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Original Article

Green synthesis and antibacterial activity of silver and gold nanoparticles using crude flavonoids extracted from *Bombax ceiba* flowers

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Abstract

The present study explores the simple and eco-friendly green synthesis of silver (AgNPs) and gold (AuNPs) nanoparticles using aqueous flower extract of Bombax ceiba, commonly known as silk cotton. The extract, rich in flavonoids, serves as both a reducing and capping agent, facilitating the synthesis of metal nanoparticles. The synthesized nanoparticles were characterized using UV spectroscopy and scanning electron microscopy (SEM), confirming their formation and stability. The antibacterial activity of the AgNPs, AuNPs, and crude flavonoids was evaluated against several bacterial strains, including Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus, using the agar well diffusion method. Our results show that AgNPs exhibit significant antibacterial activity, particularly against Gram-negative bacteria, with a marked zone of inhibition observed for S. typhi and E. coli. The inhibition zone increased with higher concentrations of AgNPs. In contrast, AuNPs and flavonoid solutions demonstrated only mild antibacterial effects, with no significant inhibition observed at lower concentrations (1–6 µL). The antibacterial efficacy of AgNPs was comparable to that of standard antibiotics, such as Azithromycin for Gram-positive bacteria and Ciprofloxacin for Gram-negative bacteria, suggesting their potential as effective antimicrobial agents. The antibacterial activity of the synthesized nanoparticles and the crude flavonoid extract highlights the promising use of Bombax ceiba flower extract in the green synthesis of metal nanoparticles with potential biomedical applications.

Keywords: Green synthesis, Phytochemical composition, Antibacterial, Antioxidant, Plant extract.

1. Introduction

Nanotechnology is the science and technology of manipulating matter on an atomic and molecular scale, particularly those that are less than 100 nm in size, where properties differ significantly from those at a larger scale [1]. One nanometer indicates one billionth of a meter or 10^{-9} meters, and the technology of such small-sized particles was first introduced by Richard P. Feynman [2]. The term nanotechnology was later defined by Professor Norio Taniguchi of Tokyo University in 1974 [3]. Nanotechnology is often described as the processing of separation, consolidation, and deformation of materials by one atom or one molecule (4). Scientists are interested in knowing how materials at small dimensions can possess different properties than those at larger scales [5]. Therefore, nanoparticles, which possess the following three important characteristics, are crucial in the field of nanotechnology: 1) Small size (less than 100 nm), 2) Unique properties due to small size, and 3) Control over the structure and composition of nanoparticles [6]. Nanoparticles possess unique optical, electrical, and biological properties and are applied in many fields such as catalysis, imaging, biosensing, drug delivery, nano-device fabrication, and nanomedicine [7].

Nanoparticles can be broadly categorized into organic and inorganic types. Organic nanoparticles include carbon-based materials, while inorganic nanoparticles include materials like gold, silver, and magnetic and semiconductor nanoparticles [8]. Metals like silver, gold, zinc, carbon, copper, palladium, aluminum, and iron have been extensively used in the synthesis of nanoparticles

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with high activity against various bacteria and fungi [9]. Many nanostructures are naturally present on Earth, such as minerals and catalysts, and play a critical role due to their unique features [10]. In recent years, metal nanoparticles have gained significant attention due to their nanoscale size, which leads to unique properties compared to bulk materials [11]. These nanoparticles exhibit size- and shape-dependent optical properties, which are useful in biomedical applications like imaging of specific target tissues, drug delivery, and bio-sensing, owing to their high surface-to-volume ratio [12]. Other metal oxides are also used for a range of applications such as catalysts, sensors, semiconductors, and in medical science [13].

Synthesis of nanoparticles using plants has become an exciting area of research due to its non-toxicity, eco-friendliness, and speed. Metal nanoparticles synthesized using plant extracts offer advantages over microbial cultures [14]. The need for environmentally friendly, non-toxic synthetic protocols for nanoparticle synthesis has led to the development of biological approaches that avoid the use of toxic chemicals as by-products. This has resulted in an increasing demand for "green synthesis" [15]. On the other hand, physical and chemical processes for the synthesis of metal nanoparticles are widely used, enabling the production of particles with desired characteristics [16]. However, these methods are often expensive, labor-intensive, and potentially hazardous to the environment and living organisms. Thus, there is a growing need for alternative, cost-effective, safe, and environmentally sound methods of nanoparticle production [17].

