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RESEARCH

Cyamopsis tetragonoloba pod extract: A green route to multifunctional silver nanoparticles with enhanced biomedical activity

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Abstract

Silver nanoparticles (AgNPs) were synthesized using *Cyamopsis tetragonoloba* (*C. tetragonoloba*) pods (CTP-NPs) powder extract. The following techniques UV–visible spectroscopy, fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) were used for the characterization of these synthesized nanoparticles. UV–Visible Spectroscopy confirmed the formation of AgNPs via a distinct Surface Plasmon Resonance (SPR) peak observed at 412 nm, indicating successful reduction. FT-IR Analysis identified key functional groups (e.g., N–H, C = O stretching) responsible for capping and stabilization, with major shifts observed at 3411 cm^{-1} and 1735.73 cm^{-1}. X-Ray Diffraction (XRD): Revealed a face-centered cubic (FCC) crystalline structure. Scanning Electron Microscopy (SEM): Illustrated a predominantly spherical morphology with average particle size 13 nm. The CTP-AgNPs IC_{50} values revealed notable variations in DPPH (183.61 µg/mL) in comparison to the ascorbic acid standard (25.7 µg/mL) and hydroxyl radical activity (53.6 µg/mL) in comparison to ascorbic acid (22.47 µg/mL). Antibacterial property of CTP-AgNPs against *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*) was proved by zone of inhibition 11 ± 0.79 mm and 12 ± 0.78 mm per 100 µg/mL respectively. In vitro anticancer assay demonstrated IC_{50} value of 18.25 µg/mL against A549 lung cancer cell line, which confirms its potent anticancer potential. Overall, the CTP mediated bio-synthesized AgNPs proved promising in vitro antioxidants by free radical scavenging, antimicrobial by zone of inhibition and anticancer by cytotoxic properties.

Keywords *Cyamopsis tetragonoloba* · Pods powder · Green synthesis · Antioxidants · Antibacterial

Extended author information available on the last page of the article

Introduction

Medicinal plants act as healing resource for local and tribal communities around the world for more than a millennium. Although it serves as the main source of drug discovery and the primary form of healthcare for around 85% of the world's population, the majority of synthetic medications are also derived from it (Fitzgerald et al. 2020). Using modern analytical procedures, the drug-resistant pathogens which shows maximum activity can be identified and isolated. New approach against the harmful bacteria which are pathogenic can be altered using the compounds obtained from medicinal plants (Vaou et al. 2021). The materials or compounds which can prevent or delay the oxidation of proteins, lipids or any other molecules by holding back free radical reaction especially the initiation or propagation are defined as the antioxidants. The compounds which are having the antioxidant activity have been derived from medicinal plants, and most of the compounds are polyphenols which include phenols, flavanoids and tannins (Merghem and Dahamna 2020).

Because of its special makeup and low toxicity, guar gum (GG), a natural polymer, has a lot of potential for application in pharmaceutical formulations. The GG can be made to meet the requirements of the medical and biological engineering fields. The *C. tetragonoloba* is a well-known legume species, mostly cultivated in a spring to summer season (Soltani et al. 2021; Tahmouzi et al. 2023). It is one among the world's most heat- and drought-tolerant row crops. 80% of the world's production comes from semi-arid regions in India and 15% from Pakistan; the remaining 5% is grown in the US (Texas and Oklahoma) and Sudan (Avola et al. 2020). Guar gum is a polysaccharide which occurs naturally called galactomannan, is made from the seeds of the *C. tetragonoloba* bean. In herbal medicine, its pods are used. The folk remedy *C. tetragonoloba* is well known. It helps with fat, artery hardening, anorexia and dyspepsia, laxative, cooling agent, digestive assistance, and most importantly respiratory diseases. Furthermore, *C. tetragonoloba* beans may be a rich source of extra phytochemicals (Jamshed et al. 2018).

Nanobiotechnology is a novel concept and area of nanotechnology that is attracting interest from all over the world. The greatest approach to lessen the adverse effects of creating and utilizing nanomaterials is through green nanotechnology, which also minimizes the likelihood of problems resulting from the application of other methods. Nanomaterials possess a wide range of properties, including optical, chemical, and thermal properties. Using nanoparticles with a much bigger surface area than their bulk form and a much smaller size is known as nanotechnology. Chemical, optical, and thermal capabilities are only a few of the many attributes of nanomaterials. Investigating several bulk materials at the nanoscale reveals that they have distinct features; their greater aspect ratio is one explanation for this phenomenon that is now understood. This can lead to an array of features for distinct nanoparticles. In response, nanoparticles have been explored as a possible substitute for theirusage in various biological applications. NPs' anti-inflammatory, biocompatibility, and antibacterial properties, effective drug delivery, bioavailability, bioactivity, anti-tumour, and organic absorption create them prevalent in medical, biological and environmental applications (Pandit et al. 2022).

Antioxidants are essential for accelerating the healing of wounds and protecting tissues from oxidative damage. Various compounds such as flavonoids, anthraquinones and naphthoquinones have been identified to possess strong antioxidant properties. By efficiently hunting of reactive oxygen species (ROS), preventing process of lipid

peroxidation, and raising concentrations of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), some herbal extracts have shown strong antioxidant properties (Yazarlu et al. 2021).

The DPPH free radical scavenging assay is a popular technique that offers a preliminary way to evaluate antioxidant activity. The FRAP test is a traditional ET-based method for measuring the reduction in ferric ion ($Fe3+$)–ligand complex, which is then converted by antioxidants into the bright blue ferrous ($Fe2+$) complex in an acidic environment (Muthukrishnan et al. 2024). Over the past four decades, a large number of novel antibiotics were developed by the pharmaceutical industry and other activities, and the overuse of these drugs has augmented the microbial resistance to them. Although overuse or misuse of antibiotics can lead to resistant illnesses and Antimicrobial Resistance (AMR) among bacterial pathogens, antibiotic resistance can also arise naturally (Bhatia et al. 2021). In many underdeveloped nations, plant materials will remain a significant part of primary healthcare as therapeutic medicines.

Nanoparticles possess distinct characteristics that make them especially valuable in cancer treatment, as they can improve the effectiveness of chemotherapy while reducing harmful effects on healthy tissues throughout the body. Various metallic nanoparticles are currently being investigated for their potential to combat different forms of cancer cells (Al-Samydai et al. 2024; Hheidari et al. 2024).

