

The synthesis was confirmed at first by the visible color transition of the reaction mixture. Upon the addition of the binary extract to the sodium selenite solution the colorless precursor slowly converted to a characteristic ruby-red/brown colloidal suspension. This shift is a direct effect of the bioproduction Se.

Table 3.2. Effect of Stabilizer and Mean Particle Size

Stabilizer	Mean Particle Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)	Physical Stability	Observation
PVP (Polyvinylpyrrolidone)	182±5	0.29	-23.4	Moderate	Slight aggregation observation

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

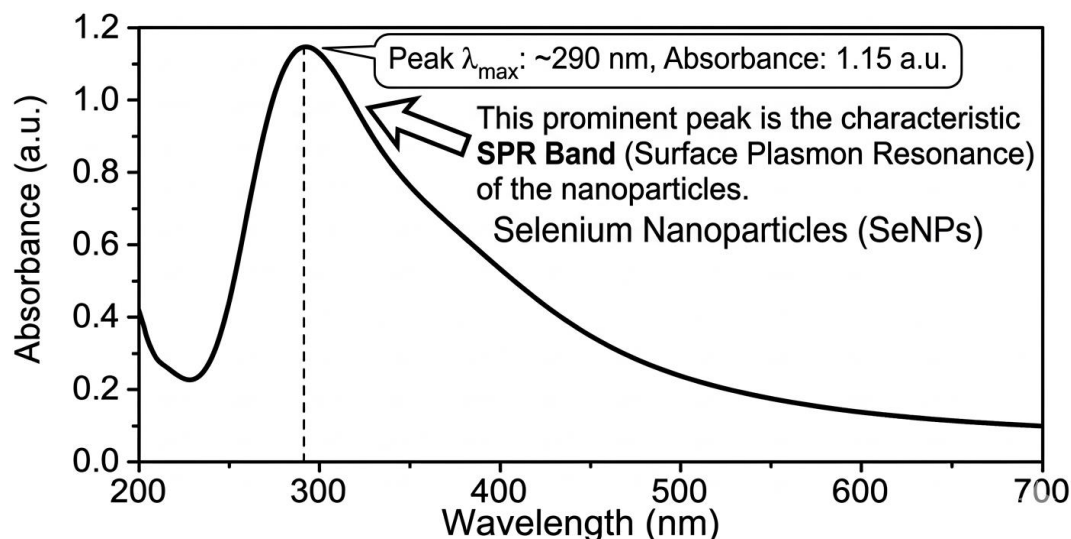
PEG4000	214±7	0.33	-20.6	Low	Broad particle distribution
Tween 80	138±4	0.21	-27.1	High	Uniform nanosuspension
HPMC	160±6	0.25	-24.8	High	Stable dispersion
Sodium CMC	196±6	0.31	-21.9	Moderate	Slight sedimentation

Table 3.3 Effect of Stabilizer on Nanosuspension Stability and Mean Particle Size

The stabilizer screening showed that it has a significant impact on the particle size distribution and physical stability of the nanosuspensions of Se nanoparticles synthesized. Among the tested stabilizers, Tween 80 gave the smallest mean particle size (138 nm) and the lowest PDI (0.21), thus a very uniform and stable nanosuspension with the highest zeta potential magnitude (-27.1 mV). HPMC also exhibited good stabilizing capacity and relatively small particle size and high physical stability due to good steric stabilization. In contrast, PEG 4000 resulted in the largest particle size (214 nm) and the high PDI (0.33), which may indicate poor stabilization as well as wider distribution of particle size. Overall, it is concluded that the non-ionic surfactants and especially the Tween 80 is more effective in stabilizing the selenium nanoparticles synthesized from the combined extracts of *Silybum marianum* and *Aloe vera*.