Over the past decades, it has been demonstrated that various biological systems, including plants, algae, diatoms, bacteria, yeast, fungi, and human cells, can transform inorganic metal ions into metal nanoparticles via the reductive capacities of the proteins and metabolites in these organisms [18]. Several studies have explored the use of plant extracts for nanoparticle synthesis, which eliminates the need for chemical ingredients, and these nanoparticles are used in pharmaceutical and biological applications [19]. Various plant parts, such as roots, stems, bark, leaves, and petals, can act as capping and stabilizing agents in the synthesis of metallic nanoparticles [20]. Plants such as Zea mays, Helianthus annuus, Oryza sativa, Saccharum officinarum, Sorghum bicolor, Azadirachta indica, Medicago sativa (Alfalfa), and Aloe vera are among those most widely used for nanoparticle synthesis [21]. Other plants, such as Emblica officinalis, Hibiscus cannabinus, Pimenta dioica, Anacardium occidentale, Hibiscus rosa-sinensis, Imperata cylindrica, and Garcinia mangostana, are also utilized in nanoparticle synthesis [22].

Plant extracts often contain various polyphenols, such as flavonoids, which are excellent reducing agents and provide natural capping and stabilizing properties [23]. Flavonoids, known collectively as Vitamin P, are a class of phenolic compounds found in plants and fungi that play a significant role in plant protection from pests and diseases [24]. Natural phenols, phenazines, biphenyls, and other pigments have remarkable health-promoting effects, such as antioxidant, anticancer, and anti-inflammatory properties [25. Bombax ceiba, a dicotyledonous angiosperm from the Bombacaceae family, is an important medicinal plant found in temperate Asia, tropical Asia, Africa, Australia, and subtropical regions [26]. The plant has high pharmacological value, and almost every part of it is used in medicine. Roots and flowers are used for a variety of ailments [27].

Bombax ceiba flowers are non-toxic and contain various biomolecules that assist in the reduction process of nanoparticles [28]. The present study was carried out to evaluate the simple and eco-friendly green synthesis of silver and gold nanoparticles using aqueous flower extract of Bombax ceiba [29]. This plant has strong antibiotic, anticancer, and anti-inflammatory properties due to its phytochemicals, which make it suitable for nanoparticle synthesis [30]. The biological synthesis of nanoparticles using Bombax ceiba flower extract has been successfully studied [31]. Various phytochemicals present in the flowers, including flavonoids and phenolic compounds, help in the reduction of metal ions into nanoparticles [32]. The synthesized nanoparticles exhibited antioxidant, antibacterial, and anti-inflammatory properties [33]. Additionally, it was observed that the Bombax ceiba flower extract was effective in synthesizing gold and silver nanoparticles, with sizes ranging from 10–100 nm [34].

2. Materials and Methods

2.1. Collection of plant materials

Fresh flowers of Bombax ceiba were collected and then washed in running tap water to purify them from dust and dried in a shaded area at room temperature for 15 days (58). Dried flowers were ground with an electric grinder machine. About 100g of powdered flowers were mixed with 200 ml distilled water in a 300 ml beaker and heated for 30 minutes. The extract was then filtered through a filtration process and the aqueous extract of flowers was collected [35].

2.2. Flavonoids extraction

The filtrate-dried powdered (100g) was mixed with 80% methanol for 24 hours with periodic shaking and then filtered through a filtration process. The filtrate obtained was then further extracted with ethyl acetate and diethyl ether, following the method of Subramanian and Nagarajan (1969) [36]. Ethyl acetate was used for bound flavonoids, whereas diethyl ether was used for free flavonoids. The ethyl acetate fraction was then hydrolyzed with 8% concentrated H_2SO_4 for 24 hours and re-extracted with ethyl acetate. The fraction obtained was repeatedly washed with distilled water to neutralize it and then kept in a water bath at 60°C to dry it.

2.2.1. Identification test for flavonoids

The following three tests—Alkali reagent test, Shinoda's test, and Zn dust with HCl solution test were carried out for identification of flavonoids. Aqueous flavonoid solutions, when treated with alkali solution, give yellow or orange color (Fig. 1). For example, a molar solution of NaOH (10%) when treated with flavonoids [37]. However, when treated with metallic magnesium or zinc and hydrochloric acid, a variable color of pink, orange, or yellow-red appears after 2-3 minutes [38, 39].