The green synthesis of AgNPs has been recognized as a better, eco-friendly alternative to the conventional physical and chemical synthesis and utilizing secondary metabolites of the different plant sources as a reducing and stabilizing agents 14(Zeheri et al., 2025). In addition, the green-fabrication of silver-based nanomaterials is especially appreciated for its improved biocompatibility and substantial therapeutic value. Moreover, the plant material predominantly composed phytochemical constitution include flavonoids, phenols and terpenoids, which may directly influence the morphology and stability of the AgNPs, they are mostly used for targeted biomedical applications like antimicrobial and anticancer treatments (Zeheri et al. 2025; Hadkar and Roopan 2025) (Zeheri et al., 2025; Hadkar, 2025). These biogenic nanomaterials not only possess a biological application and also possess specific efficacy in environmental remediation through the photocatalytic degradation of organic pollutants (Hadkar et al. 2026) (Hadkar, 2026).

Recent phytochemical studies have revealed a diverse set of bioactive secondary metabolites in addition to its well-documented galactomannan polysaccharide (guar gum), such as the flavonoid's quercetin, kaempferol, daidzein, and luteolin, and phenolic acids like sinapic, gallic, and caffeic acids. These compounds have been demonstrated to have synergistic effects in antioxidant capacity by improving free radical scavenging activity and metabolic regulation, making the plant a major lead for the development of nutraceuticals in the treatment of diseases like antidiabetic, anti-inflammatory, obesity and cancer (Asati et al. 2021; Joshi et al. 2025) (Asati et al., 2023; Joshi et al., 2025).

Although the application of *C. tetragonoloba* seeds in gum extraction and leaves in medicine has been well investigated in the existing literature, the application of its pods as a reducing agent has been largely unexplored. This research work fills this gap by utilizing the pod extract to synthesize ultra-small AgNPs with strong cytotoxic activity against A549 lung cancer cells. Given this context, this research focused on producing silver nanoparticles (AgNPs) through green synthesis using *Cyamopsis tetragonoloba* pods (CTP-NPs) powder extract. The resulting nanoparticles underwent comprehensive characterization using UV–visible spectroscopy, Fourier-transform infrared spectroscopy, X-ray diffraction, and scanning electron microscopy, followed by assessment of their antioxidant, antimicrobial and cytotoxic properties.

Materials and methods

Chemicals

Silver nitrate (99%, CAS No: 7761–88-8, made in USA), ferric chloride (98%, CAS No: 7705–08-0ADR, Made in India), Dimethyl sulfoxide (99%, SH0S600475, Made in India), potassium ferrocyanide (98%, CAS No: 13746–66-2ADR, Mande in India), ethylenediaminetetraacetic acid (EDTA) (98%, Made in India), ascorbic acid (99%, CAS No: 50–8-7, Made in India) was procured from Sigma-Aldrich. Nutrient agar, nutrient broth and fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT) dye (97%) and Thiazolyl blue tetrazolium bromide (98%−102%, CAS No: 298–93-1, made in India) were acquired from HiMedia, India. A549 cells were procured from the National Center for Cell Science (NCCS), Pune, India (Passage number ranges from 20 to 27). The bacterial species *B. subtilis* and *E. coli*. procured from Department of Microbiology Periyar University, Salem-11.

Plant sample and extraction

The fresh pods of *Cyamopsis tetragonoloba* (L.) Taub. had been collected from Trivandrum District, Kerala and authenticated by "Botanical Survey of India, Tamil Nadu Agricultural University", Tamil Nadu, Coimbatore–641003 and Authentication No: BSI/SRC/5/23/2024/Tech – 229. Collected plant was washed with distilled water and shade dried for four days. Twenty grams of coarsely powdered sample was extracted with 150 ml of water and kept at 120 rpm in an incubated shaker at 37 °C for one day. The dried extract was collected and stored at 4 °C for further analysis. The total extraction value was obtained by the formula,

$$Yield\ (\%) = (W1 \div W2) \times 100$$

W1-weight of the plant extract, W2-weight of the dried plant sample.

LC–MS analysis of *C. tetragonoloba* pods extract

The shimadzu LCMS-8030 triple quadrupole mass spectrometer coupled to an ultra-high-performance liquid chromatograph (UHPLC) were used to identification of the bioactive molecules from *C. tetragonoloba* pods extract. Separation was carried out on an acquity UPLC BEH C18 column with dimensions 1.7 µm/2.1 × 100 mm. The ionization process was conducted by utilizing an ESI probe running in both positive and negative modes of ionization for the sake of ensuring that all metabolites were captured. The ion source was run in scan mode with respect to the detection mode with the capillary voltages, nebulizer gas flow, and drying gases all being optimized for enhanced sensitivity. The data acquisition was performed using Shimadzu LabSolutions software for the identification of the secondary metabolites.

Preparation of silver nitrate nanoparticles by *C. tetragonoloba* pods

25 g of dried powdered *C. tetragonoloba* pods were diluted in 500 mL of distilled water. The mixture was then heated to 100 °C for 80 min in a boiling water bath. After cooling to room temperature, the mixture was filtered and stored. To create silver nanoparticles 0.1 mM of 500 mL silver nitrate were prepared and plant extract 10 mL was added into the silver nitrate solution under continuous stirring process until the solution colour turns into reddish brown. The fluid was centrifuged at 10,000 rpm for 15 min. The pellet was separated and placed in a petridish with a tiny amount of supernatant after centrifugation, with the supernatant being removed to extract the pellet. After the NPs had settled for at least one day, they were dried in a 55 °C oven.

Characterization

UV–Visible spectral study

The nano drop-8000 UV–Vis spectrophotometer (Thermo Scientific, United States) was used to capture the UV–Vis spectra between 200 to 800 nm appropriate to periodically track the green reduction of silver nitrate in *C. tetragonoloba* pod extracts (Hurst et al. 2006; Chi et al. 2022*)*.

FT-IR analysis

Using the Perkin-Elmer Spectrum-2000 FT-IR instrument (USA), FT-IR spectral analysis of the green synthesized CTP-AgNPs were studied. The presence of putative biomolecules responsible for the stability and reduction of the AgNPs in the 4000–400 cm^{-1} region.

XRD analysis

XRD patterns were used to examine the crystal structure of the biosynthesized CTP-AgNPs. The drop-coated silver nanoparticles on a glass substrate produced an XRD pattern that showed a wide range of Bragg's angle 2θ. Using a Cu radiation source and an ULTIMA IV-X-ray powder diffractometer manufactured by Rigaku Ltd (Japan). To find out the crystalline size of nanoparticles used the Debye–Scherrer equation, $D = K\lambda/\beta \cos \theta$.

