JOURNAL of BIOLOGICAL AND SUSTAINABILITY SCIENCES
BioSuSS

Journal of Biological and Sustainability Sciences (2024) 1:1
https://doi.org/pending,assignverysoon

ORIGINAL ARTICLE

Eco-Friendly Synthesis and Biomedical Profiling of Ampicillin-Loaded Silver Nanoparticles from *Artemisia maritima*

Abdul Sami[1], Nida Ur Rehman[1], Maryam Khan[1] Ahsan Ali[2], Siyal khan[1], Muhammad Noor[1], Mohammad Yar khan[2] Ahsan Ali Hashmi [3*]

[1]Department of Biological Sciences, International Islamic University Islamabad, Pakistan

[2]Department of Zoology, University of Balochistan, Quetta, Balochistan, Pakistan

[3]Department of Management, Glasgow Caledonian University, United Kingdom

Received: 17 July, 2024, Revised: 5 August, 2024, Accepted: 11 August, 2024, online: 14 August 2024

Abstract

This study presents the green synthesis of silver nanoparticles (AgNPs) using aqueous extracts of *Artemisia maritima* and their subsequent functionalization with ampicillin. UV-Vis spectroscopy confirmed nanoparticle formation with surface plasmon resonance (SPR) peaks at 401 nm for non-functionalized AgNPs and 457 nm for ampicillin-functionalized AgNPs (AMP-AgNPs). X-ray diffraction (XRD) analysis revealed characteristic peaks at 2θ values of 27°, 32°, 38°, 44°, and 64°, indicating the crystalline nature of the nanoparticles. FTIR analysis identified key functional groups such as hydroxyl (3262.54 cm^{-1}), aromatic alkene (1507 cm^{-1}), and amine (1023.07 cm^{-1}), confirming phytochemical involvement in nanoparticle synthesis and stabilization. Phytochemical screening confirmed the presence of flavonoids, saponins, alkaloids, and steroids. Antibacterial activity was evaluated against five clinical pathogens. At 10 µg/mL, AMP-AgNPs exhibited maximum inhibition zones of 34 mm for *Escherichia coli*, 32 mm for *Pseudomonas aeruginosa*, and 31 mm for *Klebsiella pneumoniae*, outperforming non-functionalized AgNPs, which showed inhibition zones ranging from 20–30 mm. The anticoagulant assay demonstrated that AgNPs completely inhibited blood clotting in treated samples. However, hemocompatibility tests revealed cytotoxic effects, including a reduction in hemoglobin (from 16.4 g/dL to 11.1 g/dL) and RBC counts (from 5.18 to 1.73 million/µL), with increases in platelet count (from 147 ×10³/µL to 1882 ×10³/µL) and WBC count (from 5.5 to 14.2 ×10³/µL). These findings suggest that *A. maritima*-derived AgNPs possess strong antimicrobial and anticoagulant potential, but their cytotoxic impact on blood parameters highlights the need for further in vivo studies and surface modification strategies to ensure safety in biomedical applications.

Keywords Green synthesis, Hemocompatibility, Anticoagulant, Antibiotic-Functionalized Silver Nanoparticles, Antibacterial

✉1st Author	abdulsami60789@gmail.com
✉3rd Author	maryammukaramkhan099@gmail.com
✉corresponding	aali336@caledonian.ac.uk
Published Online	14 August 2024
Crossref: doi.xxxxxxxxxxx pending	
ISSN: 000-0000 Pending	

1. Introduction

Medicinal use of plants in the treatment of diseases is one element in the history of human beings, and in fact, all the way back to early civilization. It is documented that ancient Mesopotamians, Chinese, and Egyptians used hundreds of different plant species as medicine(1). The plants are a part of contemporary pharmacognosy because of bioactive compounds like flavonoids, alkaloids, terpenoids, and phenolic constituents that are obtained in the plants. More to the point, the compounds in question have numerous pharmacological properties such as anti-inflammatory, antimicrobial, and anticancer properties(2). An outstanding example is paclitaxel, a compound which originates in the Pacific yew tree and has been widely used as a cancer drug because of its microtubule-stabilizing effect. In addition to the systemic diseases, medicinal plants have long medical used in oral health. As a case in point, the ancient chewing stick (miswak), which was chewed during the period of the Prophet Mohammed (PBUH), has antimicrobial properties, which can destroy oral pathogens(3). Plant products such as clove (*Syzygium aromaticum*) have shown strong inhibitory effects on Streptococcus mutans, a dental caries bacterium. Phytochemicals also play a role in wound

healing, burn therapy, and immunomodulation, which is also evident in the traditional Moroccan remedies(4).The broad therapeutic potential of plants, which are mentioned in religious books and confirmed by scientific research, includes such items as (*Ficus carica*), garlic (*Allium sativum*), and olive (*Olea europaea*) (5). Typical food spices such as turmeric and cinnamon have advantages in the management of glycemic statuses and immune system enhancement, as well as lowering inflammation (6).

Nanotechnology is a revolutionary technology with a wide range of applications in medicine, agriculture, and environmental science. The terminology nanoparticle was initially coined by Taniguchi in the year 1974, but currently is used to define engineered materials <100 nm, which contain special physicochemical characteristics. Nanoparticles can be used in the biomedical field as a means of targeted drug delivery, early diagnostics, and improvement of bioavailability of therapeutic agents (7). Among these numerous nanomaterials, AgNPs remain an intriguing finding due to their strong antimicrobial effect and low cytotoxicity at working doses (8). Conjugation of AgNPs with antibiotics, such as ampicillin, widens and improves the spectrum and efficacy of the application, particularly against resistant bacterial strains. Furthermore, the process of biosynthesis of nanoparticles presents a greener alternative to the traditional techniques because it uses renewable capping agents and reducing agents obtained through plants

In the green synthesis of nanoparticles, phytochemicals of plants (flavonoids, tannins, and saponins) are used as natural reducers and stabilizers. This method is environmentally friendly, it can be scaled, and it is cost-efficient (9). Nano formulated herbal drugs are more soluble and have a higher bioactivity than traditional constraints in herbal-based medicines (10). Artemisia maritima, which is also referred to as wormseed or Afsanteen-ul-Bahr is a herb that is mainly used in most traditional medicines in the Himalayan regions. It is known to contain antimalarial, antibacterial, bronchodilators and hepatoprotective activities (11). The phytochemical components of it have also made it a potential target for the production of nanoparticles by virtue of its sesquiterpenes, such as santonin, flavonoids, and essential oils. A. maritime extracts have also been proved to exert an anti-proliferative effect in non-small lung cancer via induction of apoptosis and by inhibiting cellular migration(12). Given its pharmacological profile and environmental sustainability, this study explores the synthesis of antibiotic-functionalized AgNPs using A. maritima extract. The research aims to evaluate their antimicrobial activity, Hemocompatibility, and potential as a biocompatible alternative in nanomedicine.

