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Utilization of aqueous broccoli florets extract for green synthesis and characterization of silver nanoparticles, with potential biological applications

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A R T I C L E I N F O

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A B S T R A C T

The process of creating nanoparticles using chemicals is not eco-friendly. However, a more environmentally conscious approach known as green chemistry, which involves using vegetable-mediated nanoparticle production, combines nanotechnology with biotechnology. In this study, the researchers aimed to assess the effectiveness of the green chemistry technique in producing silver nanoparticles using an liquid extract from broccoli florets (*Brassica oleracea*) under ideal environment. The successful production of silver nanoparticles was achieved through silver nitrate ($AgNO_3$) biological reduction with the help of an aqueous broccoli florets extract at a slightly acidic pH of 6–7. The silver nanoparticles occurrence was shown by a change of color that moved from colorless to reddish-brown. To characterize the green-produced nanoparticles, various analytical techniques such as Ultraviolet–Visible Spectroscopy (UV-VIS), Fourier Transform Infrared Spectroscopy (FT-IR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Energy-Dispersive X-ray Spectroscopy (EDAX) were employed. The antioxidant properties of the formed silver nanoparticles (AgNPs) were examined in vitro using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) tests. Additionally, the antibacterial properties of AgNPs against various pathogenic bacteria was evaluated. The reduction procedure was easy and simple manageable, with UV–Vis spectroscopy indicating the surface plasmon resonance (SPR) presence at 425 nm. FTIR was utilized to identify active chemical groups in the biomass before and after reduction. SEM and X-ray diffraction analyses indicated that the silver nanoparticles had an average the size of individual particles of 33 nm and exhibited a face-centered cubic (FCC) structure. EDAX analysis confirmed the occurrence of elemental silver in the nanoparticles. The study demonstrated that the biosynthesis of AgNPs led to significant variations in antioxidant activity, which was dose-dependent and showed a similar pattern to the testing of the scarfing action of the ascorbic acid against free radicals using DPPH and FRAP. The AgNPs also dispalyed firm deep-spectrum antibacterial action observed against the tested pathogenic bacteria, outperforming certain medications. Interestingly, the silver nanoparticles remained stable at ambient temperature for 25 days without precipitation, retaining their

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antioxidant and antibacterial properties. In conclusion, the research findings suggest that an aqueous extract of fresh broccoli florets can serve as a viable and environmentally friendly method for producing stable silver nanoparticles with beneficial antioxidant and antibacterial characteristics.

1. Introduction

Nanotechnology refers to the utilization of scientific principles to manipulate matter at the nanoscale. This approach broadens the scope for investigating and managing cellular-level presence of nanoparticles [1], therapeutic substance conveyance [2], cancer diagnosis by diagnostic imaging [3], artificial implants [4], HIV supresion [5], and removal of impurities from water [6]. Various chemical and photochemical methods can be employed to produce diverse nanoparticles. These techniques encompass the use of wide range of synthesis methods [7]. The groundwork of nanotechnology lies in these preparation methods, which involve minimal material conversion, utilization of harmful substances and excessive energy consumption, and time-consuming and inefficient purification processes. There is significant potential to develop more environmentally friendly manufacturing techniques for these materials. Green synthesis offers a more cost-effective and eco-friendlier substitute for conventional chemical and physical synthesis methods [8,9]. Noble metal nanoparticles have garnered considerable interest in recent times because of their exceptional optical, electrical, mechanical, magnetic, and chemical attributes, which vary greatly, as compared to bulk materials, nanoparticles give special benefits. Among these, AgNPs drives a crucial act in biological and drug sectors, owing to their enticing chemical and physical based characteristics. Throughout history, silver has been utilized for treating and preventing various ailments, especially infections. Its potent bactericidal and inhibitory capabilities, along with its broad-spectrum antimicrobial activity, have been recognized since ancient times [10]. Silver nanoparticles have been shown to exhibit anti-angiogenesis [11], anti-viral [12], anti-inflammatory [13], anti-fungal [14], and anti-inflammatory [11] effects. In recent times, there has been a surge in the popularity of using plants to facilitate the generation of noble nanoparticles in a biologically based approach.

On the basis of their inherent reduction and antioxidant prospects, various microbes such as the bacterial, fungal, and plants communities have been exploited for green synthesis of silver nanoparticles. Typically, these microorganisms facilitate the transformation of metal compounds to form nanoparticles. While there are several natural methods for producing silver nanoparticles, microorganism-assisted syntheses are currently impractical due to the requirement for highly sterile environments and maintenance. As a result, plant extracts are favored over microorganisms for this purpose because they offer greater ease of handling, lower biohazard risk, and simpler maintenance of cell cultures, making them a more preferable option [15]. It stands out as an excellent medium for producing nanoparticles because it lacks harmful synthetic compounds and includes natural capping agents to modify silver nanoparticles.

Besides, the implementation of floral concentrate in the reduction of the costs associated with isolation mechanism of smaller-scale organism isolation together with their growth compound, makes the production of nanoparticles by microorganisms more cost-effective. This motivates research on the biosynthesis of silver nanoparticles, which avails numerous benefits as compared to chemically and physically based approaches, such as environmental safety, cost-effectiveness, and greater applicability, particularly in bactericidal activities. Green production of silver nanoparticles using plant extracts has gained prominence. Plants have been proposed as a source of silver nanoparticles due to their rapidity, eco-friendliness, non-pathogenic nature, and ability to provide a straightforward approach to biosynthetic operations. The plant extracts contain various biomolecules with therapeutic properties. These biomolecules naturally possess the necessary complex structures to reduce and modulate the silver nanoparticles in the final product [16]. Furthermore, silver nanoparticles possessing antioxidant properties have found diverse applications. These nanoparticles effectively combat free radicals that induce oxidative stress, a condition resulting from oxygen-utilizing metabolic activities that upset the balance of feedback within living systems [17].

Multiple studies have examined the production of silver nanoparticles by employing a range of plant-based extracts, including broccoli. These investigations involved analyzing the lowering of silver ions and characterizing the resulting nanoparticles through application of wide range of approaches. Additionally, in certain studies, the antibacterial efficacy of the produced nanoparticles was evaluated, showcasing their potential in tackling bacterial infections [16–18,19]. All these research studies have shed light on the ability of applying the plant based extracts, including broccoli, in an environmentally friendly manner for the generation of silver nanoparticles. They showcase the thorough characterization of nanoparticles using various analytical techniques to investigate their physicochemical attributes and potential applications. Nevertheless, there may be a inadequacy of research efforts that are aimed at the green production and depiction of silver nanoparticles through the inclusion of aqueous broccoli florets extracts, or such studies might be limited in number. Therefore, the main focuses of this research approach were as follows: (i) to describe a method for producing silver nanoparticles using vegetable assistance, where the ions of silver are reduced by an aqueous extract of broccoli florets, (ii) to assess the outcomes through UV–Vis spectroscopy, FT-IR, XRD, SEM, and EDAX analyses, (iii) to conduct the DPPH and FRAP tests to evaluate the in vitro based antioxidant prospects of the bio-synthesized AgNPs from the plant extract, and (iv) to evaluate the antibacterial action of the brocken down AgNPs against specific disease causing bacteria. The researchers hypothesized that broccoli lacked antioxidant and antibacterial properties. The study's scope was confined to exploring the application of silver nanoparticles produced from broccoli, focusing on their antioxidant properties and antibacterial effects against selected microorganisms.

2. Experimental part

Materials: Broccoli for extract preparation was purchased from a nearby store, Sigma-Aldrich Chemicals provided the silver nitrate.

