

Silver nanoparticles green-synthesized ethanol-water extract of *Cleistocalyx operculatus* buds and their antimicrobial activities

Dien Trung Nguyen

Can Tho University

Thi Hong Nguyen (✉ nthong43@ctu.edu.vn)

Can Tho University

Hai Yen Hoang

Can Tho University

Thanh Huy Tran

Can Tho University

Thi Tuyet Nhung Thai

Can Tho University

Research Article

Keywords: Antimicrobial activity, Ethanol-water extract, Green synthesis, Silver nanoparticles, *Cleistocalyx operculatus* buds

Posted Date: May 10th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2739817/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Chemical Papers on August 15th, 2023. See the published version at <https://doi.org/10.1007/s11696-023-03023-8>.

1 Silver nanoparticles green-synthesized ethanol-water extract of *Cleistocalyx* 2 *operculatus* buds and their antimicrobial activities

3

4 Trung Dien Nguyen¹, Hong Thi Nguyen^{*2}, Yen Hai Hoang¹, Huy Thanh Tran¹, Nhung Tuyet-Thi Thai¹

5

6 ¹College of Natural Sciences, Can Tho University, 3/2 street, Ninh Kieu, Can Tho, 94000, Vietnam

7 ²School of Education, Can Tho University, 3/2 street, Ninh Kieu, Can Tho, 94000, Vietnam

8

9 *Corresponding author: Hong Nguyen-Thi; E-mail: nthong43@ctu.edu.vn; ORCID iD: 0000-0002-3777-0629

10

11

Abstract

12

13 In the current study, using *Cleistocalyx operculatus* bud extracts to synthesize silver nanoparticles is considered
14 a novel, eco-friendly, and low-priced process that advances chemical and physical methods. *Cleistocalyx operculatus*
15 bud extracts played a dual part in reducing and stabilizing silver nanoparticles. The formation of silver nanoparticles
16 was recognized on UV-Vis spectra at an absorption maximum of 432 nm. Transmission electron microscopy images
17 detected the quasi-spherical nanoparticle shape with an average size of 26.2 nm corresponding with water extract and
18 27.5 nm synthesized by ethanol-water extract. The obtained silver nanoparticles had moderate stability with zeta
19 potential ranging from -15.4 to -38.4 mV. The phase purity of the bio-synthesized materials was confirmed by an X-
20 ray diffraction pattern. The Fourier-transform infrared analysis demonstrated hydroxyl groups acting as stabilizing
21 agents in the extract. Silver nanoparticles synthesized from ethanol-water extract exhibited higher antibacterial and
22 anticancer activity than nanomaterials prepared with water extract. Positive-gram bacteria (*Bacillus subtilis*,
23 *Lactobacillus fermentum*, and *Staphylococcus aureus*, negative-gram bacteria (*Escherichia coli*, *Pseudomonas*
24 *aeruginosa*, and *Salmonella enterica*), and cancer cell lines (lung cancer A549, hepatic cancer Hep-G2, epidermal
25 carcinoma KB, and breast cancer MCF-7) were both inhibited by the nanoparticles synthesized with ethanol-water
extract.

26 **Keywords:** Antimicrobial activity, Ethanol-water extract, Green synthesis, Silver nanoparticles, *Cleistocalyx*
27 *operculatus* buds

28 Introduction

29 Nowadays, nanoscience is a speedily evolving field that has contributed to producing a wide range of various
30 synthesized metal nanoparticles. Metal nanoparticles are widely applied in medicine, environmental remediation, and
31 drug delivery. For instance, gold nanoparticles were recognized as safe nanocarriers widely used in drug delivery (Yang
32 et al. 2022); palladium nanoparticles were not only applied in catalysis to replace platinum nanoparticles but also were
33 materials to inhibit bacterial/cancer cells (Joudeh et al. 2022). In comparison, copper nanoparticles were used as
34 pesticides and fertilizers in agricultural activities (Hemmati et al. 2020; Bakshi et al. 2021). Due to bactericidal and
35 fungicidal activity, silver has been widely researched for synthesizing at the nanoscale and controlling microbial
36 proliferation. Generally, physical and chemical approaches were used to produce silver nanoparticles (AgNPs). Pure
37 phase and simple operation are considered outstanding advantages of these methods. However, the high cost, energy
38 consumption, and hazardous effects on the environment are considered the primary disadvantages (Ijaz et al. 2020).
39 Recently, the biogenic synthesized AgNPs have become essential for nanoparticle formation with a simple, rapid, and
40 eco-friendly route. Plant-based AgNPs were formed with phytochemicals in the extracts, including pectin, vitamins,

phenolics, and flavonoids, as reductants and stabilizers. Tyagi used *Tagetes erecta* leaf-enriched ascorbic acid and polyphenol compounds to produce AgNPs under sunlight irradiation for 30 min (Tyagi et al. 2021). Pradeep reported that AgNPs biosynthesis using *Hypericum perforatum* containing phenolic acids and flavonoids acted as efficient silver-reducing and capping agents after 5 days of reaction (Pradeep et al. 2022). Similarly, phenolics and flavonoids in the *Linacanthus nutans* leaf and stem were suggested as the main phytochemicals responsible for reducing Ag⁺ ions. Nevertheless, the presence of flavonoids and pectin in *Citrus maxima* peel extract and sunlight assisted in forming and stabilizing AgNPs (Nguyen 2020). Consequently, using plant extracts for reducing Ag⁺ provided an environmentally low-priced technology for high-scale production.

Bacteria are considered a significant threat facing medical remediation since the beginning of antibiotic-resistant bacterial due to their evolution. Drug resistance began as a complicated problem caused by the over-treatment of antibiotics and drugs in curing infectious diseases (Holmes et al. 2016). Therefore, developing innovative drugs for treating microbial pathogens is an urgent solution. The freshly evolving nanotechnology accelerated the production of metal nanoparticles, especially AgNPs, with high antibacterial activity and nonhazardous to humans at low concentrations (Xu et al. 2021). When used in low dosages, the non-toxicity of the bio-synthesized AgNPs grows to be an interesting issue for medical biotechnology. AgNPs may be a substitute for antibiotic drugs presenting better impacts on multidrug-resistant bacteria. Due to the high surface volume ratio and binding activity, nanoparticles were used as an anticancer drug (Morais et al. 2020). Banana leaf-mediated AgNPs synthesis has high potential in vitro cytotoxicity investigation in malignant cancerous cells such as the lung cancer cell line A549 and breast cancer cell line MCF-7 (Raghavendra et al. 2021). AgNPs synthesized by *Mangifera indica* seed extract get a high cytotoxic effect against cervical cancer cell line HeLa and breast cancer cell line MCF-7; less cytotoxic effect on regular fibroblast cell line (Donga et al. 2021). Green synthesized AgNPs using *Heliotropium bacciferum* extract exhibit 50% cell inhibition of breast cancer cell line MCF-7 at 5.44 µg/mL AgNPs and colorectal carcinoma cell line HCT-116 at 9.54 µg/mL AgNPs (Khan et al. 2021). Comparably, AgNPs formed by *Zingiber officinale* leaf also showed effective anticancer against pancreatic cancer cell lines (PANC-1, AsPC-1, and MIA PaCa-2) without cytotoxicity activity against normal cell line HUVEC (Wang et al. 2021).

Cleistocalyx operculatus (*C. operculatus*), an edible plant, is typically distributed in Southeast Asia and contains high amounts of phenolic compounds. The primary phytochemical leaf and bud extract of *C. operculatus* were terpenoids, flavonoids, and phloroglucinols. *C. operculatus* leaves and flower buds were utilized for inflammatory conditions, such as bruises, acne, and skin ulcers (Pham et al. 2020). Although *C. operculatus* extract contained possible reducing chemicals, the use of *C. operculatus* extract in the AgNPs synthesis has rarely been reported. For example, *C. operculatus* leaf extract was also employed as a reducing agent for synthesizing AgNPs decorated on graphene surfaces (Thi et al. 2022) and reducing Ag⁺ ions to AgNPs on the TiO₂ surface (Nguyen et al. 2022).

This work concentrated on the plant extract-synthesized AgNPs from *Cleistocalyx operculatus* buds as a reducing and stabilizing agent. The influence of ethanol-water solvent used as extraction solvent on the AgNPs formation was also evaluated. Furthermore, the antimicrobial activities of synthesized AgNPs using water extract and ethanol-water extract were compared on positive-gram bacteria (*Bacillus subtilis*, *Lactobacillus fermentum*, and *Staphylococcus aureus*, negative-gram bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enterica*), and cancer cell lines (lung cancer A549, hepatic cancer Hep-G2, epidermal carcinoma KB, and breast cancer MCF-7).