2. Materials and Methods

2.1. Synthesis of AgNPs; Extract preparation

A. maritima aerial parts were dried and turned into a fine powder. After the methods previously described to perform aqueous plant extraction, 20 g of the plant material was suspended in 200 mL of distilled water and extracted by constant stirring at 70 0 C for 1 hour by using a magnetic stirrer.(13). The suspension was filtered using muslin cloth to get rid of rough materials, then centrifuged at 3,000 rpm for 15 minutes. This supernatant was later filtered using Whatman No. 1 filter paper (three times) to get a clear extract. The extract was kept sterile at a temperature of -4 o C, at which time it was to be used later as a reducing and capping agent in the synthesis of silver nanoparticles (14).

2.2 Phytochemical Profiling

Phytochemical analysis was done under standard qualitative procedures, in order to ascertain the presence of secondary metabolites that include: flavonoids, alkaloids, tannins and phenols, steroids, Saponins, and proteins (15, 16). The Formation of foam when 5 mL of an extract is rigorously shaken confirmed the notion of Saponins being present(17). The presence of flavonoids was found through bright yellow color on the addition of NaOH and their decolorization by dilute acid(18). The presence of steroids was confirmed by a color shift to red (chloroform layer) and annoying of green fluorescence (H2SO4 layer) (19). Alkaloids were confirmed by reddish-brown precipitate that was formed by adding Wagner reagent into 2 mL of extract (20). The blue-green color observed when 2 mL of extract was combined with 2 mL 1% FeCl 3 was an indication of phenolic compounds (21). The Presence of reducing sugars was proven by a reddish-brown precipitate after running the Benedict test(22). Heat in 1% HCl to produce no crimson-red precipitate was evidence of the absence of the phlobatannins (15). A purple color produced by a combination of 1 percent CuSo4 and NaOH of the biuret reaction showed evidence of the presence of proteins (23).

2.3 Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles were synthesized by using the green chemistry approach. The AgNO 3 (Sigma-Aldrich) was dissolved into an aqueous solution (1 mM), and 50 mL of solution was transferred into the 250 mL Erlenmeyer flask. *A. maritima* was added drop by drop with constant magnetic agitation and at room temperature. During a 1-hour reaction was run darkly. The change of color, which is clear to dark brown, signified that a

nanoparticle was formed, and this could be attributed to the surface Plasmon resonance of AgNPs (24, 25).

2.4 Functionalization with Ampicillin

Ampicillin was dissolved in distilled water 25 mg dissolved in 50 mL and then added to the synthesized AgNP solution 50 mL. The suspension was kept on stirring after 3 hours at room temperature to enable electrostatic attachment and surface interaction to produce antibiotic-coupled AgNPs as has been described earlier(26).

2.5 Purification and Storage

Centrifugation at 15,000rpm for 15 minutes was carried out to collect the unloaded and ampicillin-loaded AgNPs. The obtained pellets were washed 3-4 times with deionized water to get rid of leftover silver ions and biomolecules that were not bound to silver. The purified nanoparticles were dried by heating at 80 o C in a hot air oven and kept in sterile Eppendorf tubes. Where necessary, we used lyophilization. Formation of nanoparticles was verified through UV-Visible spectroscopy based on surface Plasmon resonance behavior.(13).

3. Result

3.1 Phytochemical screening of *A. maritima*

Qualitative phytochemical analysis revealed the presence of multiple bioactive secondary metabolites in the tested extract; it is enlisted in table 1. Saponins were confirmed by the formation of persistent foam upon vigorous shaking of the extract. The presence of flavonoids was indicated by the development of a bright yellow color upon addition of sodium hydroxide, which subsequently decolorized upon acidification. Alkaloids were detected through the formation of a reddish-brown precipitate following treatment with Wagner's reagent. Steroids were identified based on a characteristic color change to red in the chloroform layer and the appearance of green fluorescence in the sulfuric acid layer. The extract also tested positive for phlobatannins, as evidenced by the development of a faint precipitate upon heating with hydrochloric acid. These findings collectively confirm the presence of saponins, flavonoids, alkaloids, steroids, and phlobatannins, indicating a rich phytochemical profile that may contribute to the biological activities of the extract.

3.2 Nanoparticle synthesis

Silver nanoparticles we obtained from the aqueous extract of *A. maritima* showed a change in color from light yellow to dark brown which indicates that the reduction of silver nitrate into silver ions and the biosynthesis of silver nanoparticles were confirmed by the absorption peak which was obtained in the region of 401 nm which confirms the formation of silver nanoparticles.

3.3 Characterization of AgNPs; UV-Vis

To analyze the absorbance of functionalized and non-functionalized Ampicillin synthesized from Artemisia maritima, UV-Vis spectroscopy was performed. AgNPs functionalized with Ampicillin showed peak at 457 nm while the non-functionalized AgNPs showed absorbance at 401nm. UV–Visible spectroscopy confirmed the formation and functionalization of AgNPs, as shown in Figure 1. AgNPs exhibited a characteristic SPR peak at 401 nm, while ampicillin-functionalized AgNPs (AMP–AgNPs)

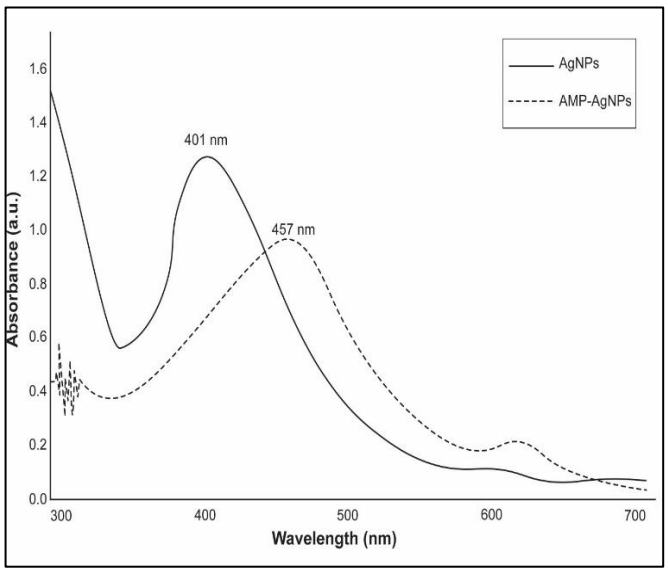

Figure 1. UV–Vis absorption spectra of biosynthesized AgNPs and ampicillin-functionalized silver nanoparticles (AMP–AgNPs). The peak at 401 nm confirms AgNP formation, while the redshift to 457 nm indicates successful functionalization with ampicillin.

showed a redshifted peak at 457 nm, indicating successful surface modification. The shift in wavelength reflects changes in particle surface chemistry and confirms effective conjugation of ampicillin to the nanoparticles.

