

## Green Synthesis of Silver Nanoparticles using *Acacia catechu* Bark Extract: Structural Characterization, Antioxidant and Antibacterial Activities and Cytotoxicity Against HT-29 Colorectal Cancer Cells

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Plant-mediated synthesis offers an alternative approach to conventional chemical methods for nanoparticle preparation. This study reports the green synthesis of silver nanoparticles (Ag NPs) using aqueous *Acacia catechu* bark extract and evaluated their structural characteristics and biological activities. The synthesized material was characterized by X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HR-TEM), selected-area electron diffraction (SAED), and UV-visible spectroscopy. XRD peaks at approximately  $2\theta = 38^\circ, 44^\circ, 64^\circ$  and  $77^\circ$  corresponded to the (111), (200), (220) and (311) planes of face-centred cubic silver. FT-IR spectra identified hydroxyl, carbonyl, aromatic and C–O-containing functional groups that may participate in silver ion reduction and nanoparticle stabilization. Electron microscopy revealed predominantly spherical nanoscale particles with some aggregation, while UV–visible spectroscopy exhibited a characteristic absorption band at 420–460 nm. Hydroxyl radical-scavenging activity increased with concentration, and the Ag NPs formulation exhibited higher activity than the bark extract and  $\text{AgNO}_3$ , reaching approximately 73% inhibition at the highest tested concentration. Disc diffusion assays recorded antibacterial inhibition zones of 11–15 mm, with the largest zone against *Pseudomonas aeruginosa*. Cytotoxicity testing against HT-29 colorectal adenocarcinoma cells revealed concentration-dependent inhibition, with higher activity for the silver-containing formulation than the bark extract alone.

**Keywords:** Silver nanoparticles, Green synthesis, Antibacterial activity, *Acacia catechu* (L.f.).

### INTRODUCTION

Silver has long been used in medicine for its antimicrobial properties and is one of the most widely investigated materials for nanoparticle production [1]. Biodegradable starches have also been employed as reducing and stabilizing agents in the silver nanoparticles (Ag NPs) synthesis. Consequently, research has increasingly focused on eco-friendly, sustainable, and cost-effective approaches using plant extracts and microorganisms [2].

Plant extracts are widely used for the green synthesis of Ag NPs due to their biological properties, availability and relatively rapid synthesis processes, which can reduce production costs [3]. *Acacia catechu* (L.f.) Willd. is a medicinal

plant traditionally used in healthcare [4]. It belongs to the family Fabaceae and is commonly known as Katha. The generic name *Acacia* originates from the Greek word *akis*, meaning “point,” while *catechu* is associated with “cutch,” a tanning extract obtained from its heartwood [5]. Black catechu contains various common bioactive compounds [6]. It has been reported to possess several pharmacological properties, including antibacterial, anticancer, anti-inflammatory, antioxidant, antidiarrheal, antipyretic, hepatoprotective and hypoglycemic activities, as well as wound-healing potential [7]. This small- to medium-sized, prickly tree can grow up to 15 m tall and is widely used in Ayurvedic formulations. Its common names include Karingali in Malayalam, Karungali in Tamil, Khair in Hindi, and Khadira in Sanskrit [8].

Previous studies have demonstrated the antibacterial potential of *A. catechu* heartwood extracts, with taxifolin identified as a contributing antibacterial compound. The plant has also exhibited broad-spectrum antibacterial and antifungal activity in *in vitro* studies [9]. Phytochemical investigations have associated the antibacterial properties of its leaves with alkaloids, carbohydrates, flavones, glycosides, phenolic compounds, saponins, steroids and tannins. Methanolic extracts have shown activity against several microorganisms, including *Bacillus cereus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*, indicating activity against both Gram-positive and Gram-negative bacteria [10]. Also, *A. catechu* exhibits antioxidant activity attributed to its flavonoid content, particularly catechin and rutin. Its free-radical-scavenging potential has been assessed using dot-blot analysis and DPPH assays, with ascorbic acid as the reference standard [11].

The aqueous heartwood extract of *A. catechu* has also been reported to exhibit chemopreventive effects, potentially associated with its polyphenolic constituents and antioxidant properties. Previous studies have investigated its antiproliferative and apoptotic effects on HeLa, COLO-205 and HT-1080 fibrosarcoma cells, along with its effects on normal human lymphocytes [12]. Cytotoxicity was assessed using MTT and trypan blue exclusion assays, while apoptosis was examined through DNA fragmentation, morphological observations and AO/EB fluorescence microscopy. The methanolic and hexane extracts demonstrated cytotoxic and antiproliferative effects, inducing cell death in HeLa and COLO-205 cells at lower concentrations [13].

