



Investigation of the Antioxidant, Antibacterial, and Food Safety Potential of Silver and Copper Nanoparticles Green Synthesized Using Pointed Cherry Laurel (*Prunus laurocerasus* L.) Extract

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Abstract

This study reports the green synthesis of silver (PFAgNPs) and copper (PFCuNPs) nanoparticles using an aqueous extract of pointed cherry laurel (*Prunus laurocerasus* L.) fruit (PF-E) and explores their applicability in food safety. The NPs were characterized via UV–Vis spectroscopy, FT-IR, XRD, SEM, EDX, and TEM confirming their successful formation and morphology. Total phenolic content (TPC), flavonoid content (TFC), and antioxidant capacity (TAC) were measured for both PF-E and the synthesized NPs. While PFCuNPs exhibited higher TPC, PFAgNPs showed superior TFC and TAC. Notably, antioxidant assays (DPPH, ABTS, FRAP) indicated stronger radical scavenging activity for PFCuNPs. Antibacterial tests against *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* revealed broad-spectrum activity of PFAgNPs, with the highest inhibition against *E. coli* (19.4 ± 0.3 mm), whereas PFCuNPs showed dose-dependent efficacy, strongest against *E. faecalis* (9.6 ± 0.2 mm). LC–MS/MS analysis of the PF-E extract revealed quinic acid (63.42 mg/g), chlorogenic acid (2.38 mg/g), protocatechuic acid (0.07 mg/g), protocatechuic aldehyde (0.04 mg/g), hesperidin (0.03 mg/g), and naringenin (0.02 mg/g) as major components. The adsorption efficiencies of the synthesized PFAgNPs and PFCuNPs were evaluated against the toxic food contaminants patulin and HMF using both standard aqueous solutions and real food systems (apple juice). In standard aqueous solutions, PFAgNPs achieved removal efficiencies of 70.96% for patulin and 52.22% for HMF, while PFCuNPs showed 61.08% and 36.38%, respectively, at 5 g/L after 240 min. In real food systems, the adsorption efficiencies decreased, with PFAgNPs and PFCuNPs removing 34.24% and 32.90% of patulin, and 39.09% and 31.87% of HMF, respectively. The adsorption kinetics of HMF and patulin onto PFCuNP and PFAgNP were found to conform to both the pseudo-first-order and pseudo-second-order kinetic models for aqueous solutions as well as real samples. These findings confirm that the biosynthesized NPs offer dual functionality as antibacterial agents and toxin adsorbents, presenting a green, sustainable approach to food safety enhancement.

Keywords Pointed cherry laurel · Silver and copper nanoparticles · Food safety · Antioxidant assays · Antibacterial tests

Introduction

Nanotechnology is a field that operates at the atomic and molecular scale, enabling the creation of advanced structures by precisely arranging individual components, often surpassing the properties of their bulk counterparts (Ergül & Çakır, 2023). Nanoparticles (NPs) are typically characterized as particles measuring between 1 and 100 nm, possessing a high surface-to-mass ratio (Banasiuk et al., 2020). As NP size decreases, their surface area and quantum effects increase, leading to enhanced optical, electrical, and magnetic properties, as well as greater reactivity (Onyia et al., 2020). These unique characteristics make NPs highly attractive for diverse applications, including medical imaging,

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electronic devices, biotechnological innovations, drug production, biochemical sensors, and stimulators (Alsamhary, 2020).

Based on morphology, size, and chemical properties, NPs can be categorized into polymeric, ceramic, liquid-based, semiconductor, metal-based, and carbon-based types. Metal NPs, in particular, exhibit remarkable optical properties owing to their well-defined surface plasmon resonance (SPR) characteristics (Khan et al., 2019). Among metal NPs, silver NPs (AgNPs) are of great interest due to their plasmonic effects and tunable optical properties (Nouri et al., 2020). They are extensively synthesized for applications in catalysis (Sharma et al., 2021), anti-inflammatory therapies (Carvalho-Silva & Dos Reis, 2024), antimicrobial applications (Basheer et al., 2023; Huq & Akter, 2021; Khane et al., 2022), anticancer strategies (Karpuz et al., 2025; Valsalam et al., 2019), drug delivery systems (Andreani et al., 2024), and biosensing technologies (Meng et al., 2019). Another prominent metal-based NP is copper NPs (CuNPs), which have garnered recent attention due to their unique chemical and physical properties (Crisan et al., 2021). CuNPs are widely employed in catalysis, paint degradation, gas sensors, and the preparation of organic–inorganic nanocomposites (Ivanova et al., 2024; Mali et al., 2023; Pakzad et al., 2019). Industrially, they serve as thermal conductors, catalysts, battery components, and solar cell materials (Al-Hakkani, 2020). In medicine, CuNPs function as anti-inflammatory and anti-arthritic potentials (Zhang et al., 2020), antibacterial agents (Jayarambabu et al., 2020; Ramzan et al., 2021), and antifungal compounds (Pariona et al., 2019). Today, the cost-effective and high-yield synthesis of AgNPs and CuNPs with such valuable properties is expected to significantly advance the development of novel nanomaterials in the rapidly growing field of nanotechnology.