Table 1: Phytochemical screening of *A. maritima*

Phytochemical Tests	Results	Observation
Test for Saponins	Positive	Foam formation

Test for flavonoids	Positive	Flavonoids present
Test for steroid Yellowish	Positive	Green fluorescence observed
Test for Alkaloid	Positive	Reddish color identified
Test for phlobatannins identification	Negative	Phlobatannins absent
Test for Phenol identification	Negative	Phenol absent
Test for carbohydrate	Negative	Carbohydrate absent
Test for Protein/Amino acid identification	Negative Protein	Absent

3.4 Fourier Transform Infrared (FTIR) Analysis

FTIR spectrum of plant-mediated AgNPs exhibited a broad spectrum of 3262.54 cm-1 corresponding to the hydroxyl (3) functional group in alcohols and functional groups. The IR band of 1507 cm-1 exhibits an aromatic alkene group. While the IR band of 1023.07 cm-1 exhibited medium-range C-N stretching showing an amine group. The FTIR spectrum of biosynthesized AgNPs showed prominent peaks at 3262.54 cm^{-1}, 1507.32 cm^{-1}, and 1023.07 cm^{-1}, corresponding to hydroxyl groups, aromatic C=C bonds, and C–N stretching vibrations, respectively as shown in figure 2. These functional groups confirm the presence of biomolecules from *A. maritima* that facilitated reduction and capping of AgNPs.

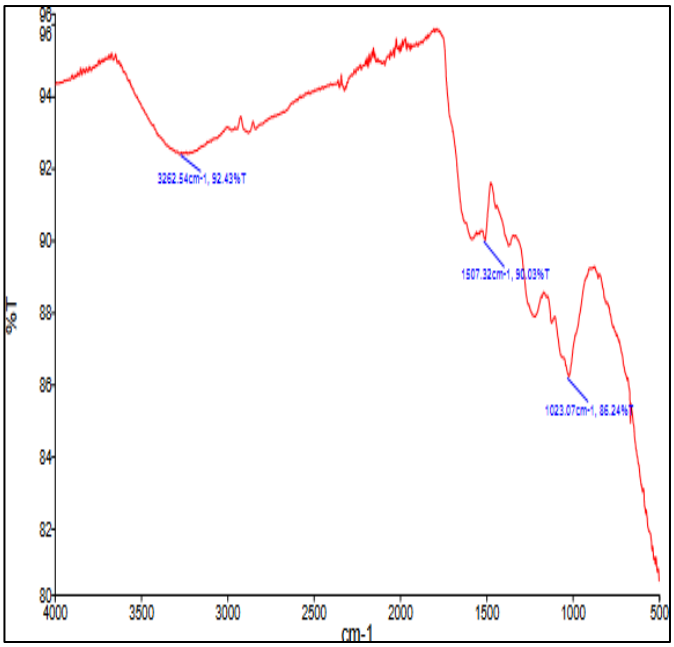

Figure 2. FTIR spectrum of *A. maritima*-synthesized silver nanoparticles showing key absorption peaks at 3262.54 cm^{-1} (–OH stretch), 1507.32 cm^{-1} (aromatic C=C), and 1023.07 cm^{-1} (C–N stretch of amines), confirming the involvement of phytochemicals in nanoparticle stabilization.

3.5 XRD diffraction

The crystalline nature of the biosynthesized silver nanoparticles was confirmed through X-ray diffraction (XRD) analysis. The XRD pattern exhibited distinct diffraction peaks at 2θ values of approximately 27°, 32°, 38°, 44°, 46°, 54°, 57°, 64°, 67°, 77°, and 81°, which correspond to the characteristic planes of face-centered cubic (fcc) silver crystals. Among these, the intense peak at around 38° is typically assigned to the (111) plane, a hallmark of metallic silver nanoparticles. These results are in strong

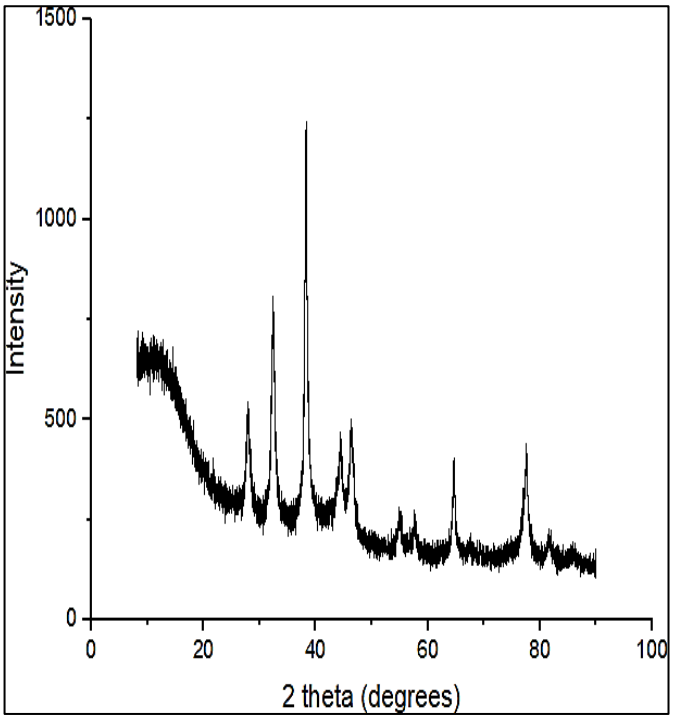

Figure 3. XRD pattern of biosynthesized silver nanoparticles from Artemisia maritima, showing characteristic peaks at 2θ values of 27°, 32°, 38°, 44°, 46°, 54°, 57°, 64°, 67°, 77°, and 81°, confirming their crystalline nature.

agreement with standard JCPDS data (No. 04-0783), supporting the successful formation of well-defined crystalline nanoparticles. Minor additional peaks may indicate the presence of organic residues from the plant extract used in the synthesis. The sharp and narrow nature of the peaks suggests high crystallinity and nanoscale particle size, which are critical for their stability and functional performance. **Figure 3** illustrates the detailed XRD pattern of the synthesized AgNPs, confirming their structural integrity and phase purity.