Preparation of Sample Extract: To eliminate surface impurities, accurate measurements of blooms for broccoli (*Brassica oleracea*) were taken and efficiently cleaned in water. After that, they were finely macerated in a blender. Once homogenized, addition of 100 ml of deionized water was made, and the blend was subjected to boiling at 80 °C for 15 min in a water bath. The resulting extract was then immediately utilized for the biosynthesis of AgNPs after being filtered through muslin cloth wire gauze (pore size 25) and Whatman paper No. 1 [18].

Formation of Silver Nanoparticles* (AgNPs)*: To synthesize AgNPs, a 1 mM $AgNO_3$ solution in water was prepared. Subsequently, 5 mL of liquefied extract materials for broccoli was added to 95 mL of $AgNO_3$ solution to initiate the creation of silver nanoparticles. The production of AgNPs was evidenced by a shift in color from transparent to reddish-brown, a finding that was subsequently corroborated through UV–Visible spectroscopy analysis [18].

2.1. AgNPs biosynthesized and characterization

Visual inspection: AgNPs were synthesized using a water-based broccoli extract as the bioreducing agent for silver nitrate. The emergence of a reddish-red hue confirmed the successful formation of silver nanoparticles. Images of the formed AgNPs can be found in the accompanying materials (Fig. 2).

UV-VIS Spectroscopy: The process of reducing $AgNO_3$ to AgNPs through the application of broccoli extract in aqueous form was explored by analyzing the Ultraviolet–Visible Spectroscopy (UV-VIS) spectra of the reaction composite. A slight portion of the reaction was diluted with deionized water, and the data was collected using a Shimadzu Dual Beam spectrometer (UV-1650 PC Model) with a 1 nm resolution [19].

FT-IR Analysis: To determine the bioactive substances that take part in both the reduction of Ag + ions as well as encapsulating the bio-reduced silver nanoparticles in the broccoli extract, FT-IR measurements were conducted. This analysis utilized KBr pellets in diffuse reflectance mode, with a resolution of 4 cm [19].

X-ray Diffraction Studies: To analyze the AgNPs reduced by broccoli, X-ray diffraction (XRD) was conducted by applying a powder and the SEIFERT JSO DEBYEFLEX -2002 X-ray diffractometer. The measurement covered an angle range of 10°–70°, and the instrument operated with CuK radiation in a −2 configuration at a 40 KV voltage and a 30 mA current. The Debye-Scherrer formula was utilized to calculate the size of the crystallite domain [20].

Scanning Electron Microscopy (SEM): The specimen was made ready by depositing a droplet of colloidal AgNPs solution onto a copper grid coated with carbon. Subsequently, it was allowed to air-dry prior to insertion into a microscope operating at 130 kV. (Hitachi –S 3400 N) [21].

Energy Dispersive X-ray Spectroscopy (EDS): Energy Dispersion Analysis (EDS) was employed to verify the existence of elemental silver. EDS utilizes the photon characteristics of light, where a solitary photon in the X-ray region has adequate energy to generate a detectable pulse of X-rays. Semiconductor material and processing circuits are utilized for the detection of these X-rays. The observations using EDS were conducted with dedicated equipment and a Scanning Electron Microscope(SEM) [21].

2.2. DPPH radical assay

Researchers tested the ability of silver nanoparticles derived from broccoli extract to counteract free radicals through the application of the DPPH (1,1-diphenyl,2-picryl-hydrazil) technique [22]. The samples' capability in the scavenging of radicals against the stable DPPH radical was assessed spectrophotometrically with the aid of an ELISA reader, employing various silver nanoparticle quantities (12.5, 25, 50, and 100 μg/ml). The colorimetric shift took place at 517 nm upon DPPH reduction, resulting in a transition from deep violet to bright yellow. Ascorbic acid acted as the positive control (reference). The percentage of inhibition was determined as provided in the provided equation:

Absorbance of $^{-}$ ve control - Absorbance of sample.

Inhibition = ×100%

Absorbance of $^{-}$ ve control.

2.3. Ferric Reducing Antioxidant Power (FRAP) assay

To create the FRAP reagent, a mixture of 20 mM iron chloride hexahydrate solution, TPTZ solution of 10 mM, and sodium acetate buffer solution of 300 mM (pH = 3.6) was combined in a proportion of 10:1:1. All the above solutions were combined to produce the active FRAP reagent solution that was applied with a prepared working solution. For the experiment, 10 μL of the extract was added together with 3 mL of the working FRAP reagent meadia in a quartz cuvette [6]. Following 30 min of spectrometer usage, a rise in light absorption at a wavelength of 593 nm was detected. The activity of reduction, assessed through application of the FRAP technique, was expressed as TE and determined by referencing the Trolox calibration curve within a concentration range of 400 to 24,000 μM.

2.4. AgNPs antibacterial action

The antimicrobial effectiveness of AgNPs was evaluated on numerous human based bacteria of disease-causing potential, including two Gram-positive strains (*Streptococcus pyogenes* and *Staphylococcus aureus*) and two Gram-negative strains (Pseudomonas aeruginosa and Serratia marcescens). These bacteria were cultured on nutrient agar slants. The assessment followed the approved protocols [23]. The validity of AgNPs, at quantities of 500, 250, 125, 62.5, and 31.25 μg/ml in aseptically formed deionized water, and the sensitivity of antibiotics against the targeted microorganisms were assessed through a disk diffusion experiment. The isolates were grown at temperature of around 25 °C for 15 min and then overnight at temperature of 37 °C. Following the procedure for incubation, the presence of zone of inhibition around the well was examined. Positive outcomes were noted by undertaking measurement on the diameter of the inhibitory area through a Vernier digital caliper [24].

3. Results and discussion

Visual Characterization: On mixing the concentrate of broccoli with an aqueous solution of 1 mM $AgNO_3$, the extract color underwent change from green to reddish-brown, signifying the production of silver AgNPs as depicted in Fig. 1 A (Brocooli plant), Fig. 1B (Brocooli extract) and Fig. 1C ($AgNO_3$ + plant extract).

3.1. Spectral techniques for identifying biosynthesized silver nanoparticles

UV-VIS Spectroscopy: It can be employed for investigating the dimensions and structure of AgNPsin water-based solutions [25]. In the UV-VIS spectrum, spherical particles typically show a solitary peak that is centered at around 420 nm. The AgNPs generated in the process of the reaction portray an absorbance peak at around 425 nm, indicating their spherical morphology. The highest peak of absorption is attributed to Mie scattering caused by the silver metal [26]. AgNPs being produced was indicated by the colour of the reaction mixture becoming yellowish. Based on the stimulation of plasmon vibrations on their surface particles, these nanoparticles are widely recognized for exhibiting vivid hues that range from pale yellow to brown [27]. (Fig. 2).

3.2. FT-IR

Fig. 3 illustrates the FT-IR spectra of AgNPs synthesis. The registered spectrum spans a wavelength range of 400 cm–4000 cm. Notable peaks at 3424 cmˆ-1 and 3433 cmˆ-1 (indicative of strong O–H structured bond) are visible, indicating the occurrence of carboxyl groups (–O-H) and secondary amides (N–H). The noted peaks suggest the existence of bound hydroxyl groups. Furthermore, the FT-IR spectrum reveals peaks at 2924 cmˆ-1, 2333 cmˆ-1, and 2856 cmˆ-1, indicating stretching of alkane bonds (C–H). Peaks at 1625 cmˆ-1 and 1383 cmˆ-1 suggest deformation of (N–H) and aromatic association of (C=C), respectively. The strong peak at 1020 cmˆ-1 is associated with protein C–N stretching vibrations. In the 700-600 cmˆ-1 range, a weak band signifies C–S stretching. These spectral bands are situated similarly to those found in native proteins [28]. Fig. 3 depicts the existence of several crucial elements engaged in transforming silver ions into AgNPs. In the IR spectra of AgNPs, several key functional groups and weaker peaks are conspicuously absent. This occurrence can be conneted to the decrease of silver ions, resulting in the disappearance and diminished intensity of these spectral bands.

