

Green Synthesis, Characterization And Antimicrobial Activity Of Silver Nanoparticles From Fruit Extract Of *Pouteria Campechiana*

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Abstract

The green synthesis of silver nanoparticles (AgNPs) using biological methods has garnered attention for its cost-effectiveness, non-toxicity, and eco-friendliness compared to traditional physical and chemical approaches. In this study, AgNPs were produced utilizing an aqueous extract from *Pouteria campechiana* fruit, with their formation confirmed through UV-visible spectroscopy. The nanoparticles were characterized by their spherical morphology. Both the *P. campechiana* fruit extract and the biosynthesized AgNPs present significant benefits over conventional chemical and physical synthesis methods, demonstrating potent antioxidant activities in various in-vitro assays. These findings highlight the efficacy of green synthesis for producing antioxidant-rich silver nanoparticles.

1. Introduction

Nanoparticles, characterized by their size of less than 100 nanometers (ranging from 1 nm to 100 nm), exhibit unique properties due to their small scale and the substantial proportion of atoms on their surface. These properties differ significantly from those of larger-scale materials. Metal nanoparticles, in particular, have been identified as key agents in combating fungal diseases in both humans and plants. Nanotechnology is a relatively recent field of science that has a diverse range of applications, including energy generation, industrial processes, and medical advancements. The concept of "nanotechnology" was first introduced in a 1974 paper by Professor Norio Taniguchi of Tokyo Science University, who described it as the precise manipulation, separation, and transformation of materials at the atomic or molecular level.

This field is rapidly growing, focusing on the creation and utilization of nanomaterials and nanoparticles across various domains such as medicine, biotechnology, and energy efficiency. Nanotechnology offers significant potential benefits, including antioxidant and antimicrobial properties that could be used to address degenerative diseases. The synthesis of nanoparticles typically involves physical and chemical techniques like lithography, ultrasonic fields, and UV irradiation, which can produce new properties depending on the size and distribution of the particles. However, some synthesis methods use non-biodegradable toxic chemicals as reducing agents, which can pose risks to both the environment and biological systems [1].

The production of nanoparticles through microorganisms often demands significant time investment for microorganism maintenance. In contrast, synthesizing nanoparticles via plant extracts is straightforward, convenient, cost-effective, and environmentally friendly [2]. Plant extracts contain various compounds and biochemicals that can serve as stabilizing and reducing agents for green nanoparticle synthesis. Silver nanoparticles, in particular, play a crucial role in antimicrobial, catalytic, and biological systems. Their significance is heightened due to the growing concern over antibiotic-resistant microbes. Although physical and chemical methods for synthesizing silver nanoparticles with specific sizes and shapes have been explored for their antimicrobial properties, their application in the biomedical field is limited due to potential toxicity.

The ratio of surface area to volume for silver nanoparticles decreases as their size increases. This leads to a rise in biological effectiveness with a greater specific surface area, owing to enhanced surface energy and catalytic activity. Numerous methods exist for nanoparticle synthesis, yet the biological approach is crucial for producing high-yield, cost-effective, and environmentally friendly nanoparticles. Gold and silver nanoparticles (AgNPs) have found extensive applications in biology, chemistry, and physics because of their unique optical, mechanical, and electronic properties. Silver, in particular, is significant in bio-nanoparticle synthesis due to its antimicrobial and antifungal properties, which help combat the growing threat of antibiotic-resistant microbes.

Already extracted nanoparticles in some plants, leaves, fruits and microorganisms are, Plants: banana peel, *cucurbita pepo* and *Malvacrispa* [3], Leaves: *Acalypha indica* [4], *Zingiber officinale* [5], *Nepenthes khasiana* [6]. Fruits: *Syzygium cumini* [7], *Punick granutum* [8], *Syzygium aromaticum* [9]. Microorganism: Malaria vector: *Anopheles stephenis* [10], Dengue vector:- *Aedes, aegypti* [11].

This study aims to synthesize silver nanoparticles (AgNPs) using an eco-friendly approach involving the aqueous fruit extract of *Pouteria campechiana* as a reducing agent to convert Ag⁺ ions to AgNPs. The resulting AgNPs were characterized using UV-Visible spectrophotometry and scanning electron microscopy. Additionally, their antioxidant,

antimicrobial, and cytotoxic properties were assessed in vitro. Plant-based green chemistry methods, which bypass the complex procedures of maintaining microbial cultures, are preferred over other environmentally friendly biological methods such as microbial techniques. The chemically stable green silver nanoparticles have shown significant potential in catalytic, antibacterial, antifungal, anticancer, and biomedical applications, with AgNPs demonstrating high potential in cancer treatment.