The present study aims to develop a simple, sustainable, and eco-friendly method for synthesizing Ag NPs using *A. catechu* bark extract as a natural reducing and stabilizing agent. Phytochemicals, including polyphenolic and other bioactive compounds, are expected to facilitate the reduction of  $\text{Ag}^+$  ions to metallic silver ( $\text{Ag}^0$ ) and contribute to nanoparticle stabilization. The study investigates the optical, structural, functional, and morphological properties of the synthesized Ag NPs and evaluates their antibacterial activity. Although previous studies have reported the synthesis of Ag NPs using *A. catechu* and examined their antioxidant and antibacterial properties, the present work further explores the synthesis process, optimizes the reaction conditions and comprehensively characterizes the resulting nanoparticles to assess their potential antibacterial applications.

## EXPERIMENTAL

Fresh bark of *Acacia catechu* (L.f.) Willd. was collected and ground into a fine powder using a grinder. Silver nitrate ( $\text{AgNO}_3$ ) was obtained from Thermo-Fisher Scientific, USA. For antioxidant activity, the reagents included 1.5 mM  $\text{FeSO}_4$  (S.D. Fine Chem Ltd., India), 6 mM  $\text{H}_2\text{O}_2$  (Molychem, India) and 20 mM sodium salicylate (Molychem, India). The reaction mixture (3 mL) consisted of 1 mL  $\text{FeSO}_4$ , 0.7 mL  $\text{H}_2\text{O}_2$  and 0.3 mL sodium salicylate. Antibacterial activity was evaluated against *S. aureus*, *E. coli*, *P. aeruginosa*, *B. cereus* and *P. mirabilis*, using pure cultures obtained from Jasmine Lab, Chennai, India [14]. Cytotoxicity was assessed using the MTT

assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; *in vitro*, USA), while acridine orange was purchased from Sigma-Aldrich, USA.

The aqueous bark extract was prepared by mixing 20 g of finely powdered bark with 100 mL of double-distilled water and boiling at 100 °C for 15 min. The resulting solution was filtered using Whatman No. 1 filter paper. For Ag NPs synthesis, different volumes of the bark extract (1, 2, 3, 4 and 5 mL) were mixed with corresponding volumes of  $\text{AgNO}_3$  solution (9, 8, 7, 6 and 5 mL), respectively. Three experimental conditions were considered (i) the boiled bark extract, (ii) the bark extract mixed with silver nitrate solution and (iii) the silver nitrate solution alone [15]. The mixtures were incubated and centrifuged at 12,000 rpm and 4 °C for 15 min. The appearance of a dark-brown colour following the addition of  $\text{AgNO}_3$  indicated nanoparticle formation. The pellets were collected, and the centrifugation and collection steps were repeated twice to obtain additional pellets. The resulting material was then subjected to characterization [16].

**Synthesis of *A. catechu* (L.f.) willd Ag NPs:** The aqueous bark extract of *A. catechu* (L.f.) Willd. was boiled at 100 °C for 15 min and mixed with  $\text{AgNO}_3$  solutions (1, 3, and 5 mM) at extract-to-silver nitrate ratios of 9:1, 8:2, 7:3, 6:4 and 5:5. A dark-brown colour developed within 15 min when 5 mM  $\text{AgNO}_3$  was mixed with the bark extract at an 8:2 ratio, indicating the formation of Ag NPs [17].

**Characterization:** X-ray diffraction (XRD) patterns were recorded using a GBC-Difftch MMA diffractometer. FT-IR spectra were obtained using a Thermo Scientific Nicolet iS10 spectrometer at a resolution of 1  $\text{cm}^{-1}$  over the range of 4000–500  $\text{cm}^{-1}$ . Surface morphology and average grain size were examined using a JEOL JSM-6360LA scanning electron microscope (SEM) operated at 20–30 kV. Transmission electron microscopy (TEM) was performed using a JEOL JEM-1230 microscope at an accelerating voltage of 100 kV. UV-Vis spectroscopy was conducted using an Ocean Optics Inc. S2000 spectrometer to investigate the synthesized nanoparticles.

**Antioxidant activity:** Hydroxyl radical-scavenging activity was evaluated using the  $\text{Fe}^{2+}/\text{H}_2\text{O}_2$  Fenton reaction [18]. The reaction mixture (3 mL) contained 1 mL of 1.5 mM  $\text{FeSO}_4$ , 0.7 mL of 6 mM  $\text{H}_2\text{O}_2$  and 0.3 mL of 20 mM sodium salicylate. The test extract was added at the desired concentrations, mixed gently, and incubated at 37 °C for 1 h. Absorbance was measured at 562 nm. Three controls were prepared:  $A_0$ , the complete reaction mixture without extract;  $A_2$ , the reaction mixture without sodium salicylate; and  $A_1$ , the reaction mixture containing the extract. Each condition was tested in triplicate, and the results were expressed as mean  $\pm$  standard deviation (SD). A lower  $A_1$  value relative to  $A_0$  indicates higher hydroxyl radical-scavenging activity, attributed to reduced salicylate hydroxylation. The hydroxyl radical activity was calculated using the formula given below:

$$\text{Scavenging (\%)} = \left( 1 - \frac{(A_1 - A_2)}{A_0} \right) \times 10$$

where  $A_0$  = absorbance without extract control,  $A_1$  = absorbance of the extract,  $A_2$  = absorbance without sodium salicylate.