Traditional synthesis of metal NPs employs physical, chemical, and mechanical methods using either top-down or bottom-up approaches (Rao et al., 2020). However, physical methods are often cost-prohibitive due to their reliance on complex equipment requiring high vacuum regulation and continuous gas flow (e.g., argon, oxygen, nitrogen). Chemical methods, meanwhile, frequently utilize toxic substances like sodium citrate and lead oxide, resulting in cytotoxic by-products (Manik et al., 2020). To address these limitations, developing eco-friendly, simple, and cost-effective alternative synthesis methods has become crucial (Tripathi & Chung, 2020). Green synthesis emerges as a superior alternative to both physical and chemical methods, offering environmental sustainability, cost efficiency, and scalability (Chandhru et al., 2019). This approach emphasizes the careful selection of solvent media, reducing agents, and non-toxic substances during NP production (Radulescu et al., 2023), while providing additional advantages in energy efficiency, product selectivity, and safety for both human

health and the environment (Hussain et al., 2024). Various biological compounds such as microorganisms and plants are used as green reducing agents for the synthesis of NPs (Yasmeen et al., 2023). In particular, the use of plant extracts for NP synthesis has attracted considerable attention due to their rapid, simple, and economically feasible procedures (Riaz et al., 2023). Various plant components (stems, fruits, roots, flowers, and leaves) contain valuable phytochemicals (e.g., polyphenols, terpenoids, polyols) that are ideal for NP synthesis (Jadoun et al., 2021). Furthermore, plant extracts facilitate control over NP size and enhance stability through mediated interactions (Javed et al., 2020).

Various plant extracts have been successfully utilized in the synthesis of AgNPs and CuNPs for biotechnology and nanotechnology applications. Recently, AgNPs and CuNPs synthesized from various parts (such as flowers, leaves, roots, and fruits) of many plant species have been reported, including *Berberis vulgaris* (Behravan et al., 2019), *Abelmoschus esculentus* (Devanesan & AlSalhi, 2021), *Salvia spinosa* (Pirtarighat et al., 2019), *Rosa damascena* (Peron et al., 2021), *Cicer arietinum* (Baran et al., 2022), *Moringa oleifera* (Bindhu et al., 2020), *Ageratum houstonianum* Mill. (Chandraker et al., 2020), *Curcuma longa* (Jayarambabu et al., 2020), Ginger lily leaf extract (Nagore et al., 2020), strawberry fruit extract (Hemmati et al., 2020), *Phoenix dactylifera* L. (Al Mutairi et al., 2022), *Fragaria ananassa* (Bayat et al., 2021), and *Rhus coriaria* L. (Sumaq) (Ismail, 2020).

As evidenced by previous studies, while numerous plants and their parts have been employed in NP production, the exploration of plants with distinct biological properties remains ongoing. One such candidate is the cherry laurel (*Laurocerasus officinalis* Roem., Rosaceae family [syn: *Prunus laurocerasus* L., *Prunoideae* subfamily]) (Baltacı et al., 2024), a locally abundant and economical fruit tree native to Turkey's Black Sea region (Halasz et al., 2021). The natural distribution area of the species worldwide, apart from the eastern regions of the Black Sea, includes the Taurus Mountains, the Caucasus, and the northern and eastern parts of the Marmara region (Yilmaz et al., 2023). The common cherry laurel fruits (CLFs) in Turkey's Eastern Black Sea Region include sweet cherry (Kiraz), sour cherry (Sivri), and wild cherry (Yabani). These CLFs originate from distinct wild cherry tree varieties, each exhibiting unique morphological, cytological, and phylogenetic traits (Baltacı et al., 2024). This study selected pointed CLFs due to their abundance in Turkey's Eastern Black Sea Region, accessibility, and cost-effectiveness. Notably, to our knowledge, CLFs have not yet been explored for NP synthesis in the literature, presenting a novel opportunity for research.