2.2.2. Preparation of flavonoids aqueous solution

Two grams of dry flavonoid mass were dissolved in 80ml distilled water. It was vigorously shaken and placed on an electric heater for better dissolution with continuous stirring [40].

2.3. Preparation of 1 milliMolar solution of silver nitrate ($AgNO_3$)

A silver nitrate molar solution was prepared (gm/L) and kept on a magnetic stirrer for 15 minutes at room temperature. Then, 31 µl of the aqueous molar solution of $AgNO_3$ was mixed with 969 µl distilled water using a 1000 µl micropipette, resulting in a concentration of 1mM silver nitrate solution (65) [41].

2.3.1. Synthesis of silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were synthesized by reducing the freshly prepared 1 mM silver nitrate with the aqueous flavonoid extract. 1ml of aqueous solution of 1mM $AgNO_3$ was reduced using 1, 2, and 3 ml of flavonoids, respectively (i.e., increasing the concentration of flavonoids while keeping the silver nitrate concentration constant) at room temperature for 10-30 minutes, resulting in a dark or dark brown solution indicating the formation of silver nanoparticles [42]. The color change indicates the synthesis of silver nanoparticles [43].

2.4. Preparation of 1 milliMolar Solution of Gold chloride ($AuCl_3$)

Similarly, a molar solution of gold chloride was prepared (g/L) in distilled water and kept on a magnetic stirrer for 25 minutes at room temperature to mix the crystals of gold chloride in the solution. After this, 31 µl of the aqueous molar solution of $AuCl_3$ was mixed with 969 µl distilled water using a 1000 µl micropipette, resulting in a concentration of 1mM gold chloride solution [44].

2.4.1. Synthesis of gold nanoparticles (AuNPs)

The same process was repeated for the synthesis of gold nanoparticles as used for silver nanoparticles. AuNPs were synthesized by reducing the freshly prepared 1mM auric chloride with the aqueous flavonoid extract. 1 ml of aqueous solution of 1mM $AuCl_3$ was reduced using 1, 2, and 3 ml of flavonoids, respectively (i.e., increasing the concentration of the plant extract while keeping the gold chloride concentration constant) at room temperature for 10-30 minutes. The mixture was kept on a magnetic stirrer until the color changed into pink or reddish-brown, indicating the formation of gold nanoparticles. Generally, the color change revealed the bio-reduction of metal oxides into metal nanoparticles [45, 46].

2.5. Characterization

Green synthesized AuNPs and AgNPs were characterized using UV-Vis spectroscopy and SEM analysis. The formation of silver and gold NPs from the 1mM solution of silver nitrate and auric chloride was confirmed by UV-visible spectral analysis. AgNPs and AuNPs have free electrons that give rise to a surface plasmon resonance (SPR) absorption band [47, 48], due to the combined vibration of electrons of metal NPs in resonance with the light wave (49). The SPR spectra for AgNPs were obtained in the range of 230-270 nm, and for AuNPs, in the range of 230-320 nm [50]. SEM analysis showed the morphology of gold and silver NPs, which appeared spherical, with silver particles initially in an aggregated form. This reveals that the silver particles are slightly agglomerated, with a size range of 0.2-1 nm. Gold NPs were spherical and ranged from 1nm in size [51].

2.5.1. Simple drying method of aqueous nanoparticles

Sometimes, characterization requires a dried sample of nanoparticles. Therefore, using a water bath is the easiest and simplest method for drying. The aqueous NPs were placed in the water bath at 60°C to evaporate volatile liquids and water bodies [52].

2.4. Antibacterial activity of green synthesized nanoparticles

Green synthesized nanoparticles were tested for their antibacterial activity using five bacterial strains: Salmonella typhi, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa. Both the agar well diffusion method and the colony-forming unit method were used to determine antibacterial activity. However, the agar well diffusion method was preferred [53].

2.7 Culture media

Mueller Hinton agar medium (table 1) was used for culturing and growth of all microorganisms used in the present study. Nutrient broth media was used for inoculation, shaking, incubation and standardization of microorganisms.

2.7.1. Preparation of media

Fresh Nutrient broth media was prepared (13g/liter) for inoculation and incubation of all kinds of microbial growth. Bacterial strains were inoculated into fresh sterilized Nutrient broth and incubated for the 24-hour cycle at 37°C. After that, Nutrient agar media was prepared in distilled water (38g/L) and sterilized at 15 psi pressure and 121 °C for 15 minutes along with the required Petri plates and other materials. After sterilization, the media was poured into each sterilized Petri plate in concentration of about 20ml under the laminar flow hood in order to avoid contamination. The media was allowed to be solidified in Petri plates for about 10-20 minutes [54].