SEM analysis

After being synthesized, the CTP-AgNPs were observed using by scanning electron microscopy (SEM) (JEOL JSM-IT500, Japan). During the imaging procedure, the images were collected using a cover slip. The magnifying glass used, and the overall size of the contents were all included in the photographs themselves.

Bio-activity of synthesize AgNPs

DPPH (2, 2-diphenyl-1-picrylhydrazyl) antioxidant assay

DPPH is a deep purple, stable chromogen radical that is easily obtained commercially and doesn't need to be generated beforehand. One milliliter of 0.8 mM DPPH solution was

combined with one milliliter of CTP-AgNPs at different concentrations (40 to 200 µg/mL). After giving the mixture a good shake, it was allowed to stand for half an hour. Antioxidants contribute electrons to neutralize the DPPH radical in the DPPH scavenging assay, which causes the DPPH solution's color to shift and be detected at 517 nm. The effectiveness of the antioxidant is indicated by the extent of color change. IC_{50}, or the effective concentration of the antioxidant required to inhibit the DPPH concentration free radical by 50%, is a common way to express the antioxidant activity found using the DPPH scavenging method (Geetha et al. 2014).

$$\%RSA = (Abs\ control - Abs\ sample)/Abs\ conrol \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP test is a traditional ET-based method for measuring the reduction in ferric ion (Fe^{3+})–ligand complex, which is then converted by antioxidants into the bright blue ferrous (Fe^{2+}) complex in an acidic environment. The CTP-AgNPs sample was taken at various concentrations (20 to 100 µg/mL) and phosphate buffer is added to all the tubes. Along with 2.5 mL of 1% ferrocyanide. Then the contents are covered using aluminium foil and incubated at 37 °C for 20 min. After incubation, 10% TCA is added for about 2.5 mL to each tube. And centrifuge them at 3000 rpm for 5–6 min, 205 mL of the supernatant was collected to that 2.5 mL of distilled H_2O was added. Then 0.5 mL of ferric chloride was added to all the tubes. The blue color developed was read at 700 nm. The blank consists of about 1 ml of water (Prati et al. 2010).

Hydroxyl Free Radical Scavenging Assay (HRSA)

A chosen antioxidant sample's ability to scavenge hydroxyl radicals is measured by calculating the area under the fluorescence decay curve, both with and without the antioxidant present. The result is represented as Trolox equivalents per unit of the antioxidant (Muthukrishnan et al. 2022). One millilitre of iron EDTA solution, half a millilitre of EDTA solution, one millilitre of DMSO, and half a millilitre of ascorbic acid were added to CTP-AgNPs samples in different concentrations (25 to 100 µg/mL). For fifteen minutes, the mixture was incubated at 80 to 90 °C in a boiling water bath. Following incubation, 3 millilitres of Nash reagent and 1 millilitre of ice-cold TCA were added, and the reaction mixture was kept at room temperature for 15 min. At 412 nm, the absorbance was measured. The % hydroxyl free radical hunting action is calculated by the following formula:

$$\%HRSA = (Abs\ control - Abs\ sample/Abs\ control \times 100)$$

Antibacterial activity of CTP-AgNPs

CTP-AgNPs were used to test antibacterial activity against *B. subtilis* and *E. coli*. For the antibacterial activity, the pour plate and well diffusion procedures were used (Muthukrishnan et al. 2022). Bacterial strains are plated in nutrient agar medium and micro pipette tips were used to make well. Three different concentrations (25–100 µg/mL) of CTP-AgNPs and for positive control the tetracycline (1 mg/mL) were loaded into the wells respectively. Then the petri plates were maintained at 37˚C in incubator

for one day, after incubation period the inhibition zone was measured and values were expressed as millimetres (Mm).

Evaluation of anticancer properties of silver nanoparticles

The cytotoxic effects of synthesized AgNPs were evaluated against lung carcinoma cells (A549) using the MTT colorimetric assay methodology. A549 cells were seeded in RPMI medium containing the following supplements: 10% fetal bovine serum, 10 mM sodium pyruvate, 10 mM HEPES buffer, 10 mg/L bovine insulin, 4.5 g/L glucose and 1% antibiotic solution. Cell cultures were incubated under standard conditions (37 °C, 5% CO_2 atmosphere with 95% humidity) in a controlled environment incubator for 24 h. After incubation, cells were treated with AgNPs at various concentrations of control (0), 25, 50, 100, 150 and 200 µg/mL in triplicates for 24, and 48 h. Following a 48 h incubation period, cell viability was assessed by adding 20 µL of MTT reagent (prepared at 5 mg/mL concentration in phosphate-buffered saline) to each well. The plates were then incubated for an additional 4 h under the same environmental conditions to allow formazan crystal formation. Then the culture medium was removed and substituted with 100 µL of dimethyl sulfoxide (DMSO) in each well to solubilize the formazan crystals. Absorbance readings were obtained using an ELISA microplate reader at a wavelength of 620 nm to determine optical density values.

Statistical analysis

The findings were demonstrated as the average ± SD of three repeats. A two-way ANOVA and Bonferroni test were employed in the statistical studies to examine significant differences ($p < 0.05$) using GraphPad Prism, version 10.0.0 (GraphPad Software, Boston, USA).

Results and discussions

Quantitative analysis

The initial quantitative findings of the total water extraction values exposed a significant percentage value of 22.5 ± 0.9%. The medicinal plants are a rich source of bioactive com pounds and they are used for the treatment of various diseases. In the qualitative analysis, the aqueous extracts of *C. tetragonoloba* pod have more potential and lower expensive for extracting more bioactive compounds than other solvents. Extraction yield indicates its potential therapeutic use.

LC–MS analysis

Chemical profiling of the ethanolic leaf extract from *C. tetragonoloba* pod was conducted using LC–MS/MS analysis, revealing a diverse array of bioactive secondary metabolites.