Experimental

Materials

The precursor for the AgNPs formation was silver nitrate (AgNO₃, Merck), while double-distilled water/ethanol-water was considered a solvent for extracting. *Cleistocalyx operculatus* buds (*C. operculatus*) were collected from a

local grocery store. The extract was prepared using *C. operculatus* buds extracted in 100 mL double-distilled water/ethanol-water at T °C for t min. The heterogeneous solutions were refrigerated to room temperature, purified with Whatman filter paper, and centrifuged at 6800 rpm for 60 min to eliminate unsolvable solids. The resulting filtrate was stored in an amber-colored glass container in the dark, labeled as Ext-W (extraction in water) and Ext-E (extraction in ethanol-water), and stored at 5°C for the AgNPs synthesis. The obtained extract played both reductants and stabilizers for AgNPs synthesis.

Extract of C. operculatus buds assisted in the AgNPs formation

The extract of *C. operculatus* buds was used as a green, reducing, and stabilizing agent to synthesize AgNPs. Therefore, parameters, including the concentration of *C. operculatus* buds, extraction temperature, and time that affected the phytochemicals of the extract, were studied in detail. Silver nitrate (0.0085 g) was soluted into 50 mL of the extract to maintain an Ag⁺ concentration of 1 mM. The synthesized solutions were prepared in an amber-colored glass container to avoid reducing Ag⁺ ions by light irradiation and continuously stirred at room temperature. The pale yellow solutions were converted from yellow to red-orange with a gradually increasing reaction time, indicating that AgNPs formed (Soto et al. 2019). Investigating the effect of numerous parameters on the AgNPs formation, several experiments were carried out with varying concentrations of *C. operculatus* buds (2.5, 5.0, 7.5, 10.0, and 12.5 g/L), extraction temperatures (30, 40, 50, 60, and 70 °C), and extraction time (10, 20, 30, 40, and 50 min). AgNPs samples using water extract were denoted as AgNPs-W, while AgNPs-E samples were obtained from the ethanol-water extract.

Measurements

UV-visible absorption spectra (UV-Vis) of the biosynthesized AgNPs were recorded on a Jasco model V730 spectrophotometer in the 300-700 nm range. X-Ray diffraction (XRD) was analyzed on a Bruker D2 Phaser instrument with CuK_α excitation ($\lambda = 1.5418 \text{ \AA}$) operating at 40 kV and 40 mA. Zeta potential measurements of the synthesized solutions were carried out using a Zetasizer SZ-100 (Horiba, Japan) at 25 °C. Transmission electron microscopy (TEM) images of the prepared AgNPs were obtained using a JEOL model JEM-1400 instrument operating at 120 kV. The particle-size distribution of the produced AgNPs was measured by ImageJ software of TEM images and estimated Origin 9.0 with the Gauss method. Nicolet 6700 Fourier transform infrared (FTIR) instrument was used to verify the presence of functional groups stabilized by the as-prepared AgNPs. The efficiency synthesis was determined by converted Ag⁺ concentration using the inductively coupled plasma optical emission spectroscopy (ICP-OES).

Analysis of phenolic components in the extract

The presence of phenolic in the *C. operculatus* bud extracts was quantified by the Folin-Ciocalteu method with alteration (Chandra et al. 2014). The samples containing 250 μL of extract, 250 μL of water, and 250 μL of Folin-Ciocalteu phenol reagent were prepared, respectively. After stirring and maintaining room temperature for 5 min, 250 μL of 10% w/w Na₂CO₃ solution was introduced, followed by heating up at 50 °C for 20 min. The absorbance of the analyzed sample was determined on UV-Vis spectra at a wavelength of 765 nm. The water-soluble and ethanol-water-soluble phenolic content was shown in mg gallic acid equivalents (GAE) per g of *C. operculatus* buds. Each test was accomplished thrice for repetitions; means and standard deviations were calculated using excel software.

Antibacterial activity

The reference bacterial strains used for the antibacterial assay were positive grams: *Bacillus subtilis* ATCC 6633 (*B. subtilis*), *Lactobacillus fermentum* N4 (*L. fermentum*), *Staphylococcus aureus* ATCC 13709 (*S. aureus*) and negative grams: *Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 15442 (*P. aeruginosa*), and *Salmonella enterica* ATCC 35664 (*S. enterica*) using the well-diffusion method. Tryptic soy broth and tryptic soy agar were employed for bacteria. The pathogenic cultures were subcultured and incubated at 37 °C for 24 h to attain 5×10^5

CFU/mL using MacFarland's standard. Ampicillin and cefotaxime were used to control positive-gram and negative-gram bacteria, respectively. 96-well microplates evaluated the 50% inhibition concentration IC_{50} of the synthesized AgNPs and the absorbance was quantified at 630 nm.

Antiproliferative activity on cancer cell lines

Various categories of cancer cell lines, such as lung cancer (A549), hepatic cancer (Hep-G2), epidermal carcinoma (KB), and breast cancer (MCF-7), were assessed for the proliferative effect of the synthesized AgNPs. Dulbecco's modified Eagle's medium (DMEM), complemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, was accustomed to subculture the cell lines in an incubator with 5% CO_2 . Cells were stained with trypan blue and living cells were counted on a hemocytometer. The density of cancer cells was calibrated to 3×10^4 CFU/well and the suspension was seeded into a 96-well plate for 72 h.

A549, Hep-G2, KB, and MCF-7 cell lines were tested with varied concentrations: 0.12, 0.47, 1.88, and 7.50 μ M for biosynthesized AgNPs-W while concentrations of AgNPs-EW were 0.15, 0.59, 2.38, and 9.50 μ M. Dimethyl sulfoxide (DMSO) was used as the solvent to dilute the concentrations. After treatment, the cells were incubated for 72 h with 5% CO_2 . After that, 100 mg/mL of the MTT reagent was added, and the mixture was incubated at 37 $^{\circ}C$ for 4 h. The generated formazan was solubilized by adding 100 μ L of DMSO. In this experiment, Ellipticine was used as a control sample. The absorbance was quantitatively measured at 540 nm on a 96-well plate.

The percentage of the bacteria/cell growth inhibition was calculated as follows (Kamiloglu et al. 2020)

$$I = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100 \quad (1)$$

where I is the percentage of cell growth inhibition. A_{sample} , A_{blank} , and A_{control} are the absorbance of the tested, blank, and control samples, respectively.

Each test in the present investigation was performed thrice for repetitions. Means and standard deviations were calculated using GraphPad Prism software.

Results and Discussion

Synthesizing AgNPs with water extract of *C. operculatus* buds (AgNPs-W)

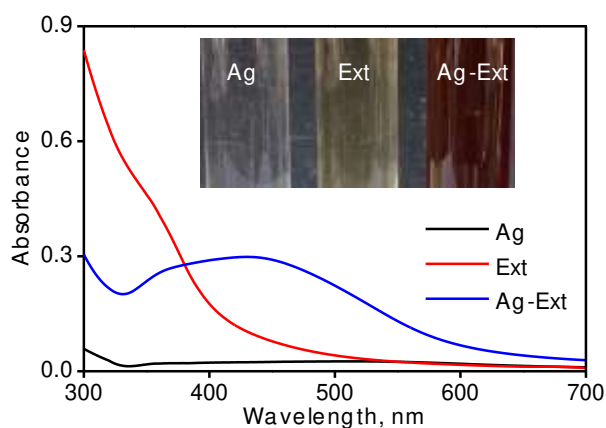


Figure 1. The UV-visible spectra of $AgNO_3$ solution (Ag), water extract of *C. operculatus* buds (Ext), and $AgNO_3$ dissolved water extract of *C. operculatus* buds (Ag-Ext).