**Cytotoxicity assay:** HT-29 cells were cultured in RPMI medium supplemented with 10% fetal bovine serum, penicillin/

streptomycin (250 U/mL), gentamicin (100 µg/mL) and amphotericin B (1 mg/mL). The cytotoxicity of the extract and synthesized Ag NPs was evaluated at different concentrations.

## RESULTS AND DISCUSSION

**XRD analysis:** The XRD pattern of the pristine *A. catechu* extract (Fig. 1a) exhibits a broad diffuse hump centred at approximately  $2\theta = 20\text{--}25^\circ$ , characteristic of predominantly amorphous or poorly crystalline organic compounds. This feature may be associated with polyphenols, flavonoids, tannins, sugars, and other plant-derived biomolecules that lack long-range crystalline order [19]. The XRD pattern of the *A. catechu*-mediated Ag NPs (Fig. 1b) displays distinct diffraction peaks at approximately  $2\theta = 38^\circ, 44^\circ, 64^\circ$  and  $77^\circ$ , corresponding to the (111), (200), (220), and (311) planes, respectively, of face-centred cubic (fcc) silver. These reflections confirm the crystalline nature of the synthesised Ag NPs. Minor additional peaks, if present, may originate from residual phytochemicals, impurities, or secondary crystalline phases; their precise identification requires further analysis [20]. The observed diffraction features support the formation of crystalline Ag NPs through the use of *A. catechu* extract, whose phytochemicals may participate in the reduction of  $\text{Ag}^+$  ions and the subsequent stabilization of the nanoparticle surfaces.

**FT-IR analysis:** The FT-IR spectra of pristine *A. catechu* extract and *A. catechu*-mediated Ag NPs (Fig. 2) reveal functional groups that may participate in silver ion reduction and nanoparticle stabilization. The broad absorption band at approximately  $3500\text{--}3300\text{ cm}^{-1}$  is attributed to O–H stretching vibrations of phenolic and alcoholic groups, indicating the presence of polyphenolic compounds [21]. The band at  $1650\text{--}1600\text{ cm}^{-1}$  may be associated with aromatic C=C vibrations or C=O stretching in flavonoids and tannins, while the peaks at  $2950\text{--}2920\text{ cm}^{-1}$  correspond to the aliphatic C–H stretching. Absorption bands in the  $1100\text{--}1050\text{ cm}^{-1}$  region are

assigned to C–O or C–O–C stretching vibrations of alcohols and ethers. Changes in peak positions and intensities, along with the appearance of additional bands after Ag NPs synthesis, may reflect interactions between these functional groups and the nanoparticle surfaces [22]. These spectral changes support the possible involvement of *A. catechu* phytochemicals in the reduction of  $\text{Ag}^+$  to  $\text{Ag}^0$  and the capping and stabilization of the resulting nanoparticles.

**SEM analysis:** The surface morphology of the dried *A. catechu* bark extract was examined using scanning electron microscopy (SEM). The low- and high-magnification micrographs (Fig. 3a-d) reveal elongated, rod-shaped structures with typical dimensions ranging from 45 to 80 µm. Particle agglomeration is evident in the micrographs. SEM analysis was performed primarily to examine the morphology and surface features of the dried plant material [23,24].

**HR-TEM analysis:** The morphology and crystalline characteristics of the synthesised nanoparticles were examined using HR-TEM (Fig. 4a-b). The low-magnification images reveal well-dispersed, nearly spherical nanoparticles with some aggregation, confirming the formation of nanoscale structures [25]. The high-resolution images display distinct lattice fringes, providing evidence of crystalline ordering. The SAED pattern (Fig. 4c) exhibits distinct diffraction spots arranged in concentric rings, supporting the polycrystalline nature of the sample. The diffraction rings are consistent with reflections from different crystallographic planes and agree with the XRD results [26]. These observations confirm the nanoscale morphology and crystalline nature of the synthesised material. The assignment of a spinel structure, however, should be retained only if supported by the identified lattice spacings and phase-specific diffraction data.