XRD: X-ray diffraction (XRD) patterns of AgNPs were acquired through measurements by applying a powdered X-ray diffractometer (SEIFERT JSO DEBYEFLEX 2002) within an angle range of 10°–70°. As shown in Fig. 4, the presented data exhibit multiple Bragg reflections comparable to the (111), (200), and (220) lattice plane sets, which are characteristic of face-centered cubic silver and can be accurately indexed. These peaks align with those specified in the Powder Diffraction Joint Committee Standards (file No. 04–0783), providing solid evidence for the formation of crystalline AgNPs [29]. Additionally, employing the Scherrer formula and using the Full Width at Half Maximum (FWHM) derived from the diffraction peaks, the average diameter of the AgNPs is estimated to

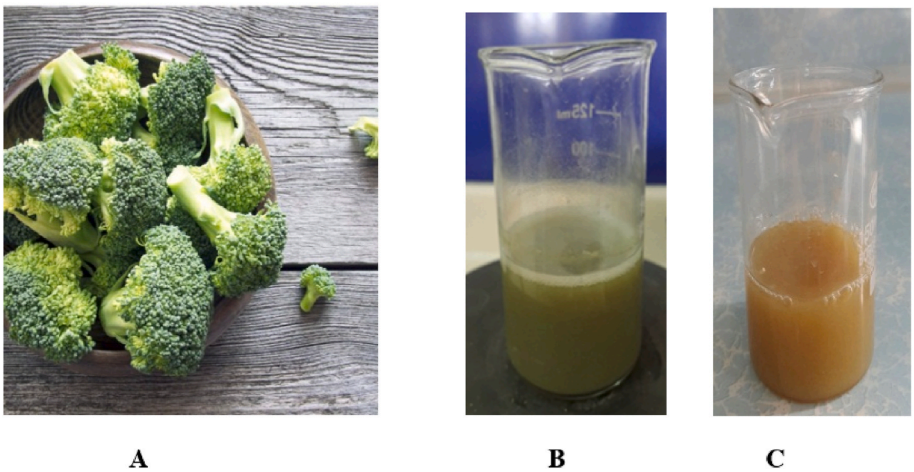

Fig. 1. A-Brocooli plant B- Brocooli extract C-($AgNO_3$ + plant extract).

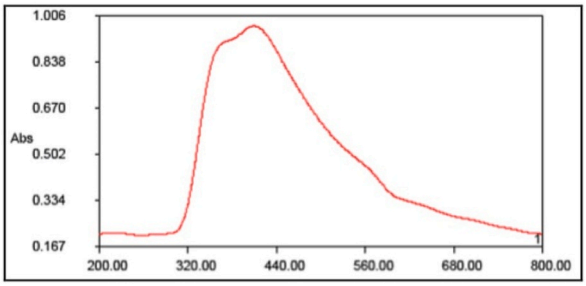

Fig. 2. UV–Vis Spectrum for AgNPs synthesis by Broccoli extract.

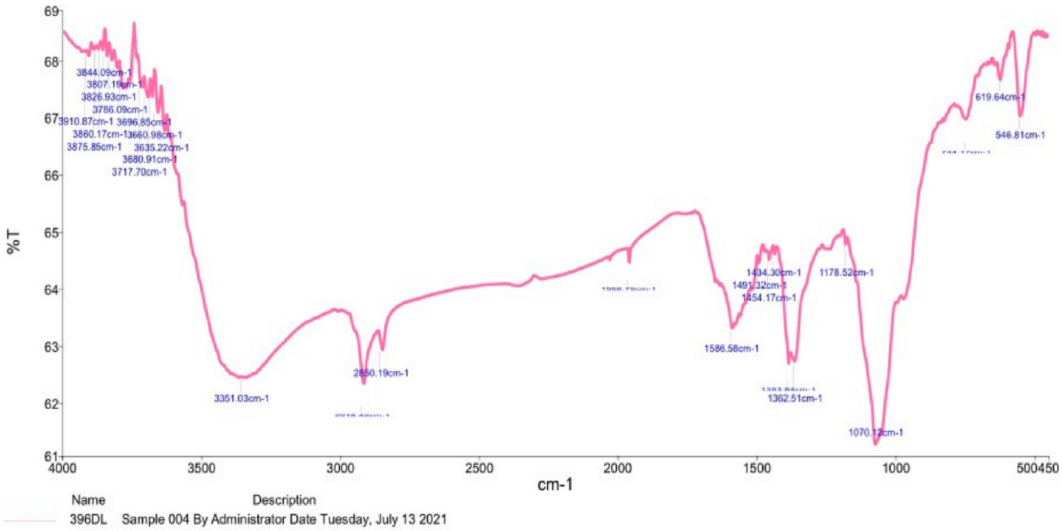

Fig. 3. FT-IR of AgNPs synthesis by Broccoli extract.

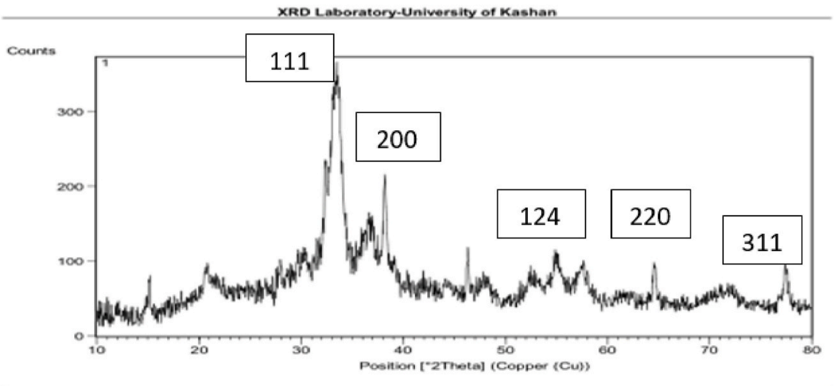

Fig. 4. XRD of AgNPs synthesis by broccoli extract.

be approximately 46 nm. This information confirms the crystal form and synthesized AgNPs magnitude:

$$D = 0.89\lambda/\beta \cos \theta$$

The XRD relies on several important parameters such as the wavelength of the Cu target, the Full Width at Half Maximum (FWHM) of the diffraction peak, the diffraction angle, and the mean grain size represented by D. These essential factors are frequently utilized in XRD to discern a material's chemical composition and crystal structure, as depicted in Fig. 4. Due to its ability to analyze these key parameters, XRD has become a widely used technique in materials characterization and identification [30].

SEM Studies: The morphology and magnitude of the AgNPs were thoroughly examined through the application of scanning electron microscopy (SEM). As indicated in the findings presented in Fig. 5, the synthesized nanoparticles displayed a well-defined shape which was of spherical form, with an estimated approximately cross-sectional diameter of 33 nm. These observations align with previous reports in the scientific literature, reinforcing the consistency of the obtained results. The use of SEM allowed for a detailed and visual assessment of the nanoparticles' structural characteristics, providing valuable insights into their shape and size, which can be beneficial for further applications and research [31,32].

