Bio-fabrication of silver nanoparticles (AgNPs) using Antioxidants-rich Syzygium cumin L. leaves extract with evaluation of its antibacterial, and anti-inflammatory activity

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ABSTRACT

This work included the green synthesis of silver nanoparticles (AgNPs) utilizing an aqueous extract of Syzygium cumin L. leaves. The aqueous extract was analyzed qualitatively and quantitatively for phytochemical content. The morphological features, optical characteristics, and surface properties of synthesized AgNPs were examined using X-ray diffraction (XRD), transmission electron microscopy (TEM), ultraviolet-visible (UV-Vis) absorption spectroscopy, and Fourier transform infrared spectroscopy (FTIR). the analysis revealed that the concentrations of flavonoids, tannins, phenolics, and flavonols were 194.77±0.55 µg RT /g, 46.02±0.53 µg TAE/g, 107.25.92±0.5 µg GAE/g, and 32.6±0.67 µg RT /g, respectively. The plant extract demonstrated antioxidant activity, with an IC50 value of 13.54±1.1 µg/mL-1, attributed to its high content of flavonoid and phenolic compounds, suggesting its potential as a natural source of antioxidants. The TEM study demonstrated that the bio-synthesized AgNPs exhibited a remarkable level of homogeneity in relation to their surface morphology. The mean diameter of the particles were around ~55 nanometers. The green synthesized Ag-NPs showed a notable antibacterial activity against several bacterial species. The diameter of the inhibition zones ranged from 26 to 28 mm against six different bacterial isolates, namely *S. aureus, S. haemolyticus, E. faecalis, A. baumannii, K. pneumoniae,* and *E. coli*. The AgNPs had a dose-dependent impact on the stabilization of the red blood cell in human (HRBC) membrane. This led to a varying degree of prevention of hemolysis, ranging from 77.6 ± 1.1% to 95.2 ± 1.4%. The present work introduces a straight forward and economically viable approach for synthesizing AgNPs using environmentally friendly processes, intending to use them as antibacterial agents.

Keywords: Syzygium cumin L; silver nanoparticles; antioxidant; anti-inflammatory; antibacterial and hemolysis**.**

INTRODUCTION

Oxidative stress and antibiotic resistance pose substantial issues in the field of contemporary medicine, with wide-ranging implications for the well-being of the general population. The two issues are intricately linked and present a potential challenge to the efficacy of traditional treatments and the general welfare of individuals. Inflammation frequently results in the production of both anti-inflammatory and pro-inflammatory mediators, including chemokines, tumor necrosis factor, cytokines, inducible enzymes such inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2), which have important regulatory functions (Putra & Murniasih, 2016). Therefore, recent research has focused on various pathways and mediators implicated in inflammatory states and painful (Cavalcante-Silva et al., 2012). The need for finding new drugs act as anti-inflammatory is further exacerbated by the fact that, despite the arsenal of anti-inflammatory agents that is currently available, which is typically comprised of non-steroidal anti-inflammatory drugs, glucocorticoids and immunosuppressants, the treatment appears to be insufficiently effective and is also limited by numerous undesirable side effects (A Mohamed et al., 2017).

Nanotechnology and nanoscience make a lot of contributions to the world, and expanded its involvement in many fields such as biology, chemistry, physics, engineering and medicine. Novel metallic nanomaterials are widely studied because of their specific characteristics. Many researchers have focused on the optimization of metal nanoparticles for many effective and safe applications (Bar et al., 2009; Kajani, Bordbar, Esfahani, Khosropour, & Razmjou, 2014; Khalil, Ismail, El-Baghdady, & Mohamed, 2014; Kumar, Palanichamy, Roopan, & Spectroscopy, 2014; Alshawwa et al., 2022). Silver nanoparticles have achieved more recognition. Its specific properties are

high chemical stability, electrical conductivity, and its antimicrobial properties (Ahmed, Ahmad, Swami, Ikram, & sciences, 2016; Mekky et al., 2021; V. K. Sharma, Yngard, Lin, & science, 2009).

Furthermore, Silver nanoparticles are widely used as effective ant microorganisms agents and shown better antimicrobial performance than usual bulk silver materials (Dhand et al., 2016; Han et al., 2020; Meydan et al., 2022; Oves et al., 2022; Salem, Ali, Reyad, Abd-Elsalam, & Hashem, 2022). Antimicrobial effect of silver nanoparticles has been widely used in the food sector, textiles, and a variety of environmental applications (Ahmed, Ahmad, Swami, & Ikram, 2016; Bourgonje et al., 2023; Huang et al., 2020), because of the silver nanoparticles colloidal solutions have low toxicity to human cells, excellent low volatility and thermal stability, AgNPs are quickly replacing other materials as the preferred choice for use in medicine, a variety of animal husbandry, industries, health, accessories, and the military. Ag-NPs have shown highly effective antifungal activity as well as potential antibacterial effects against pathogenic organisms such *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus haemolyticus, Enterococcus faecalis, Acinitobacter baumanni*, *Klebsiella pneumoniae* and *Escherichia coli* (Manimaran et al., 2023; Shashiraj et al., 2023; Yuan, Peng, & Gurunathan, 2017).