The study utilized both positive and negative ('+'ve and '−'ve) ionization modes to capture a comprehensive chemical fingerprint, with the resulting chromatograms providing a visual map of the plant's internal chemistry (Fig. 1). The detected molecule information based on high-resolution mass spectrometry in both positive and negative ionization modes is shown in Tables 1 and 2. The significant phytoconstituents, including phenolic acid, flavonoid, flavonoid glycoside, Terpenoid, organic acid and flavanol were existing in methanol extract of *C. tetragonoloba* pod in '+'ve and '−'ve ionization modes of LC–MS/MS analysis. Some of the bioactive compounds recorded in '+'ve ionization mode such as luteolin-O-glycoside, apigenin $[M+H]^+$ and sinapic acid (fragment/small phenolic) and the major bioactive compounds in '−'ve ionization modes quinic acid, caffeoylquinic acid, apigenin, catechin/epicatechin, coumaric acid derivatives and chrysin derivative. According to phytochemical studies on the *C. tetragonoloba* pod extracts, it has been found to contain a high number of phenolic acids, flavonoids, saponins, and tannins, in addition to its prominent galactomannan polysaccharide, guar gum. Recent reports have also stressed that the bioactive compounds apigenin and luteolin O-glycosides, possess strong antioxidant, antidiabetic, and anti-inflammatory properties through their ability to modulate oxidative stress and inhibit key enzymes of carbohydrate metabolism, such as alpha-glucosidase (Joshi et al. 2025) (Joshi et al., 2025).

FTIR of *C. tetragonoloba* pod aqueous extracts

Characteristic peaks in the mid infra region (4000–500 cm^{-1}) have been found in the spectrum. This indicates the presence of organic functional groups usually found in polymeric materials. The broad peak at 3302 cm^{-1} indicates the presence of O–H stretching vibration of the hydroxyl group and N–H stretching vibration of amines/amides, indicating hydrogen bonding. The peak at 2922 and 2860 cm^{-1} indicates weak C-H stretching of sp^3 hybridization. The peak at 1620 and 1530 cm^{-1} indicates C = O stretching of Amide I and N–H bending/C-N stretching of Amide II, indicating the presence of proteins. The peak at 1387 and 1232 cm^{-1} indicates C-H bending of methyl groups and C-O stretching of Phosphatase. The peak at 1045 and 880 cm^{-1} indicates C-O stretching of alkoxy and C-H out-of-plane bending, indicating carbohydrates and cellulose (Fig. 2).

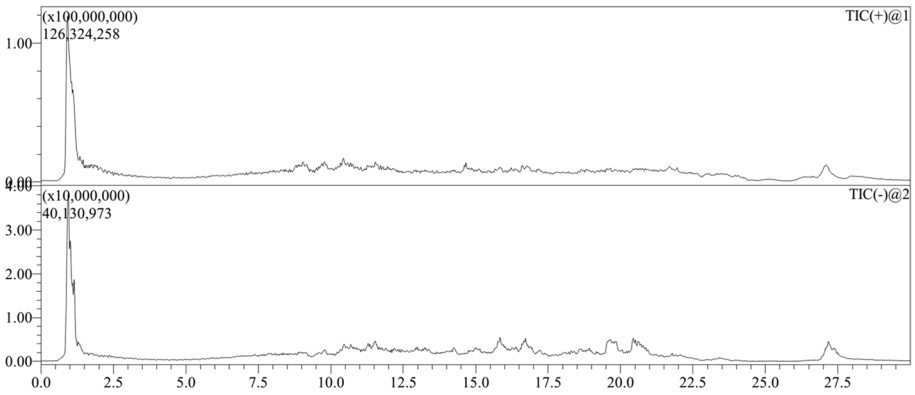

Fig. 1 LC–MS/MS base peak chromatogram of *Cyamopsis tetragonoloba* pod extract in positive ionization mode and negative ionization mode

Table 1 Compound list of *Cyamopsis tetragonoloba* pod extract in positive ionization mode

Peak no	RT (min)	Base peak (m/z)	Tentative compound	Compound class	Literature support
1	1.051	221	Sinapic acid fragment/small phenolic	Phenolic acid	Clifford et al., J Agric Food Chem
2	9.01	430	Flavonoid glycoside	Flavonoid	Cuyckens & Claeys, J Mass Spectrom
3	9.717	210	Phenolic derivative	Phenolic	Harborne, *Phytochemical Methods*
4	11.51	269	Apigenin $[M+H]^+$	Flavonoid	Zhou et al., Food Chem
5	14.663	354	Luteolin-O-glycoside	Flavonoid glycoside	Fabre et al., Phytochemistry
6	17.402	297	Isorhamnetin fragment	Flavonoid	Wolfender et al., Phytochem Anal
7	21.739	391	Terpenoid derivative	Terpenoid	Wink, Plant Secondary Metabolites

Table 2 Compound list of *Cyamopsis tetragonoloba* pod extract in negative ionization mode

Peak no	RT (min)	Base peak (m/z)	Tentative compound	Compound class	Literature support
1	1.029	191	Quinic acid $[M–H]^-$	Organic acid	Clifford et al., J Agric Food Chem
2	10.481	269	Apigenin $[M–H]^-$	Flavonoid	Justesen, J Chrom A
3	11.338	329	Caffeoylquinic acid	Phenolic acid	Clifford et al
4	14.261	313	Chrysin derivative	Flavonoid	Harborne
5	15.816	295	Coumaric acid derivative	Phenolic acid	Mattila et al
6	16.691	297	Flavonoid fragment	Flavonoid	Fabre et al
7	19.826	279	Catechin/Epicatechin	Flavanol	Lin et al., Food Chem
8	20.586	255	Phenolic compound	Phenolic	MassBank

UV–visible studies

When silver nanoparticles (AgNPs) were isolated from *Cyamopsis tetragonoloba* pods, their absorption property was noted. The UV–visible spectrophotometer examination revealed the strong absorbance peak, which was linked to the Ag nanoparticles' surface plasmon resonance (Fig. 3). At 422 nm, absorption was detected with a stated value. The CTP-AgNPs has dual functions as a reducing and stabilizing agent. The presence of many bioactive compounds and their functional groups in the CTP-AgNPs may be the reason of the bio reduction and capping (Spiegel et al. 2020).