EDAX: The EDAX pattern, presented in Fig. 6, provided evidence of the crystalline nature of the AgNPs generated by reducing silver ions with fresh aqueous broccoli extract. The EDS spectrum was captured using the spot-profile mode, allowing for detailed elemental analysis. A distinctive optical absorption peak corresponding to metallic AgNPs was clearly observed at 3 KeV, indicating their characteristic absorption behavior. The stronger indicators originating from Ag atoms were highly pronounced, while indicators emanating from C, O, and P atoms appeared relatively weaker. Based on the EDS signals, the silver nanoparticles produced through the reduction by aqueous broccoli extract contained a weight proportion of elemental Ag amounting to 73.76%. This EDAX and EDS analysis offered significant information pertaining to the configuration and properties of the synthesized AgNPs, underscoring the effectiveness of the reduction process using the natural extract [31,32].

DPPH assay:

The antioxidant potential of the biosynthesized AgNPs was evaluated through the application of the DPPH test, with ascorbic acid employed as the control substance. The AgNPs synthesized in this investigation demonstrated comparable free radical scave action when compared to the control. Remarkably, the highest antioxidant action of the AgNPs was observed at quantities of 200, 100, 50, and 25 μg/ml, achieving a scavenging rate of 75.2% at 200 μg/ml. At concentrations of 100, 50, and 25 μg/ml, the AgNPs exhibited antioxidant activities of 68.01%, 53.52%, and 36.04%, respectively. Similarly, the same concentrations of ascorbic acid exhibited antioxidant activities of 80.00, 70.40, 56.60, and 40.09%, respectively. These findings highlight the potential of the biosynthesized AgNPs as efficient antioxidants, with their scavenging capabilities comparable to that of ascorbic acid, which is well-known for its antioxidative properties. The DPPH assay allowed for the quantitative assessment of the AgNPs' antioxidant potential at various concentrations, providing valuable insights into their potential application in combating free radicals and oxidative stress-related conditions. (Fig. 7).

The AgNPs synthesized through the use of broccoli extract displayed a DPPH free radical scavenging activity profile akin to that of ascorbic acid at dosages of 200, 100, and 25 μg/ml. At these specific concentrations, notable variations ($P \leq 0.05$) were observed in comparison to the control group. The obtained findings provide evidence that the DPPH free radical scavenging action of AgNPs is influenced by the dosage, signifying a dose-dependent relationship. The observed similarities in scavenging activity between AgNPs and ascorbic acid at certain doses suggest that the biosynthesized AgNPs possess promising antioxidative capabilities, comparable to a well-established antioxidant like ascorbic acid. The indicated results support the understanding about the potential applications of AgNPs in combating free radicals and oxidative stress-related conditions. Additionally, the dose-dependent nature of the scavenging activity highlights the significance of optimizing the concentration of AgNPs for specific therapeutic or preventive purposes.

Furthermore, the process of oxidation in phenolic compounds results in the creation of quinoid compounds, which have the ability to be adsorbed onto the surfaces of nanoparticles, thereby contributing to the stability of the nanoparticle suspension [33,34]. It is widely recognized that phenolic compounds often play specific role directed in exhibiting antioxidant action [35]. Plant-derived phenolics are acknowledged for their antioxidant properties owing to their redox characteristics, which enable them to act as effective singlet oxygen quenchers, reducing agents, and providers of hydrogen. These intrinsic redox properties of phenolic compounds make them valuable contributors to the overall antioxidant potential of nanoparticles and their suspensions. The adsorption of quinoid compounds on the nanoparticle surfaces further enhances the stability of the suspension, supporting the overall efficacy of the antioxidant activity displayed by plant phenolics and their interaction with nanoparticles [36].

Nanoparticles offer promising advantages in addressing vascular alterations, specifically endothelial dysfunction produce by the action of oxidative intolerant [37]. Endothelial dysfunction can lead to a decline in the availability of nitric oxide (NO), adversely affecting the regulation of vascular tone and becoming a contributing factor in the progression of cardiovascular disorders. Hence,

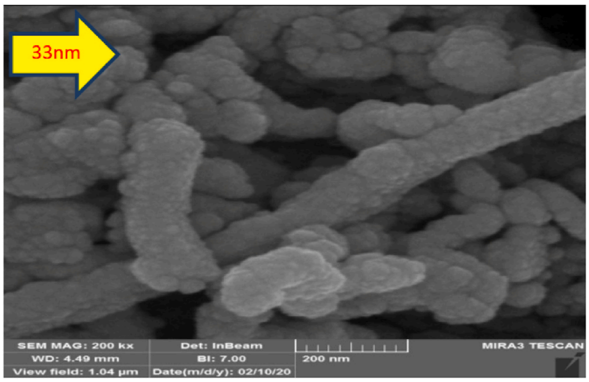

Fig. 5. SEM of AgNPs synthesis by broccoli extract.

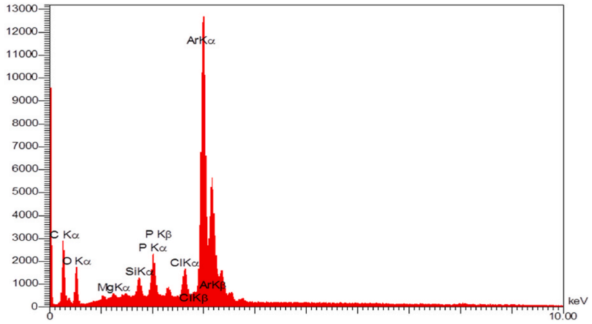

Fig. 6. EDS of AgNPs synthesis by Broccoli extract.

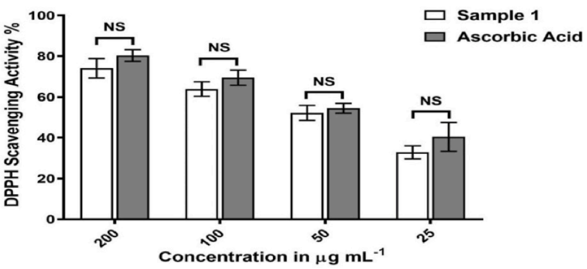

Fig. 7. DPPH of AgNPs synthesis by Broccoli extract.

nanoparticles possessing antioxidant characteristics, as synthesized in the present study, exhibit considerable potential in treating vascular dysfunction that are connected with conditions like hypertension, diabetes, or atherosclerosis. By mitigating the harmful effects of oxidative stress on endothelial cells, these nanoparticles can help restore the proper functioning of the endothelium and improve NO bioavailability, ultimately supporting cardiovascular health and reducing the risk of related diseases. This highlights the potential of nanoparticles with antioxidant capabilities as an inventive therapeutic approach within the area of cardiovascular medication and health.