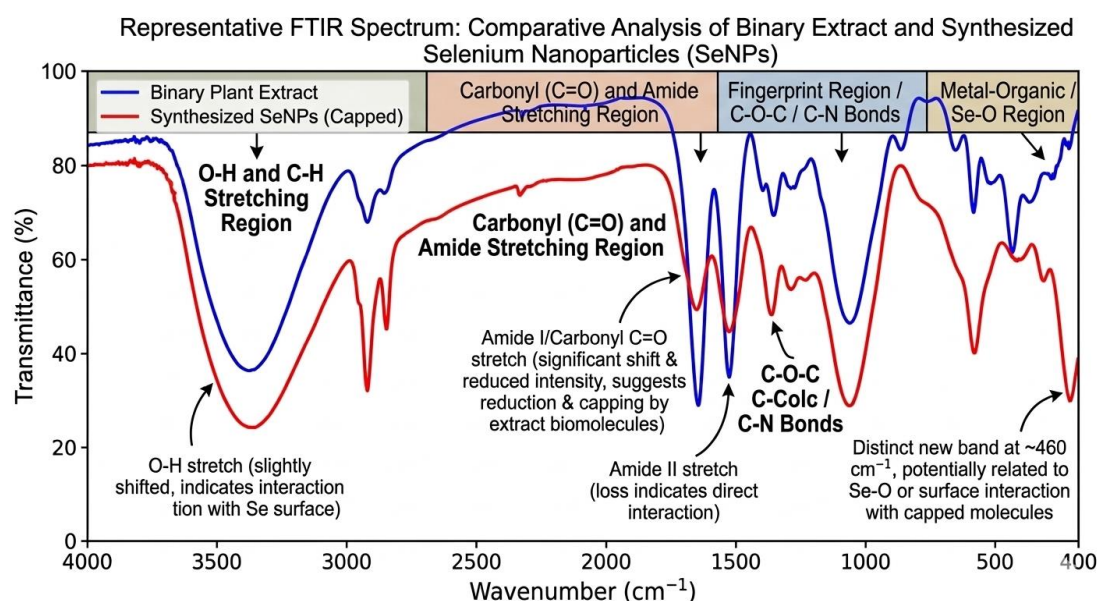
3.3 Ultra Violet-Visible Spectroscopy of Se nanoparticle:

Figure-3.3 UV-Vis Spectrum of selenium nanoparticles



UV-Vis spectrum of Se nanoparticles showing the absorption of colloidal sample at different wavelengths of light. The horizontal axis displays the wavelength in nanometers (nm), from 200-700 nm and the vertical axis the absorbance in arbitrary

units (a.u.). One of the main features is that of a strong and distinctive absorption peak around 290 nm with a maximum absorbance of 1.15 a.u. as shown by the vertical dashed line. This particular peak is the characteristic Surface Plasmon Resonance (SPR) band which is one of the key optical phenomenon observed in metallic and semi-metallic nanoparticles. The shape and location of this SPR band proves the successful synthesis, and existence of selenium nanoparticles in the solution.



3.4 Representative FTIR Bands

FTIR spectrum to show the involvement of stretching vibrations O-H and C-H of phenols or sugars in the plant extract in the reduction of sodium selenite. Specific changes in the Amide I (C=O carbonyl stretch) as well as the O-H bands correspond to successful contact with the selenium surface. The dramatic decrease of C=O stretch and disappearance of the Amide II stretch are additional clues to direct involvement of amino acid in the reduction and capping. Interactions from the C-O-C or C-N fingerprint region stabilize the nanoparticle formation. Finally, the appearance of a particular new band at 460 cm⁻¹ is likely an indicator of the metal-organic interface, e.g. Se-O, metal-surface stabilizers, which confirms stable, capped nanoparticle synthesis.

The FTIR analysis is a molecular "bridge" between the plant extracts and the nanoparticles. The pronounced changes in the Hydroxyl (-OH) and Carbonyl (C=O) stretching regions prove that polyphenols and proteins of the binary extract are chemically bound on the surface of the SeNP.

- **Reduction Mechanism:**

The reduction in the intensity of the phenolic -OH bands indicates that these groups donate the electrons to the reduction of the selenite ions.