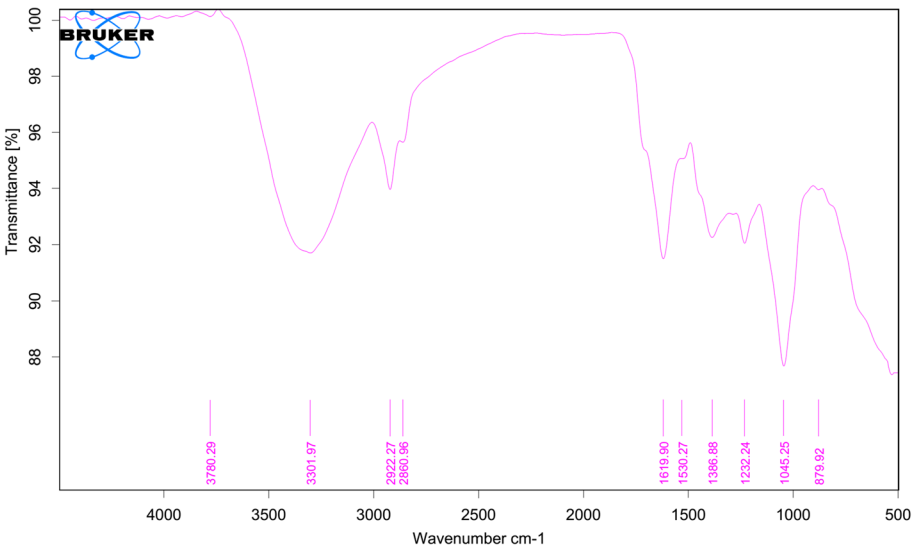

Fig. 2 FT-IR analysis of *Cyamopsis tetragonoloba* pod extract

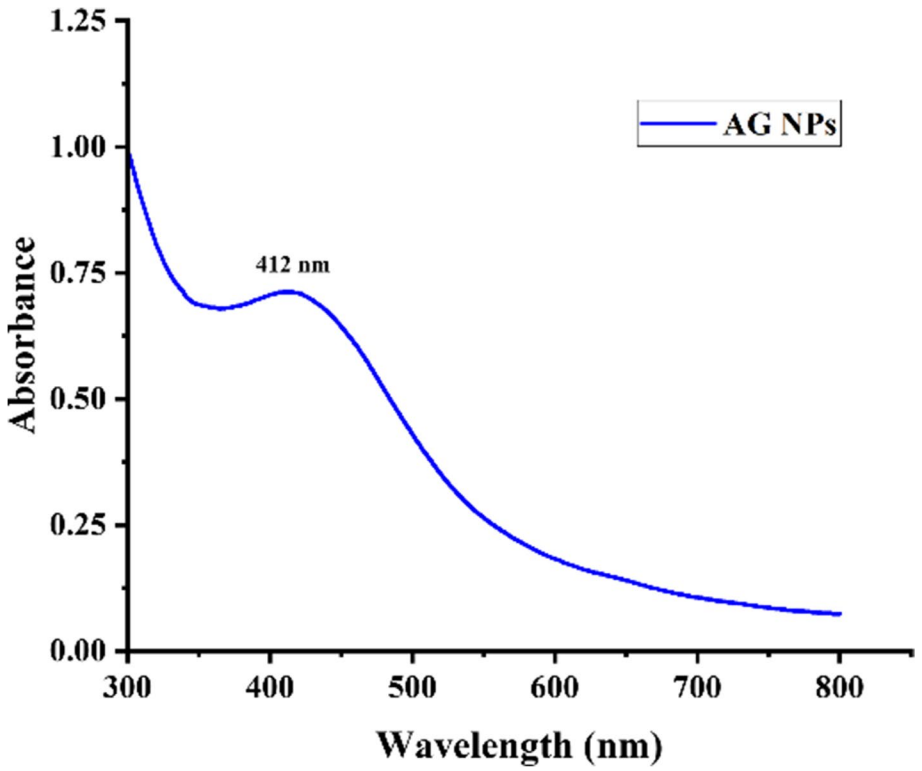

Fig. 3 UV Spectral Analysis of CTP-AgNPs

FT-IR studies

To determine which functional groups in *Cyamopsis tetragonoloba* are in charge of stabilizing and decreasing the produced nanoparticles (AgNPs), FT-IR analysis was performed. FT-IR analysis showed the spectral chart for functional groups identified in *C* CTP-AgNPs Amine or Amide (P1) 3411.35 cm^{-1} (N–H); Alkane (P2-P3) 2925.77- 2853.93 cm^{-1} (C-H); Carbonyl (P4) 1735.73 cm^{-1} (C = O); Alkene (P5- P6) 1642.03 −1416.97 cm^{-1} (C = C; Methane (P7) 1384.63 cm^{-1} (CH3). Nitrogen dioxide (P8) 1338.78 cm^{-1} (NO2); Fluorocarbon (P9- P10) 1272.84–1112.82 cm^{-1} (C-F); Methylene (P11) 876.81 cm^{-1} ($=CH_2$); Carbon chloride (P12) 780.66 cm^{-1} (C–Cl); Carbon bromide (P13) 621.72 cm^{-1} (C–Br) bonds. The spectrum of the CTP-AgNPs (Fig. 4) showed the broad peak is the higher energy area point out the existence of hydroxyl group in CTP. Whereas the sharp peaks indicate at could be due to stretching vibration C-H for alkenes, weak peaks ascribed to the unsaturated aromatic C = C (Nakamura et al. 2020). While C = O of esters, ketones (or) acids. The peaks ranging comes 621–780. The peaks in the spectrum of CTP-AgNPs was similar to that of plant extract, but the lower intensity indicates capping of the synthesized nanoparticles.