To investigate the reducing/stabilizing role of *C. operculatus* buds extract, several samples such as 1 mM $AgNO_3$ solution (Ag), water extract of *C. operculatus* buds (Ext), and $AgNO_3$ dissolved with water extract of *C. operculatus* buds (Ag-Ext) were prepared. After stirring at ambient temperature for 24 h, all as-prepared samples were recorded from 300 to 700 nm on a UV-Vis spectrophotometer. The AgNPs bio-fabrication could be determined by a

155 chromatic transformation of the reaction substrate since the color conversion from yellowish to brown-orange indicated
 156 a reduction of Ag^+ ion to Ag^0 (Figure 1). The formation of AgNPs surface plasmon at 432 nm with an intensity of 0.299
 157 on Ag-Ext. These results agreed well with the results in the literature that indicated the characteristic peak at 434 nm
 158 belongs to the AgNPs formation (Erdogan et al. 2019). Adsorption peak was absent in the case of *C. operculatus* buds
 159 extract (Ext) and AgNO_3 solution (Ag). The bioreduction of AgNPs involved phytochemicals such as alkaloids,
 160 phenolics, terpenoids, flavonoids, amino acids, and alcohol (Shaikh et al. 2021). Therefore, the current result may be
 161 interpreted based on the reducing effect of phenolics, quantified to 2.43 ± 0.12 mg GAE/g for the 7.5 g/L of *C.*
 162 *operculatus* bud water extract. For optimizing the AgNPs synthesis, the following influential factors: concentrations of
 163 *C. operculatus* buds water extract (2.5, 5.0, 7.5, 10.0, and 12.5 g/L), extraction temperature (30, 40, 50, 60, and 70 °C),
 164 and extraction duration (10, 20, 30, 40, and 50 min) were optimized in detail.

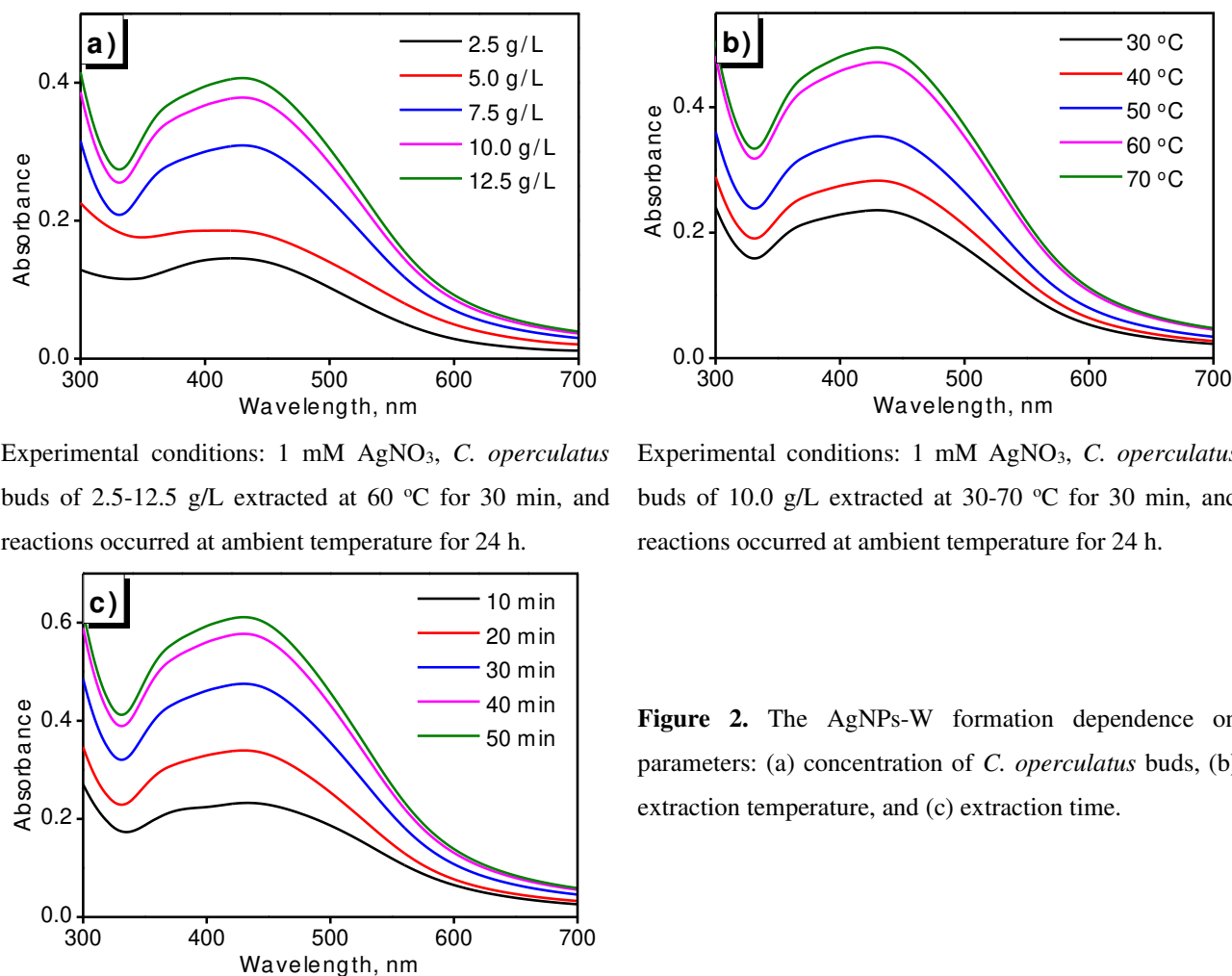


Figure 2. The AgNPs-W formation dependence on parameters: (a) concentration of *C. operculatus* buds, (b) extraction temperature, and (c) extraction time.

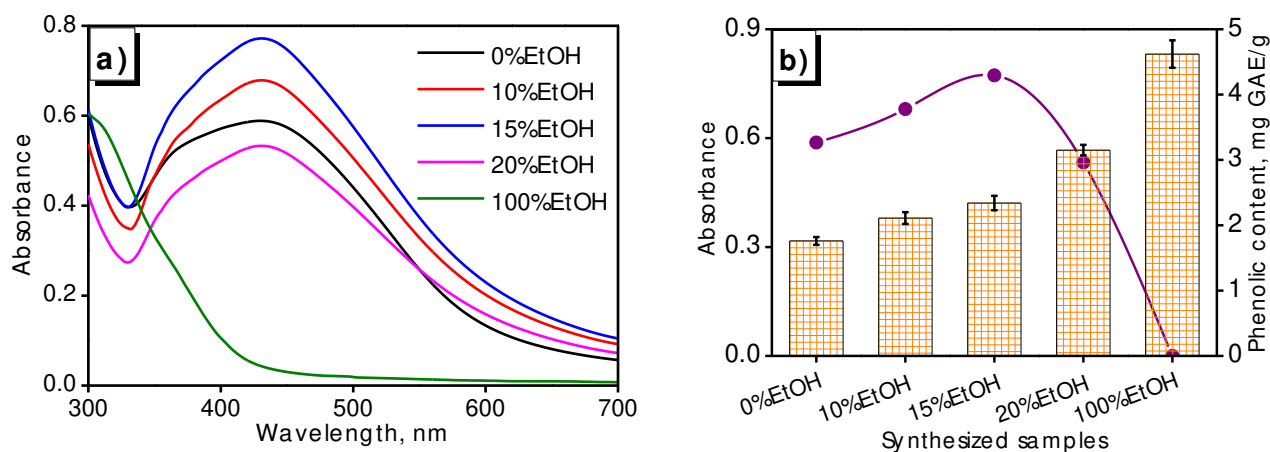
165 The concentration of *C. operculatus* bud extract is a vital parameter affecting the AgNPs formation. To optimize
 166 a suitable concentration of *C. operculatus* bud extract, the influence of *C. operculatus* bud extract on the AgNPs
 167 synthesis was studied in a range of 2.5-12.5 g/L. The UV-Vis spectra of the obtained with several *C. operculatus* bud
 168 concentrations were illustrated in Figure 2a. The absorbance intensity enhanced and shifted to a lower wavelength as *C.*
 169 *operculatus* buds increased from 2.5 to 12.5 g/L. The lack of reducing/stabilizing agents was at low extract
 170 concentrations, leading to little AgNPs formation and aggregation. In contrast, more AgNPs were formed in the
 171 presence of numerous reductants, and the formed AgNPs were also well stabilized by stabilizers at high extract

172 concentrations (Mishra et al. 2020). Therefore, the low absorbance intensity of AgNPs peak was observed at lower than
 173 5.0 g/L of *C. operculatus* bud extract. In comparison, the high values reached the range concentrations of *C.*
 174 *operculatus* bud extract from 7.5 to 12.5 g/L. However, there was a slight rise in intensity when the concentration of *C.*
 175 *operculatus* buds shifted from 10-12.5 g/L. Based on the results in Figure 2a, 10 g/L of *C. operculatus* buds was
 176 selected as an optimal value in additional tests.