2.7.2. Agar well diffusion method

After 10-20 minutes of media solidification in Petri plates, Culture bacteria from broth media were added into each Petri plate of a soft agar media, using method of

Table 1. Chemical Composition of Mueller agar media used in the experiment.

Mueller agar composition		
S/no	**Composition**	**gram/liter**
1	Beef extract	2.0g
2	Acid Hydrolysate of Casein	17.5g
3	Starch	1.5g
4	Agar	17.0g
5	Total	38

Table 2. Representation of chemical composition of nutrient broth media used in the cultured.

Nutrient broth modified		
S/no	**Composition**	**gm/liter**
1	Gelatin peptone	5.0g
2	Beef extract	1.0g
3	Yeast extract	2.0g
4	Sodium chloride	5.0g
5	Total	13

swabbing (Swab) and streaking with inoculation loop until to be sure that bacteria was mixed perfectly. The media is then punched with 6mm cork borer-making wells [54]. Different concentrations with variable volumes of flavonoids and synthesized nanoparticles were added in respective wells (1µl, 3µl and 6µl,). Then all the plates were incubated at $37C^0$ for 24 hours in incubators. A zone of inhibition was measured after incubation period.

3. Results

3.1. Silver and gold nanoparticles synthesis

Using the eco-friendly, simple and viable synthetic method, the present study deals with the synthesis of silver and gold nanoparticles. Synthesis took place at room temperature, using aqueous crude extracts (flavonoids) of *Bombax ceiba* flower, as a green reducing and stabilizing agent, acts as an antioxidant agent. The formation of AuNPs and AgNPs was noticed with the change in the color of solutions. Aqueous solution of flavonoids of *B. ceiba* flower was added to 1mM solution of silver nitrate and gold chloride solution with various concentrations of flavonoids i.e. 1, 2, 3ml. While keeping constant the 1mM ratio of silver nitrate and gold chloride, color of a reaction rapidly changes from yellow-brown to dark brown indicating formation of silver nanoparticles as shown in (Figure.1). Similarly change in color from pink to reddish brown specifies synthesis of gold nanoparticles (Figure.2), due to reduction process and excitation of surface Plasmon vibrations, within a few minutes to hours. Color change indicates nanoparticle synthesis.

3.2. Characterization of nanoparticles

3.2.1. SEM analysis

The size and shape of the synthesized and stabilized AgNPs and AuNPs were characterized with the help of scanning electron Microscopy (SEM) to visualize the shapes and measure the diameter of nanoparticles. Fig.3 (a-e) shows SEM analysis of AgNPs. Similarly, Fig.4 (a-e) showed the SEM micrograph of AuNPs. SEM analysis suggests that the particles are dispersed with flavonoid extract and were mostly spherical in shape. Great variation is found among the particle sizes and their average size is estimated to be 0.2 µm to 1 µm.

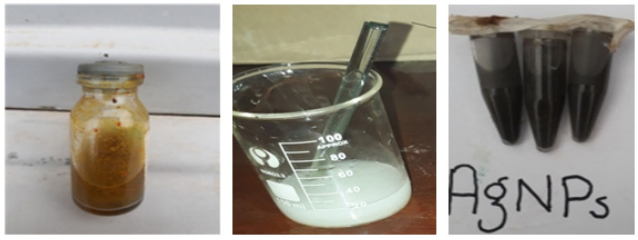

Fig. 1. Representation of Stock solution and synthesis of Silver NPs (AgNPs).

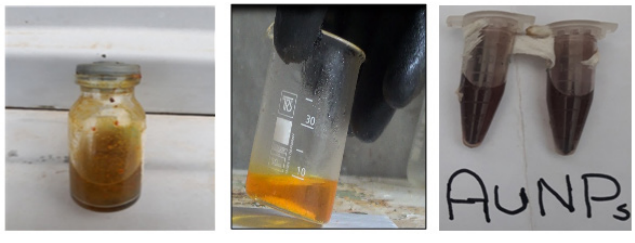

Fig. 2. Preparatory stock solution and synthesis of gold nanoparticles.