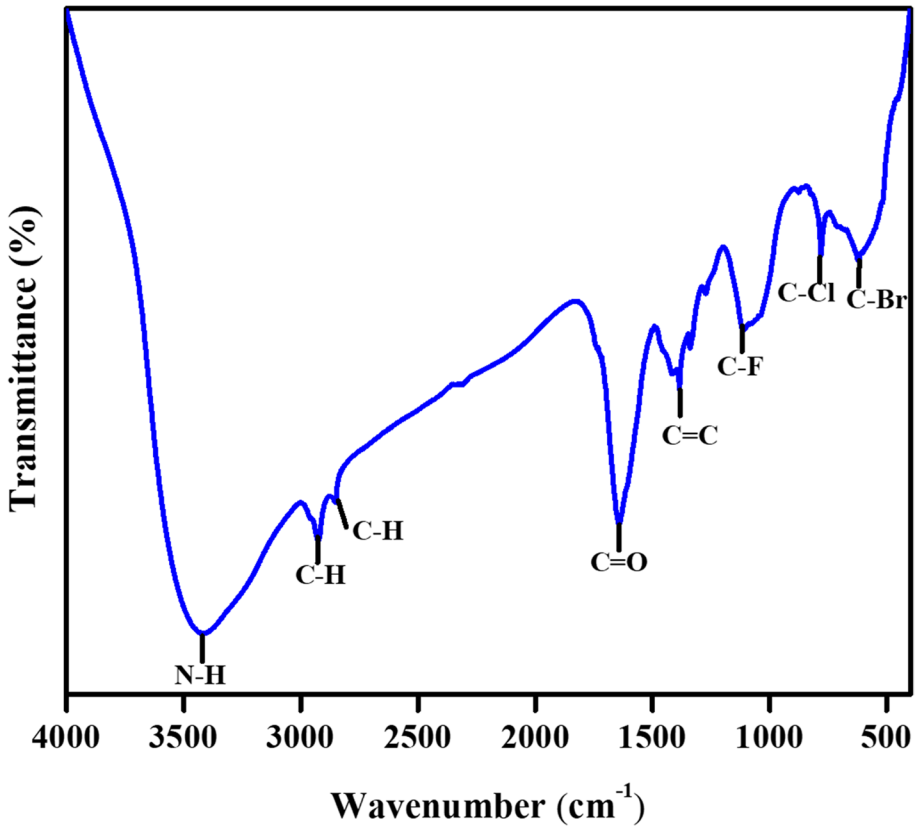

Fig. 4 FT-IR analysis of CTP-AgNPs

XRD studies

Similarly, X-ray diffraction (XRD) study revealed a number of characteristic diffraction peaks. These included the thin and sharp peaks at 2θ = 38.18º, 46.23º, 64.47º and 77.47º which were attributed to (111), (200), (220) and (311) respectively. Figure 5 displays the AgNPs' XRD pattern. The presence of sets of lattice plains was clearly indicated by various bragg reflections, and furthermore, they can be indexed as the Face Centred Cubic Centre (FCC) structure of AgNPs (JCPDS file no. 04 0783) (Pattnaik et al. 2023). Accordingly, the XRD pattern to clear AgNPs formed using *Cyamopsis tetragonoloba* pods extracts were essentially crystaline in nature. In addition to the braggs peak representative of FCC AgNPs, an additional unassigned peak was obtained, which suggested that the surface nanoparticles synthesized by the *C. tetragonoloba* extract is where the bioorganic phase crystallization occurs. The average crystallite size of synthesized AgNPs is 22.79 nm.

Morphological studies

The description to ascertain the size, shape, and structural morphology of green produced Ag nanoparticles, SEM examination is utilized. The result observation reveals the observed

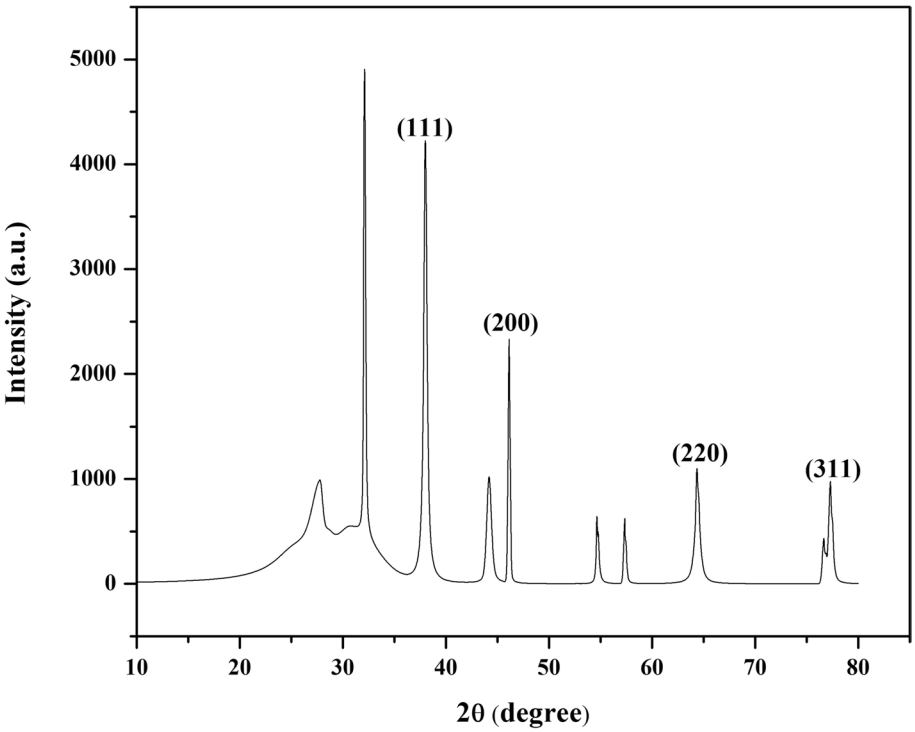

Fig. 5 XRD analysis of CTP-AgNPs

structural morphology of CTP-AgNPs was predominantly notified mostly polydispersed aggregation of spherical cuboidal and oval structure (Fig. 6). Free amino, carbohydrate, and hydroxyl groups that may be interested in the AgNPs surface could be the cause of the generated CTP-AgNPs stability. In addition, by creating separate, transparent structures, the medium's protein aids stabilize and inhibits agglomeration. According to the SEM analysis, the synthesized CTP-AgNPs average particle size was 37 nm. The average crystallite size of synthesized AgNPs was calculated using the XRD pattern and the Debye–Scherrer equation, which gave a value of 22 nm. However, the average physical particle size was observed using the SEM image, which gave a value of 37 nm. This is scientifically justified since the XRD measures the internal crystalline scattering domain, which is the metallic core. However, the larger particle size observed using the SEM image which may be the presence of amorphous non-crystalline phytochemicals on nanoparticles.

Antioxidant properties

AgNPs of *Cyamopsis tetragonoloba* demonstrated strong concentration-dependent antioxidant activity in the % of inhibition of DPPH/ascorbic acid, FRAP and HRSA. The AgNPs had a comparable reduction capacity from minimum to higher concentration (10–250 µg/mL) in different free radical scavenging activities measured when compared with the known standard.