Green nanotechnology offers a sustainable alternative to traditional synthesis processes by harnessing natural and renewable resources, specifically plant extracts. Plant extracts are known to possess a diverse range of bioactive chemicals that exhibit properties like as reduction, stabilization, and templating, which make them suitable for the production of nanomaterials. In addition to diminishing the dependence on harmful chemicals, this approach also presents the possibility of developing nanoproducts that are both biodegradable and biocompatible. Polyphenols compounds in plant extracts give antioxidant, antiobesity, antitumor, anticarcinogenic and anti-inflammatory properties (P. Tang et al., 2018; Zhang et al., 2019; Mekky et al., 2023). Additionally, the enzymes, flavonoids, proteins and other biomolecules found in the aqueous or ethanolic solutions of plant extracts can be used as single or dual-action capping and reducing agents in the manufacturing process of nanoparticles in a green way (Shaik et al., 2018). Similar to similar nanostructures, silver nanoparticles tailoring in shape and size, which is dependent on the chemical make-up and functional groups present in the plant extract, affects their physicochemical qualities (Saied et al., 2023). Because it is more affordable, environmentally friendly, and nontoxic than chemical and physical processes, green production of silver nanoparticles is preferred (Erci, Cakir-Koc, Isildak, & biotechnology, 2018). Silver nanoparticles synthesized via of green plant extracts possess unlimited antioxidant, anti-inflammatory and antimicrobial activity on many human pathogens. Nanoparticles are mostly used in drug delivery applications for treatment of tumer diseases (Hariharan et al., 2020).

One botanical species that warrants attention is *Syzygium cumin* L., most often referred to as Cumin. The therapeutic virtues of *Syzygium cumin* L. have been widely acknowledged and it has been extensively utilized in several traditional medicine systems. The botanical specimen demonstrates a diverse array of phytochemical constituents, encompassing flavonoids, phenolic compounds, terpenoids, and alkaloids, which collectively contribute to its medicinal capacity (Kirtikar, Basu, & Blatter, 1975). The phytochemical constituents found in *Syzygium cumin* L. exhibit significant potential in the manufacture of silver nanoparticles (AgNPs). The distinctive chemical features of these substances can effectively aid in silver ions reduction and subsequent stability of the resultant nanoparticles. Moreover, it should be noted that these phytochemicals exhibit intrinsic biological characteristics, including antioxidant, anti-microorganisms, and anti-inflammatory effects. These features have the potential to augment the therapeutic capabilities of the produced AgNPs. *S. cumini* is a medicinal plant, the parts of which have been pharmacologically proven to possess antibacterial, hypoglycaemic, anti-HIV, and antidiarrheal activity (Bhuiyan, Mia, & Rashid, 1996). The ethanolic extract of *S. cumini* seeds lowered the level of blood glucose of alloxan induced diabetic rats which also has antibacterial activity (Chhikara et al., 2018; Joshi, Paudel, Upreti, & Phytochemistry, 2019; Yadav et al., 2011).

Taking into account the aforementioned factors, the main objective of our research is to examine the process of bio-fabricating silver nanoparticles (AgNPs) by the utilization of leaf extract derived from *Syzygium cumin* L. The study aims to characterize the produced AgNPs in terms of their size, structure, and stability, while also assessing their antioxidant, antibacterial, and anti-inflammatory properties. Additionally, the research will investigate the possible uses of these silver

nanoparticles manufactured by environmentally friendly methods in several industries.

MATERIALS AND METHODS

Materials

Silver nitrate (AgNO3, 99%) was obtained from Sigma-Aldrich. Agar Nutrient medium, Muller-Hinton, and Nutrient Broth were also collected from Sigma-Aldrich German. All glassware was thoroughly cleaned with sterile double distilled water and then dried in an electric oven to remove any lingering impurities. Six isolates of bacteria: Staphylococcus aureus, Staphylococcus haemolyticus, Enterococcus faecalis, Acinitobacter baumanni, Klebsiella pneumoniae, and Escherichia coli were collected from the Microbiology Department, Faculty of Medicine, Cairo University, Egypt through the proper protocol. and identified depending according to Bergey's manual via morphological cultural, and biochemical characteristics (Vos et al., 2011).

Plant sample collection

S. cumini L. Skeels leaves were collected from one of the gardens at Al-Azhar University, Egypt (July 2023). The plant authentication and identification by staff of Plant Taxonomy, Department Botany and Microbiology, Faculty of Science, Al-Azhar University, Egypt. A voucher specimen was prepared and stored in the Desert Research Center herbarium, Cairo. The leaves were gently washed with double distilled water then were shade dried for 15 days at room temperature till constant weight and powdered using an electric grinder.

Preparation of S. cumini aqueous extract

The powder of leaves dried in air of S. cumini (L.) Skeels (100 g) were extracted by double distilled water (1000 mL-1) at room temperature for 24 hours. Afterwards, the mixture was filtered via Whatman filter paper and rotary evaporator was used for concentration (Final yield 13.61%). The crude dry extract was kept at 4 °C until future use.

Preliminary phytochemical screening

Standard procedures (Harborne, 1998; Shinde et al., 1999), were followed in the tests for the phytochemical constituents of S. cumini L. aqueous extract. The following constituents were tested for carbohydrates, phenol, tannins, flavonoids, saponins, glycosides, alkaloids, anthraquinon, cardiac glycosides, sterols and terpenes.

Quantitative determination of phytochemicals

Total phenolic content

The quantification of total phenolic acids in the aqueous extract was conducted using the Folin-Ciocaleau technique as described by (Makkar, 2003). To ensure proper distribution, 50 µL of the phenolic extract into several test tubes. The contents of each test tube should be filled to a volume of 1 mL-1 with distilled water. For each test tube, including the blank, add 0.5 mL-1 of Folin-Ciocalteu reagent (1N). Subsequently, it is recommended to let the substance to remain undisturbed at ambient temperature for aduration of 5 min. Subsequently, include a volume of 2.5 mL-1 of Na2CO3 (5%) into each of the test tubes, including the blank. Subsequently, the sample should be subjected to incubation for a duration of 40 min at ambient temperature, while ensuring absence of light exposure. The OD725 nm absorbance of the calibration curve for gallic acid, which had been dissolved in methanol, was detected using a spectrophotometer. The total phenolic content was determined by calculating the mean ± standard deviation (n=3; all samples were evaluated in triplicate) and reported as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Total flavonoid content