**UV-Vis spectrophotometer:** The colour of the reaction mixture changed from yellow to orange and finally to dark brown (Fig. 5a-c) confirmed the formation of Ag NPs. The UV-Vis absorption spectrum exhibited a characteristic absorption band in the 420–460 nm range, attributed to the surface plasmon resonance of Ag NPs [27]. The antioxidant

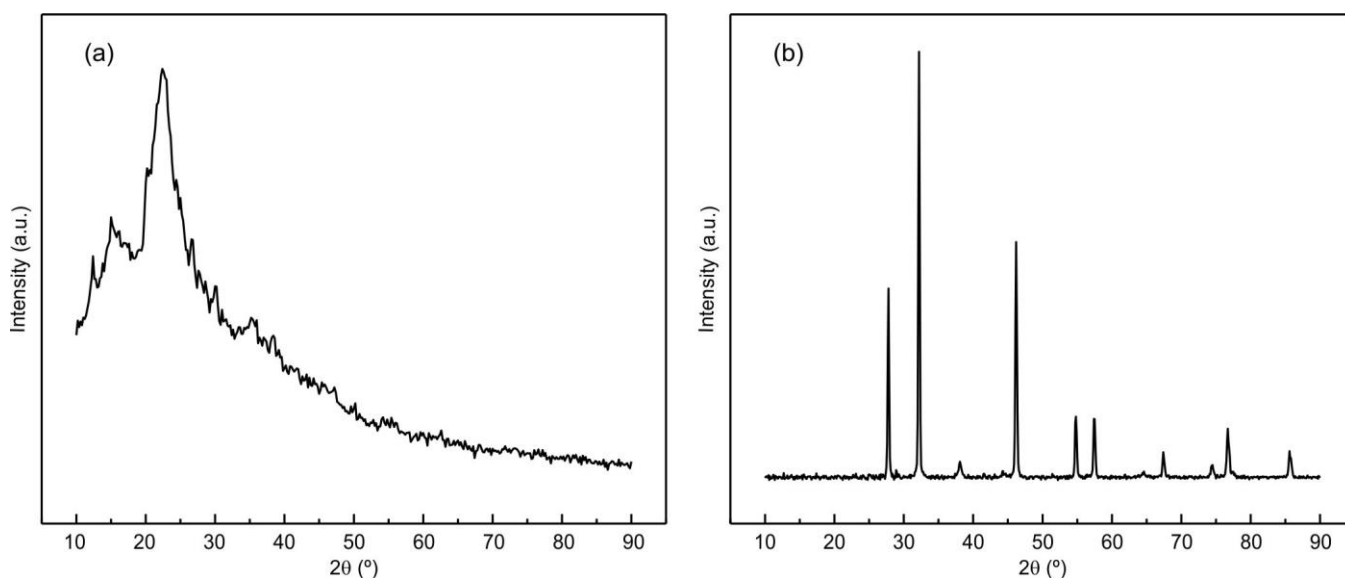


Fig. 1. XRD patterns of (a) *Acacia catechu* (L.f) wild extract and (b) *Acacia catechu* extract with silver nanoparticles

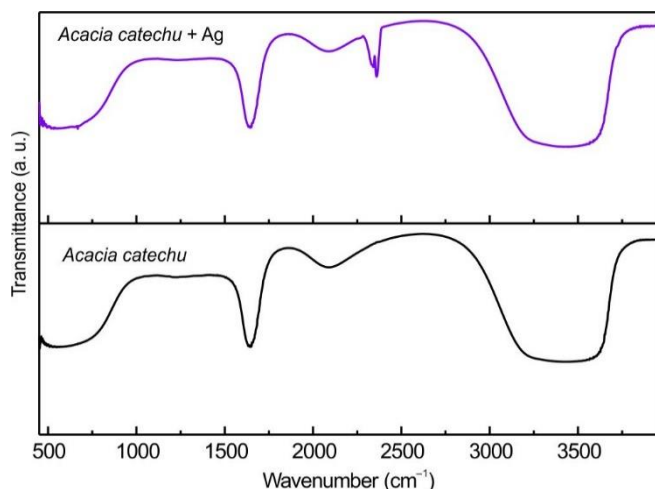


Fig. 2. FT-IR spectra of *A. catechu* (L.f) willd and *A. catechu* (L.f) willd with silver nanoparticles