FRAP assay:

The antioxidant potential of the biosynthesized AgNPs was evaluated using the FRAP test, with ascorbic acid employed as the reference compound for comparison. The AgNPs produced in the current research exhibited a free radical scavenging activity similar to that of ascorbic acid. Notably, the highest antioxidant activity of the AgNPs was observed at quantities of 200, 100, 50, and 25 μg/ml, reaching 68.3% within 200 μg/ml. At quantities of 100, 50, and 25 μg/ml, the AgNPs demonstrated antioxidant activities of 71.5, 43.1, and 31.8%, respectively. Similarly, at the same concentrations, ascorbic acid displayed antioxidant activities of 75.00, 61.40, 48.50, and 40.1%, respectively. These results indicate that the biosynthesized AgNPs possess significant antioxidative capabilities, comparable to a well-established antioxidant like ascorbic acid. The FRAP test allowed for a quantitative assessment of the AgNPs' ability to scavenge free radicals at various concentrations, providing valuable insights into their potential application as antioxidants in combating oxidative stress-related conditions. The observed dose-dependent nature of the antioxidant activity underscores the importance of optimizing the concentration of AgNPs for specific therapeutic or preventive purposes. These findings contribute to the understanding of the antioxidative properties of the biosynthesized AgNPs and their potential use as alternative antioxidants in various operations (Fig. 8).

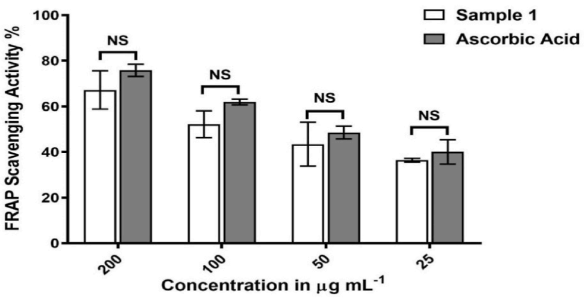

Fig. 8. DPPH of AgNPs synthesis by Broccoli extract.

The antioxidant activity of AgNPs, synthesized using broccoli extract, demonstrated a consistent pattern similar to that of ascorbic acid at dossings of 200, 100, 75, and 25 μg/ml. Notably, these concentrations exhibited significant statistical variations ($P \leq 0.05$) when in comparison to the group under control. These findings imply that the DPPH free radical scavenging activity of AgNPs is influenced by the dosage administered. The observed similarities in scavenging activity between AgNPs and ascorbic acid at varying doses suggest that the biosynthesized AgNPs possess considerable potential as antioxidants, comparable to the widely recognized antioxidative properties of ascorbic acid. The DPPH assay allowed for a quantitative assessment of the antioxidant capabilities of AgNPs at different concentrations, revealing their effectiveness in combating free radicals. This dose-dependent relationship is essential to consider when optimizing the concentration of AgNPs for specific applications in oxidative stress-related conditions. The ability of AgNPs to scavenge free radicals at multiple concentrations emphasizes their potential as alternative antioxidants with possible therapeutic and preventive implications [38–41].

In the process of nanoparticle formation, secondary metabolites play crucial roles as capping agents. These secondary metabolites encompass terpenoids, flavonoids, soluble proteins, and phenolics, which are derived from the plant extracts [42]. Notably, polyphenols present in the plant extracts possess electron-donating capabilities, facilitating the biological based reduction of $Ag+$ ions to Ag°, thereby initiating the synthesis of AgNPs. Furthermore, these polyphenols act as stabilizing agents, ensuring the stability of the resulting AgNPs. Similarly, water-soluble flavonoids, another class of secondary metabolites found in plants, actively participate in reducing silver ions during the build-up of AgNPs. Their presence and contributions are vital in ensuring the successful formation of stable and functional AgNPs with potential applications in various fields, including nanomedicine and nanotechnology. The involvement of these natural compounds in the biosynthesis of AgNPs highlights the sustainable and environmentally friendly nature of this approach, paving the way for greener and more biocompatible nanomaterial production [43]. The antioxidant capabilities of the extract obtained from plants can be ascribed to the availability of essential phytochemicals, as well as flavonoids, polyphenols, saponins, terpenoids, and vitamins. Within the context of our research, we detected significant amounts of these bioactive compounds in the broccoli extract. These phytochemicals are known for their ability to neutralize harmful free radicals, thereby providing potential health benefits and supporting the antioxidant defense system of living organisms. The diverse array of phytochemicals in the broccoli extract contributes to its overall antioxidant potency, making it a valuable natural resource for various uses within the areas of medicine, nutrition, and food production. The observation of substantial quantities of these phytochemicals in our indicates the ability of broccoli extract as a rich origin and supplier of antioxidants and may open avenues for future investigations into its health-promoting properties [16]. The heightened antioxidant potential indicated in the present research can be attributed to the binding of phytochemicals to the larger surface area of AgNPs. This increased surface area provides more active sites for the attachment of these bioactive compounds, enhancing their overall antioxidant capacity. Moreover, the enhanced bioactivity can be further attributed to the electrostatic synergy that exists between the phytochemicals that are negatively charged and the positively or neutrally charged AgNPs. This electrostatic attraction leads to a strong affinity between the phytochemicals and the AgNPs, promoting their efficient incorporation onto the nanoparticle surfaces and augmenting their antioxidant effects. As a result of this synergistic interplay, the AgNPs serve as carriers, effectively delivering and stabilizing the bioactive compounds, thereby boosting their antioxidant capabilities. This interaction between phytochemicals and AgNPs showcases the potential of nanotechnology-based approaches in enhancing the bioactivity and therapeutic applications of natural antioxidants, paving the way for innovative developments in the field of nanomedicine and biomedical research [44].

Pathogenic bacteria isolated from burn and wound patients:

Four different pathogens were isolated and identified from various locations in patients, comprising two Gram-negative bacteria and two bacteria of gram positive in nature. To ensure accurate identification, all isolates underwent initial confirmation using biochemical tests. Subsequently, the Vitek-2 compact system (Biomérieux) was employed to further verify the identification of these pathogens. The Vitek-2 system is an automated platform that utilizes advanced technologies and databases to rapidly and accurately identify microbial species based on their unique biochemical characteristics. This combination of traditional biochemical tests and modern automated systems ensures a reliable and precise identification of the isolated pathogens, which is crucial for appropriate treatment and management of infections.

3.3. Susceptibility profiles of the isolated bacteria

The bacteria obtained from diverse sites in patients underwent susceptibility testing to assess their response to commonly used antibiotics for combating these specific bacterial strains. The sensitivity testing involved the utilization of five distinct antibiotic discs: ceftriaxone (CRO-30μg), ampicillin (AM-10 μg), cephalothin (KF-30μg), chloramphenicol (C-30μg), and meropenem (MEM-10μg). The test results revealed that all bacterial isolates exhibited resistance to the antibiotics tested. These findings indicated that the selected antibiotic discs failed to effectively inhibit the growth and proliferation of any of the bacterial isolates under investigation, based on the standards provided by the Clinical and Laboratory Standards Institute (CLSI). The identification of antibiotic resistance patterns is crucial in guiding clinicians to choose appropriate treatment options and implement measures to control the spread of multidrug-resistant bacterial infections. This data emphasizes the importance of regular surveillance of antibiotic resistance trends to ensure optimal patient care and address the global health challenge posed by antimicrobial resistance, Figure −9.

CRO = ceftriaxone AM = ampicillin KF = cephalothin.

C = chloramphenicol MEM = meropenem.

Through our comprehensive analysis, we found that all bacterial isolates displayed resistance to the tested drugs. Each individual isolate demonstrated complete resistance to ceftriaxone, ampicillin, cephalothin, chloramphenicol, and meropenem, highlighting the multidrug-resistant (MDR) nature of all the studied bacteria. However, it is noteworthy that meropenem exhibited some degree of effectiveness against the bacterial isolates, making it the sole exception among the tested antibiotics. Despite this, the overall prevalence of multidrug resistance among the bacterial isolates is a cause for concern and underscores the urgency of implementing prudent antibiotic stewardship practices and infection control measures to combat the rise of MDR pathogens. Identifying the antibiotic resistance patterns of these bacterial strains is crucial for formulating effective treatment strategies and ensuring patient safety. This underscores the importance of continuous surveillance of antimicrobial resistance to guide clinical decision-making and promote the rational use of antibiotics. Efforts to discover new antimicrobial agents and alternative treatment modalities are crucial in the battle against the emergence and dissemination of MDR bacteria [45].