The quantification of flavonoids in the aqueous extract of S. cumini L. was conducted using the aluminum chloride technique as outlined by (Zhishen, Mengcheng, & Jianming, 1999). A volume of 500 µL of the extract was aliquoted into a series of test tubes. Ensure that each test tube is filled with 1 mL of distilled water. Next, introduce 150 µL of a 5% NaNO2 solution into each tube and allow for incubation at ambient temperature for a duration of 5 min. The experimental procedure involves the addition of 150 µL of AlCl3 solution with a concentration of 10% to each of the test tubes. Subsequently, the test tubes are to be incubated at room temperature for a duration of 6 minutes. To each of the test tubes, add a volume of 2 mL per 1 mL of NaOH solution with a concentration of 4%. Prepare the test tubes by filling them with 5 mL-1 of distilled water. The test tubes were well mixed by vortexing and then left undisturbed for aduration of 15 min at ambient temperature. The standard calibration curve of rutin was used in this study. The rutin standard was dissolved in methanol and its measurement was conducted using a spectrophotometer at a wavelength of 510 nm. Total flavonoid content was calculated as the mean ± standard deviation (n=3; All samples were analyzed in three replications) and

expressed in mg of rutin equivalent per gram of dry weight (mg QE/g DW).

Total tannin content

The quantification of tannins was conducted using the Folin-Denis spectrophotometer technique, as described by (Makkar, 2003). A total of 0.5 ml of plant sample and 0.5 ml of distilled water were combined with 0.1 g of polyvinyl polypyrrolidone under low temperature conditions to induce precipitation of tannins. Subsequently, the tubes were incubated for a duration of 4 h at a temperature of 4 °C. Subsequently, subject the tubes to centrifugation at a speed of 3000 rpm for a duration of 10 min, while maintaining a temperature of 4°C. The liquid portion of the solution exclusively comprises phenolic compounds that are not classified as tannins. A volume of 100 µL of non-tannin phenolics extract was extracted from the sample and combined with 0.5 mL of Folin-Ciocalteu reagent (1 N). The resulting mixture was then diluted to a final volume of 1 mL using distilled water, including the blank. The test tubes were well mixed and afterwards left undisturbed for a duration of 5 minutes at ambient temperature. Subsequently, a volume of 2.5 mL-1 of a 5% Na2CO3 solution was introduced into each of the test tubes, including the control sample. The test tubes were thereafter subjected to agitation and placed in an environment with a constant temperature for a duration of 40 minutes, while being shielded from light. The standard calibration curve of tannic acid, dissolved in methanol, was used for measurement using a spectrophotometer at a wavelength of 725 nm. The tannin content was determined by calculating the mean ± standard deviation (n=3; all samples were evaluated in triplicate) and reported as milligrams of tannic acid equivalent per gram of dry weight (mg TAE/g DW).

Total flavonol content

The quantification of the total flavonols content in the aqueous extract of S. cumini L. was conducted using the aluminium chloride colorimetric technique, as outlined by (Miliauskas, Venskutonis, & Van Beek, 2004). The experimental procedure involves the addition of 2 mL-1 of AlCl3 and 6 mL of CH3COONa. Subsequently, the specimen was subjected to incubation at a temperature of 20 °C for a duration of 2 h. The samples were analyzed using a UV-Vis spectrophotometer, and the readings were taken at a wavelength of 440 nm. The total flavonols content was determined by calculating the mean ± standard deviation (n=3; all samples were evaluated in three replications) and reported as milligrams of rutin equivalent per gram of dry weight (mg rutin/g DW).

Evaluation of antioxidant ability using DPPH radical scavenging method

Scavenging of free radical ability of aqueous extract of S. cumini was determined by 1, 1- diphenyl-2-picryl hydrazyl known by (DPPH). 0.1 mM DPPH solution was prepared in ethanol to put it briefly. 3 ml of various extracts in ethanol, each at a different concentration (1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.8 and 3.9 µg/ mL-1), were mixed with 1 mL-1 of this solution. Only those extracts that are soluble in ethanol are employed in this study, and different concentrations of t extracts were prepared using the dilution procedure. The mixtures were vigorously shaken and it was allowed to stand for 30 minutes in room temperature. In the final step, a (UV-VIS) Milton Roy spectrophotometer was used to detect absorbance at OD517 nm. Ascorbic acid had been used as the reference standard compound, and three replications of the experiment were performed. The log dose inhibition curve was used to calculate the IC50 of a sample, which is the concentration needed for inhibiting 50 % of DPPH free radicals. The lower the absorbance of the reaction mixture was indicative of a more active Free Radical activity. The following equation has been used for calculating the percentage of DPPH scavenging
effect:

$$\text{DPPH scavenging effect (\%) or \% inhibition} = A0 - A1 / A0 \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where A1 was the Absorbance in presence of test or standard sample and A0 was the Absorbance of control reaction.

Green synthesis of AgNPs using S. cumin leaves extract

A 1 mM/50 mL aqueous solution of AgNO3 is often introduced into 5 mL of S. cumin leaf extract with vigorous agitation inside a 250 ml reaction vessel. The reaction temperature was elevated to 90 °C for a duration of one hour. Following a duration of one hour, the observed transformation in color exhibited a shift from a light green hue to a yellowish brown shade, serving as a visual indication of the synthesis of AgNP.