177 Figure 2b showed the UV-Vis spectra of the obtained AgNPs at various extraction temperatures (30-70°C) for 30
 178 min. At the same time, the concentration of the *C. operculatus* bud extract was kept constant at 10 g/L and reaction
 179 mixtures were conducted at ambient temperature for 24 h. It was observed that 60-70 °C was a beneficial temperature
 180 for preparing extract with an AgNPs absorbance of 0.470 compared to different temperatures. Generally, the solubility
 181 of the total phenolics increased as the extraction temperature increased, resulting in more phenolic contents being
 182 extracted. Therefore, numerous reductants and stabilizers were produced at 60-70 °C, leading to high absorbance for
 183 samples synthesized with *C. operculatus* bud extracted. The results were similar to those reported in the literature,
 184 which showed that higher than 50 °C was enough temperature to extract total phenolic contents (Liaudanskas et al.
 185 2017; Zhong et al. 2019). Nevertheless, there was an insignificant difference in the AgNPs absorbance between the
 186 samples prepared at 60 and 70 °C. Thus, the suitable temperature for extracting *C. operculatus* bud to synthesize AgNPs
 187 was 60 °C in the present study.

188 Another factor affecting the extraction efficiency of phenolics was the extraction time, so the extraction
 189 duration's influence on the AgNPs formation was also studied in the range of 10-50 min. A considerable increase in the
 190 absorbance of the bio-synthesized samples was observed by prolonging the extraction duration in a range of 10-50 min
 191 (Figure 2c). It means increasing the extraction time from 10 to 50 min resulted in forming more phytochemicals to
 192 produce a higher quantity of AgNPs. This trend was similar to a study by Vishwasrao on the *Kalipatti sapota* fruit for
 193 synthesizing AgNPs (Vishwasrao et al. 2019). To create AgNPs efficiently, the appropriate duration selected for
 194 extracting was 40 min in the present work. Based on the obtained results from Figure 2, it can be concluded that the
 195 optimal parameters were carefully chosen for synthesizing AgNPs-W as 10.0 g/L of *C. operculatus* buds extracted at 60
 196 °C for 40 min.

197 **Synthesizing AgNPs with ethanol-water extract of *C. operculatus* buds (AgNPs-E)**



198 **Figure 3.** The AgNPs-E formation dependence on ethanol-water extracts (EtOH). Experimental conditions: 1 mM
 199 AgNO₃, 10.0 g/L of *C. operculatus* bud ethanol-water extracted at 60 °C for 40 min, and reactions occurred at ambient
 200 temperature for 24 h.

201 López-Miranda extracted *Sargassum* in three solvents: water, ethanol, and ethanol-water for synthesizing
 202 AgNPs. The obtained indicated that ethanol-water was more favorable for the AgNPs formation than water and the
 203 nonpresence of AgNPs used ethanol extract. According to López-Miranda, nanoparticles obtained from the ethanol-

water extract had a three-time higher concentration than the water extract due to a higher content of reducing and stabilizing agents (López-Miranda et al. 2021). In another study by Rahman, the investigation of AgNPs synthesis from ethanol-water extracts using *Phyllanthus niruri* showed that the presence of ethanol increased the solubility of phytochemicals in the extract. However, a large amount of ethanol was disadvantageous for forming AgNPs (Rahman et al. 2022). The AgNPs-E formation dependence on ethanol-water extracts was demonstrated in Figure 3a. There was a similarity to the observations of López-Miranda and Rahman with this present work. The presence of ethanol produced more phenolics in the extract (Figure 3b). When the phenolic content exceeded, it was unfavorable for forming AgNPs due to the competition of the complexation process between Ag⁺ ions and phenolics. The highest AgNPs concentration at 15% ethanol-water (v/v) corresponded to 2.34 mg GAE/g, the AgNPs concentration decreased at 20% ethanol-water in the presence of phenolics of 3.15 mg EAG/g, and the absence of AgNPs was recognized on a sample containing 100% ethanol-water (4.62 mg EAG/g of phenolics). The formation of AgNPs and complexes in the presence of phenolics was illustrated in Figure 4. Gallic acid was characterized as a typical substance for the phenolics present in the extracts of *C. operculatus* bud. The coordination of Ag⁺ ions with gallic acid was involved in the carboxylate and adjacent hydroxyl groups (Frešer et al. 2021; Fedenko et al. 2022). Temporarily, the reduction of Ag⁺ ions into Ag⁰ was due to phenolic hydroxyl groups (Ismail et al. 2019). The obtained results proposed that 15% ethanol-water was a suitable solvent for AgNPs formation from *C. operculatus* bud. The AgNPs formed from 1 mM AgNO₃ and 10.0 g/L of *C. operculatus* bud 15% ethanol-water were denoted as AgNPs-E.

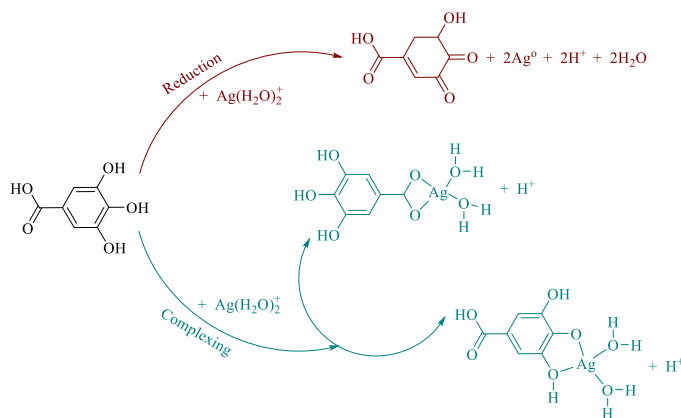


Figure 4. Competition between reduction and complexation of Ag⁺ ions with gallic acid.

The synthesis efficiency of AgNPs using water and ethanol-water extract was determined at the optimal conditions following formula (2) and the calculated values were presented in Table 1. The synthesis efficiency for AgNPs-W was 59.5% and 75.6% was obtained from AgNPs-E green-synthesis. From Table 1, more reducing agents are produced for reducing Ag⁺ ions to AgNPs in the ethanol-water extract, improving the synthesis efficiency contrasted with water extract.

$$H = \frac{C_o - C}{C_o} \times 100 \quad (2)$$

where H is the efficient synthesis of AgNPs; C_o and C are Ag⁺ concentrations in the precursor and the bio-synthesized AgNPs solution, respectively.

Table 1. Ag⁺ concentration in the precursor and bio-synthesized solution.

Solution	Ag ⁺ concentration, mg/L	Synthesis efficiency, %
Precursor	104.9	
AgNPs-W	42.5	59.5
AgNPs-E	25.6	75.6

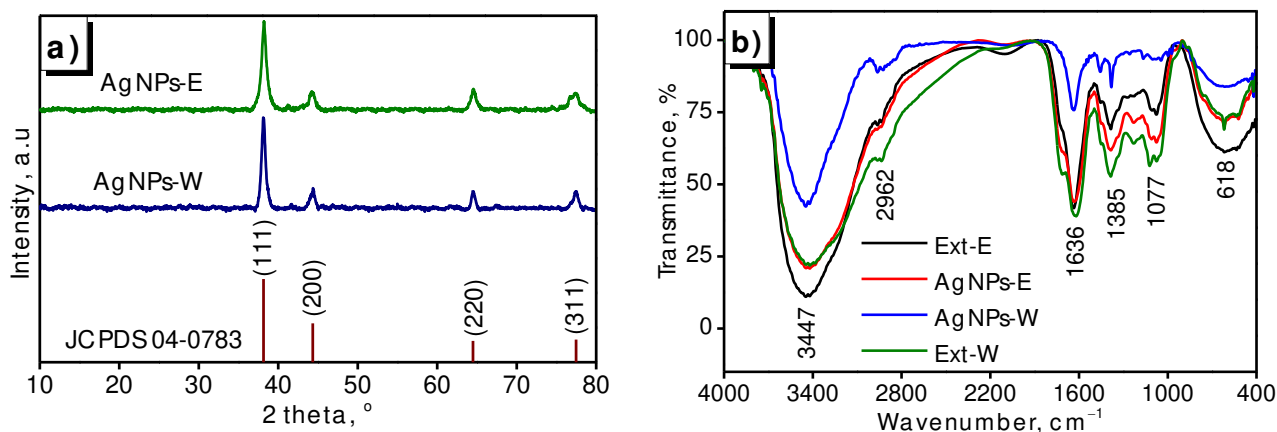


Figure 5. XRD patterns (a) and FTIR spectra (b) of the synthesized AgNPs: the water extract of *C. operculatus* buds (Ext-W), the ethanol-water extract of *C. operculatus* buds (Ext-E).

Characteristics of AgNPs-E/AgNPs-W and their antibacterial/anticancer activity

Table 2. The phase composition and crystallite size of the bio-synthesized AgNPs-W and AgNPs-E.