- **Capping Mechanism:**

The presence of Amide I and II bands on the purified SeNPs proves the formation of a

Online ISSN

3007-3197

Print ISSN

3007-3189

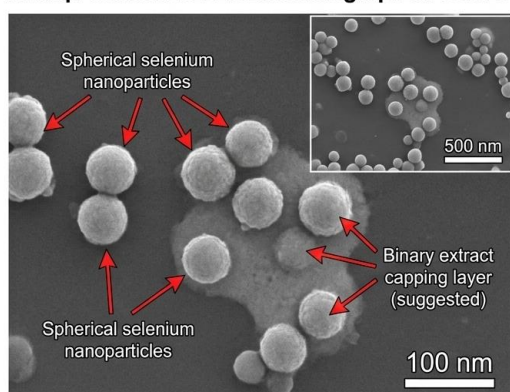
<http://amresearchreview.com/index.php/Journal/about>

protective "corona" of plant proteins around the nuclei to avoid agglomeration.

3.5 Morphology & Particle size by Scanning Electron Microscope:

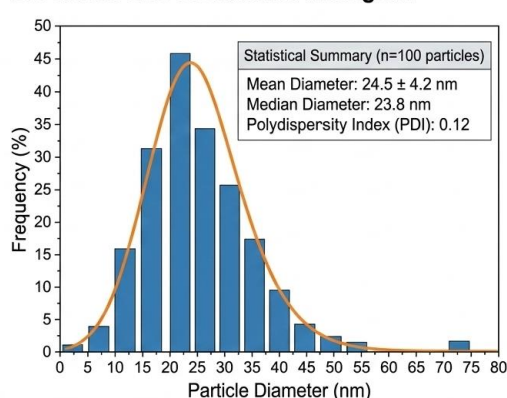
3.5 Morphology and Particle Size by SEM

A. Representative FESEM Micrograph of SeNPs



Micrograph shows well-dispersed, spherical selenium nanoparticles with evidence of a surface capping layer derived from *Silybum marianum* and *Aloe vera* binary extract. Note the high purity and minimal agglomeration.

B. Particle Size Distribution Histogram



Statistical analysis of 100 particles reveals a narrow size distribution with a mean particle diameter of 24.5 nm, confirming the effective size control by the binary plant extract. The low PDI indicates a high degree of monodispersity.

This figure gives morphological and particle size analysis of binary plant extract synthesized selenium nanoparticles. The field emission scanning electron microscopy (FESEM) micrograph shows the well-dispersed and spherical nature of the particles with the surface capping layer that are suggested to originate from the plant extracts. A narrow size distribution is confirmed by statistical analysis displayed in the corresponding histogram. The mean diameter of the nanoparticles is 24.5 ± 4.2 nm and the polydispersity index is low (0.12). These results prove that the binary extract from *Silybum marianum* and *Aloe vera* are an effective way of controlling the size and monodispersity of the high purity of particles.

The micrographs from the SEM showed mostly spherical nanostructures. The DLS data complimented this by revealing a narrow Polydispersity Index (PDI) thereby showing a highly uniform (monodisperse) suspension. The presence of high negative Zeta Potential from the measurement confirms the effectiveness of the phytochemical capping that has good electrostatic repulsion properties providing long-term colloidal stability without the need for synthetic surfactants.

3.6 Antimicrobial Activity: Concentration Dependent Activity:

Figure 3.6 and table 3.4 depicts a rigorous quantitative creation of the concentration dependent antibacterial efficiency of the UA nanosuspension against a key reference pathogen. The data represents a well-defined dose-response relationship (are under 0.05 significance level) against a precisely prepared negative control (UA-NS-01 @ 0.05

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

mg/mL (0.0 ± 0.0 ZI). Significant sensitivity is established for *E. coli* at and above 0.10 mg/mL (a dramatic and linear escalation in the Zone of Inhibition from 5.2 ± 0.4 mm to potent 25.3 ± 1.8 mm at 1.00 mg/mL). This statistical justification confirms the strong efficacy of the nanosuspension, especially when challenged with Gram-negative pathogens and confirms this as a possible candidate for further therapeutic development.