Characterization of Green synthesis of AgNPs

The verification or assessment of the change in color of the reaction mixture was conducted by visual observation after the reduction of Ag+ to silver nanoparticles using an extract derived from S. cumin leaves, as

outlined in the study by (Korbekandi, Ashari, Iravani, & Abbasi, 2013). In order to determine the optical absorption properties of ultraviolet (UV) light, a JASCOVIS-730 dual beam spectrophotometer was used. In addition to an increase in wavelength of about 0.2 nm, the absorption spectrum has been recorded between 200 and 900 nm. The micrographs of the samples were analyzed using the EOL MODEL 1200EX Transmission Electron Microscope, running at a voltage of 120kV. X-ray diffraction (XRD) experiments were conducted using a Powder Diffractometer (Ultima IV, Rigaku, Japan) equipped with a Cu target emitting Kα1 radiation at a wavelength of 1.54060 Å. The measurements were performed within the 2θ range of 20-70 degrees. An X-ray scan was conducted using the 2θ/θ continuous mode, with a scanning rate of 2 degrees per minute and a step size of 0.02. At a voltage of 40 kilovolts (kV) and a current of 40 milliamperes (mA), both the voltage and current in the tubes remained constant. The Fourier transform infrared spectrometer (FT-IR) was acquired within the spectral region of 400 to 4000 cm−1 with the FT/IR model JASCO-6700 spectrophotometer.

Antimicrobial activity

The synthesized Ag-NPs were used with standard agar well diffusion method to test antimicrobial activity. According to (Perez, Ineichen, Seals, Michalsky, & Stewart, 1990), human pathogenic Gram-negative bacteria (A. baumannii, K. pneumoniae and E. coli) and Gram-positive bacteria (S. aureus, S. haemolyticus, and E. feacalis) As test specimens, they were used. A pure cultures of the test specimens were sub-cultured in nutrient broth and the strain was uniformly spread on sterilized petri plates with Muller-Hinton agar. In the plates, which were sterilized using a sterilized cork-borer, an annular well of 6 mm diameter was made. In order to monitor antibacterial activity the well was filled with 100 µl of AgNPs, and plates were incubated for 37oC for 24h. in order to measure inhibition zones.

Estimation of the in vitro anti-inflammatory activity using HRBC membrane stabilization method

Healthy volunteers' fresh whole blood (3 mL-1) was centrifuged at 3000 rpm for 10 minutes. in heparinized tubes. The red blood pellets were dissolved in the typical saline supernatant volume. Quantifying the volume of the dissolved red blood pellets, a 40% v/v solution in isotonic buffer (10 mM sodium phosphate buffer, pH 7.4) was created. The buffer solution contains 1.17 g Na2HPO4, and 9 g NaCl in 1 L of distilled water. The supernatant from reconstituted red blood cells was utilized according to (Shinde et al., 1999). The hypotonic solution (5 mL-1) with successive dosages of silver nanoparticles (1000, 800, 600, 400, 200 and 100 µg/ mL-1) were placed in centrifuge tubes in duplicate pairs, this included increasing quantities of the same isotonic solution (5 mL-1) as above. 200 g/ml of indomethacin was dissolved in 5 ml of distilled water for the control tubes. Each tube received 0.1 mL-1 of the erythrocyte suspension. The mixtures were incubated for 1 hour in 37°C then centrifuged at 1300 rpm for 3 minutes. Spectrophotometer evaluated supernatant hemoglobin absorbance (OD) at 540 nm. Heamolysis in distilled water samples were assumed to be 100% to compute the percentage (Anosike, Obidoa, & Ezeanyika, 2012). The AgNPs hemolysis inhibition percentage was calculated as follows: $\%\ \text{Inhibition of haemolysis} = 1 - ((OD2 - OD1)/(OD3 - OD1)) \times 100$ (2)

Where OD1 is absorbance for test sample in the isotonic solution, OD2 is absorbance of the test samples in the hypotonic solution, and OD3 is absorbance of the control samples in the hypotonic solution.

RESULTS AND DISCUSSIONS

The preliminary phytochemical screening of *S. cumini* leaves extract

The extraction of bioactive chemicals from plants is influenced by many aspects, for example the kind of differing polarity solvents, pH levels, duration of extraction, and extraction temperature, in addition to the chemical composition of the plant samples. According to López and Tangil (2011), the solvent and the chemical characteristics of the sample are considered the two most significant components when subjected to identical time and temperature circumstances. (López, Rico, Rivero, & de Tangil, 2011). Table 1 illustrates that the aqueous extract of *S. cumini* exhibited a higher concentration of phytochemical components. The leaves of *S. cumini* contain several phytochemicals, including carbohydrates, flavonoids, saponins, tannins, glycosides, phenols, proteins, triterpenoids, steroids, and fixed oils and fats. Nevertheless, the presence of alkaloids and anthraquinone was not detected in any of the leaf extracts. The findings of this study align with the research conducted by Ramos et al. (2017) and Rajkumar et al. (2021), which indicated that *S. cumini* leaves possess notable bioactive compounds that confer antioxidant properties. Furthermore, these compounds have been

shown to exhibit antibacterial, anticancer, anti-diabetic, and various other therapeutic effects (Rajkumar, Jayasinghe, & Vinotha, 2021; Ramos & Bandiola, 2017). The findings of the phytochemical screening conducted in this work on S. cumini are consistent with earlier research conducted in many nations (Aziz & Banerjee, 2018; Kamal, 2014; Murti et al., 2012; Prabakaran, Shanmugavel, & Research, 2017).