3.2.2. UV-visible spectrophotometer

The formation of gold and silver nanoparticles from 1mM solution using flavonoids was confirmed by UV-visible spectral analysis at the range interval of 200-1000nm. The gold nanoparticles show Plasmon resonance

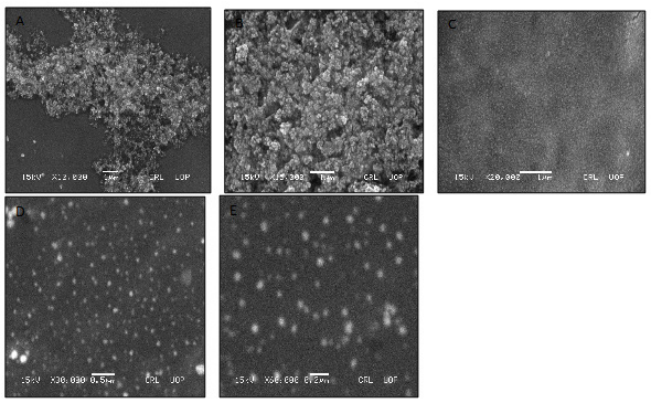

Fig. 3. a-e. Representation of scanning electronic Micrograph of Silver Nanoparticles.

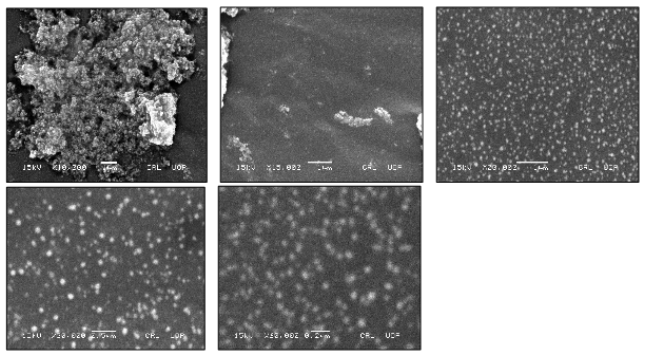

Fig. 4. a-e. SEM analysis of gold nanoparticles aggregated form with flavonoids and later mono-dispersed.

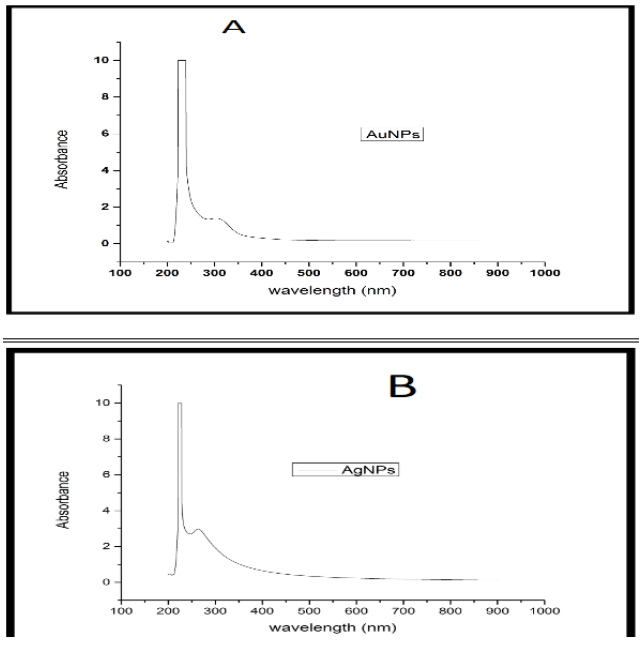

Fig. 5. (a, b,). UV spectral analysis of gold and silver NPs in the visible region

at 230 nm to 350 nm in the visible region with pink-red or yellow color. The UV-visible spectrum of gold NPs is shown in Figure 5 (a). The absorbance spectra of the silver NPs were observed at 230-270 nm as shown in Figure 5 (b) with dark brown in color, which are very close to the peak position indicating no agglomeration of silver nanoparticles and the fact that the NPs are well dispersed in the aqueous solution. The peaks indicate the formation of spherical shape NPs.