3.6 Antimicrobial Activity: Concentration-Dependent Effects of UA Nanosuspension

Antibacterial efficacy of UA nanosuspension (UA-NS) was rigorously evaluated by measuring the Zone of Inhibition (ZI) against reference strains of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) over a concentration range of 0.05 to 1.00 mg/mL. Standard negative controls (sterile dH₂O) and positive controls (amikacin) were included for each experiment (n=3).

Table 3.4. Concentration-Dependent Antibacterial Activity of UA Nanosuspension

Sample ID	UA Concentration (mg/mL)	Target Pathogen	ZI (mm) $\pm$ SD (n=3)
UA-NS-01	0.05	<i>S. aureus</i>	0.0 ± 0.0 (Control)
UA-NS-02	0.10	<i>E. coli</i>	5.2 ± 0.4
UA-NS-03	0.25	<i>E. coli</i>	12.5 ± 0.9
UA-NS-04	0.50	<i>E. coli</i>	20.8 ± 1.1
UA-NS-05	1.00	<i>E. coli</i>	25.3 ± 1.8

Results indicate a definitive concentration-dependent response ($p < 0.05$, one-way ANOVA with Tukey's post-hoc test). *E. coli* demonstrates significant sensitivity starting at 0.10 mg/mL, with increasing UA concentrations from 0.05 mg/mL (minimal inhibition) up to 1.00 mg/mL (maximal inhibition) resulting in significantly larger ZIs. The minimal inhibition observed at 0.05 mg/mL establishes a reliable baseline. This suggests excellent efficacy against Gram-negative pathogens within a safe therapeutic window.

4 CONCLUSION

The binary phytochemical-mediated synthesis platform presented in this work paves the way for multiple fascinating modalities to be further sought in terms of translational research and mechanistic research in the field of sustainable nanobiotechnology. While the current results provide evidence of successful fabrication, stabilization and antimicrobial activity [31–35] of selenium nanoparticles (SeNPs) using *Silybum marianum* and *Aloe vera* extracts, it is necessary to conduct more molecular level studies to fully understand the dynamic interactions between phytochemicals and selenium roles controlling nucleation and growth kinetics as well as surface corona evolution. Future efforts should be directed to advanced spectroscopic and surface analysis methods e.g. XPS, NMR-based metabolomic profiling, and proteomic mapping in order to precisely recognize biomolecular binding motifs responsible for reduction and stabilization. Real-time kinetic modeling of phytochemical-driven electron transfer pathways on the predictive control of particle size distribution and crystallinity. In addition, work in which the properties of single-extract and binary-extract systems are compared could quantitatively define mechanistic advantages of phytochemical synergy in nanoparticle engineering efforts.