Sample	Position 2θ, °	FWHM	Particle size, nm	Average particle size, nm
AgNPs-W	38.15	0.555	15.1	16.4
	44.32	0.628	13.7	
	64.55	0.443	21.2	
	77.43	0.650	15.7	
AgNPs-E	38.15	0.645	13.0	12.8
	44.32	0.693	12.4	
	64.55	0.618	15.2	
	77.43	0.964	10.6	

XRD patterns studied the structures of AgNPs-W and AgNPs-E. The XRD pattern with 2θ values extending in a range of 10-80° symbolized four peaks involving diffraction from the (111), (200), (220), and (311) planes (Figure 5a). Diffraction of the (111) plane on samples: AgNPs-W and AgNPs-E was also greatest. The peaks were indexed based on silver's face-centered cubic (FCC) lattice and were completely consistent with the standard data JCPDS No 04-0783. The average crystallite size was assessed from the FWHM of the diffraction peak by applying the Debye-Scherrer formula

$$D = \frac{K \times \lambda}{\beta \times \cos \theta} \quad (3)$$

where K represents Scherrer's constant ($K = 0.94$), D signifies crystallite size, β expressed in radians is the full width at half maximum (FWHM) of the peak, and θ denotes the position of the diffraction peak. The FWHM was estimated from the Gaussian function fitted with four peaks (111), (200), (220), and (311) by the software Origin 9.0. The average crystallite size ranged from the highest for AgNPs-W (16.4 nm) to the lowest for AgNPs-E (12.8 nm) in Table 2.

Figure 5b showed the FTIR spectra of the purified AgNPs and the extract of *C. operculatus* buds by different solvents. The purified nanoparticles exhibited absorption peaks at 1077, 1385, 1636, 2926, and 3447 cm^{-1} due to C–O–C symmetrical stretching, cyclic C–C=C, OH bending of water, C–H stretching, and O–H stretching, respectively (Elhawary et al. 2020). The peaks with slight shifts were observed in the purified AgNPs compared with extracts. After AgNPs formation, there was also a decline in peak intensity compared with the extract. From the FTIR spectra, it may be inferred that the phytoconstituents in the extract were in charge of the reduction. Further, the extracts formed a layer on the surface of the obtained AgNPs that prevented agglomeration (Abbai et al. 2016).

254 TEM images observed the shapes and sizes of the synthesized AgNPs in various synthetic solvents. The typical
 255 TEM of water-synthesized AgNPs-W and ethanol-water-synthesized AgNPs-E are shown in Figure 6a and 6b. The
 256 TEM images showed a more uniform formation of spherical on AgNPs-E than AgNPs-W. The average particle size was
 257 27.5 nm for AgNPs-W and 26.2 nm for AgNPs-E. The particle-size distribution of the synthesized solutions was also
 258 represented in Figure 6c and 6d. The particle size of the AgNPs-W sample ranged from 8 nm to 54 nm, while that of the
 259 AgNPs-E sample occupied sizes of 10 to 38 nm.

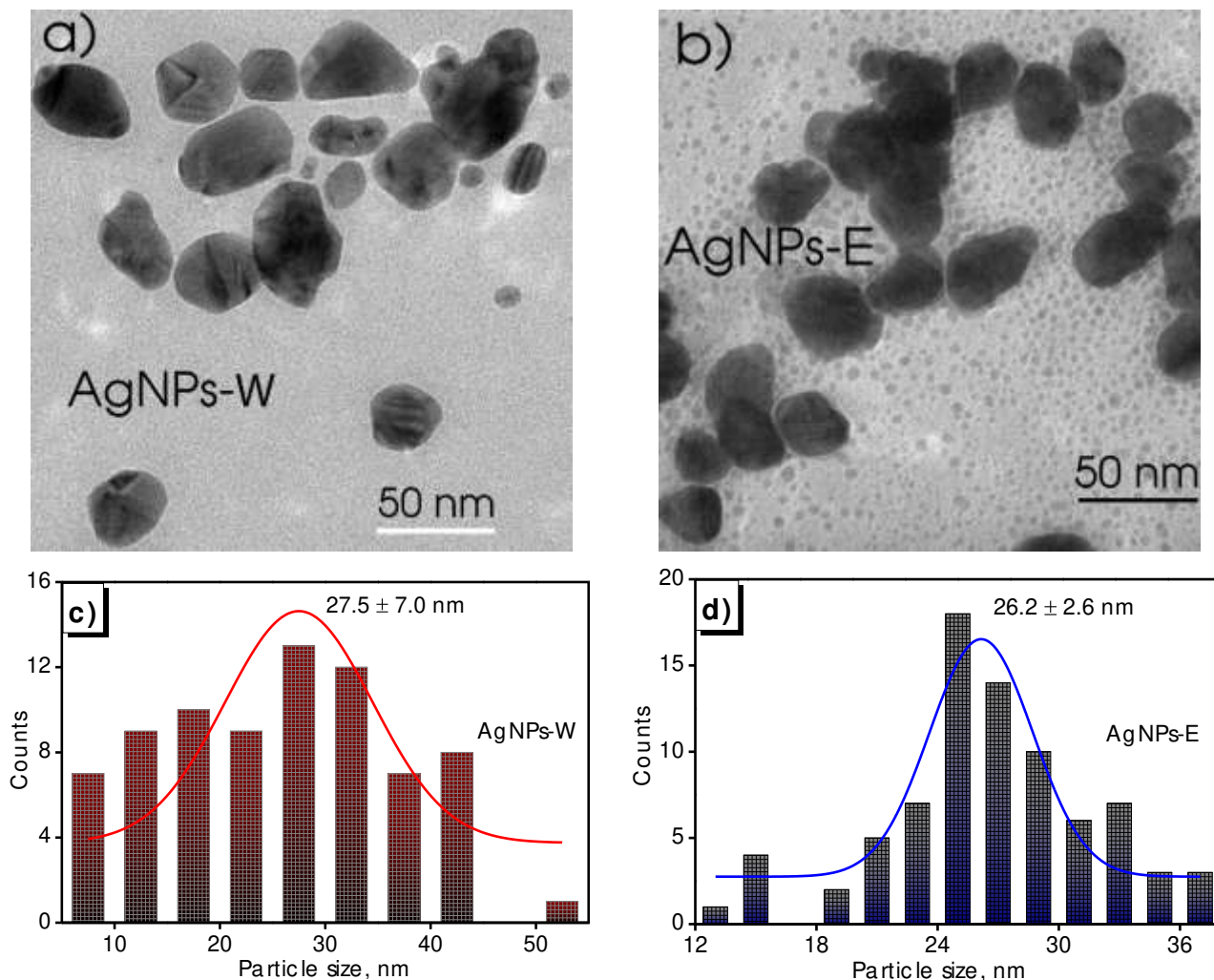


Figure 6. TEM images (a, b) and distribution size (c, d) of the synthesized AgNPs.

260 AgNPs obtained from water and ethanol-water extracts were both negatively charged (Figure 7). The zeta
 261 potential of AgNPs-W and AgNPs-E was -38.4 and -15.4 eV, respectively. The presence of a stabilizing agent was
 262 considered a determinant of a material's surface charge (Mehta et al. 2014). For example, starch formed the surface of
 263 AgNPs charge-neutral (Abbaszadegan et al. 2015), chitosan created a positively charged surface (Dara et al. 2020), and
 264 phenolics conferred a negative charge on the composites (Jayeoye et al. 2022). The zeta potential of AgNPs-W differed
 265 from AgNPs-E due to phytochemicals in the extracts. In addition, more phenolics are participating in reducing Ag^+ to
 266 Ag^0 on AgNPs-E compared to AgNPs-W through the reaction efficiency (Table 1), leading to fewer phenolics as
 267 stabilizing agents for AgNPs-E than AgNPs-W. Therefore, the zeta potential of AgNPs-E lowered charge-negative than
 268 AgNPs-W.
 269

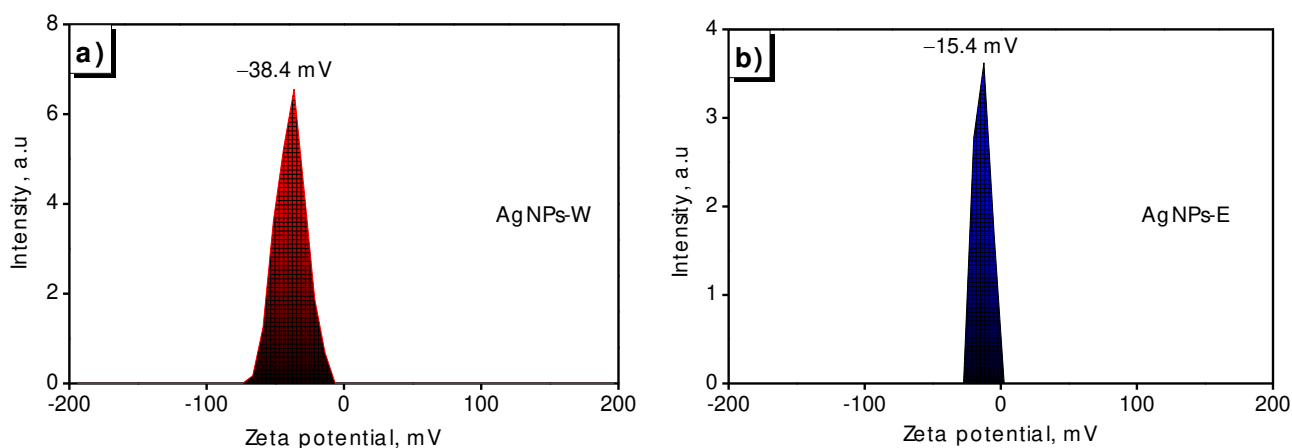


Figure 7. The zeta potential of the synthesized AgNPs-W (a) and AgNPs-E (b).