3.4. AgNPs antimicrobial action against selected pathogenic bacteria

In order to evaluate the antibacterial efficacy of the produced AgNPs against a varieties of pathogenic bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Streptococcus mutans*, the inhibitory zone was meticulously measured in various dimensions. The inhibitory zone represents the region of bacterial growth inhibition surrounding the AgNP-impregnated discs on an agar plate. The measurement of this zone provides crucial insights into the extent to which the AgNPs can hinder the growth and proliferation of the targeted pathogenic bacteria. The assessment of the inhibitory zone's size against each bacterial strain aids in determining the relative sensitivity or resistance of these microorganisms to the AgNPs, contributing to the overall understanding of the nanoparticles' potential as effective antimicrobial agents. These findings have significant implications for the development of novel antibacterial strategies, especially in light of the global challenges posed by antibiotic resistance and the urgent need for alternative approaches to combat bacterial infections shown in Fig. (10) and Fig. (11).

The silver nanoparticles (AgNPs) have been demonstrated to possess robust and wide-ranging antibacterial activity, effectively targeting multidrug-resistant microorganisms. In our study, we conducted a comparative analysis of the action of various antibiotics on the bacterial isolates. The findings, illustrated in Fig. 9, revealed that not all selected bacterial isolates responded favorably to the antibiotics used. Interestingly, AgNPs exhibited substantially larger inhibitory zone widths in comparison to certain antibiotics, particularly at higher concentrations of nanoparticles. This observation highlights the potent antibacterial potential of AgNPs, surpassing the inhibitory effects of some conventional antibiotics against the tested bacterial strains. The broader spectrum of antibacterial activity displayed by AgNPs is particularly promising in the context of combating multidrug-resistant microorganisms, which have become a significant global health concern. This study underscores the relevance of exploring alternative antimicrobial agents, such as AgNPs, to address the growing problem of resistant nature of antibiotics and to encourage the efforts on the production of more effective therapeutical ways. Within quantities of 500 μg/ml, the sample organisms under the test displayed the highest inhibitory effect, resulting in a maximum zone of inhibition measuring 20 mm against Escherichia coli. Remarkably, even at a dosage as low as 31.25 μg/ml, the AgNPs exhibited good sensitivity in inhibiting bacterial growth. Among the chosen antibiotics, Streptococcus mutans and Escherichia coli exhibited the highest levels of resistance as isolates. Notably, the means of activity underlying the antibacterial potency of AgNPs entails the induction of reactive oxygen species (ROS) release, such as superoxide. This rapid release of ROS leads to a disruptive impact on the potential action of the bacterial cell membrane, effectively compromising the structural stability of the microorganisms. In addition to this membrane disruption, the presence of AgNPs also contributes to the degradation of essential biomolecules within the bacterial cells, further accentuating their antimicrobial activity. This multifaceted mode of action makes AgNPs a promising candidate in the production of effective therapeutic ways for deterring the bacterial infections, especially in the context of dealing with antibiotic-resistant strains and persistent microbial threats [46].

Despite numerous research efforts, the precise antibacterial mechanism of action exerted by silver nanoparticles (AgNPs) remains to be definitively established. Fig. 9 outlines several theories that have been proposed in an attempt to elucidate the antibacterial effects of AgNPs. One prevailing theory posits that the eradication of bacteria occurs through the production of reactive oxygen species (ROS) upon the formation of $Ag +$ ions from the AgNPs. These released $Ag +$ ions are believed to interact with sulfhydryl groups in proteins, resulting in their denaturation and consequent disruption of essential cellular processes within the bacterial cells. Additionally, another proposed mechanism involves the direct connection of AgNPs to the bacterial cell surface, subsequently leading to cellular damage and destruction. The binding of AgNPs to the bacteria may alter the cell membrane's integrity, provoke structural damage, and disturb vital cellular functions. These potential mechanisms collectively contribute to the bactericidal activity of AgNPs, culminating in the inhibition of bacterial growth and proliferation. Although further research is needed to fully comprehend the complex interactions between AgNPs and bacteria, these proposed mechanisms shed light on the diverse and multifaceted ways through which AgNPs display their antibacterial effects. As our understanding of the antibacterial mechanisms continues to evolve, AgNPs hold tremendous potential as promising candidates for combating bacterial infections and addressing the escalating concern of antimicrobial resistance [47]. Similarly, several postulated mechanisms contribute to the antibacterial effects of silver nanoparticles (AgNPs). One of these proposed mechanisms entails the attachment of AgNPs onto cell walls of bacteria, where they interact and hinder membrane permeability. This disruption of the cell membrane compromises its integrity, causing death of the cell. Another proposed pathway suggests that AgNPs interact with enzymes and amino acids within the bacterial cells, triggering the production of reactive oxygen species (ROS). These ROS, in turn, induce cellular stress and damage essential biomolecules, ultimately causing cell dysfunction and breakdown. Moreover, AgNPs' interactions with specific sites rich in phosphorus and sulfur within the bacterial cell nucleus can potentially induce damage to the bacterial DNA. This DNA damage can lead to genetic alterations and cell death, further contributing to the overall antibacterial activity of AgNPs. These diverse mechanisms collectively contribute to the bactericidal effects of AgNPs and underscore their potential as effective antimicrobial agents against various pathogenic bacteria. As our understanding of these mechanisms deepens, the rational design and application of AgNPs in combating bacterial infections can be further optimized, paving the way for innovative solutions to address the growing challenges posed by antibiotic resistance., Fig. 12 [48].

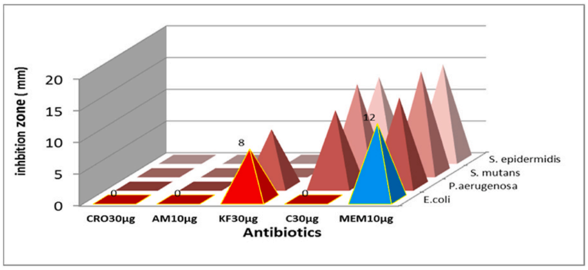

Fig. 9. Antibacterial action of AgNPs on *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Streptococcus mutans*.

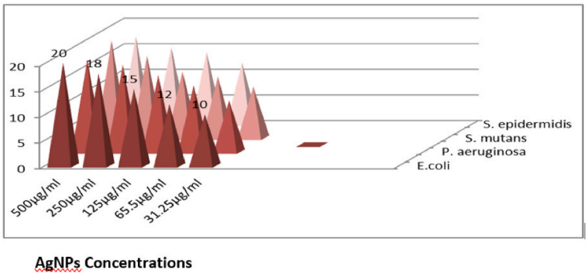

Fig. 10. Antibacterial action of AgNPs against pathogenic Bacteria.