3.3. Essay of antibacterial activity

Antibacterial activity of as-prepared gold and silver nanoparticles along with crude flavonoids was determined by using Agar well diffusion method against some gram-positive and gram-negative bacteria. Muller-Hinton cultural media was used for all kinds of bacterial strains. The bacterial test organisms were cultured on sterilized nutrient broth (13g/L) and incubated for 24 hours before the start of experiments. Then the Nutrient agar media (38g/L) was prepared in distilled water and autoclaved at $137C^0$ for 15 minutes. The media is then poured into Petri plates, allowing for solidification and using cotton swabs to spread culture bacteria all over the solidified agar media under laminar flow hood. Using micropipette, the test samples (NPs and flavonoids) were then applied in respective wells of agar plate in various conc. and incubated for 24 hours. Five bacterial strains including *Salmonella typhi, Escherichia coli, Pseudomonas aurogi-nosa, Bacillus subtalis and Steplococcus .aurous* were used in the present study for all kinds of experiments.

3.4. Growth studies

After incubation period, diameter of the zone of inhibition and growth of bacteria was measured in mm caused by flavonoids and NPs. Table 4 shows the distinct significant zone of inhibition of AgNPs. It was observed that the inhibition zone increased as increased the concentration of green synthesized AgNPs. Gram-negative bacteria, *Salmonella typhi* and *Escherichia coli* show greater inhibitory effects against AgNPs than other bacterial strains. It might be due to their cell wall composition. It was also observed that AuNPs and Flavonoids solution showed no inhibitory effects against the used microorganism, per agar plate at the respective conc. of 1µl, 3µl and 6µl (table 5). Gold nanoparticles with the concentration of 1:1-3:3 and flavonoids were added to the cultures for each test isolate on agar plate but the inhibitory result, observed was mild as shown in Petri plates**)** for gold NPs and show flavonoids Petri plates**.** Hence we might be right to say that the result was positive mostly. The unseen inhibition result might be due to scientific error, weak gold NPs or flavonoids and nature of bacteria.

3.3.1. Standardized antibiotics (Positive control)

Azithromycin is an antibiotic useful for the treatment of some gram-positive bacterial infections. It inhibits protein synthesis by impairing the elongation of the peptidyl chain by binding to the 50S ribosome during bacterial infections (78). Similarly, Ciprofloxacin is a class of antibiotics used for a number of gram-negative bacterial diseases. It inhibits bacteria by blocking the active sites of protein synthesis (79).

Diffusion that measures the inhibition zone diameter provides estimates of the susceptibility of bacteria to antibacterial compounds. The zone size is determined using a standardized test method (Broth and Agar medium) (80). Muller Hinton agar preparation, sterilization, spreading of bacteria, and well methods, as previously used, are the same for the positive control of bacteria except for Azithromycin and Ciprofloxacin antibiotics (Table 6), which are used instead of NPs. Antibiotic Azithromycin is used for gram-positive bacteria while Ciprofloxacin is used for gram-negative bacteria in concentrations of 6, 12, and 18 µl in each respective well against bacterial strains.

4. Discussion

Medicinal plants play an important role in human life by providing a base for the pharmaceutical industry due to certain phytochemical constituents screened for various methods. Phytochemical screening means testing a whole plant or parts of the plant quantitatively and qualitatively for the presence of various secondary metabolites such as phenols, alkaloids, tannins, flavonoids, etc. [55]. These naturally active chemicals have immense importance in medicines, cosmetics, and food, and provide health benefits for humans [56]. In the present work, we explored the green synthesis of gold and silver nanoparticles using flavonoids, a secondary metabolite of Bombax ceiba flower extract, and their antimicrobial activity. Recently, flavonoids and phenolic compounds have been recognized for their wide spectrum of pharmacological activities, especially in the reduction of metallic oxides into respective nanoparticles seen in many plants [57]. Bombax ceiba flowers were phytochemically tested for their secondary metabolites and methods of extraction. One of these is flavonoids, which act as reducing, stabilizing, and capping agents and reduce metal oxides into nanoparticles. The work is focused on the synthesis of gold and silver nanoparticles using the aqueous solution of flavonoids from *Bombax Ceiba* flowers. Both silver and gold nanoparticles were synthesized at room temperature. Upon the addition of aqueous flavonoid solutions with various concentrations (1 ml, 2 ml, 3 ml…) to the 1 mM solution of silver nitrate and gold chloride, the color of the solution gradually changed to dark or dark brown, indicating the formation of AgNPs [58]. Similarly, the 1 mM solution of gold chloride, when treated with flavonoid solution, was observed visually to change color from yellow to pink or reddish brown, indicating the synthesis of AuNPs within minutes to hours [59, 60]. The NPs synthesis is due to the action of flavonoids obtained from B. ceiba flowers, which are excellent reducing agents and are used mostly for nanoparticles and nanotechnology.