Table 3. IC₅₀ of the green-synthesized AgNPs on bacteria and cancer cells.

Bacteria/cancer cells		AgNPs-W, pM	AgNPs-E, pM
Positive-gram bacteria	<i>S. aureus</i>	3.280 ± 0.250	0.210 ± 0.020
	<i>B. subtilis</i>	0	3.440 ± 0.040
	<i>L. fermentum</i>	0	2.690 ± 0.110
Negative-gram bacteria	<i>S. enterica</i>	0	2.150 ± 0.180
	<i>E. coli</i>	0.830 ± 0.050	0.072 ± 0.001
	<i>P. aeruginosa</i>	0.170 ± 0.020	0.030 ± 0.002
Cancer cells	KB	0.860 ± 0.060	0.860 ± 0.030
	Hep-G2	0.410 ± 0.030	0.180 ± 0.010
	A549	1.220 ± 0.080	1.480 ± 0.040
	MCF7	0.230 ± 0.020	1.870 ± 0.060

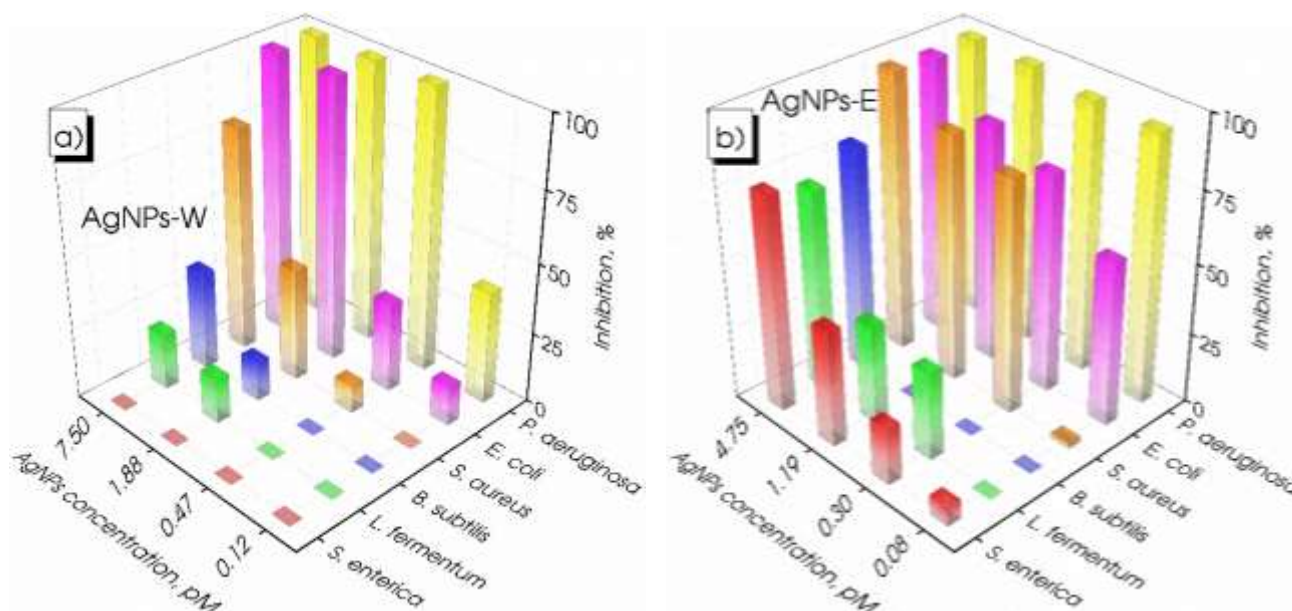


Figure 8. Antibacterial activity of the green-synthesized AgNPs-W (a) and AgNPs-E (b) at the numerous AgNPs concentrations.

AgNPs were highly antimicrobial against various microorganisms, including bacteria, fungi, and cells (Rathi Sre et al. 2015) (Braithwaite et al. 2014). In the present work, the as-prepared AgNPs-W and AgNPs-E were tested for

inhibitory activity against human pathogenic microorganisms by measuring the half-maximal inhibitory concentration (IC₅₀). Inhibitory efficiency and IC₅₀ values of AgNPs-W and AgNPs-E were represented in Table 3 and Figure 8. The results revealed that the produced AgNPs inhibited well against *P. aeruginosa* with IC₅₀ = 0.170 pM for AgNPs-W and IC₅₀ = 0.030 pM for AgNPs-E. Moreover, AgNPs-W with 0.120-7.500 pM ineffectively inhibited the growth of *B. subtilis*, *L. fermentum*, and *S. enterica*. In contrast, the growth of gram-negative and gram-positive bacteria was hindered by AgNPs-E. The sensitivity of pathogenic bacteria with AgNPs-E was as follows: *P. aeruginosa* (IC₅₀ = 0.030 pM) > *E. coli* (IC₅₀ = 0.072 pM) > *S. aureus* (IC₅₀ = 0.210 pM) > *S. enterica* (IC₅₀ = 2.150 pM) > *L. fermentum* (IC₅₀ = 2.690 pM) > *B. subtilis* (IC₅₀ = 3.440 pM). The results demonstrated that the AgNPs-E exhibited promising antibacterial activity against the clinical pathogens compared to AgNPs-W.

Compared with the antibacterial activity, the inhibitory activity against cancer cells of AgNPs-W and AgNPs-E was high, with the inhibitory efficiency reaching > 90%. Evaluating the antiproliferative effects of the plant-assisted AgNPs in detail, numerous concentrations of nanoparticles were introduced to cancer cell lines, such as A549, Hep-G2, KB, and MCF-7. The results showed a continuous decrease in cell growth with an accelerating concentration of AgNPs (Figure 9). Table 3 showed that the bio-synthesized AgNPs-W were more toxic toward MCF-7 than the cells (A549, Hep-G2, and KB). In contrast, AgNPs-E exhibited significant anticancer activity against the liver cell line Hep-G2.

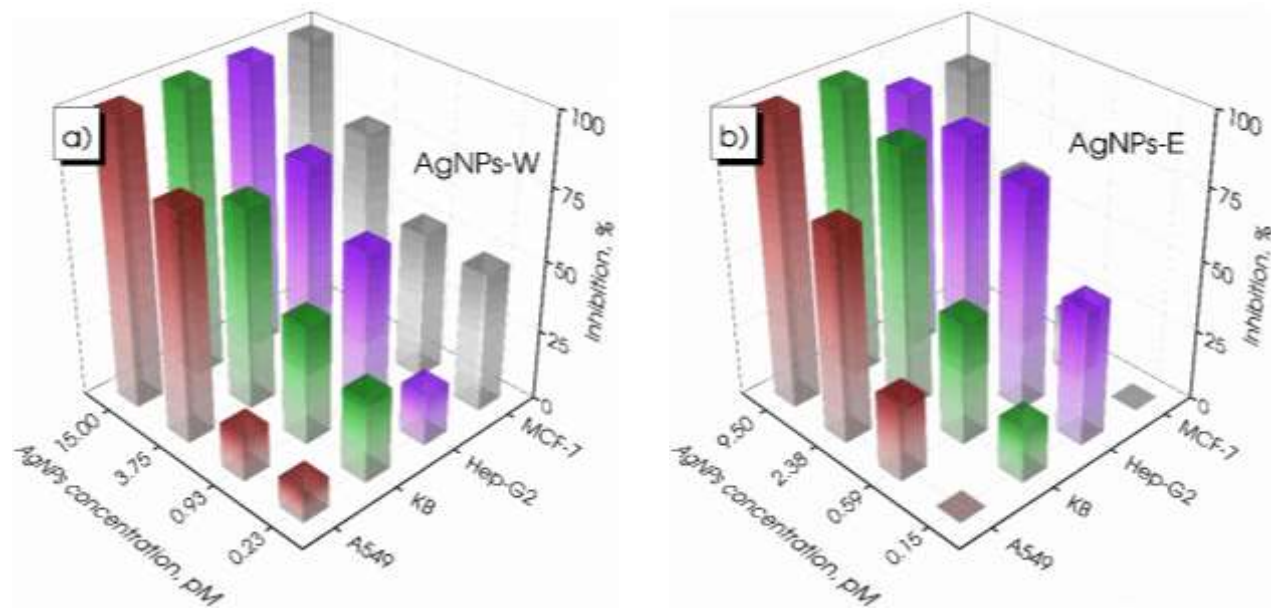


Figure 9. Anticancer activity of the bio-synthesized AgNPs-W (a) and AgNPs-E (b) at the several AgNPs concentrations.