3.6 Kinetic Stability of AgNPs

The effect of different temperature ranges on absorption spectra was determined by UV. At 50 degrees Celsius, the value for lambda was 401 nm and at 70 °C and 100°C, the values were 394 nm and 379 nm respectively. Drop in wavelength with rise in temperature is due to the formation of AgNPs of decreasing size showing homogenous dispersion. The time parameter kinetics of AgNPs were also determined. The intensity of absorption increases as contact time increases showing reduction pf Ag as a result colloidal particles enhances. The UV–Vis spectra

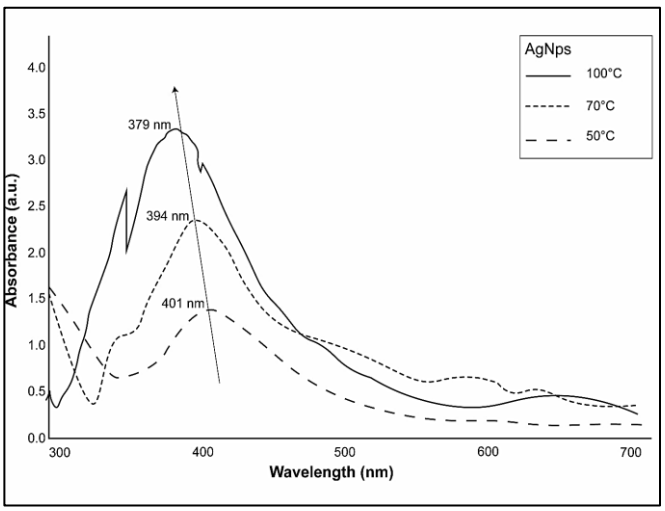

Figure 4. UV–Vis absorption spectra of AgNPs synthesized at different temperatures (50 °C, 70 °C, and 100 °C), showing a blue shift in surface plasmon resonance (SPR) peaks from 401 nm to 379 nm with increasing temperature, indicating reduced particle size and enhanced homogeneity.

(Figure 4) show a progressive blue shift in the SPR peak from 401 nm at 50 °C to 379 nm at 100 °C, indicating the formation of smaller, more monodispersed nanoparticles at elevated temperatures. This thermal dependency highlights the role of reaction conditions in modulating nanoparticle size and optical properties. Figure 5 further illustrates the time-dependent progression of nanoparticle formation. As the reaction time increased from 1 to 3 hours, the SPR peak blue-shifted from 422 nm to 401 nm, indicating continued reduction of silver ions and the generation of smaller, well-dispersed AgNPs. The increasing absorbance also reflects higher nanoparticle yield over time.

3.7 Antibacterial activity

The antibacterial activity was performed among antibiotic functionalized with ampicillin an d non-antibiotic (not functionalized with ampicillin) against several strains i.e., *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Escherichia coli*. The higher activity was

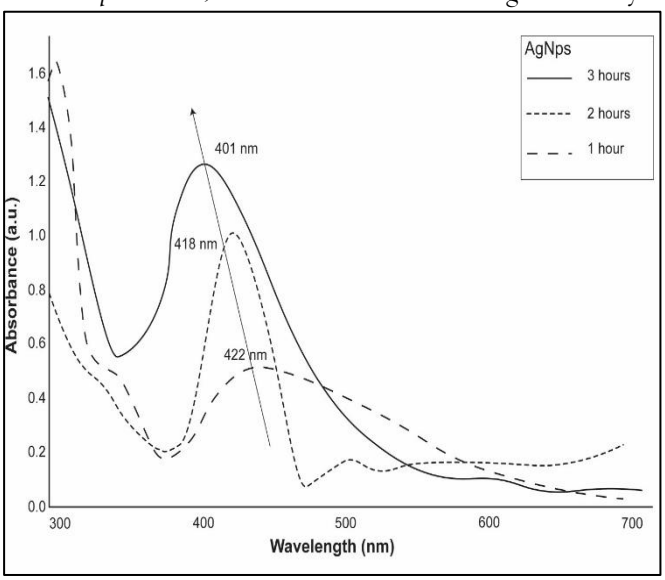

Figure 5. UV–Vis absorption spectra of AgNPs synthesized over different reaction times (1, 2, and 3 hours). The surface plasmon resonance (SPR) peak shifts from 422 nm to 401 nm with longer reaction times, indicating enhanced reduction and formation of smaller, more stable nanoparticles.

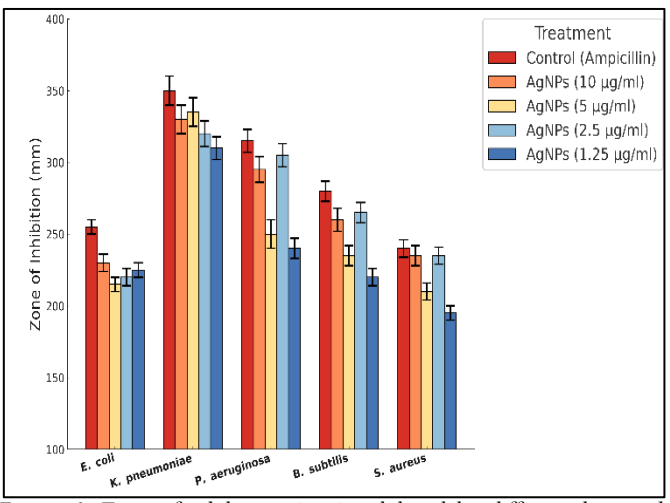

Figure 6. Zone of inhibition (mm) exhibited by different bacterial strains treated with varying concentrations of silver nanoparticles (AgNPs) and ampicillin (control).

obtained against antibiotic functionalized with ampicillin. AgNPs demonstrated effective antimicrobial activity against *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtills* and *Staphylococcus aurea*. The enhanced antibacterial activity of AMP-AgNPs against selected bacterial species is visually represented in Figure 6.