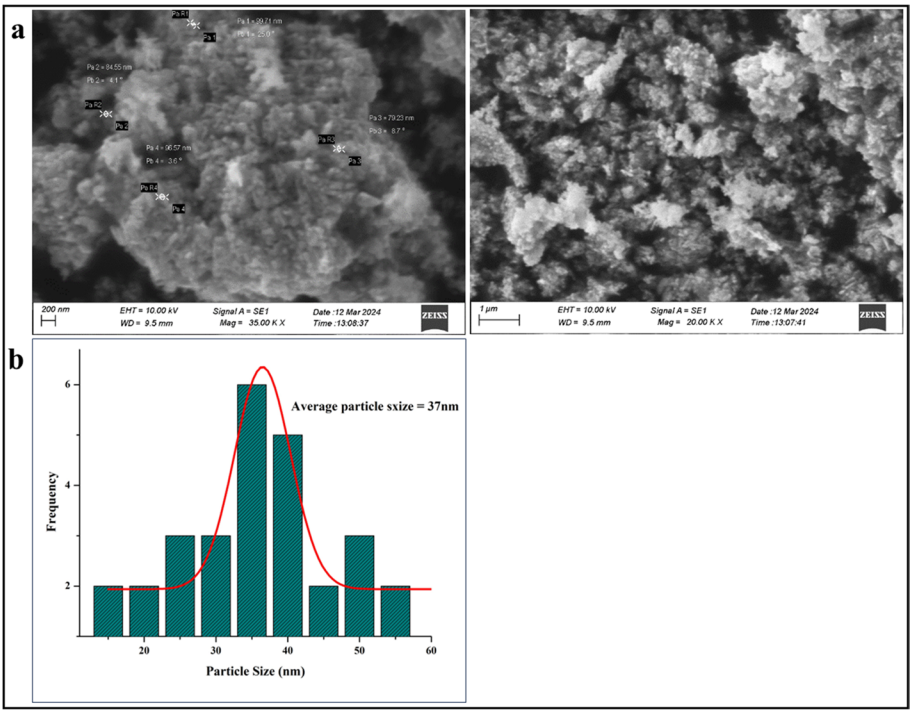

Fig. 6 SEM analysis of CTP-AgNPs

At higher concentrations of 200 µg/mL, 100 µg/mL, and 100 µg/mL, respectively, CTP-AgNPs and ascorbic acid shown significant scavenging effects on the DPPH (Fig. 7A), FRAP (Fig. 7B), and Hydroxyl Radical (Fig. 7C). For DPPH, it showed $39.21 \pm 0.46\%$ inhibition (CTP-AgNPs) and $87.1 \pm 0.54\%$ inhibition (Ascorbic acid); for hydroxyl radical scavenging activity, it showed $47.05 \pm 0.47\%$ inhibition (CTP-AgNPs) and $59.01 \pm 0.21\%$ inhibition (Ascorbic acid). The CTP-AgNPs IC50 values revealed notable variations in DPPH (183.61 µg/mL) in comparison to the ascorbic acid standard (25.7 µg/mL) and hydroxyl radical activity (53.6 µg/mL) in comparison to ascorbic acid (22.47 µg/mL). A lower IC_{50} value is linked to a stronger capacity to scavenge free radicals (Muthukrishnan et al. 2023). The CTP-AgNPs demonstrated a strong ability to scavenge free radicals in a concentration-dependent manner.

Antibacterial activity

Depending on the concentration (25–100 µg/mL), these CTP-AgNPs demonstrated positive inhibitory effect; nonetheless, they were still less efficient than conventional antibiotics. CTP-AgNPs have demonstrated antibacterial properties against strains of bacteria, including *E. coli* and *Bacillus* sp (Fig. 8). The inhibitory zones (measured in Mm) that developed by the nanoparticles and the positive control (tetracycline antibiotic) are displayed in Table 3. The presence of phytochemicals that function as reducing and stabilizing agents during the eco-friendly synthesis of AgNPs results in enhanced bactericidal properties (Khan et al. 2025; Murugan et al. 2025).

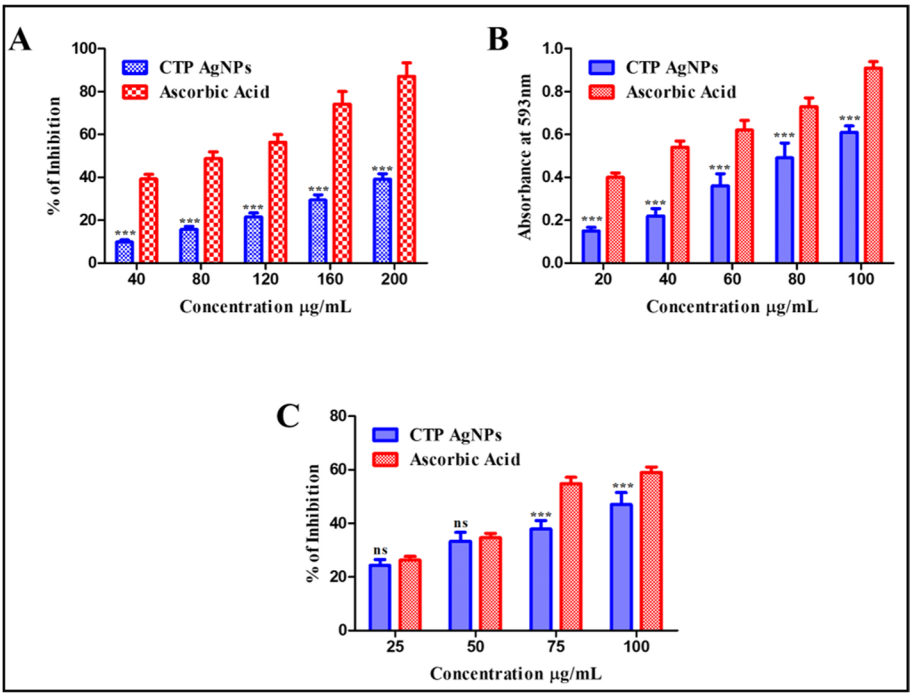

Fig. 7 In vitro radical scavenging activity of CTP-AgNPs synthesized using *Cyamopsis tetragonoloba* pod extract. A. The scavenging rate of CTP AgNPs to DPPH, B. The scavenging rate of CTP-AgNPs to FRAP and C. The scavenging rate of CTP-AgNPs to hydroxyl radical. The statistically significant levels were ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, $^{ns}p > 0.05$

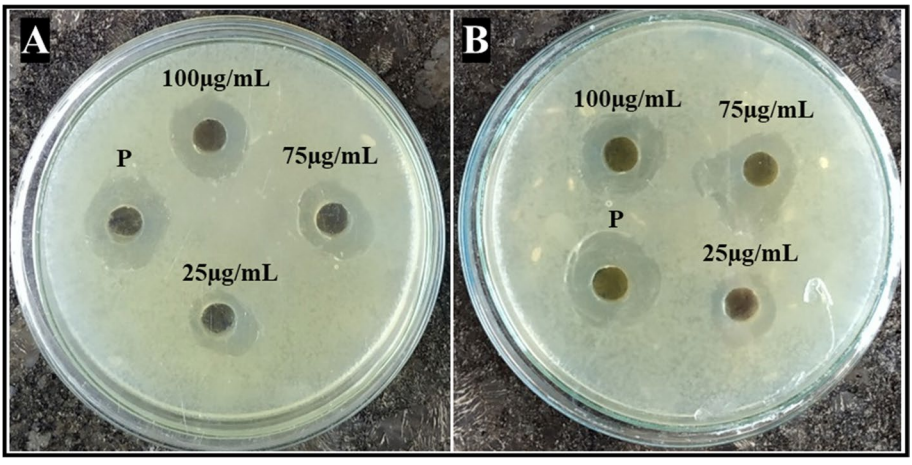

Fig. 8 Antibacterial effect of CTP-AgNPs against human bacterial pathogens; **A**) *E. coli* and **B**) *B. subtilis*. P- positive control