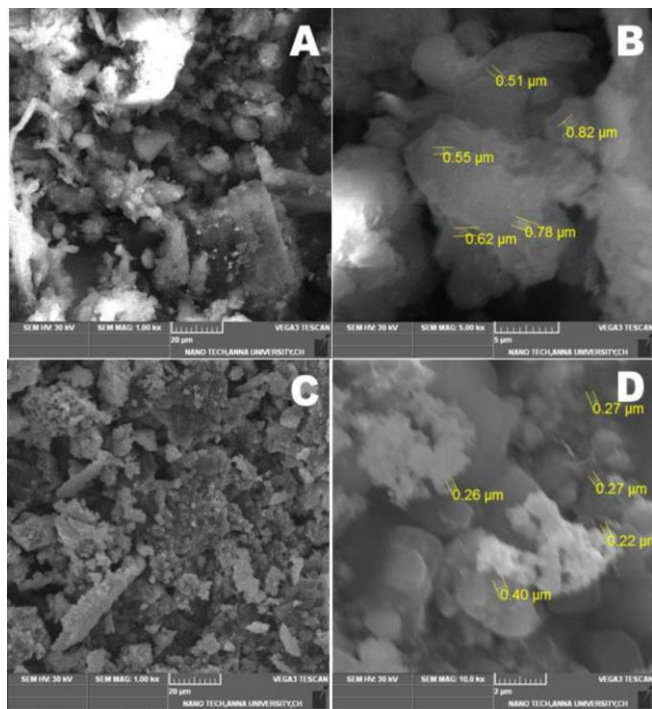


Fig. 3. SEM images of *A. catechu* (L.f) willd bark extract particles (a) low and (b) high magnification image of *A. catechu* (L.f) willd bark extract with silver nanoparticles (c) low and (d) high magnification

activity of the aqueous *A. catechu* bark extract was evaluated *in vitro* using hydroxyl radical-scavenging assays (Fig. 5a-b). Previous studies reported dose-dependent radical-scavenging activity, with an  $IC_{50}$  value of  $455.2 \pm 15.48$  mg/mL. The extract exhibited hydroxyl radical-scavenging activity comparable to that of ascorbic acid [28].

**Antioxidant activity:** The hydroxyl radical-scavenging activity increased with concentration in all the tested groups, revealing a concentration-dependent antioxidant response (Table-1). The activity was relatively high at the initial concentration of 0.2 and approached a plateau at 0.8-1.0, particularly for *A. catechu*-mediated Ag NPs. The Ag NPs exhibited higher scavenging activity than the pristine *A. catechu* extract at every tested concentration, with the highest improvement of approximately 13% at 0.2 and a sustained advantage of approximately 5% at 1.0. The mean enhancement of approximately 10.3% suggests that nanoparticle formation may improve the antioxidant performance of the plant extract, although further interaction studies are required to establish synergistic effects.  $AgNO_3$  exhibited the lowest mean activity (38.4%), underperforming the plant extract by approximately 18% on average. This difference suggests that ionic silver does not provide the same antioxidant response as the synthesized nanoparticles under the tested conditions.

TABLE-1  
PERCENTAGE OF SCAVENGING EFFECT OF DIFFERENT CONCENTRATION OF BARK, BARK + Ag AND  $AgNO_3$

| Conc. | Scavenging effect (%) |             |          |
|-------|-----------------------|-------------|----------|
|       | Sample                | Sample + Ag | $AgNO_3$ |
| 0.2   | 40                    | 53          | 33       |
| 0.4   | 52                    | 55          | 35       |
| 0.6   | 57                    | 63          | 39       |
| 0.8   | 65                    | 67          | 40       |
| 1.0   | 68                    | 73          | 45       |

The enhanced activity of the Ag NPs may be associated with interactions between the nanoparticle surfaces and bio-active phytochemicals, potentially affecting electron-transfer processes and the accessibility of reactive functional groups. The lower activity of  $AgNO_3$  may reflect differences in the chemical interactions of ionic silver with extract constituents; these mechanisms require further investigation. Ascorbic acid was used as the positive control, and the antioxidant

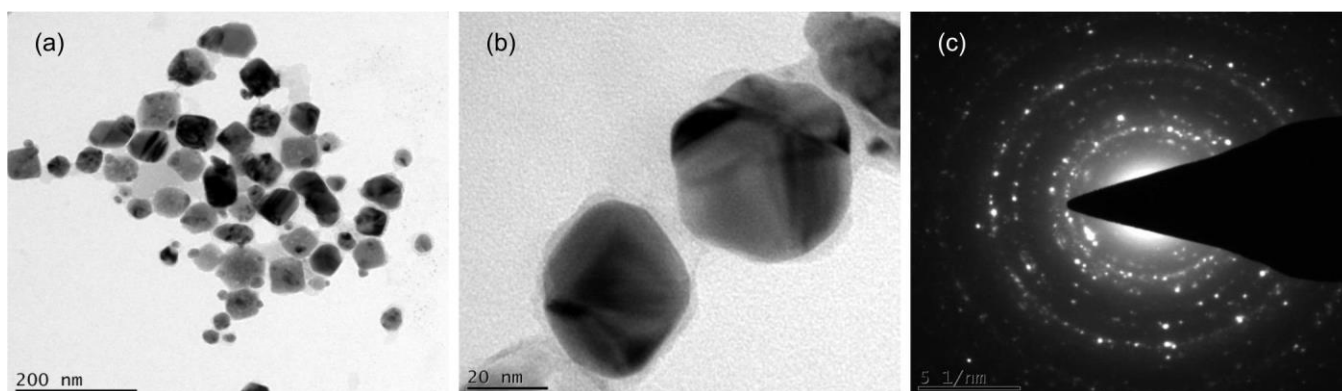


Fig. 4. HR-TEM images of *A. catechu* (L.f) willd using silver nanoparticles (a) low, (b) high magnification and (c) SAED pattern