AgNPs have significant and diverse applications [12]. Historically, silver's disinfecting properties have been utilized in areas ranging from traditional medicine to culinary tools. Research indicates that AgNPs are non-toxic to humans and highly effective against bacteria, viruses, and other eukaryotic microorganisms, even at low concentrations, without causing any side effects [13, 14]. While small amounts of silver are safe for human cells, they are deadly to microorganisms. The antimicrobial properties of AgNPs make them suitable for various household uses, such as disinfecting textiles, treating water, storing food, in home appliances, and in medical devices [15]. In the medical field, the primary application of silver and AgNPs is in topical ointments to prevent infections in burns and open wounds [16]. Currently, the biological synthesis of nanoparticles using plant extracts is being explored by researchers, who are also testing their antimicrobial properties [17].

AgNPs also possess significant applications. Historically recognized for their disinfecting properties, silver has been used in traditional medicines and culinary items. AgNPs exhibit potent antimicrobial activity against bacteria, viruses, and other microorganisms at low concentrations without adverse effects on human cells. This characteristic makes them suitable for applications in textiles disinfection, water treatment, food storage containers, home appliances, and medical devices. Notably, silver and AgNPs are utilized in medical industry applications such as topical ointments for preventing infections in burns and wounds [16].

Different approaches are used to synthesize silver nanoparticles, including reverse micelles [18], thermal decomposition of silver compounds [19, 20], radiation methods [21], electrochemical techniques [22], chemical synthesis [23], and microwave-assisted methods [24]. However, chemical synthesis can sometimes leave harmful residues on nanoparticles, which might negatively impact their medical use [25]. Currently, silver-based topical dressings are extensively used for treating infections in wounds [16] and chronic ulcers [26], and for preventing oxidation and discoloration of materials. Overall, nanotechnology holds the promise of transformative advancements across a wide range of fields such as information technology, energy, environmental science, healthcare, national security, food safety, and transportation [27-29].

Pouteria campechiana, commonly known as the canistel or egg fruit, is an evergreen tree found in various countries including India, Costa Rica, Brazil, the United States, Australia, Indonesia, and Sri Lanka [30, 31]. The tree bears orange-yellow fruits known as yellow sapotes, which are edible. Canistel trees can reach heights of up to 10 meters, and the size and shape of the fruit can vary depending on the cultivar. Each fruit weighs approximately up to 400 grams and contains one to six large brown seeds. The canistel fruit, scientifically known as *Pouteria campechiana*, is prized for its versatility and nutritional benefits. Its flesh, resembling a hard-boiled egg yolk in texture [32], can be enjoyed fresh or processed into various culinary delights such as jams, pancakes, and flour. Blended with milk and other ingredients, the ripe pulp makes delicious shakes and enriches custards and ice creams [33]. Rich in carbohydrates, amino acids, carotenoids, polyphenolics, flavonoids, and Vitamins B and C, canistel has a long history of use in traditional medicine [34]. The bark decoction serves as an antipyretic and aids in treating skin ailments, while unripe fruits help manage diarrhea. Canistel's leaves boast anti-inflammatory properties, and its seeds are employed in ulcer remedies. Modern research highlights its broad pharmacological benefits, including antimicrobial, anti-obesity, anti-inflammatory, and antioxidant activities [35]. Originating from the Mexican town of Campeche [36], this fruit is known by various names worldwide, including chesa or tiessa in the Philippines [37], Lauulu or Lawalu in Sri Lanka, and Lamut Khamen in Thailand [34].

The research focused on employing an eco-friendly approach to synthesize silver nanoparticles, using the aqueous fruit extract of *Pouteria campechiana* as a natural reducing agent to convert Ag⁺ ions into silver nanoparticles. The characterization of the synthesized nanoparticles was carried out using a UV-Visible spectrophotometer and a scanning electron microscope. Additionally, the study evaluated the in-vitro antioxidant, antimicrobial, and cytotoxic activities of these green-synthesized silver nanoparticles.