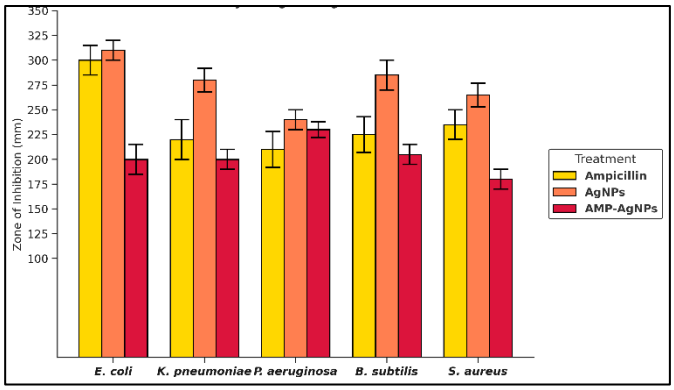

Figure 7. Zone of inhibition (mm) observed for five bacterial strains (*E. coli, K. pneumoniae, P. aeruginosa, B. subtilis, and S. aureus*) treated with Ampicillin, biosynthesized AgNPs, and ampicillin-functionalized silver nanoparticles (AMP-AgNPs).

The zone of inhibition for each bacterium was measured in micrometers (5) at concentrations of 10, 5, 2.5 and 1.25 µg/ml. At 10 µg ml, in case of AMP-AgNPs the zone of inhibition for *E. coli* was 34 mm, for *Klebsiella pneumoniae* it was 31 mm, for *Pseudomonas aeruginosa* it was 32 mm, for *Bacillus subtilis* it was 30 mm, and for *Staphylococcus aureus* it was 29 mm as shown in the Figure 7. The maximum zone of inhibition in case of non-functionalized Ampicillin AgNPs is shown by *E. coli* and it was 30 mm followed by *Bacillus subtilis* at 27 mm, *Klebsiella pneumonia* at 26 mm, *Staphylococcus aureus* at 24 mm, and *Pseudomonas aeruginosa* at 20 m.

3.8 Anticoagulant assay

The anticoagulant activity of AgNPs synthesized from Artemisa maritima was analyzed to check whether they interfere with coagulant process or not. Normal blood samples form clot however blood on which nanoparticles were added did not show any coagulation.

3.9 *In vitro* hemocompatibility of AgNPs

In vitro hemocompatibility of AgNPs synthesized from Artemisa maritima was analyzed to check the effects of Ag on blood parameters. The hemoglobin, and red blood cells showed sudden decrease, while white blood cells and platelets increased. This reveals cytotoxic effects of AgNPs on blood parameters.

Table 4.4: Effects of Artemisia maritime-synthesized AgNPs on Complete Blood Profile

Test	**Result**		**Units**	Reference Range
	Normal	Nanoparticles treatment		
Hemoglobin	16.4	11.1	g/dl	M: 13.0- 18.0, F:11.5-16.5
Red Blood Cells count	5.18	1.73	million/µl	M: 4.5-6.5, F: 4.0-5.8
Platelet Count	147	1882	103/µl	150,000-400,000
White Blood Cells count	5.5	14.2	103/µl	4,000-11,000
Differential Count				
Neutrophils	63.4	-	%	40-75
Lymphocytes	28.6	41.8	%	20-45
Monocytes–basophils–eosinophils mixed (MXD)	8.0	-	%	02-10
Hematocrit test (Hct)	48.9	17.2	%	M: 41-50%, F: 36-44%
Platelet-large cell ratio (P-LCR)	26.5	14.4	%	15-35
Absolute Values				

Mean platelet value (MPV)	10.1	7.6	fl	7-9
Mean corpuscular volume (MCV)	94.4	99.4	fl	76-96
Mean corpuscular hemoglobin (MCH)	31.7	64.2	pg	27-31
Mean corpuscular hemoglobin concentration (MCHC)	**33.5**	**64.5**	**g/dl**	32-35

4. Discussion

4.1 Phytochemical screening of *A. maritima*

The qualitative phytochemical screening of *Artemisia maritima* demonstrated the presence of significant secondary metabolites including saponins, flavonoids, alkaloids, steroids, and phlobatannins. This profile is consistent with previous studies reporting the rich phytochemistry of *Artemisia* species, supporting their wide use in traditional medicine. The detection of saponins, confirmed by persistent foam formation, suggests potential anti-inflammatory, antimicrobial, and immunomodulatory activities. Saponins have also been shown to exhibit cytotoxic properties relevant to cancer prevention and cholesterol regulation (27). This finding aligns with the therapeutic applications of *A. maritima* in folk medicine. Flavonoids, which produced a yellow coloration under alkaline conditions, are notable antioxidants. Their presence supports previous reports of the antioxidant capacity of *Artemisia* species and highlights their role in mitigating oxidative stress and inflammation-related diseases (28). Additionally, flavonoids may contribute to hepatoprotective and cardioprotective effects observed in traditional uses. The identification of alkaloids via reddish-brown precipitate formation with Wagner's reagent underscores a class of compounds known for analgesic, antimicrobial, and antimalarial properties. This confirms a possible pharmacological basis for the plant's use in treating infectious and parasitic conditions. Steroids were detected as evidenced by characteristic color changes in the Liebermann-Burchard reaction. Plant steroids are recognized for their hormonal modulation and anti-inflammatory activities, which may enhance membrane stability and contribute to the plant's medicinal efficacy. Phlobatannins were also present, suggesting astringent and antimicrobial effects. These condensed tannins have been associated with wound healing and free radical scavenging, indicating additional therapeutic potentials. Overall, the phytochemical composition of *A. maritima* substantiates its use in traditional medicine and warrants further investigation. Future research should include quantitative phytochemical assays, isolation and characterization of active compounds, and comprehensive pharmacological testing to validate therapeutic applications.

4.2 Synthesis of AgNPs

In this study, the green synthesis of AgNPs was successfully achieved using the aqueous extract of *A. maritima*. The synthesis process was visually confirmed by a distinct color change from light yellow to dark brown, a well-documented indicator of nanoparticle formation due to surface plasmon resonance (SPR); the collective oscillation of conduction electrons in response to incident light. This phenomenon confirms the reduction of silver ions (Ag^{+}) to elemental silver (Ag^{0}), mediated by phytochemicals within the *A. maritima* extract, such as flavonoids, phenolics, and saponins, which act as natural reducing and capping agents (29-32).