There were numerous studies using plant extracts for synthesizing AgNPs. However, *C. operculatus* bud extract has not yet been investigated for forming AgNPs and the use of ethanol-water as a solvent in the AgNPs synthesis is still limited. Table 4 compared the results in the present work with the literature that used ethanol-water as a solvent for extraction. The ethanol concentration used to extract *C. operculatus* in the present work was much lower than in other studies. Moreover, our study has spherical particles with an average size of 28 nm, similar to the results of AgNPs, which used the ethanol-water extract of *Murraya paniculata* leaf, *Sargassum*, and *Phyllanthus niruri*. Predominantly, the synthesis efficiency of AgNPs (75.6%) using the ethanol-water extract of *C. operculatus* was determined. The synthesized AgNPs displayed inhibitory activity against some cancer cell lines such as KB, Hep-G, A549, and MCF7.

Table 4. Evaluation of the results in the present research with the recent literature using ethanol-water extract for AgNPs synthesis.

Plant	Reaction conditions	Shape, size	Antimicrobial activity	Synthesis efficiency	Reference
<i>Murraya paniculata</i> leaf	40% ethanol-water extract, 1 mM AgNO ₃ , light-irradiation, room temperature	Spherical and oval, 20-35 nm	<i>Candida albicans</i> and <i>Pseudomonas aeruginosa</i>	–	(Antropova et al. 2021)
<i>Sargassum</i>	50% ethanol-water extract, 10 mM AgNO ₃ , room temperature	Polyhedral, 26 nm	<i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i>	–	(López-Miranda et al. 2021)
<i>Phyllanthus niruri</i>	50% ethanol-water extract, 2 mM AgNO ₃ , room temperature	–	–	–	(Rahman and Sutoyo 2022)
<i>Cleistocalyx operculatus</i>	15% ethanol-water extract, 1 mM AgNO ₃ , room temperature	Spherical, 28 nm	<i>Bacillus subtilis</i> , <i>Lactobacillus fermentum</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella enterica</i> , lung cancer A549, hepatic cancer Hep-G2, epidermal carcinoma KB, and breast cancer MCF-7	75.6%	This work

Conclusions

The synthesis of AgNPs using *C. operculatus* buds by a green route had several advantages, such as an economical, efficient, and eco-friendly advantage of locally available and easy-to-find raw plants. The plant-mediated AgNPs synthesis is bio-compatible for a wide-ranging selection of biomedical applications. An ethanol-water solvent is used more effectively for extracting reducing and stabilizing agents present in the extract of *C. operculatus* buds than an aqueous solvent. The obtained AgNPs are the negatively charged sphere in an average of 26.2 nm for AgNPs-W and 27.5 nm for AgNPs-E. The synthesis efficiency of AgNPs-E (75.6%) was higher than that of AgNPs-W (59.5%). Furthermore, the fabricated AgNPs exhibited high bacteria and antiproliferative effects on various human cancer cell lines, especially AgNPs-E. The formed AgNPs using ethanol-water are practical applications in antibacterial/inhibiting human cancer cells. Additionally, *C. operculatus* buds were introduced as adequate plant-enriched phenolics for synthesizing AgNPs.

Acknowledgments

This work was supported by Can Tho University, Vietnam, No-TSV2022-142.

Conflict of Interest

There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Reference

1. Abbai R, Mathiyalagan R, Markus J, Kim YJ, Wang C, Singh P, Ahn S, Farh Mel A, Yang DC (2016) Green synthesis of multifunctional silver and gold nanoparticles from the oriental herbal adaptogen: Siberian ginseng. *Int J Nanomedicine* 11:3131–3143. <https://doi.org/10.2147/ijn.S108549>
2. Abbaszadegan A, Ghahramani Y, Gholami A, Hemmateenejad B, Dorostkar S, Nabavizadeh M, Sharghi H (2015) The effect of charge at the surface of silver nanoparticles on antimicrobial activity against gram-positive and gram-negative bacteria: A preliminary study. *J Nanomater* 2015:720654. <https://doi.org/10.1155/2015/720654>
3. Antropova IG, Revina AA, Oo PM, Kurakina ES, Butorova IA, Magomedbekov EP (2021) Synthesis of silver nanoparticles using reactive water-ethanol extracts from *Murraya paniculata*. *ACS Omega* 6:8313–8321. <https://doi.org/10.1021/acsomega.1c00019>
4. Bakshi M, Kumar A (2021) Copper-based nanoparticles in the soil-plant environment: Assessing their applications, interactions, fate and toxicity. *Chemosphere* 281:130940. <https://doi.org/10.1016/j.chemosphere.2021.130940>
5. Braithwaite MC, Tyagi C, Tomar LK, Kumar P, Choonara YE, Pillay V (2014) Nutraceutical-based therapeutics and formulation strategies augmenting their efficiency to complement modern medicine: An overview. *J Funct Foods* 6:82–99. <https://doi.org/10.1016/j.jff.2013.09.022>
6. Dara PK, Mahadevan R, Digita PA, Visnuvinayagam S, Kumar LRG, Mathew S, Ravishankar CN, Anandan R (2020) Synthesis and biochemical characterization of silver nanoparticles grafted chitosan (Chi-Ag-NPs): in vitro studies on antioxidant and antibacterial applications. *SN Appl Sci* 2:665. <https://doi.org/10.1007/s42452-020-2261-y>
7. Donga S, Chanda S (2021) Facile green synthesis of silver nanoparticles using *Mangifera indica* seed aqueous extract and its antimicrobial, antioxidant and cytotoxic potential (3-in-1 system). *Artif Cells Nanomed Biotechnol* 49:292–302. <https://doi.org/10.1080/21691401.2021.1899193>
8. Elhawary S, Hala EH, Mokhtar FA, Sobeh M, Mostafa E, Osman S, El-Raey M (2020) Green synthesis of silver nanoparticles using extract of *Jasminum officinal* L. leaves and evaluation of cytotoxic activity towards bladder (5637) and breast cancer (MCF-7) cell lines. *Int J Nanomedicine* 15:9771–9781. <https://doi.org/10.2147/IJN.S269880>
9. Erdogan O, Abbak M, Demirbolat GM, Birtekocak F, Aksel M, Pasa S, Cevik O (2019) Green synthesis of silver nanoparticles via *Cynara scolymus* leaf extracts: The characterization, anticancer potential with photodynamic therapy in MCF7 cells. *Plos One* 14:0216496. <https://doi.org/10.1371/journal.pone.0216496>
10. Fedenko VS, Landi M, Shemet SA (2022) Metallophenolomics: A novel integrated approach to study complexation of plant phenolics with metal/metalloid ions. *Int J Mol Sci* 23:11370. <https://doi.org/10.3390/ijms231911370>