Determination of phytochemical screening of *S. cumini* leaves extract

Most phenolic acids are mostly found in ester form, attached to cell walls. They may also be present as esters and glycosides (Aliyu, Musa, Oshanimi, Ibrahim, & Oyewale, 2008). We investigated the contents of total phenolic acids, total flavonols, total flavonoids, and total tannins in the aqueous leaves extract of S. cumini L. (Figure 1). In terms of quantitative analysis, the S. cumini exhibited a significantly higher flavonoid content (194.77 μg RT /g) compared to the total phenolic acids (107.25 μg GAE/g) and tannin content (46.2 μg TAE/g). The analysis revealed that the overall concentration of flavonols was shown to be quite low, measuring at 32.6 μg RT /g. The findings presented align with the previously documented outcomes provided by (Prasad, Gurav, & Prasad; Tambe, Pedhekar, Harshali, & Development, 2021). Nevertheless, these observations also suggest that the effectiveness of extraction is contingent upon the polarity of the solvents. The present study has shown that the alterations in the composition of bioactive compounds to S. cumini extract have been substantiated. These changes have been found to be influenced by the chemical polarity qualities of the solvent, which in turn affect the qualitative structure and physicochemical activity of the extracts as confirmed in previous reports by (Gaweł-Bęben et al., 2015). Phenolic acids, flavonols, and flavonoids are known for their potent antioxidant properties and their ability to bind with proteins, therefore inhibiting the activity of the amylase enzyme. Hence, the regulation of glycemic index in food products and the participation in the cellular free radical defence mechanism, leading to the mitigation of excessive oxidative stress in human cells during unfavourable circumstances, have been observed (Al-Ishaq, Abotaleb, Kubatka, Kajo, & Büsselberg, 2019; Shahidi & Ambigaipalan, 2015; Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018) .Furthermore, Tannins are well recognised as anti-nutritional compounds. Nevertheless, several tannins have shown therapeutic attributes, including anti-carcinogenic and antibacterial effects, etc. (Chung et al., 1998).

Antioxidant Capacity of *S. cumini* leaves extract

The antioxidant properties of the plant extract were assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test, which measures the ability of antioxidants to scavenge free radicals. The results are shown in Figure 2. In a dose-dependent way, the percentage of antioxidant activity shown an increase across all dosages investigated. Ascorbic acid is often used as a prescription drug. The data are the mean of triple experiments and are reported as the mean ± standard error of the mean. The study revealed that the plant extract had significant scavenging action, as determined by DPPH tests, with an IC50 value of 13.54±1.1 μg/mL-1. The ascorbic acid used as the standard positive control has an IC50 value of 3.45±0.2 μg/mL-1. These findings may be attributed to the elevated levels of phenolic compounds and flavonoids present in the plant extract. The antioxidant activity of phytoconstituents is positively correlated with their antioxidant nature and abundance, notably in the case of phenolics. Phenolics are known to be produced in large numbers in response to the harsh environmental fluctuations in the plant's habitat (Ivan & Oprica, 2013; Rakhmankulova et al., 2015).

Characterization of AgNPs extract

UV-Vis spectroscopy

Synthesized AgNPs characterization was performed using aqueous extracts of *S. cumini* leaves was conducted using UV-Vis spectroscopy. Surface plasmon resonance (SPR) occurring at about 430 nm, together with the presence of color fluctuations in the reaction mixture, renders UV-Vis spectroscopy a widely used method for monitoring the synthesis of AgNPs (Figure 3A).

XRD pattern

Figure 3B depicts the XRD patterns obtained for the AgNPs created by a green synthesis method known as the cold technique. This approach involves the use of $AgNO_3$ and an extract derived from *S. cumini* leaves. The observed patterns demonstrate intensities and peaks that are characteristic of cubic-crystalline AgNPs. XRD pattern obtained from the reference sample, identified by the COD Card Number [96-901-3054], was compared to the XRD pattern that was fitted to the data. An additional peak is seen in the presence of AgO nanoparticles, namely

monoclinic Ag_3O_4–COD Card Number [96-151-0026]. The graphic shown depicts the discernible angles, crystal systems, and Miller's index. The solution underwent oxidation when being exposed to air for a certain period of time. The process of oxidation resulted in the development of heightened intensities in the aforementioned peaks, which displayed certain orientations that may be linked to the existence of nano silver oxide. According to a previous study done by (Vazquez-Muñoz et al., 2019).

FTIR spectra

The FTIR spectra of the dehydrated AgNPs were analyzed, and the corresponding findings are shown in Figure 3C. The peculiar absorption bands seen in the high-frequency region of 3500 to 3000 cm^{-1} may be attributed to the O-H stretching vibration and the presence of phenolic chemicals responsible for the reduction of Ag^{+} to Ag^{0}. The spectral band seen at a wavenumber of 1660 cm^{-1} is due to the stretching of –CH and C = C bonds in aromatic compounds. The presence of C–O–C resulting from the synthesis involving the use of *S. cumini* and $AgNO_3$ solution might potentially be associated with the emergence of new bands at 1070 cm^{-1} on the AgNPs. This observation is consistent with the findings reported by (Balavijayalakshmi, Ramalakshmi, & technology, 2017; Farahani, Hamdi, & Mirzaee, 2022). Additionally, a spectral peak corresponding to a beam originating from the group (-CH) was detected at a frequency of 635 cm^{-1}.

TEM

The present study included the examination of the AgNPs' topology using TEM, the results are shown in . The resulting AgNPs exhibited a spherical morphology and were found to aggregate, with an average size ranging from approximately 40 to 60 nm .The size of the biogenic silver nanoparticles seen in this research was similar to the dimensions reported in a recent investigation conducted by (Hawsawi et al., 2023; Masum et al., 2019). In their work, it was revealed that the utilization of *Phyllanthus emblica* fruit extract facilitated the synthesis of silver nanoparticles with an average diameter of 48.1 nm. Skandalis et al. (2017) used of freshly derived *Arbutus unedo* leaf extract has been employed for the synthesis of green silver nanoparticles, exhibiting comparable dimensions. The synthisised nanoparticles exhibitd a typical size range of 40 to 58 nm in terms of diameter (Skandalis et al., 2017).