4.3 Characterization of AgNPs; UV-Vis

UV-Visible spectroscopic analysis further validated the synthesis and stability of the biosynthesized AgNPs, revealing a characteristic SPR absorption peak at approximately 401 nm. This wavelength is consistent with previous reports for spherical silver nanoparticles obtained via green synthesis. The relatively sharp and symmetric nature of the absorption peak suggests a narrow particle size distribution and favorable colloidal stability, which are crucial for maintaining antimicrobial efficacy and preventing aggregation (33). The functionalization of these nanoparticles with ampicillin resulted in a notable redshift of the SPR peak to 457 nm. Such shifts are commonly

interpreted as indicative of successful surface modification, reflecting changes in the local dielectric environment and possible increases in particle size or aggregation state. This functionalization likely enhances the stability and broadens the antimicrobial spectrum of the AgNPs by combining the intrinsic antibacterial properties of silver with the antibiotic activity of ampicillin. The use of *A. maritima* extract provides an eco-friendly and sustainable alternative to conventional physical and chemical methods of nanoparticle synthesis, which often involve hazardous chemicals and high energy consumption. The dual role of the plant phytochemicals as reducing and stabilizing agents eliminates the need for external surfactants or chemical stabilizers, simplifying the synthesis process and minimizing potential environmental impact (33, 34). These findings confirm that *A. maritima* extract can effectively mediate the green synthesis of stable and functionally enhanced AgNPs. These results encourage further exploration of such biosynthesized nanoparticles for antimicrobial, pharmaceutical, and biomedical applications, particularly in combating antibiotic-resistant pathogens.

4.4 FTIR Analysis

FTIR spectroscopy was used to identify functional groups involved in the reduction, stabilization, and capping of AgNPs. The spectrum showed a broad peak at 3262.54 cm^{-1}, characteristic of O–H stretching vibrations, indicating the presence of hydroxyl-rich biomolecules such as flavonoids, phenols, and tannins that likely contribute as reducing and capping agents. A notable absorption at 1507 cm^{-1} corresponded to C=C stretching in aromatic alkenes, suggesting aromatic compounds possibly polyphenols or aromatic amino acids that may stabilize AgNPs via π-electron interactions. The peak at 1023.07 cm^{-1} was attributed to C–N stretching from amine groups, likely derived from proteins or amino acids in the extract, which enhance nanoparticle stability through electrostatic and steric effects (35).

These findings confirm that multiple phytoconstituents participate in the biosynthesis and surface modification of AgNPs. The presence of hydroxyl, aromatic, and amine groups supports the role of plant biomolecules in reducing Ag^+ ions to elemental silver and capping the formed nanoparticles, contributing to their stability. Additionally, the bioactive surface coatings implied by these functional groups may improve the biocompatibility, antioxidant properties, and biomedical applicability of the synthesized AgNPs.

4.5 XRD diffraction

XRD analysis confirmed the crystalline nature of synthesized AgNPs. Distinct diffraction peaks at 2θ values of approximately 27°, 32°, 38°, 44°, 46°, 54°, 57°, 64°, 67°, 77°, and 81° indicate a well-defined crystalline structure. The prominent peak near 38° corresponds to the (111) plane of face-centered cubic (fcc) silver, with additional peaks at 44°, 64°, and 77° supporting the fcc phase, consistent with JCPDS standards (No. 04-0783).

Other peaks may arise from silver oxide impurities or phytoconstituent residues, reflecting the polycrystalline composite nature introduced by green synthesis and biomolecule capping. The sharp, intense peaks suggest high crystallinity and small particle size, crucial for nanoparticle stability and functional activity (31, 32). These XRD results complement UV-Vis data, confirming that *A. maritima*-mediated reduction produces stable, crystalline AgNPs suitable for biomedical and antimicrobial use.

4.6 Kinetic Stability of AgNPs

The kinetic stability of biosynthesized AgNPs was evaluated by UV-Vis spectroscopy, revealing their response to temperature and reaction time. Increasing temperature from 50°C (401 nm) to 70°C (394 nm) and 100°C (379 nm) resulted in a progressive blue shift of the SPR peak. This suggests that higher temperatures facilitate faster nucleation and limit particle growth, yielding smaller, more uniformly dispersed AgNPs. Concurrently, time-dependent analysis showed increased absorbance intensity with prolonged reaction time, indicating a continuous reduction of Ag^+ ions and higher nanoparticle yield (35, 36). These findings highlight the thermodynamic and kinetic favorability of *A. maritima*-mediated synthesis under mild heating, offering control over particle size and enhancing colloidal stability. Such kinetic robustness and temperature-dependent size

control are crucial for reproducible, scalable green synthesis, making these AgNPs promising for industrial and biomedical applications requiring thermal resilience and extended shelf-life (35, 36).

4.7 Antibacterial activity:

The antibacterial assays demonstrated that ampicillin-functionalized AgNPs (AMP-AgNPs) showed significantly enhanced antimicrobial activity against both Gram-positive and Gram-negative bacteria compared to non-functionalized AgNPs. This improvement is likely due to a synergistic interaction between the intrinsic bactericidal properties of silver nanoparticles and the β-lactam antibiotic, ampicillin, which may facilitate better penetration of bacterial membranes and simultaneous targeting of multiple cellular pathways (37). Among tested strains, *E. coli* was the most susceptible to AMP-AgNPs, showing the largest inhibition zone (34 mm) at 10 µg/mL, followed by *P. aeruginosa, Klebsiella pneumoniae, B. subtilis*, and *S. aureus*. Even non-functionalized AgNPs displayed broad-spectrum antimicrobial activity, with inhibition zones ranging from 20 mm to 30 mm, underscoring the inherent oxidative stress induction and membrane disruption mechanisms of AgNPs (37, 38). The pronounced susceptibility of Gram-negative bacteria such as *E. coli* and *P. aeruginosa* to AgNPs aligns with previous findings attributing this phenomenon to their thinner peptidoglycan layers and negatively charged outer membranes, which enhance nanoparticle interaction. These results highlight that antibiotic functionalization not only potentiates the antibacterial efficacy of AgNPs but also presents a promising approach to target multidrug-resistant pathogens. The enhanced activity of AMP-AgNPs against clinically relevant Gram-negative strains suggests significant potential for their application in tackling resistant infections, emphasizing the value of combining nanomaterials with conventional antibiotics for improved antimicrobial therapies (37, 38).