2. Materials and Methods

2.1 Materials

P. campechiana fruit were collected and basic plant identification was done. Silver nitrate used for the synthesis of silver nanoparticles *P.campechiana* (Egg fruit) extract was used. The cultures of bacteria were used for antimicrobial activity. For the study of characterization of nanoparticles UV-vis spectral, SEM (Scanning Electron Microscope) was used for the analysis of synthesized nanoparticles.

2.2 Preparation of fruit sample

The *P. campechiana* fruit was first rinsed with distilled water before its outermost layer was removed. Subsequently, the fruit was cut into small, fine pieces and dried in a hot air oven at 80°C for 48 hours. After 18 hours, the fruit pieces were

taken out and ground into powder using a pestle and mortar. 10g of the powdered fruit was then combined with 100 mL of double distilled water and boiled for about 20 minutes on a hot plate while being stirred continuously. The boiled mixture was then filtered using Whatman No. 1 filter paper, and the resulting filtrate was stored in a refrigerator for future use [38].



Figure 1. Preparation of *P. campechiana* fruit sample; (a) Digital image of the whole *P. campechiana* fruit. (b) The fruit cut pieces dried in a hot air oven at 80°C for 48 hours. (c) The dried fruit pieces ground into powder using a pestle and mortar.

2.3 Biogenic synthesis of AgNPs

20 mL of a 1% (w/v) aqueous fruit extract was combined with 20 mL of a 1 mM AgNO₃ solution. The mixture was placed in a rotor with magnetic beads, and a noticeable color change occurred. Over 48 hours, the solution darkened and eventually turned dark brown. The nanoparticles were then separated by centrifuging the mixture at 15,000 rpm for 20 minutes. The resulting loose pellet of nanoparticles at the bottom of the centrifuge tube was resuspended in a small amount of deionized water and centrifuged again at 15,000 rpm for 5 minutes. Alcohol was added to the dispersion, and the mixture was placed in a hot air oven for evaporation. After the evaporation process, crystals were obtained.

2.4 Phyto-chemical screening

A chemical analysis was conducted on the extract of the chosen plant using established methods to determine its constituents. The aqueous fruit extract underwent various qualitative preliminary tests to identify the phytochemicals present [39].

2.4.1 Test for Tannins

A Ferric chloride test is conducted to detect the presence of tannins. About 0.5 g each of the dried powdered plant samples were boiled in 20 ml of water and then filtrate through Whatman filter paper no 42. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black colouration [40].

Experiment	Observation	Inference
0.5g of dried fruit sample + 20ml of water, the mixture boiled and filtered + 0.1% ferric chloride.	Brownish green colour was observed	Tannin is present

2.4.2 Test for Flavonoids

To assess the presence of flavonoids in the fruit sample, an ammonia test was conducted. A portion of the aqueous filtrate from the fruit extract was mixed with 5 ml of dilute ammonia solution, followed by the addition of 1 ml of concentrated H₂SO₄. The appearance of a yellow color that faded upon standing suggested the presence of flavonoids.

Experiment	Observation	Inference
Aqueous filtrate of fruit extract + 5 mL of dilute ammonia solution + 1 mL of Conc. H ₂ SO ₄	Yellow colour disappeared	Flavonoid is present

2.4.3 Test for Steroids:

2 mL of acetic anhydride was mixed with 0.5 g of ethanolic extract from each sample, followed by the addition of 2 mL of H_2SO_4 . A color change from violet to blue or green in certain samples indicated the presence of steroids [41].

Experiment	Observation	Inference
2 mL of acetic anhydride + 0.5g ethanolic extract of sample with 2 mL H_2SO_4 .	Colour altered from violet to blue.	Steroid is present

2.4.4 Test for Cardiac Glycosides (keller-killani test)

To test for deoxy sugars, indicative of cardenolides, 5 mL of the extract was combined with 2 mL of glacial acetic acid containing a drop of ferric chloride solution. The mixture was then gently layered with 1 mL of concentrated sulfuric acid. A brown ring at the interface between the two liquids suggests the presence of deoxy sugars. Additionally, a violet ring may form below the brown ring, and a greenish tint may appear throughout the acetic acid layer [42].

Experiment	Observation	Inference
5ml fruit extract + 2mL glacial acetic acid + drop of ferric chloride solution + 1 mL H_2SO_4	No brown ring formed	Glycoside is absent

2.4.5 Test for Terpenoids (salkowski test)

To test for terpenoids, 5 mL of the extract was combined with 2 mL of chloroform. Concentrated sulfuric acid (3 mL) was then gently added to create a distinct layer. The formation of a reddish-brown color at the interface indicated a positive result for the presence of terpenoids [43].