Antimicrobial activity

The agar well test is a commonly used method in microbiology to evaluate the antimicrobial activity of various substances. The results of the preliminary antimicrobial screening indicate that the AgNPs (6 mg/disc) exhibited significant activity against all tested Gram-negative (*A. baumannii, K. pneumoniae* and *E. coli*) and Gram-positive (*S. aureus*, *S. haemolyticus*, and *E. feacalis*) bacteria, as shown by the observed inhibitory zone (Table 2 and Figure 4). A negative control was conducted by introducing 100 µl of untreated pure distilled water into a standard well and afterwards monitoring the absence or presence of inhibitory zones across various microbial strains. Hence, the efficacy of AgNPs remained unaltered when exposed to distilled water. Furthermore, significant antibacterial efficacy was shown against *S. aureus* (28 mm), *S. haemolyticus* (27 mm), and *E. faecalis* (27 mm), with *A. baumannii, K. pneumoniae* and *E. coli* exhibiting the greatest susceptibility among the tested bacterial strains, displaying the smallest inhibitory zone of 26 mm. In addition, it was observed that the standard antibiotic drug Levofloxacin (with a minimum inhibitory concentration of 5 µg mL^{-1}) exhibited an inhibition zone against various pathogens. The pathogens, namely *S. aureus* and *S. haemolyticus*, displayed the lowest inhibition zone of 13 mm each. The tested concentration of Levofloxacin demonstrated inhibition zones of 14 mm against *E. faecalis* and *K. pneumonia* each, Furthermore, *A. baumannii*, and *E. coli* demonstrated inhibition zones 14 and 15 mm of Levofloxacin, respectively. The obtained data reveals that the inhibition zone measurements indicate a higher level of antibacterial efficacy of AgNPs against Gram-positive bacteria compared to Gram-negative bacteria. The observed results can be ascribed to the morphological disparities among these bacteria. Specifically, Gram-negative bacteria possess an outer phospholipidic membrane that contains structural lipopolysaccharide components. This distinction in morphology may account for the varying sensitivity of these bacteria to antibacterial agents, as it renders the cell wall resistant to chemical interventions (A. A. Hamad et al., 2022; Mugweru et al., 2016; Nostro, Germano, D'angelo, Marino, & Cannatelli, 2000). The observed effects may be attributed to the sustained release of silver ions by silver nanoparticles, which might potentially serve as

a method for eradicating microorganisms (Qamer et al., 2021; Wright et al., 2022). Moreover, as a result of electrostatic attraction and a strong affinity for sulphur proteins, silver ions have the capability to stick to both the cytoplasmic membrane and the cell wall (S. Tang & Zheng, 2018). According to Santos et al. (2018), the integrity of the bacterial envelope may be compromised by the presence of linked ions, leading to an increase in permeability of the cytoplasmic membrane (Santos, Figueiredo, Azevedo, Braeckmans, & De Smedt, 2018). According to Hamad et al. (2020), the ingestion of free silver ions by cells may result in the inactivation of respiratory enzymes (A. Hamad, Khashan, Hadi, Polymers, & Materials, 2020). This, in turn, can lead to the reactive oxygen species generation and subsequent lack of adenosine triphosphate synthesis. (Singh, Garg, Pandit, Mokkapati, & Mijakovic, 2018) have shown that reactive oxygen species may facilitate the beginning of DNA alterations and cell membrane rupturing. According to Makvandi et al. (2020), the presence of silver ions may lead to complications in DNA replication, cell development, and eventually, the demise of microbial cells (Makvandi et al., 2020).This is attributed to the interaction between silver ions and sulphur and phosphorus, which are crucial components of DNA. According to Sharma et al. (2021), the denaturation of ribosomes in the cytoplasm by silver ions may also inhibit protein production (D. Sharma, Gulati, Sharma, & Chaudhary, 2022). The release of silver is more likely to occur in smaller AgNPs with a spherical shape, as seen in the present work, due to their larger surface area. The nanoscale dimensions of AgNPs facilitate their ability to traverse bacterial cell walls and induce modifications to the cellular membrane structure. The denaturation of the cytoplasmic membrane may lead to the rupture of organelles and the probable lysis of cells (Uddin, Parimi, Bollu, Bhatt, & Suresh, 2022).

Anti-inflammatory activity of AgNPs through HRBC method *in vitro*

Inflammation refers to the physiological response of tissue or organ damage, characterized by the manifestation of clinical discomfort, swelling, increased temperature, and redness. Hence, it is linked to the process of membrane lipid peroxidation and subsequent development of various clinical states. Hence, the control of lysosomal membrane impairment is the pivotal factor in mitigating the inflammatory response. Numerous plant-derived chemicals, together with their secondary metabolites, have been shown to facilitate the synthesis of nanoparticles, hence augmenting cellular contacts and promoting the stabilization of cell membranes. The present investigation demonstrates that the AgNPs display dose-dependent human red blood cell (HRBC) membrane stabilization, as well as inhibition of hemolysis, as shown in Figure 5. The anti-inflammatory ability of AgNPs was seen to range from 77.6 ± 1.1% to 95.2 ± 1.4% when the hypertonic solution was exposed to HRBC samples. These results are closed to those mentioned by (David et al., 2014).

CONCLUSION

The co-sedimentation approach was used to enhance the efficiency of AgNPs by using *S. cumini* extract from leaves to create silver nanomaterials. The UV-Visible analysis of AgNPs exhibited surface plasmon resonance at a wavelength of 430 nm. FTIR and XRD analyses revealed distinct peaks and intensities in the obtained patterns, which are indicative of the presence of AgNPs with cubic-crystalline. The dimensions of the AgNPs were around ~55 nm. Additionally, TEM analysis revealed that the synthesized AgNPs had a high degree of uniformity in terms of their surface morphology, exhibiting a spherical shape and consistent size. The AgNP had a dose-dependent effect on HRBC membrane stabilization, resulting in a range of inhibition of hemolysis from 77.6 ± 1.1% to 95.2 ± 1.4%. Additionally, the AgNPs exhibited antibacterial ability against both Gram-positive and Gram-negative pathogens that were selected for this study. In general, the use of biosynthesized AgNPs is being explored as a promising substitute for steroids and other immunosuppressant medications in the treatment of inflammation.