4.8 Anticoagulant assay

This study demonstrated that AgNPs show significant anticoagulant activity, as evidenced by complete inhibition of blood coagulation compared to normal samples. The anticoagulant effect is likely mediated through AgNP interactions with clotting factors or platelet surface receptors, disrupting the enzymatic cascade essential for fibrin clot formation. Previous reports suggest that silver nanoparticles can bind to thiol groups of coagulation proteins, inducing structural alterations or functional inhibition (39). Additionally, AgNPs may interfere with platelet aggregation and calcium-dependent signaling pathways involved in coagulation (39). These findings extend the biomedical applicability of *A. maritima*-derived AgNPs, presenting potential for use in blood-contacting medical devices, surface coatings, or therapeutics targeting thrombotic conditions. Nonetheless, further mechanistic studies employing coagulation assays such as activated partial thromboplastin time (aPTT), prothrombin time (PT), and platelet function tests are essential to delineate the precise mode of action and evaluate safety prior to clinical translation.

4.9 In vitro hemocompatibility of AgNPs

The in vitro hemocompatibility assessment of *A. maritima*-synthesized AgNPs showed decreased hemoglobin and RBC counts, with increased WBC and platelet levels, indicating cytotoxic effects on blood components. The decline in hemoglobin and RBCs likely results from erythrocyte membrane disruption or oxidative damage mediated by reactive oxygen species (ROS) generated by AgNPs, leading to hemolysis and compromised cell integrity. Concurrently, elevated WBC and platelet levels suggest a pro-inflammatory or immunostimulatory reaction, possibly triggered by the recognition of nanoparticles as foreign materials (40). These findings underscore the importance of thorough toxicological assessments when considering *A. maritima*-derived AgNPs for biomedical applications involving direct blood contact. While these nanoparticles exhibit promising antimicrobial and anticoagulant activities, their hematological impact necessitates careful dose optimization and potential surface modification strategies to improve biocompatibility and safety profiles.

Conclusion

This study demonstrates the successful green synthesis of silver nanoparticles (AgNPs) using aqueous extracts of *Artemisia maritima* and their subsequent functionalization with ampicillin. The phytochemical profiling confirmed the presence of multiple bioactive compounds that likely facilitated the reduction and stabilization of AgNPs. Characterization via UV-Vis, FTIR, and XRD affirmed the crystalline, functionalized nature of the nanoparticles. The AMP-AgNPs exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacterial

strains, with notably enhanced efficacy compared to non-functionalized nanoparticles—suggesting a synergistic antimicrobial effect. The anticoagulant assays showed that AgNPs effectively inhibited blood clotting, indicating potential utility in blood-contacting medical devices or therapies targeting thrombotic disorders. However, hemocompatibility analysis revealed cytotoxic effects, including a significant reduction in red blood cells and hemoglobin, alongside elevated white blood cell and platelet counts, raising concerns regarding their direct systemic application. These findings highlight the therapeutic promise of *A. maritima*-derived AgNPs, while also emphasizing the importance of surface modification and dosage optimization to ensure safety and clinical applicability. Further studies focusing on in vivo toxicity, pharmacokinetics, and mechanistic exploration of their biological effects are necessary to translate these biosynthesized nanoparticles into effective biomedical solutions.

Funding: No Funding is avail for this research

Data Availability Statement: The data supporting the findings of this study are available in the Lab manual at the Infectious Disease lab, International Islamic University Islamabad, Islamabad.

Acknowledgments: We acknowledge Department of Biological Sciences, International Islamic University Islamabad, Pakistan, Department of Zoology, University of Balochistan, Pakistan and Department of Management, Glasgow Caledonian University, United Kingdom, for facilitating and providing chemicals, lab access, resources and his administrative support throughout this project.

Conflicts of Interest: Authors declare there is no conflict of interest.

References

1. Petrovska BB. Historical review of medicinal plants' usage. Pharmacognosy reviews. 2012;6(11):1.
2. AL-Dulaimi DW, Faisal SF, Baharetha HM, Majid A. Cytogenetic an experimental monitoring test for plant extracts. IOSR J Pharm Biol Sci. 2017;12(1):100-5.
3. Haque MM, Alsareii SA. A review of the therapeutic effects of using miswak (Salvadora Persica) on oral health. Saudi medical journal. 2015;36(5):530.
4. Merrouni IA, Elachouri M. Anticancer medicinal plants used by Moroccan people: Ethnobotanical, preclinical, phytochemical and clinical evidence. Journal of Ethnopharmacology. 2021;266:113435.
5. Ahirwar RK, Kumar Srivastava A, Yadav D, Bhoi DK, Jangde R. Exploration of Ethnomedicinal Plants for Drug Discovery in High-Throughput Omics Era. Ethnomedicinal Plants for Drug Discovery: Current Developments: Springer; 2024. p. 423-42.
6. Dubey D, Sharma AK, Agrawal K, Singh M, Sanduja M, Snehi V, et al. Scientific Update on the Pharmacognostic and Pharmacological Properties of Brassica juncea. Free Radicals and Antioxidants. 2023;13(1):3-9.
7. Adetuyi BO, Olajide PA, Omowumi OS, Adetunji CO. Application of Plant-Based Nanobiopesticides as Disinfectant. Handbook of agricultural biotechnology. 2024;1:63-130.
8. Asif M, Yasmin R, Asif R, Ambreen A, Mustafa M, Umbreen S. Green synthesis of silver nanoparticles (AgNPs), structural characterization, and their antibacterial potential. Dose-Response. 2022;20(2):15593258221088709.
9. Jeetkar T, Khataokar S, Indurkar A, Pandit A, Nimbalkar M. A review on plant-mediated synthesis of metallic nanoparticles and their applications. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2022;13(3):033004.
10. Das S, Sharangi AB. Nanotechnology: A potential tool in exploring herbal benefits. Functional Bionanomaterials: From Biomolecules to Nanoparticles: Springer; 2020. p. 27-46.
11. Hussain A, Hayat MQ, Sahreen S, ul Ain Q, Bokhari SA. Pharmacological promises of genus Artemisia (Asteraceae): A review: Pharmacological promises of genus Artemisia. Proceedings of the Pakistan Academy of Sciences: B Life and Environmental Sciences. 2017;54(4):265–87-–87.
12. Tang J, Guo Z, Wen Y, Ao S, Wang J. Herbal extract of Artemisia maritima induces selective anti-tumor effects in three human lung carcinoma cells (H1299, NCI-H1437, PC-14) through activating apoptosis-related proteins, G2/M cell cycle arrest and suppression of cell migration. Journal of BU ON: Official Journal of the Balkan Union of Oncology. 2020;25(2):869-74.
13. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules. 2009;14(6):2167-80.
14. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnology advances. 2013;31(2):346-56.
15. Harborne A. Phytochemical methods a guide to modern techniques of plant analysis: springer science & business media; 1998.
16. Njoya H, Onyeneke CE, Okwuonu C, Erifeta G. Phytochemical, proximate and elemental analysis of the African mistletoe (Tapinanthus preussii) crude aqueous and ethanolic leaf extracts. extraction. 1993;21:22.
17. Ariwaodo J, Ugwu R, Odega C, Weriateide A, Obiajulu L, Olu O. Examining the Phytochemicals in Leaves of Pterocarpus Species in Nigeria. Kastamonu University Journal of Forestry Faculty.24(3):329-38.
18. Ye W-C. Phytochemical studies on medicinal plants: Euphorbia ebracteolata, Pulsatilla chinensis, and Gymnema sylvestre: Hong Kong University of Science and Technology (Hong Kong); 2001.