Experiment	Observation	Inference
5ml fruit extract + 2 mL chloroform + 3 mL con. H_2SO_4	Reddish brown colour formed	Terpenoid is present

2.4.6 Test for Saponin

About 2 grams of the powdered sample were heated with 20 mL of distilled water in a water bath, then filtered. Subsequently, 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously, resulting in a stable, lasting froth. To this frothy solution, 3 drops of olive oil were added, and after vigorous shaking, an emulsion formed [44].

Experiment	Observation	Inference
10 ml aqueous filtrate of fruit extract + 5 mL distilled water shake well, froth is mixed with 3 drop of olive oil	Emulsion formed	Saponin is present

2.5 Synthesis and separation of AgNPs

For the synthesis of silver nanoparticles 20 mL of fruit extract was added to the 20 mL of AgNO_3 solution. Then the mixture was kept in magnetic stirrer for 48 hours the colour was changed into light brown colour at first 24 hours. After 48hours the solution was fully turned into dark brown colour this way the distinct colour was observed. Then the solution was centrifuged at 1500 rpm for 20 min then the pellet was collected at the bottom of the centrifugation tube. The pellets were collected by using acetone alcohol followed by drying in a watch glass and the nanoparticles were then stored at -4°C for further use.

2.6 Characterization of AgNPs

2.6.1 Scanning electron microscopy-energy dispersive X-ray spectrometer (SEM-EDX)

Silver particles were initially centrifuged at 10,000 rpm for 30 minutes. The resulting pellet was re-suspended in 10 mL of ethanol and then subjected to three washes with sterile distilled water. After washing, the pellet was dried in an oven. Thin films were prepared by dispersing the dried sample at a concentration of 10 mg/mL and applying it to carbon-coated copper grids for analysis. The scanning electron microscopy (SEM) was conducted using the Zeiss EVO 18-EDX special edition, which is compatible with EDX systems. Image magnification software, tailored for SEM, was utilized to examine the particle size and texture of the nanoparticles, confirming the successful formation of silver nanoparticles [38].

2.6.2 UV-Visible spectra analysis of nanoparticles

The process of reducing pure silver ions was monitored by analysing the UV-Visible spectrum of the reaction at various time points [45]. For this analysis, 1 mL of distilled water was used as a blank, and 1 mL of the reaction mixture was analysed. The spectrophotometer was set to a resolution of 1 nm, covering the wavelength range from 400 to 650 nm.

2.6.3 X-Ray diffraction (XRD)

The silver nanoparticle sample will be analysed using an X-Pert Pro X-Ray diffractometer. The device will be set to a voltage of 40 kV and a current of 30 mA, utilizing Cu K α radiation. Measurements will be taken over a range of 200-800.

2.7 Antibacterial assay

Materials: Nutrient agar media, nutrient broth, biosynthesized Ag nanoparticles

Method: The antibacterial effectiveness of AgNPs was assessed using the Kirby-Bauer disc diffusion method [46]. For bacterial cultivation, nutrient broth/agar was prepared with 1g of beef extract, 1g of peptone, and 0.5g of NaCl dissolved in 100 mL of double-distilled water. The nutrient agar plates were seeded with cultures of *Streptococcus pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Xanthomonas campestris*, and *Bacillus subtilis* and incubated overnight at 36°C. Sterile 5mm Whatman filter discs were impregnated with varying concentrations of AgNPs (15 μ g, 25 μ g, 50 μ g, 75 μ g, and 100 μ g) and placed on the bacterial lawns on the agar plates. After incubation at 37°C for 24 hours, the zones of inhibition were measured.

3. Result and Discussion

Silver ions in aqueous solution were reduced to AgNPs upon the addition of egg fruit extract and subsequent incubation. Initially, the mixture displayed a yellow color for up to 24 hours (as shown in Figure 8). After 48 hours, it transformed to a dark brown (as depicted in Figure 9). This color change was attributed to the surface plasmon resonance of the formed AgNPs. In contrast, control experiments that did not include fruit extract in the silver nitrate solution showed no color change, even after one week.

3.1 Phytochemical test

Different chemical tests were carried on extract of selected plant using standard procedure to identify the constituents or the aqueous fruit extract was subjected to various qualitative preliminary experiments in order to identify the phyto-constituents present in fruit extract as reported under the section 2.4. The presence and absence of various phytochemicals are presented in the Table 1.