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Table 1: The preliminary phytochemical screening of *S. cumini* L. leaves by aqueous extract.

	Constituents	Tests	*S. cumini* (L.) Skeels aqueous extract
1	Carbohydrates	Molisch,s test	+
2	Phenol	Ferric chloride test	+
3	Tannins	Ferric chloride test	+
4	Flavonoids	Lead acetate test	+
		$AlCL_3$ test	+
5	Saponins	Froth test	+
6	Glycosides	Glycosides test	+
		Conc. H_2SO_4 test	+
7	Alkaloids	Dragendroff's test	-
		Wagner,s test	-
		Hager,s test	-
8	Anthraquinone	Borntrager,s Test	-
9	Sterols and terpenes	Salkawskis test	+
10		Legal,s test	+
	Cardiac glycosides	Keller Killini test	+

(+) mean present, (-) mean absent.

Table 2: Diameter of inhibition zones of AgNPs on various microbial strains

No.	Isolate Name	Antibacterial activity (mm)		
		AgNPs	+ve	-ve
1	*Staphylococcus aureus*	28	13	0
2	*Staphylococcus haemolyticus*	27	13	0
3	*Enterococcus faecalis*	27	12	0
4	*Acinetobacter baumannii*	26	14	0
5	*Klebsiella pneumoniae*	26	14	0
6	*Escherichia coli*	26	15	0

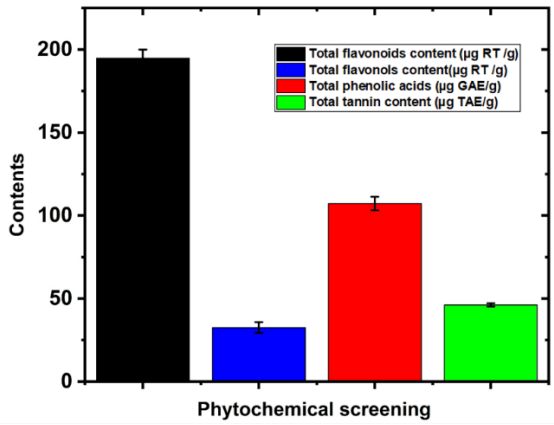

Figure 1: Contents of major bioactive compounds (total flavonoids content, total flavonols content, total phenolic acids content and total tannin content in aqueous leaves extract of *S. cumini* L

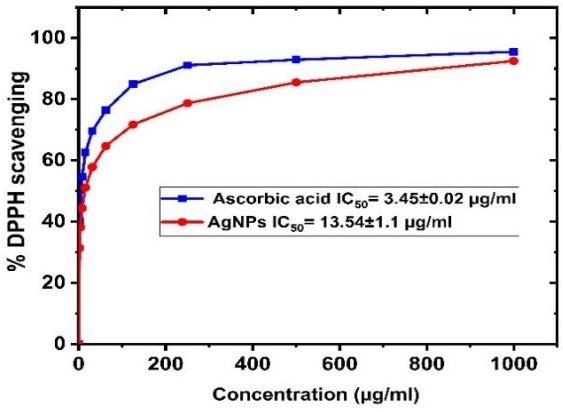

Figure 2: Antioxidant activities of *S. cumini* leaves extract using DPPH assay. Data are presented as Mean ± SE (*n*=3).

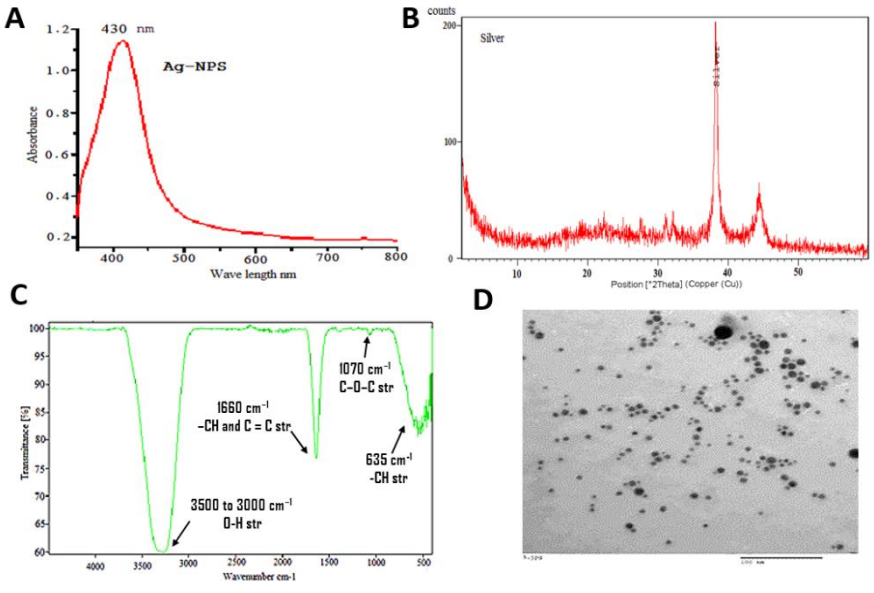

Figure 3: (A) The UV-Vis spectroscopy (B) XRD pattern. (C) FTIR spectra, and (D) the morphological surface as evaluated by TEM (scale bar a length of 100 nm) of AgNPs sample using a green synthesis process at ambient temperature.