19. Edeoga HO, Okwu D, Mbaebie B. Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology. 2005;4(7):685-8.
20. Pithayanukul P, Ruenraroengsak P, Bavovada R, Pakmanee N, Suttisri R, Saen-Oon S. Inhibition of Naja kaouthia venom activities by plant polyphenols. Journal of ethnopharmacology. 2005;97(3):527-33.
21. Li X-M. Protective effect of Lycium barbarum polysaccharides on streptozotocin-induced oxidative stress in rats. International journal of biological macromolecules. 2007;40(5):461-5.
22. Cowan ST. Cowan and Steel's manual for the identification of medical bacteria: Cambridge university press; 1993.
23. Aschaffenburg R, Rook J. Section C. Dairy Chemistry and Physics: Part I. Chemistry and Physical Chemistry. Journal of Dairy Research. 1949;16(3):390-438.
24. Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. Journal of advanced research. 2016;7(1):17-28.
25. Mohammed Fayaz A, Balaji K, Girilal M, Kalaichelvan P, Venkatesan R. Mycobased synthesis of silver nanoparticles and their incorporation into sodium alginate films for vegetable and fruit preservation. Journal of agricultural and food chemistry. 2009;57(14):6246-52.
26. Singh R, Magesh S, Rakkiyappan C. Formation of fenugreek (Trigonella foenum-graecum) extract mediated Ag nanoparticles: mechanism and applications. Int J Bioeng Sci Technol. 2011;2(3):64-73.
27. Özbilgin S, Acıkara ÖB, Akkol EK, Süntar I, Keleş H, İşcan GS. In vivo wound-healing activity of Euphorbia characias subsp. wulfenii: Isolation and quantification of quercetin glycosides as bioactive compounds. Journal of Ethnopharmacology. 2018;224:400-8.
28. Wu J, Zhao X, Sun Q, Jiang Y, Zhang W, Luo J, et al. Synergic effect of PD-1 blockade and endostar on the PI3K/AKT/mTOR-mediated autophagy and angiogenesis in Lewis lung carcinoma mouse model. Biomedicine & Pharmacotherapy. 2020;125:109746.
29. Akhter MS, Rahman MA, Ripon RK, Mubarak M, Akter M, Mahbub S, et al. A systematic review on green synthesis of silver nanoparticles using plants extract and their bio-medical applications. Heliyon. 2024;10(11):e29766.
30. Essghaier B, Hannachi H, Nouir R, Mottola F, Rocco L. Green Synthesis and Characterization of Novel Silver Nanoparticles Using Achillea maritima subsp. maritima Aqueous Extract: Antioxidant and Antidiabetic Potential and Effect on Virulence Mechanisms of Bacterial and Fungal Pathogens. Nanomaterials [Internet]. 2023; 13(13).
31. Dhaka A, Chand Mali S, Sharma S, Trivedi R. A review on biological synthesis of silver nanoparticles and their potential applications. Results in Chemistry. 2023;6:101108.
32. Esther Arland S, Kumar J. Green and chemical syntheses of silver nanoparticles: Comparative and comprehensive study on characterization, therapeutic potential, and cytotoxicity. European Journal of Medicinal Chemistry Reports. 2024;11:100168.
33. Oselusi SO, Sibuyi NRS, Meyer M, Meyer S, Madiehe AM. Phytofabrication of silver nanoparticles using Ehretia rigida leaf aqueous extract, their characterization, antioxidant and antimicrobial activities. Materials Today Sustainability. 2025;29:101059.
34. Brown AN, Smith K, Samuels TA, Lu J, Obare SO, Scott ME. Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and methicillin-resistant Staphylococcus aureus. Appl Environ Microbiol. 2012;78(8):2768-74.
35. Pasieczna-Patkowska S, Cichy M, Flieger J. Application of Fourier Transform Infrared (FTIR) Spectroscopy in Characterization of Green Synthesized Nanoparticles. Molecules [Internet]. 2025; 30(3).
36. Wan Mat Khalir WKA, Shameli K, Jazayeri SD, Othman NA, Che Jusoh NW, Hassan NM. Biosynthesized Silver Nanoparticles by Aqueous Stem Extract of Entada spiralis and Screening of Their Biomedical Activity. Front Chem. 2020;8:620.
37. More PR, Pandit S, Filippis A, Franci G, Mijakovic I, Galdiero M. Silver Nanoparticles: Bactericidal and Mechanistic Approach against Drug Resistant Pathogens. Microorganisms. 2023;11(2).
38. Fahim M, Shahzaib A, Nishat N, Jahan A, Bhat TA, Inam A. Green synthesis of silver nanoparticles: A comprehensive review of methods, influencing factors, and applications. JCIS Open. 2024;16:100125.
39. Hajtuch J, Iwicka E, Szczoczarz A, Flis D, Megiel E, Cieciórski P, et al. The Pharmacological Effects of Silver Nanoparticles Functionalized with Eptifibatide on Platelets and Endothelial Cells. Int J Nanomedicine. 2022;17:4383-400.
40. de la Harpe KM, Kondiah PPD, Choonara YE, Marimuthu T, du Toit LC, Pillay V. The Hemocompatibility of Nanoparticles: A Review of Cell-Nanoparticle Interactions and Hemostasis. Cells. 2019;8(10).