The particle size and morphology of the as-prepared gold and silver nanoparticles were examined by scanning electron microscopy (SEM). The size and shape of the synthesized NPs were visualized by SEM, and the diameter of nanoparticles was measured. Fig. 3a shows the SEM analysis of AgNPs. Similarly, Fig. 1b shows the SEM micrograph of AuNPs. SEM analysis suggests that the particles are dispersed with flavonoid extract and are mostly spherical in shape. Hence, it may be understood that the experimental conditions (pH, temperature, and the optimum concentration of Ag and Au) will achieve monodispersity and uniform shape. A great variation is found among the particle sizes, and their average size is estimated to be 0.2 µm to 1 µm. The biological molecules could possibly perform the dual function of the formation and

Table 3. Microorganisms used in the present work based on the prevalence.

Name of Microorganism	Nature	Temp. Required (°C) for growth	Time required for growth
Escherichia coli	Gram-negative	37	24 hrs
Staphylococcus aureus	Gram-negative	37	24 hrs
Pseudomonas aeroginosa	Gram- negative	37	24 hrs
Bacillus subtilus	Gram-positive	37	24 hrs
Salmonella typhus	Gram-negative	37	24 hrs

Table 4. Showed zone of inhibition in mm of green synthesized AgNPs using *Bombax ceiba* crude Flavonoids from flower.

SS/no	S	Name of bacteria used	Zone of inhibition in mm			Incubation period
			1µl	3µl	6µl	
11	1	*Salmonella typhi*	5	7	15	24 hrs
22	2	*Escherichia coli*	10	5	13	24 hrs
33	3	*Pseudomonas aero*	6	8	5	24 hrs
44	4	*Bacillus subtalis*	5	6	8	24 hrs
55	5	*S. aureus*	5	5	6	24 hrs

Table 5. Antibacterial activity of AuNPs and Flavonoids at various concentrations.

	SS/no	Name of bacteria used	Flavonoids and AuNPs Concentration			Incubation period
			1µl	3µl	6µl	24 hrs
1		*Salmonella typhi*	–	–	–	**24 hrs**
	22	*Escherichia coli*	–	–	–	**24 hrs**
	33	*Pseudomonas aero*	–	–	–	**24 hrs**
	44	*Bacillus subtilis*	–	–	–	**24 hrs**
	55	*S. aureus*	–	–	–	**24 hrs**

Table 6. Positive control of bacteria using standard antibiotics Azithromycin and Ciprofloxacin

Name of microorganism	Standard Antibiotic used	Nature	Conc. and Average ZOI			Temp. Required for growth (°C)	Time required for growth
			6 µl	12 µl	18 µl		
Escherichia coli	Ciprofloxcin	Gram-negative	23.33	24.6	25	37	24 hrs
Staphylococc-us aureus	Ciprofloxcin	Gram-negative	24	27	35.3	37	24 hrs
Pseudomonas aeroginosa	Ciprofloxcin	Gram- negative	23.66	29	29.3	37	24 hrs
Bacillus subtilus	*Azithromycin*	Gram-positive	31.6	30.6	32	37	24 hrs
Salmonella typhus	Ciprofloxcin	Gram-negative	25.33	26.6	27.3	37 C^0	24 hrs

stabilization of silver and gold NPs in the aqueous medium [61, 62]. The formation and stability of prepared gold and silver NPs were also monitored on a UV-visible spectrophotometer [63]. The surface plasmon resonance spectra for AuNPs are observed at 230 and 350 nm with a pinkish-reddish color, while for AgNPs, the absorption is at 230-270 nm with a dark brown color in the visible region. Ag and Au NPs have free electrons, which give rise to surface plasmon resonance absorption bands due to the combined vibration of electrons of metal nanoparticles in resonance with light waves. The peaks observed for gold and silver NPs are comparable to the literature reports [64].