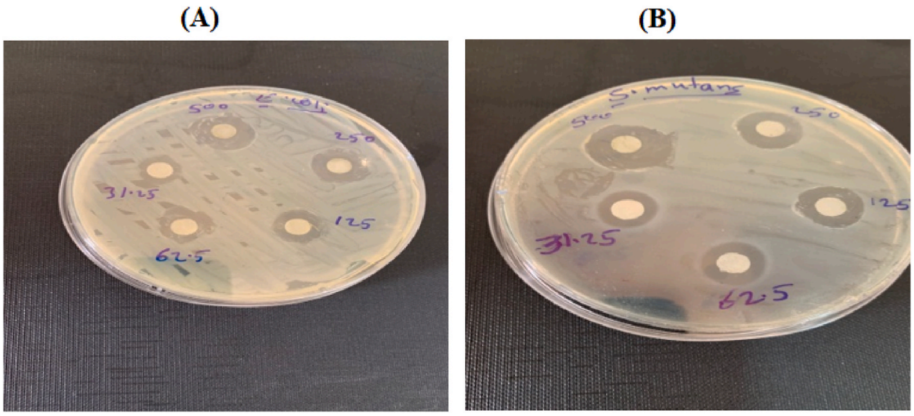

Fig. 11. Inhibition zone of Gram –ve (A) and +ve (B) bacterial growth as a result of antibacterial activity of AgNPs.

4. Conclusion

This research introduces a simple and favorable environmentally acceptable means for the synthesis of AgNPs utilizing aqueous extracts derived from the root barks of a medicinal broccoli variety. The FTIR spectra reveal shifting peaks, indicating the involvement of numerous active and functional groups from plant secondary metabolites, which act as conclusive and balancing agents during the nanoparticle formation process. Detailed analyses employing XRD and FESEM confirm the AgNPs' characteristics, revealing a size of approximately 33 nm and a face-centered cubic crystalline structure, with a well-defined spherical structure.

EDX examination further provides insight into the elemental composition of the nanoparticles, showing relative abundance of potassium, oxygen, silver, and carbon. Additionally, the synthesized AgNPs exhibit heightened antioxidant and antibacterial activities, further demonstrating their potential for various biomedical and medicinal applications.

Further investigations exploring the influence that arise from nanoparticle size, shape, and specific phytochemicals on the physicochemical properties would contribute to optimizing the biological activities of AgNPs. This pursuit holds significance in advancing the understanding of nanomaterial-biological interactions and in designing tailored AgNPs with enhanced therapeutic effects.

In conclusion, this eco-friendly and straightforward biosynthetic approach represents a promising substitute eans ordinary chemically and physically basedmethods for AgNP production. The utilization of plant-mediated synthesis showcases the potential of natural sources in providing biocompatible and biologically active nanoparticles for diverse applications in medicine, biotechnology,

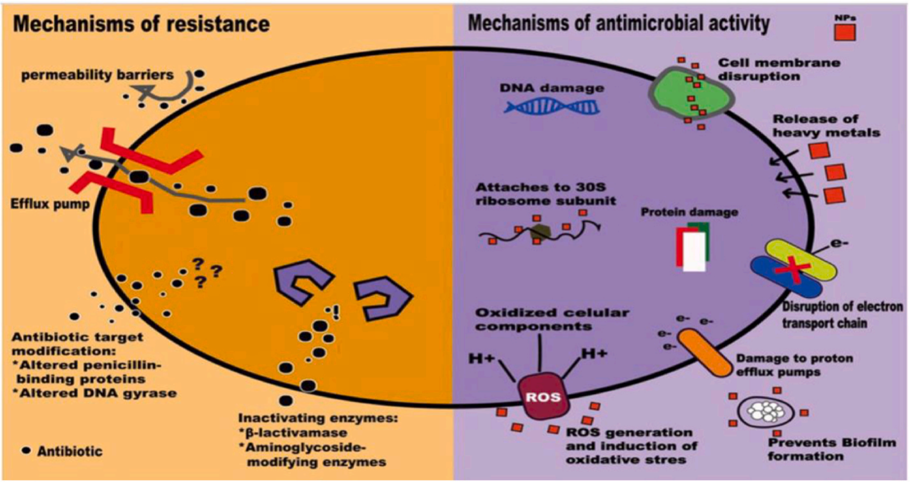

Fig. 12. Mechanisms of antimicrobial resistance and actions of nanoparticles [51, 52].

and other fields of research. The continuous exploration of such green synthesis strategies opens new horizons for sustainable nanotechnology, contributing to the development of innovative solutions to address various global challenges in health and environmental domains.

Author contribution statement

Lubna Abdulazeem, Abdullah Alasmari, Metab Alharbi, Abdulrahman Alshammari, Ziyad Muhseen: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] L. Du, H. Jiang, X. Liu, E. Wang, Biosynthesis of gold nanoparticles assigned by E. coli DH5ά and its applications on direct electrochemistry communication 598 (2) (2007) 181–192.
[2] D. John, J. Paul, A. Born, Drug delivery and nanoparticles; Application and hazards, Journal nanomedicines 3 (2) (2008) 133–149.
[3] K. Kairemo, P. Ebra, K. Bergstron, E.K.J. Pavwels, Nanoparticles in cancer Current radiopharma 5 (27) (2008) 30–36.
[4] A.R. Adini, M. Redlich, R. Teene, Medical application of inorganic fullerene like nanoparticles, J mat chem 6 (4) (2011), https://doi.org/10.1039/CIJM11799H.
[5] J.L. Elechiguena, J.L. Burt, J.R. Morones, A.C. Bragado, G.O. GaX, H.H. Lara, Interaction of Ag nanoparticles with HIV -1, Nanotechnology 3 (7) (2005) 1–10.
[6] J.R. Morones, J.L. Elechiguena, A. Canacho, K. Holt, J.B. Kouri, J.T. Ramfrez, M.J. Yacaman, The bactericidal effect of silver nanoparticles, Nanotechnology 4 (16) (2005) 2346–2353.
[7] V. Parashar, R. Parashar, B. Sharma, A. C, Pandey Digest of jouran of nano materials and bio structure 4 (1) (2009) 45–50.
[8] M. Kowshik, S. Ashtaputre, S. Kharazzi, W. Vogel, J. Urban, S.K. Kulkarni, K.M. Panikar, Extracellular synthesis of silver tolrent strain MKY3, Nanotechnology 14 (6) (2003) 95–100.
[9] K. Nabikha, R. Kathiersan, A. Raj, M. Alikunhi, A. Nabeel, Synthesis of antimicrobial silver nanoparticles by callus and leaf extract from salt marsh plants, Sesuvium portulacastrum L, Colliods and Surface: Biointerfaces 79 (7) (2009) 488–493.
[10] S.S. Shankar, A. Rai, B. Ankamwar, A. Singh, A. Ahmad, M. Sastry, Nat. Mater. 3 (3) (2004) 482–488.
[11] J.V. Rogers, C.V. Parkinson, Y.W. Choi, J.L. Speshock, S.M. Hussain, A Preliminary Assessment of Silver Nanoparticle Inhibition of Monkeypox Virus Plaque Formation 3 (10) (2008) 129–133.
[12] S. Gurunathan, K. Lee, K. Kalishwaralal, S. Sheikpranbabu, R. Vaidyanathan, S. Eom. Biomater. 30 (42) (2009) 6341–6350.