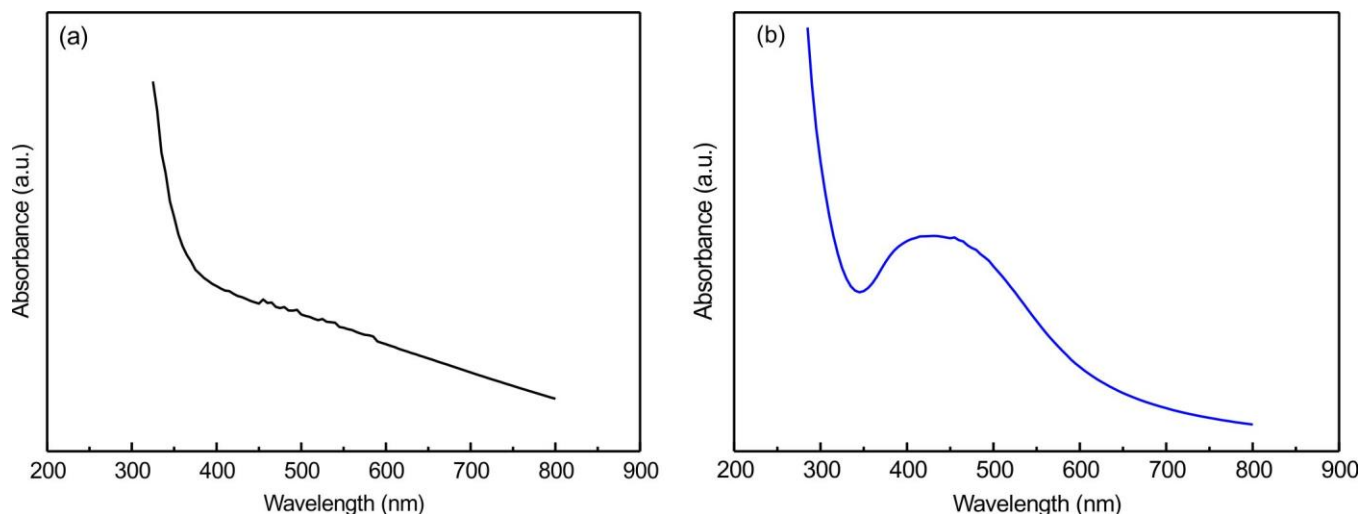


Fig. 5. UV-Vis spectra of (a) *A. catechu* (L.f) willd and (b) *A. catechu* (L.f) willd with silver nanoparticles

activity of the synthesized Ag NPs was evaluated at different concentrations. Percentage inhibition was calculated, and the  $IC_{50}$  value was determined from the concentration-dependent inhibition curve. Comparison with ascorbic acid provides a quantitative basis for evaluating the antioxidant potential of the synthesized nanoparticles. The highest scavenging activity, approximately 73%, was recorded for *A. catechu*-mediated Ag NPs at 1.0, while the relatively stable response between 0.8 and 1.0 suggests that the assay response was approaching a plateau. This behaviour may be related to limitations in the availability of reactive species or changes in the reaction kinetics under the assay conditions.

**Antibacterial activity:** The antibacterial activity of the synthesized Ag NPs was evaluated using the disc diffusion method. Paper discs impregnated with 20  $\mu$ L of each test sample were placed on bacterial culture plates. The results revealed more pronounced inhibition with the *A. catechu* bark extract–Ag NP formulation than with  $AgNO_3$  or the bark extract alone [29–31]. Among the tested organisms, *P. aeruginosa* exhibited the largest inhibition zone. No inhibition zone was observed for the bark extract control, which indicate negligible antibacterial activity under the tested conditions. The inhibition zones ranged from 11–15 mm for the Ag NPs formulation, compared with 8–13 mm for  $AgNO_3$ , while the bark extract alone showed no measurable inhibition beyond the 6 mm disc diameter (Table-2). The maximum zone diameter of 15 mm was recorded against *P. aeruginosa*. These findings indicate enhanced antibacterial activity of the Ag NPs formulation against the tested bacteria. Since recognized antibiotic controls, such as ciprofloxacin, gentamicin or streptomycin, were not included, the results should be considered preliminary; comparison with standard antibiotics would provide a more complete assessment of antibacterial efficacy.