Formation of silver and gold NPs from 1 mM solution of silver nitrate and auric chloride with flavonoids solution increases with an increase in reaction time. AgNPs and AuNPs have free electrons, which give rise to surface plasmon resonance (SPR) absorption bands, or due to the combined vibration of electrons of metal NPs in resonance with light waves [65].It is well known that Ag+ ions and AgNPs have strong antimicrobial effects. Since Klabunde et al. (2002) demonstrated that reactive metal oxide NPs show excellent bactericidal effects [66]. It is of great interest to investigate the use of other inorganic NPs as antibacterial materials. The application of silver and gold NPs as antimicrobial agents was investigated by growing bacterial strains on agar plates, all supplemented with gold and silver NPs. *Bombax ceiba* flower crude flavonoids-mediated synthesized silver and gold nanoparticles demonstrated inhibitory activity against bacteria *E. coli, S. aureus, P. aeruginosa, S. typhi, B. subtilis,* and *B. subtilis*. Among these, silver nanoparticles showed strong inhibitory effects against all bacteria, with an average zone size of

9 mm (Fig. 7a-e). The AgNPs were found to exhibit greater biocidal action compared to the gold NPs against the test microorganisms [67]. This may be due to the higher surface activity of the silver NPs compared to gold NPs and flavonoids [68]. *Salmonella typhi* and *E. coli* exhibited stronger inhibition than other bacteria. It was observed that the inhibition zone increased as the concentration of AgNPs increased from 1 µl-10 µl [69].

Several factors play a critical role in determining the size, shape, and stability of nanoparticles, and their optimization is essential for improving nanoparticle synthesis. X-ray diffraction (XRD) and dynamic light scattering (DLS) are common techniques used to assess the crystalline structure and particle size distribution of nanoparticles. Previous studies have shown that the synthesis conditions, such as pH, temperature, and reaction time, significantly influence the properties of nanoparticles [70, 71]. For instance, the pH of the synthesis solution can affect the reduction of metal ions and the formation of nanoparticles, as acidic or basic conditions may lead to variations in particle size and morphology [72]. Additionally, the extraction temperature and time are critical in maximizing the yield and stability of plant-derived flavonoids, which in turn affect the nanoparticle synthesis process [73]. While the current study utilized standard methods, it did not explore the effects of these parameters on nanoparticle properties, and we suggest this as a limitation for future work.

Flavonoids themselves and synthesized AuNPs showed less or no inhibitory effects, and the results were mild in most cases [74]. It has been found that the antimicrobial effects of AuNPs and flavonoids may be associated with the characteristics of certain bacterial species, with gram-positive and gram-negative bacteria having differences in their membrane structures [75]. The thicker the cell membrane structure (peptidoglycan), the easier and more specific it is to use AgNPs as an antibacterial agent [76]. However, the mechanism of the inhibitory action of the metal NPs is not yet clearly understood. However, literature revealed that the electrostatic attraction between the negatively charged bacterial cell and the positively charged NPs is crucial for the activity of the nanoparticles as bactericidal material [77]. The antibacterial activity of AgNPs on gram-negative bacteria is closely related to the formation of pits in the cell membrane where AgNPs accumulate, ultimately causing cell death [78].

The use of *Bombax ceiba* flower extract for the synthesis of stable gold and silver nanoparticles offers an easy, low-cost, rapid, eco-friendly, and one-pot approach. It has been observed that the flavonoids present in *B. ceiba* flower extracts exhibit strong antioxidant and capping properties, which help stabilize silver and gold oxides into AuNPs (gold nanoparticles) and AgNPs (silver nanoparticles). The green synthesis of metallic nanoparticles utilizing phytochemicals has garnered significant attention from scientists due to their remarkable antibacterial and antifungal activities, as well as their potential applications in medicine, catalysis, industry, bioimaging, and theranostic agents. Characterization techniques such as UV spectroscopy and transmission electron microscopy (TEM) confirm the formation and stability of the green synthesized AgNPs and AuNPs, revealing an average size range of 0.2-1 µm, which is highly promising for biological applications. The synthesized AgNPs demonstrated strong antibacterial activity against various bacterial strains, including *E. coli*, *P. aeruginosa*, *S. typhi*, *Bacillus subtilis*, and *S. aureus*, as assessed by the agar well diffusion method.

5. Recommendation

We recommend that future studies incorporate additional characterization techniques, such as X-ray diffraction (XRD) and dynamic light scattering (DLS), to provide more detailed information on the crystalline structure and size distribution of the synthesized nanoparticles. Stability testing should also be conducted, ideally using UV-Vis spectroscopy over time, to assess the long-term stability of the nanoparticles. Additionally, optimizing synthesis parameters like pH, temperature, and reaction time experimentally would help in controlling the size, shape, and stability of the nanoparticles, ultimately enhancing reproducibility and scalability for practical applications.

Conflict of interest

All the authors mentioned in the manuscript have no conflict in the research work and compilation.

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