Sl No.	Experiment	Result
1	Tannins	Present
2	Flavonoids	Present
3	Steroids	Present
4	Terpenoides	Present
5	cardiac glycosides	Absent
6	Saponin	Present

Table 1: Various phytochemicals present in the aqueous fruit extract of *P. Campechiana*.

3.2 UV-Visible analysis

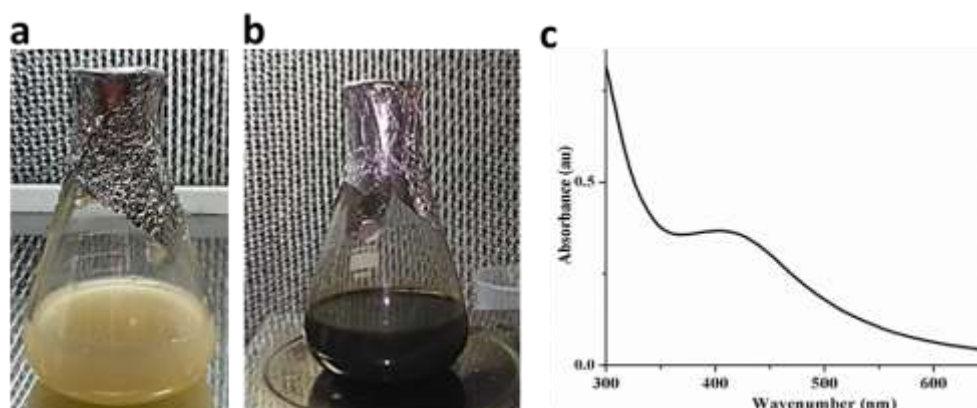


Figure 2. Aqueous fruit extract of *P. Campechia* and AgNO_3 mixture initial (a) and after 24 hours (b). (c) UV-Visible spectra of AgNPs produced from *P. campechiana* fruit extract.

AgNPs exhibit a yellowish-brown color in aqueous solutions due to surface plasmon resonance. As various fruit extracts from *Pouteria campechiana* were introduced into an aqueous silver nitrate solution, the color evolved from a pale yellow (Figure 2a) to yellowish-brown, then to reddish-brown (Figure 2b), and ultimately to a colloidal brown, indicating the formation of AgNPs. The UV-vis spectra, recorded 24 hours after the reaction commenced, is depicted in (Figure 2c). The spectra reveal that the absorption maxima of the AgNPs, which are in the range of 400 to 460 nm, are due to surface plasmon resonance, with a notable peak observed at 420 nm.

3.3 SEM analysis

The SEM images depicted in Figures 3 reveal that varying fruit extracts led to the formation of AgNPs with different shapes. Specifically, extracts from *P. campechiana* produced AgNPs that were mostly spherical and oval. This variation in nanoparticle morphology is likely attributed to the differing amounts and types of capping agents present in the various fruit extracts. This observation is further corroborated by the shifts and changes in peak areas observed in the FTIR analysis.