Table 3 Antibacterial Activity of Biosynthesized CTP-AgNPs Antibacterial effect by proving zone of inhibition of CTP-AgNPs against two human bacterial pathogens

Pathogens	Concentration (µg/mL)/ Inhibition zone (mm)			
	25	75	100	Standard (Tetracycline)
E. coli	$6 \pm 0.21^{***}$	$9 \pm 0.48^{***}$	11 ± 0.79^{ns}	12 ± 0.31
B. subtilis	$6 \pm 0.27^{***}$	$9 \pm 0.86^{***}$	12 ± 0.78^{ns}	13 ± 0.69

The statistically significant levels were ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, $^{ns}p > 0.05$

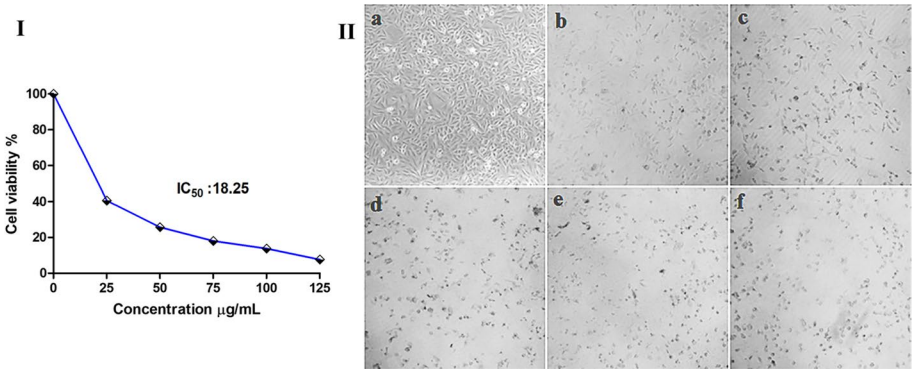

Fig. 9 Cytotoxicity activity of Synthesized AgNPs. I. Cell viability results with IC_{50} value. II. Cells after exposure to varying concentrations of nanoparticle, highlighting dose-dependent cytotoxicity against A549 cells. **a**) Control, **b**) 25, **c**) 50, **d**) 100, **e**) 150 and **f**) 200 µg/mL

Cytotoxic property of AgNPs

The cytotoxic effects of silver nanoparticles (AgNPs) were assessed using A549 cell lines across a concentration range of 25–200 µg/ml. The findings demonstrated a clear concentration-dependent response in the treated cells, particularly at higher concentrations (Fig. 9 II). Regarding cellular mortality, an AgNP concentration of 18.25 µg/ml was adequate to achieve 50% cell death (Fig. 9 I). The cellular responses to nanoparticles in vitro are influenced by both the specific cell line utilized and the physicochemical characteristics of the nanoparticles themselves. Accumulating research evidence suggests that environmentally-synthesized or biogenic nanomaterials show promise for diverse biomedical uses, including oncological applications, with reduced adverse effects (Saravanan et al. 2019; Sati et al. 2025; Keskin et al. 2025). Critical parameters including particle dimensions, morphology, surface area, aggregation behavior, purity levels, dissolution characteristics, stabilizing agents, surface electrical properties, structural modifications, and bioavailability all contribute to the anticancer efficacy of nanoparticles (Jabeen et al. 2025; Barabadi et al. 2019). The surface characteristics of nanoparticles, encompassing their composition, geometry, functionalization, and electrical charge, play crucial roles in determining their cytotoxic potential against cancer cells (Barabadi et al. 2018; Rasmussen et al. 2018).

Conclusion

When the bio reduction process began, the UV spectral peak at 400–450 nm was sharp and stable, highlighting the efficient synthesis of AgNPs, create a strong capping of nanoparticles in the extract of *Cyamopsis tetragonoloba* pods with silver nitrate. FT-IR analysis revealed the CTP-AgNPs exhibited thirteen peaks a wide range of functional groups namely Amine or Amide, Alkane, Carbonyl, Alkenes, Methane, Nitrogen dioxide, Fluorocarbon, Methylene, Carbon chloride respectively. The structural morphology of the formation in CTP-AgNPs made from the extract of *Cyamopsis tetragonoloba* pods was clearly visualized by the SEM analysis. CTP-AgNPs demonstrated significant antioxidant radical scavenging action in vitro, while DPPH demonstrated high antioxidant capability. All examined bacterial strains (*E. coli B. Subtilis*) were found to be susceptible to the antibacterial activity of CTP-AgNPs. Significantly, our research reveals that silver nanoparticles (AgNPs) exhibit toxic effects against human lung adenocarcinoma A549 cells, leading to cellular mortality. Consequently, we suggest that subsequent investigations involving these nanoparticles should incorporate animal model studies and comprehensive safety assessments for potential therapeutic applications. Overall, the CTP mediated bio-synthesized AgNPs proved promising antioxidants by free radical scavenging, antimicrobial by zone of inhibition and anticancer by cytotoxicity properties.

Author contribution Braivy Anto: conceptualization, methodology, data curation, investigation, formal analysis, writing Suriyavathana Muthukrishnan: conceptualization, methodology, investigation, editing Anandhi E.: methodology, data curation, investigation, formal analysis, writing Thamaraiselvi Ganesan: methodology, data curation, investigation, formal analysis, writing Indumathi K.P.: methodology, data curation, investigation, formal analysis, writing Rasiravathanahalli Kaveriyappan Govindarajan: methodology, data curation, investigation, formal analysis, writing Sivakumar Murugesan: conceptualization, methodology, investigation, editing Sivakumar Loganathan: methodology, data curation, investigation, formal analysis, writing Marcello Iriti: conceptualization, methodology, investigation, editing, supervision.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval None.

Conflict of interest The authors declare no competing interests.

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