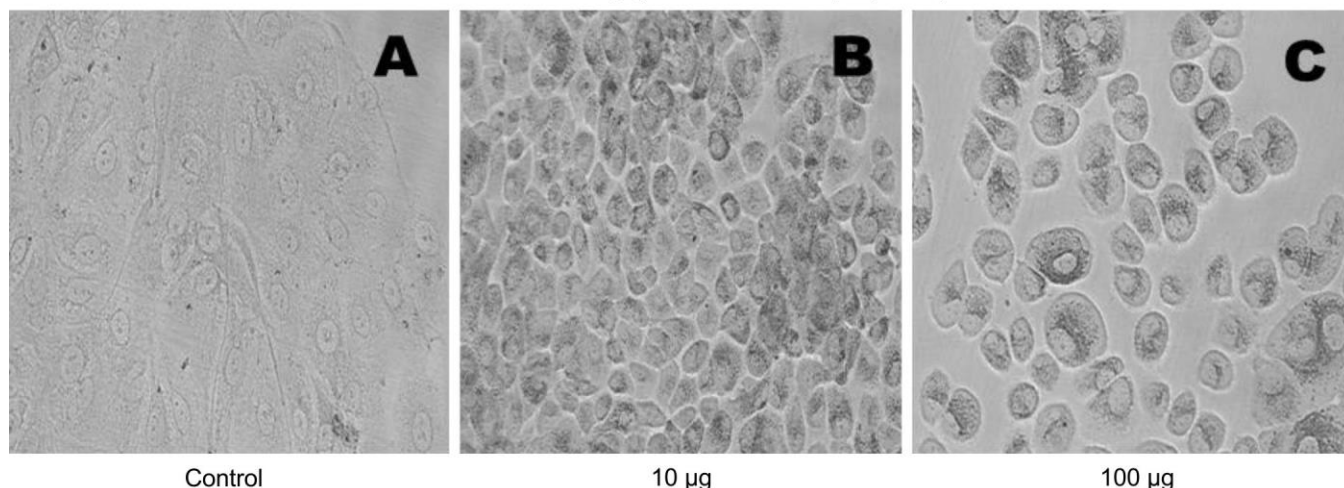
Previous studies have reported antibacterial activity in *A. catechu* extracts against both Gram-positive and Gram-negative bacteria. Heartwood extracts contain bioactive constituents such as taxifolin, while methanolic extracts have been reported to act against *B. subtilis*, *S. aureus*, *S. typhi*, *E. coli* and *P. aeruginosa*, as well as *C. albicans* [32]. Another study reported antibacterial activity of *A. catechu* pigment extracts against

TABLE-2  
Comparative Antibacterial Activity of  $AgNO_3$ ,  
Ag NPs–*Acacia catechu* and *Acacia catechu* Extract

| Organism                      | Sample                         | Zone diameter  |
|-------------------------------|--------------------------------|----------------|
| <i>Staphylococcus aureus</i>  | $AgNO_3$                       | 8 mm           |
|                               | Ag NPs + <i>Acacia catechu</i> | 11 mm          |
|                               | <i>Acacia catechu</i>          | 6 mm (no zone) |
| <i>Pseudomonas aeruginosa</i> | $AgNO_3$                       | 13 mm          |
|                               | Ag NPs + <i>Acacia catechu</i> | 15 mm          |
|                               | <i>Acacia catechu</i>          | 6 mm (no zone) |
| <i>Bacillus cereus</i>        | $AgNO_3$                       | 11 mm          |
|                               | Ag NPs + <i>Acacia catechu</i> | 12 mm          |
|                               | <i>Acacia catechu</i>          | 6 mm (no zone) |
| <i>Proteus mirabilis</i>      | $AgNO_3$                       | 10 mm          |
|                               | Ag NPs + <i>Acacia catechu</i> | 11 mm          |
|                               | <i>Acacia catechu</i>          | 6 mm (no zone) |
| <i>Escherichia coli</i>       | $AgNO_3$                       | 10 mm          |
|                               | Ag NPs + <i>Acacia catechu</i> | 12 mm          |
|                               | <i>Acacia catechu</i>          | 6 mm (no zone) |

*Citrobacter* at concentrations ranging from 100 to 400  $\mu$ g/mL, with larger inhibition zones at higher concentrations [33]. The present findings are consistent with the reported antibacterial potential of *A. catechu*, particularly against *P. aeruginosa*.