11. Frešer F, Hostnik G, Tošović J, Bren U (2021) Dependence of the Fe(II)-gallic acid coordination compound formation constant on the pH. *Foods* 10:2689. <https://doi.org/10.3390/foods10112689>
12. Hemmati S, Ahany Kamangar S, Ahmida A, Zangeneh MM, Zangeneh A (2020) Application of copper nanoparticles containing natural compounds in the treatment of bacterial and fungal diseases. *Appl Organomet Chem* 34:5465. <https://doi.org/10.1002/aoc.5465>
13. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJV (2016) Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet* 387:176–187. [https://doi.org/10.1016/S0140-6736\(15\)00473-0](https://doi.org/10.1016/S0140-6736(15)00473-0)
14. Ijaz I, Gilani E, Nazir A, Bukhari A (2020) Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. *Green Chem Lett Rev* 13:223–245. <https://doi.org/10.1080/17518253.2020.1802517>
15. Ismail M, Gul S, Khan MI, Khan MA, Asiri AM, Khan SB (2019) Medicago polymorpha-mediated antibacterial silver nanoparticles in the reduction of methyl orange. *Green Process Synth* 8:118–127. <https://doi.org/10.1515/gps-2018-0030>
16. Jayeoye TJ, Eze FN, Olatunji OJ, Tyopine AA (2022) Synthesis of biocompatible Konjac glucomannan stabilized silver nanoparticles, with *Asystasia gangetica* phenolic extract for colorimetric detection of mercury (II) ion. *Sci Rep* 12:9176. <https://doi.org/10.1038/s41598-022-13384-x>
17. Joudeh N, Saragliadis A, Koster G, Mikheenko P, Linke D (2022) Synthesis methods and applications of palladium nanoparticles: A review. *Front Nanosci* 4:1062608. <https://doi.org/10.3389/fnano.2022.1062608>
18. Kamiloglu S, Sari G, Ozdal T, Capanoglu E (2020) Guidelines for cell viability assays. *Food Front* 1:332–349. <https://doi.org/10.1002/fft2.44>
19. Khan MS, Alomari A, Tabrez S, Hassan I, Wahab R, Bhat SA, Alafaleq NO, Altwaijry N, Shaik GM, Zaidi SK, Nough W, Alokail MS, Ismael MA (2021) Anticancer potential of biogenic silver nanoparticles: A mechanistic study. *Pharmaceutics* 13:707. <https://doi.org/10.3390/pharmaceutics13050707>
20. Liaudanskas M, Zymonė K, Viškelis J, Klevinskas A, Janulis V (2017) Determination of the phenolic composition and antioxidant activity of pear extracts. *J Chem* 2017:7856521. <https://doi.org/10.1155/2017/7856521>
21. López-Miranda JL, Esparza R, González-Reyna MA, España-Sánchez BL, Hernandez-Martinez AR, Silva R, Estévez M (2021) Sargassum influx on the Mexican Coast: A source for synthesizing silver nanoparticles with catalytic and antibacterial properties. *Appl Sci* 11:4638. <https://doi.org/10.3390/app11104638>
22. Mehta M, Shah J, Khakhkhar T, Shah R, Hemavathi KG (2014) Anticonvulsant hypersensitivity syndrome associated with carbamazepine administration: Case series. *J Pharmacol Pharmacother* 5:59–62. <https://doi.org/10.4103/0976-500x.124428>
23. Mishra AK, Tiwari KN, Saini R, Kumar P, Mishra SK, Yadav VB, Nath G (2020) Green synthesis of silver nanoparticles from leaf extract of *Nyctanthes arbor-tristis* L. and assessment of its antioxidant, antimicrobial response. *J Inorg Organomet Polym Mater* 30:2266–2278. <https://doi.org/10.1007/s10904-019-01392-w>

24. Morais M, Teixeira AL, Dias F, Machado V, Medeiros R, Prior JAV (2020) Cytotoxic effect of silver nanoparticles synthesized by green methods in cancer. J Med Chem 63:14308–14335. <https://doi.org/10.1021/acs.jmedchem.0c01055>
25. Nguyen TH, Hoang NH, Van Tran C, Nguyen PTM, Dang TD, Chung WJ, Chang SW, Nguyen DD, Senthil Kumar P, La DD (2022) Green synthesis of a photocatalyst Ag/TiO₂ nanocomposite using *Cleistocalyx operculatus* leaf extract for degradation of organic dyes. Chemosphere 306:135474. <https://doi.org/10.1016/j.chemosphere.2022.135474>
26. Nguyen VT (2020) Sunlight-driven synthesis of silver nanoparticles using pomelo peel extract and antibacterial testing. J Chem 2020:6407081. <https://doi.org/10.1155/2020/6407081>
27. Pradeep M, Kruszka D, Kachlicki P, Mondal D, Franklin G (2022) Uncovering the phytochemical basis and the mechanism of plant extract-mediated eco-friendly synthesis of silver nanoparticles using ultra-performance liquid chromatography coupled with a photodiode array and high-resolution mass spectrometry. ACS Sustain Chem Eng 10:562–571. <https://doi.org/10.1021/acssuschemeng.1c06960>
28. Pham GN, Nguyen TTT, Nguyen-Ngoc H (2020) Ethnopharmacology, phytochemistry, and pharmacology of *Syzygium nervosum*. Evid Based Complement Alternat Med 2020:8263670. <https://doi.org/10.1155/2020/8263670>
29. Raghavendra N, Hublikar LV, Patil SM, Bhat P (2021) Microwave assisted biosynthesis of silver nanoparticles using banana leaves extract: Phytochemical, spectral characterization, and anticancer activity studies. J Water Environ Nanotechnol 6:49–61. <https://doi.org/10.22090/jwent.2021.01.005>
30. Rahman RA, Sutoyo S (2022) Synthesis and characterization of silver nanoparticles from ethanol extract of Meniran (*Phyllanthus niruri* L.) using bioreduction method. Int J Progress Sci Technol 34:32–40. <https://doi.org/10.52155/ijpsat.v34.2.4616>
31. Rathi Sre PR, Reka M, Poovazhagi R, Arul Kumar M, Murugesan K (2015) Antibacterial and cytotoxic effect of biologically synthesized silver nanoparticles using aqueous root extract of *Erythrina indica* lam. Spectrochim Acta, Pt A: Mol Biomol Spectrosc 135:1137–1144. <https://doi.org/10.1016/j.saa.2014.08.019>
32. Shaikh WA, Chakraborty S, Owens G, Islam RU (2021) A review of the phytochemical mediated synthesis of AgNP (silver nanoparticle): the wonder particle of the past decade. Appl Nanosci 11:2625–2660. <https://doi.org/10.1007/s13204-021-02135-5>
33. Soto KM, Quezada-Cervantes CT, Hernández-Iturriaga M, Luna-Bárcenas G, Vazquez-Duhalt R, Mendoza S (2019) Fruit peels waste for the green synthesis of silver nanoparticles with antimicrobial activity against foodborne pathogens. LWT 103:293–300. <https://doi.org/10.1016/j.lwt.2019.01.023>
34. Tyagi PK, Tyagi S, Gola D, Arya A, Ayatollahi SA, Alshehri MM, Sharifi-Rad J (2021) Ascorbic acid and polyphenols mediated green synthesis of silver nanoparticles from *Tagetes erecta* L. aqueous leaf extract and studied their antioxidant properties. J Nanomater 2021:6515419. <https://doi.org/10.1155/2021/6515419>
35. Thi HPN, Thi KTP, Thi LN, Nguyen TT, Nguyen PTM, Vu TT, Le HT, Dang TD, Huynh DC, Mai HT, La DD, Chang SW, Nguyen DD (2022) Green synthesis of an Ag nanoparticle-decorated graphene nanoplatelet nanocomposite by using *Cleistocalyx operculatus* leaf extract for antibacterial applications. Nano-Struct Nano-Objects 29:100810. <https://doi.org/10.1016/j.nanoso.2021.100810>

36. Vishwasrao C, Momin B, Ananthanarayan L (2019) Green synthesis of silver nanoparticles using sapota fruit waste and evaluation of their antimicrobial activity. *Waste and Biomass Valorization* 10:2353–2363. <https://doi.org/10.1007/s12649-018-0230-0>
37. Wang Y, Chinnathambi A, Nasif O, Alharbi SA (2021) Green synthesis and chemical characterization of a novel anti-human pancreatic cancer supplement by silver nanoparticles containing *Zingiber officinale* leaf aqueous extract. *Arab J Chem* 14:103081. <https://doi.org/10.1016/j.arabjc.2021.103081>
38. Xu Z, Zhang C, Wang X, Liu D (2021) Release strategies of silver ions from materials for bacterial killing. *ACS Appl Bio Mater* 4:3985–3999. <https://doi.org/10.1021/acsabm.0c01485>
39. Yang Z, Wang D, Zhang C, Liu H, Hao M, Kan S, Liu D, Liu W (2022) The applications of gold nanoparticles in the diagnosis and treatment of gastrointestinal cancer. *Front Oncol* 11:819329. <https://doi.org/10.3389/fonc.2021.819329>
40. Zhong L, Yuan Z, Rong L, Zhang Y, Xiong G, Liu Y, Li C (2019) An optimized method for extraction and characterization of phenolic compounds in *dendranthema indicum* var. *aromaticum* flower. *Sci Rep* 9:7745. <https://doi.org/10.1038/s41598-019-44102-9>