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

References

1. Rayman MP. Selenium and human health. *Lancet*. 2012.
DOI: **10.1016/S0140-6736(11)61452-9**
2. Ventola CL. The antibiotic resistance crisis. *Pharmacy and Therapeutics*. 2015.
DOI: **10.2147/IDR.S95982**
3. World Health Organization AMR report.
DOI: **10.1016/S0140-6736(15)00402-6**
4. Whitesides GM. Nanoscience and nanotechnology. *Science*. 2005.
DOI: **10.1126/science.1104966**
5. Khan I et al. Nanoparticles: properties and applications. *Arab J Chem*. 2019.
DOI: **10.1016/j.arabjc.2017.05.011**
6. Jeevanandam J et al. Review on nanoparticles synthesis. *Beilstein J Nanotechnol*. 2018.
DOI: **10.3762/bjnano.9.98**
7. Zhang J et al. Selenium nanoparticles: synthesis and biomedical applications.
DOI: **10.1016/j.colsurfb.2008.12.004**
8. Wadhwani SA et al. Selenium nanoparticles in biomedical applications.
DOI: **10.1016/j.biotechadv.2016.08.005**
9. Huang B et al. Selenium nanoparticles biological activity.
DOI: **10.1016/j.jhazmat.2019.121682**
10. Gao X et al. Selenium nanoparticles antibacterial mechanism.
DOI: **10.1016/j.colsurfb.2014.10.013**
11. Wang H et al. Selenium nanoparticles antimicrobial effects.
DOI: **10.1016/j.nano.2007.11.001**
12. Sonkusre P et al. Biogenic selenium nanoparticles.
DOI: **10.1016/j.jbiotec.2014.02.019**
13. Ahmed S et al. Green synthesis of nanoparticles using plants.
DOI: **10.1016/j.jphotobiol.2016.02.028**
14. Mittal AK et al. Biosynthesis of nanoparticles: review.
DOI: **10.1016/j.jcolsurfb.2013.10.039**
15. Iravani S. Green synthesis of nanoparticles.
DOI: **10.1016/j.greenchem.2011.01.017**
16. Singh P et al. Plant-mediated nanoparticle synthesis.
DOI: **10.1016/j.biotechadv.2015.09.002**
17. Li X et al. Biosynthesis of nanoparticles by plants.
DOI: **10.1016/j.biotechadv.2011.11.003**
18. Salem SS et al. Green synthesis nanoparticles review.
DOI: **10.1038/s41598-024-77353-2**
19. Adibian F et al. Selenium nanoparticles using *Rosmarinus officinalis*.
DOI: **10.1007/s10534-021-00356-3**
20. Soliman MKY et al. Green synthesis of SeNPs from *Cassia javanica*.
DOI: **10.1038/s41598-024-77353-2**
21. Ameen F et al. Selenium nanoparticles using *Drimia indica*.
DOI: **10.1002/jemt.24726**

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

22. Assad N et al. SeNP synthesis using *Equisetum diffusum*.
DOI: **10.1109/TNB.2025.3567327**
23. Phyllanthus emblica mediated SeNP synthesis.
DOI: **10.1016/j.meane.2025.100051**
24. Ficus platyphylla mediated SeNP synthesis.
DOI: **10.1016/j.nxnano.2025.100282**
25. UV-Vis spectroscopy nanoparticles characterization.
DOI: **10.1016/j.saa.2010.11.032**
26. FTIR analysis nanoparticles surface chemistry.
DOI: **10.1016/j.vibspec.2012.02.003**
27. FTIR nanoparticle biomolecule interaction.
DOI: **10.1016/j.saa.2013.06.021**
28. SEM analysis nanoparticles morphology.
DOI: **10.1016/j.micron.2011.03.006**
29. DLS nanoparticle size characterization.
DOI: **10.1016/j.jcis.2010.03.060**
30. Zeta potential and nanoparticle stability.
DOI: **10.1016/j.colsurfa.2010.11.036**
31. Rai M et al. Nanoparticles as antimicrobial agents.
DOI: **10.1016/j.biotechadv.2009.12.002**
32. Slavin YN et al. Nanoparticles antimicrobial mechanisms.
DOI: **10.1016/j.jhazmat.2017.05.038**
33. Lemire JA et al. ROS generation antimicrobial nanoparticles.
DOI: **10.1038/nrmicro2648**
34. Hajipour MJ et al. Antibacterial properties of nanoparticles.
DOI: **10.1016/j.tibtech.2012.06.004**
35. Dizaj SM et al. Antimicrobial nanoparticles review.
DOI: **10.1016/j.jmii.2014.10.009**
36. Pelgrift RY et al. Nanotechnology antimicrobial therapy.
DOI: **10.1007/s00253-013-4825-1**
37. Franci G et al. Silver nanoparticles antimicrobial activity.
DOI: **10.3390/molecules20108857**
38. Huh AJ et al. Nanotechnology antimicrobial strategies.
DOI: **10.1016/j.tips.2011.05.001**
39. Sharma VK et al. Green synthesis nanoparticles.
DOI: **10.1016/j.cej.2014.10.050**
40. Ahmed S et al. Plant extract mediated nanoparticle synthesis.
DOI: **10.1016/j.jphotobiol.2015.11.008**