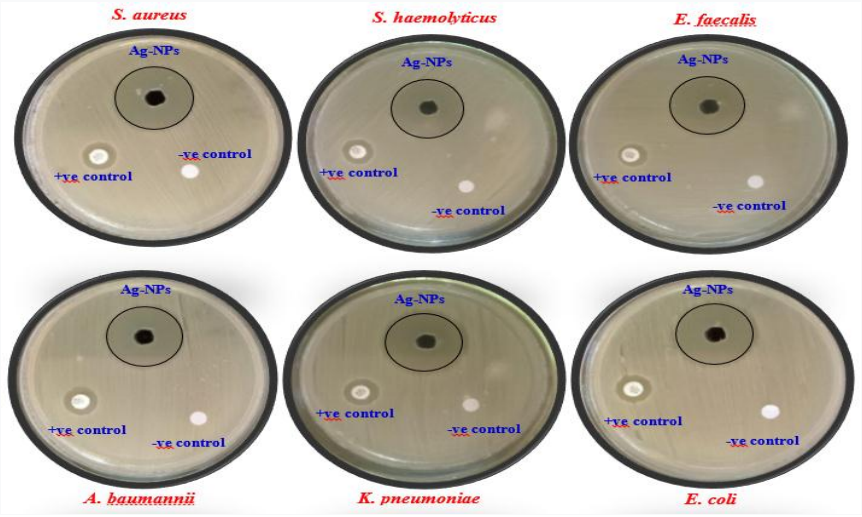

Figure 4: Antimicrobial activity of AgNPs against isolated bacteria.

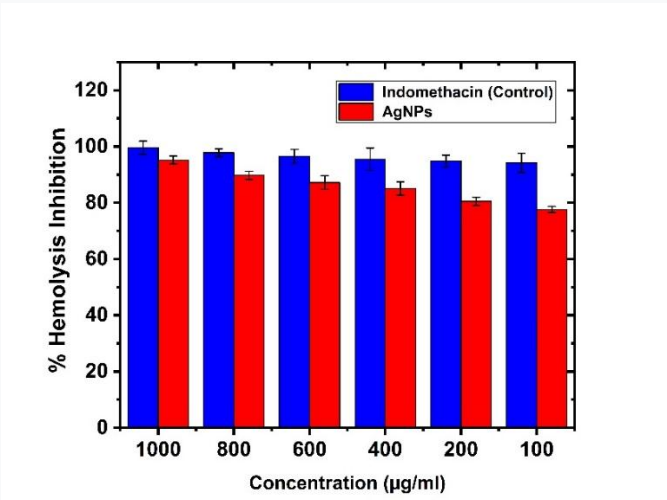

Figure 5: Effect of Stander Indomethacin and AgNPs on HRBC hemolytic and membrane stabilization

التصنيع الحيوي لجسيمات الفضة النانوية (AgNPs) باستخدام مستخلص أوراق نبات البامبوزيا الغني بمضادات الأكسدة مع تقييم نشاطه المضاد للبكتيريا والمضاد للالتهابات

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الملخص العربي

شمل هذا العمل التوليف الأخضر لجسيمات الفضة النانوية باستخدام المستخلص المائي لأوراق البامبوزيا تم تحليل المستخلص المائي نوعيا وكميا للمحتوى الكيميائي النباتي. تم فحص السمات المورفولوجية والخصائص البصرية والخصائص السطحية لجزيئات الفضة المُصنّعة باستخدام المجهر الإلكتروني النافذ ، حيود الأشعة السينية ، التحليل الطيفي لامتصاص الأشعة فوق البنفسجية والمرئية ، ومطياف تحويل فورييه للأشعة تحت الحمراء. أظهر التحليل أن تراكيز مركبات الفلافونويد والعفص والفينولات والفلافونول كانت 194.77±0.55 ميكروجرام روتين /غم وزن جاف ((DW، 0.53±46.02 ميكروجرام حمض التانيك /غم DW، 0.5±107.25.92 ميكروجرام حمض الغاليك /جم DW و 32.6 ± 0.67 ميكروجرام روتين / جم DW على التوالي. أظهر المستخلص النباتي نشاطًا مضادًا للأكسدة، بقيمة IC50 تبلغ 13.54 ± 1.1 ميكروجرام/مل-1، ويعزى ذلك إلى محتواه العالي من المركبات الفينولية والفلافونويدات، مما يشير إلى إمكاناته كمصدر طبيعي لمضادات الأكسدة. أظهرت دراسة المجهر الإلكتروني النافذ أن جزيئات الفضة النانوية أظهرت مستوى ملحوظًا من التجانس فيما يتعلق بتشكل سطحها. كان متوسط قطر الجزيئات حوالي 55 نانوميتر. أظهرت جزيئات الفضة النانوية الخضراء المركبة نشاطًا مضادًا للبكتيريا ملحوظًا ضد العديد من الأنواع البكتيرية. تراوح قطر مناطق التثبيط من 26 إلى 28 ملم ضد ستة عزلات بكتيرية مختلفة وهي S. aureus، S. haemolyticus، E. faecalis، A. baumannii، K.pneumoniae، و E. coli. كان لجزيئات الفضة النانوية AgNPs تأثير يعتمد على الجرعة على استقرار غشاء خلايا الدم الحمراء البشرية. وأدى ذلك إلى درجة متفاوتة من الوقاية من انحلال الدم، تتراوح من 77.6 ± 1.1% إلى 95.2 ± 1.4%. يقدم العمل الحالي نهجًا مباشرًا ومجديًا اقتصاديًا لتجميع جزيئات الفضة النانوية باستخدام عمليات صديقة للبيئة، بهدف استخدامها كعوامل مضادة للبكتيريا.

الكلمات الاسترشادية: البامبوزيا, جسيمات الفضة النانونية, مضادات الاكسدة, مضاد للالتهابات, مضاد للبكتيريا, انحلال الدم.