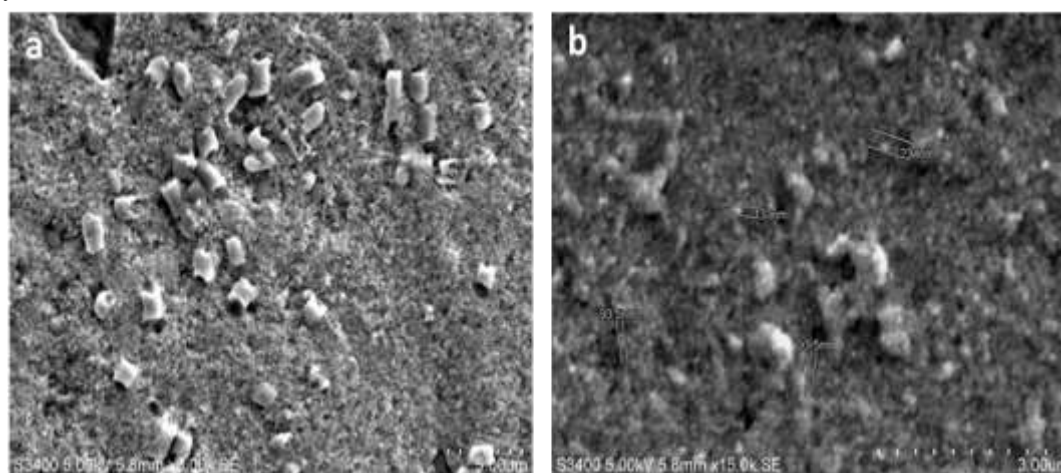
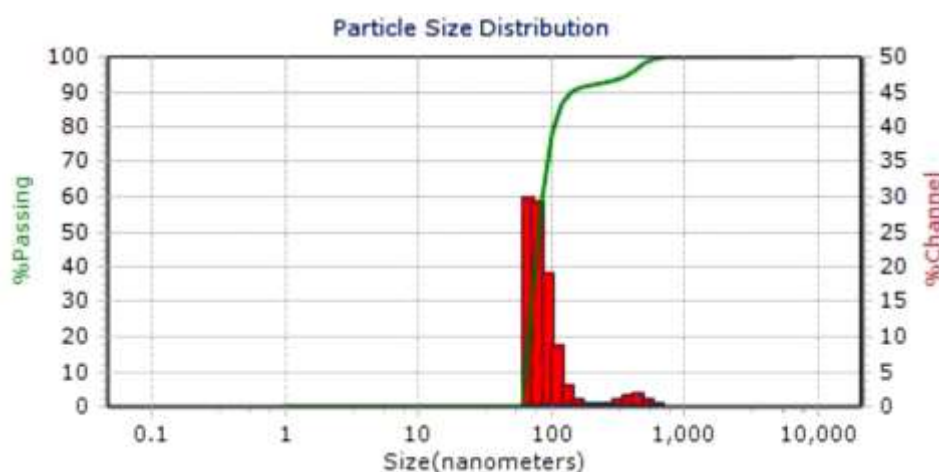


Figure 3. SEM images (a and b) of AgNPs obtained from *Pouteria campechiana* extract.

3.4 DLS Analysis

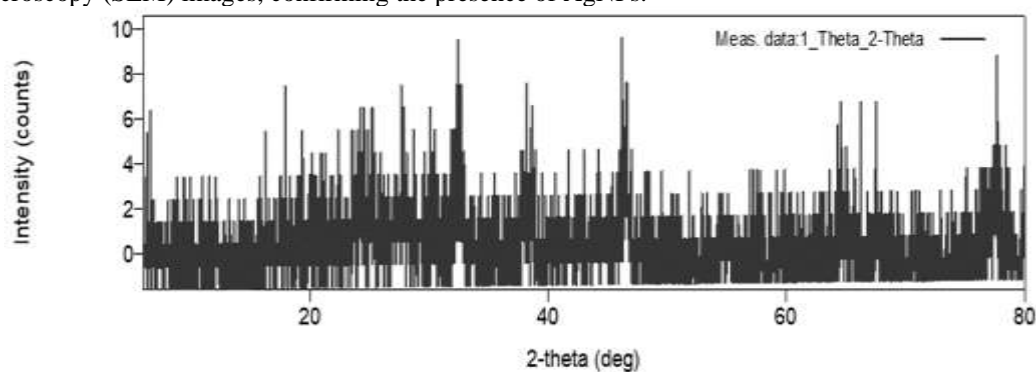
The data obtained in the DLS particle size analysis indicated that the silver nanoparticles obtained from *P. campechiana* extract were in the range of 96-578 nm (Figure 4). Whereas stability of AgNPs obtained from *Pouteria campechiana* extracts were determined by zeta potential measurement. Zeta potential value -5.1mv for *P. campechiana* AgNPs was obtained (Table 2). This suggested that the surface of the nanoparticles was negatively charged that dispersed in the medium.

Zeta potential analysis of <i>Pouteria campechiana</i> AgNPs	
Mobility	-0.40u/ s/ V/ cm
Zeta potential	-5.1mv
Charge	-0.02357 fC
Polarity	Negative
Conductivity	96 uS/ cm

Table 2: Zeta potential analysis of *P. campechiana* AgNPs.**Figure 4.** Particle size distribution by dynamic light scattering from *P. campechiana* AgNPs.

3.5 XRD Analysis

The X-ray diffraction (XRD) technique was employed to analyse the phase of the synthesized *P. campechiana* AgNPs, with the result illustrated in Figure 5. The XRD pattern revealed distinct peaks corresponding to the cubic phase of silver (JCPDS No. 3-921). This XRD data strongly supports the findings from UV-visible spectroscopy and scanning electron microscopy (SEM) images, confirming the presence of AgNPs.