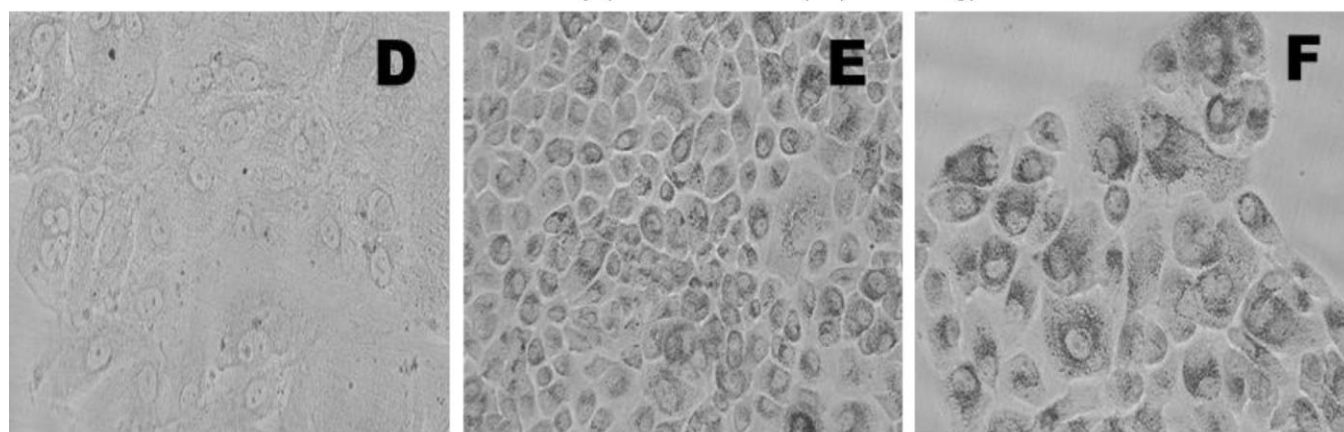
**Cytotoxicity assay on cell lines:** The cytotoxic potential of aqueous *A. catechu* (L.f.) Willd. bark extract and its silver nitrate formulation were evaluated against HT-29 human colorectal adenocarcinoma cells. As shown in Fig. 6, both treatments exhibited cell inhibition at 100  $\mu$ g [34]. The concentration-dependent results in Fig. 7 further show that cell inhibition increased across the tested concentrations (1 ng, 10 ng, 100 ng, 1  $\mu$ g, 10  $\mu$ g and 100  $\mu$ g). Inhibition was minimal at 1–10 ng but increased at 100 ng and above. The silver nitrate formulation consistently showed higher inhibition than the bark extract alone, with substantial cytotoxicity at 10 and 100  $\mu$ g. The solvent control showed negligible inhibition. These findings are consistent with previous reports of cytotoxic responses in HT-29 cells exposed to *A. catechu*-based preparations [35,36]. Although Ag NPs have been associated

HT29–MTT assay (*Acacia catechu* (L.f) willd)

Control

10 µg

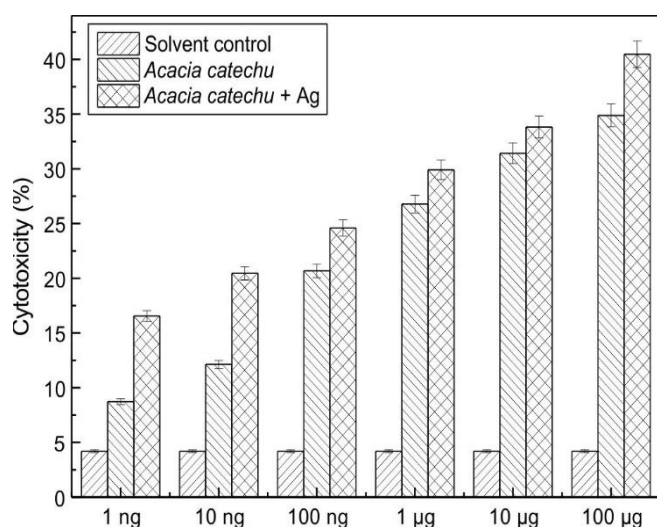
100 µg

HT29–MTT assay (*Acacia catechu* (L.f) willd + Ag)

Control

10 µg

100 µg

Fig. 6. MTT assay of cell line HT29 in *Acacia catechu* (L.f) willd bark extract and with silver nitrate in 10 µg and 100 µgFig. 7. Concentration-dependent cytotoxicity of *Acacia catechu* bark extract and silver nitrate-treated extract in HT29 cells

with oxidative stress, mitochondrial dysfunction, DNA damage and apoptosis, these mechanisms were not directly examined in the present study. The absence of standard anticancer drug

controls and selectivity testing against normal cells limits interpretation of the results.

### Conclusion

The present investigation establishes that the bark extract of *Acacia catechu* (L.f.) Willd serves as an efficient biogenic precursor for the synthesis of silver nanoparticles (Ag NPs). Structural and compositional analyses performed using XRD, FT-IR and HR-TEM confirmed the presence of multiple phytoconstituents within the bark extract, which act as reducing and stabilizing agents during nanoparticle formation. The synthesized Ag NPs were further characterized by UV-Vis spectroscopy, which validated their plasmonic resonance and SEM imaging, which revealed predominantly elongated rod-like morphologies. The biologically derived Ag NPs demonstrated pronounced antimicrobial efficacy, particularly against *P. aeruginosa*, in comparison to other tested bacterial strains. In addition, the nanoparticles exhibited significant antioxidant and anticancer activities, underscoring their potential therapeutic relevance. The findings support the use of *A. catechu* bark extract for the green synthesis of Ag NPs and indicate that the resulting formulation exhibits antioxidant, antibacterial and cytotoxic activities under the tested conditions.

# CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

# DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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