[13] B.J. Wiley, S.H. Im, J. McLellan, A. Siekkinen, Y. Xia, Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis, J. Phys. Chem. B 110 (32) (2006) 15666–15675.
[14] P.L. Nadworny, J. Wang, E.E. Tredget, R.E. Burrell, Nanomedicine: Nanotech. Biol. Med. 4 (32) (2008) 241–251.
[15] K. Kalishwaralal, V. Deepak, S.R.K. Pandia, M. Kottaisamy, M.K.S. Barath, B. Kartikeyan, S. Gurunathan, Biosynthesis of silver and gold nanoparticles using *Brevibacterium casei*, Colloids Surf. B Biointerfaces 77 (2) (2010) 257–262.
[16] N. Kulkarni, U. Muddapur, Biosynthesis of metal nanoparticles: a review, J Nanotechnol (2014) 8.
[17] S. Tdesco, J. Blasco, H. Doyle, G. Redmond, Oxidative stress and toxicity of gold nanoparticles in Mytilus edulis, J: Aquat. Toxicol. 100 (2010) 178–186.
[18] G. caroling, S. kumari tiwari, A. mercy ranjitham, R. suja, Biosynthesis of silver nanoparticles using aqueous broccoli extract characterization and study of antimicrobial, cytotoxic effects, J. Asian J Pharm Clin Res. 6 (4) (2013) 165–172.
[19] B. Stuart, Infrared Spectroscopy: Fundamentals and Application, Analytical Techniques in the Sciences", Wiley Publications., 2004, p. 248.
[20] B.L. Dutrow, X-Ray Powder Diffraction (XRD)", Geochemical Instrumentation and Analysis, 1997, pp. 349–359.
[21] S. Sadhasivam, P. Shanmugam, M. Veerapandian, R. Subbiah, K. Yun, Biogenic synthesis of multidimensional gold nanoparticles assisted by Streptomyces hygroscopicus and its electrochemical and antibacterial properties, Biometals 25 (2) (2012) 351–360.
[22] N. Kumar, A. Mueen, R. Dang, A. Husain, Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone, J. Med. Plants Res. 2 (2008) 361–364, 5656.
[23] Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational, Supplement. CLSI Document M02-A10 and M07-A8, Clinical and Laboratory Standards Institute, Texas, 2012.
[24] CLSI, Performance standards for antimicrobial susceptibility testing, in: CLSI Supplement M100S, Clinical and Laboratory Standards Institute, Wayne, PSA, 2020.
[25] J.A. Creighton, D.G. Eadont, Ultra violet –visible absorption spectra of the colloidal metallic elements, J. Chem Soc., Faraday Trans. 87 (1991) 3881–3891.
[26] K. Aoki, J. Chen, N. Yang, H. Nagasava, Nanochemistry. Langmuir. 19 (2003) 9904.
[27] S. Kapoor, Preparation, characterization, and surface modification of silver particles, Langmuir 14 (1998) 1021–1025.
[28] I.D.G. Macdonald, W.E. Smith, Orientation of cytochrome C adsorped on a citrate- reduced silver colloid surface, Langmuir 12 (1996) 706–713.
[29] T. Swanson, Natl. Bur. Stand. (U.S.), Circ 539 (No.1) (1953) 23.
[30] C. Udayasoorian, K.R. Vinoth Kumar, M. Jayabalakrishnan, Extracellular synthesis of silver nanoparticles using leaf extract of Cassia auriculata, Dig J Nanomater Bios 6 (1) (2011) 279–283.
[31] S.P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry, Synthesis of gold nanotriangles and silver nanoparticles using aloevera plant extract, Biotechnol. Prog. 22 (2006) 577.
[32] J.S. devi, B.V. Bhimba, R. Krupa, Invitro anticancer activity of silver nanoparticles synthesized using the extract of gelidiella sp, Int J Pharm Pharm Sci 4 (Suppl 4) (2012) 710–715.
[33] W. Wang, Q. Chen, C. Jiang, D. Yang, X. Liu, S. Xu, One step synthesis of biocompatible gold nanoparticles using gallic acid in the presence of poly-(Nvinyl-pyrrolidone), Colloids Surf. A Physicochem. Eng. Asp. 301 (2007) 73–79.
[34] L. Abdulazeem, M.J. AL Jassani, M.A. Al-Sheakh, Free radical scavenging and antioxidant activity of silver nanoparticles synthesized from *Cuminum cyminum* (cumin) seed extract, August, Research J. Pharm. and Tech. 14 (8) (2021). ISSN 0974-3618.
[35] J.M. Awika, L.W. Rooney, X. Wu, R.L. Prior, L.C. Zevallos, Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products, J. Agric. Food Chem. 51 (2003) 6657–6662.
[36] S.T. Chang, J.H. Wu, S.Y. Wang, P.L. Kang, N.S. Yang, L.F. Shyur, Antioxidant activity of extracts from Acacia confusa bark and heartwood, J. Agric. Food Chem. 49 (2001) 3420–3424.
[37] M.D. Mauricio, S. Guerra-Ojeda, P. Marchio, Nanoparticles in medicine: a focus on vascular oxidative stress, Oxid. Med. Cell. Longev. (2018) 20.
[38] W. Wang, Q. Chen, C. Jiang, D. Yang, X. Liu, S. Xu, One-step synthesis of biocompatible gold nanoparticles using gallic acid in the presence of poly-(Nvinyl-pyrrolidone), Colloids Surf. A Physicochem. Eng. Asp. 301 (2007) 73–79.
[39] J.M. Awika, L.W. Rooney, X. Wu, R.L. Prior, L.C. Zevallos, Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products, J. Agric. Food Chem. 51 (2003) 6657–6662.
[40] S.T. Chang, J.H. Wu, S.Y. Wang, P.L. Kang, N.S. Yang, L.F. Shyur, Antioxidant activity of extracts from Acacia confusa bark and heartwood, J. Agric. Food Chem. 49 (2001) 3420–3424.
[41] M.D. Mauricio, S. Guerra-Ojeda, P. Marchio, Nanoparticles in medicine: a focus on vascular oxidative stress, Oxid. Med. Cell. Longev. (2018) 20.
[42] V. Kumar, S. Singh, B. Srivastava, R. Bhadouria, R. Singh, Green synthesis of silver nanoparticles using leaf extract of Holoptelea integrifolia and preliminary investigation of its antioxidant, anti- inflammatory, antidiabetic and antibacterial activities, J. Environ. Chem. Eng. 7 (3) (2019) 103094–103097.
[43] A. Afreen, R. Ahmed, S. Mehboob, et al., Phytochemical-assisted biosynthesis of silver nanoparticles from Ajuga bracteosa for biomedical applications, Mater. Res. Express 7 (7) (2020) 1–14.
[44] M.S. Akhtar, M.K. Swamy, A. Umar, A.A. al Sahli, Biosynthesis and characterization of silver nanoparticles from methanol leaf extract of Cassia didymobotyra and assessment of their antioxidant and antibacterial activities, J. Nanosci. Nanotechnol. 15 (12) (2015) 9818–9823.
[45] M.Z. Hamelian, M.M. Angeneh, A. Amisama, K. Varmira, H. Veisi, Green synthesis of silver nanoparticles using Thymus kotschyanus extract and evaluation of their antioxidant, antibacterial and cytotoxic effects, J. WILEY Appl. Organometal Chem. (2018) 1–8, https://doi.org/10.1002/aoc.4458.
[46] Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational, Supplement. CLSI Document M02-A10 and M07-A8, Clinical and Laboratory Standards Institute, Texas, 2021.
[47] R.H. Ahmed, D.E. Mustafa, Green synthesis of silver nanoparticles mediated by traditionally used medicinal plants in Sudan, Int. Nano Lett. 10 (1) (2020) 1–14.
[48] H. Veisi, S. Hemmati, H. Shirvani, H. Veisi, Green synthesis and characterization of monodispersed silver nanoparticles obtained using oak fruit bark extract and their antibacterial activity, Appl. Organomet. Chem. 30 (6) (2016) 387–391.