**Figure 5.** XRD spectra of AgNPs synthesized from fruit *P.campechiana* aqueous extract.

3.6 EDS analysis of nanoparticles

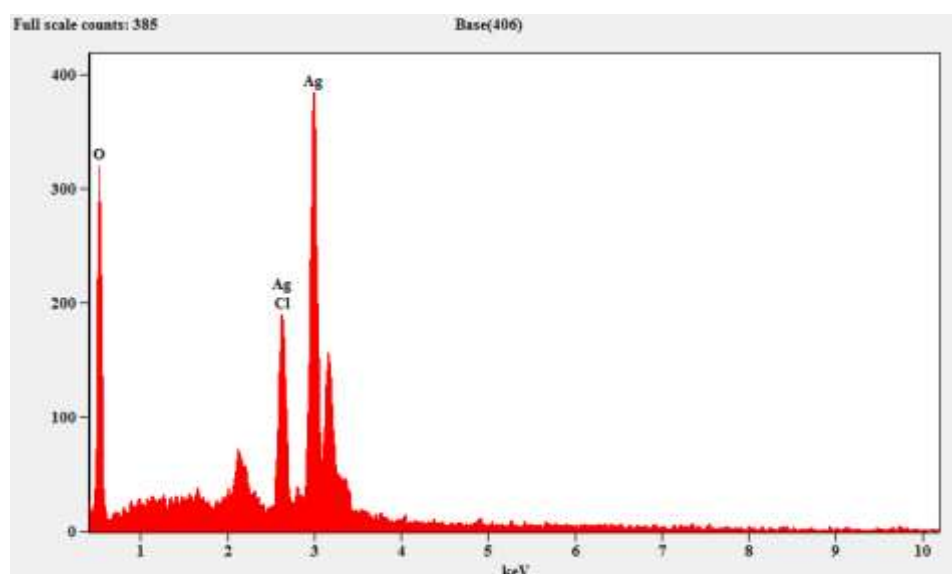


Figure 6. EDS spectra of the AgNPs synthesized from fruit *P.campechiana* aqueous extract.

Figure 6 displays the EDS spectra for the AgNPs powder derived from *P. campechiana* fruit extract. The prominent peaks observed in the EDS spectra reveal a significant concentration of silver element in the nanoparticles, indicating the absence of contamination. The pronounced optical absorption peak at 3 keV can be attributed to the surface plasmon resonance effect associated with the AgNPs nanocrystalline structure.

3.7 Antibacterial assay

AgNPs synthesized from fruit *P.campechiana* aqueous extract were fairly toxic to *Salmonella typhi*, *Streptococcus pneumoniae*, *Xanthomonas campestris*, *Pseudomonas auriginosa* and *Bacillus subtilis*. Zone of inhibition around silver nanoparticle impregnated disc for individual bacterial culture are shown in below table.

Sample	Diameter of inhibition zone(mm)				
	<i>Salmonella typhi</i> ,	<i>Streptococcus pneumoniae</i>	<i>Xanthomonas campestris</i> ,	<i>Pseudomonas auriginosa</i>	<i>Bacillus subtilis</i> .
Distilled Water	0	0	0	0	0
Tetracycline	22	20	21	20	19
Green silver nano particle	8	6	10	11	12

Antibacterial assay of AgNPs synthesized from fruit *P.campechiana* aqueous extract.

Conclusion

This study explores the synthesis, characterization, and antimicrobial properties of AgNPs produced using *Pouteria campechiana* fruit extract. This eco-friendly biogenic method is non-toxic, simple, cost-effective, and free of harmful chemicals. The results indicate that the fruit extract effectively facilitates the reduction and stabilization of silver nanoparticles. After incubating the mixture for 72 hours, a brown color developed. UV-Visible spectroscopy revealed an absorption peak at 200 nm. The nanoparticles exhibited sizes ranging from 96 to 578 nm. Characterization of the biosynthesized AgNPs was performed using SEM, DLS, EDS, and XRD techniques. From a technical perspective, the biogenic AgNPs demonstrated significant antibacterial and antioxidant activities, suggesting their potential utility in various applications, including pharmaceuticals, biomedical fields, and industrial uses such as bandages and food and water storage. Future research should focus on optimizing models to enhance these applications.

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