Recently, nanotechnology applications in food safety have gained significant attention as a research priority (Alaca & Güvenliçi, 2021; Zhou et al., 2023). The World Health

Organization (WHO) emphasizes food safety as both a critical global health issue and a major contributor to reduced economic efficiency (WHO, 2022). In the food sector, nanotechnology offers innovative solutions for processing, packaging, safety monitoring, and quality control (Alaca & Güvenliçi, 2021). These advancements have accelerated efforts to address microbial contamination while enhancing toxin detection, shelf-life extension, and packaging strategies (Fadiji et al., 2023).

A pressing concern in food safety is the increasing prevalence of chemical and biological pollutants, particularly mycotoxins, which are highly toxic secondary metabolites produced by specific fungal species (Joshi et al., 2021). Patulin, one of the most hazardous mycotoxins, is a toxic compound classified as a mycotoxin, which refers to secondary metabolites produced by fungi such as *Penicillium*, *Aspergillus*, *Mucor*, and *Fusarium* (Agriopoulou et al., 2020). Patulin-producing fungi frequently contaminate fruits like apples, peaches, plums, and pears (Pushparaj et al., 2023), leading to trace residues in derived products. Apple-based foods such as juices, wines, compotes, and purees are especially susceptible, primarily due to *Penicillium expansum* (*P. expansum*), the fungus responsible for apple Valsa canker and blue mold (Li et al., 2022). Chronic exposure to patulin is associated with genotoxicity, neurotoxicity, and carcinogenesis (Saleh & Göktepe, 2019), and the WHO classifies it as a genotoxic compound (Notardonato et al., 2021). These toxic substances can exert deleterious effects on various organs, particularly the nervous and immune systems as well as the gastrointestinal tract and possess the potential to induce both acute and chronic toxicity (Mahato et al., 2021).

Hydroxymethylfurfural (HMF) is another critical food safety contaminant, classified as a toxic aldehyde generated during thermal processing of sugary foods or improper storage (Sahebi et al., 2025). Several studies have indicated that high concentrations of HMF in foods may elicit cytotoxic effects, potentially causing irritation to the eyes, upper respiratory tract, skin, and mucous membranes (Choudhary et al., 2021; Farag et al., 2020). Animal investigations have suggested that HMF can act both as an initiator and promoter of colon carcinogenesis, warranting its consideration as a potentially carcinogenic compound (Mirza Alizadeh et al., 2025). Furthermore, 5-sulfo-methyl-2-furfuraldehyde, a metabolite of HMF, has been reported as a highly reactive intermediate capable of interacting with DNA and exhibiting genotoxic potential under in vitro conditions (Choudhary et al., 2021).

To our knowledge, this is the first study to synthesize AgNPs and CuNPs using aqueous extract of pointed CLF and to evaluate their antibacterial, antioxidant, and toxin adsorption efficiencies, with a particular focus on patulin and HMF. Additionally, there is no comparative study addressing the different application potentials of green-synthesized

AgNPs and CuNPs in various biological fields. The synthesized NPs were characterized in terms of size, morphology, distribution, crystallinity, and surface structure using X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), and Fourier-transform infrared spectroscopy (FT-IR). The phytochemical composition of the PF-E extract was analyzed using liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS). The phenolic compounds in PF-E and the synthesized NPs were quantified using total phenolic content (TPC), flavonoid content (TFC), and antioxidant capacity (TAC) assays. Antioxidant activities were further evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) methods. Antibacterial properties were tested against Gram-positive and Gram-negative bacterial strains. Finally, the adsorption efficiencies of the synthesized AgNPs and CuNPs were evaluated against two foodborne contaminants, patulin and HMF, known for their toxic and carcinogenic effects, using both standard aqueous solutions and real food matrix (apple juice) samples. Furthermore, the potential of these NPs to adsorb and remove the targeted toxins was investigated. Overall, the synthesized NPs were assessed with respect to their antibacterial, antioxidant, and toxin-removal properties, and were subjected to a comprehensive, multi-dimensional evaluation regarding their potential relevance to food safety.

Materials and Methods

Materials

The experimental procedures exclusively involved analytical-grade reagents, with all solutions formulated using ultrapure water to maintain consistency and purity. Chemicals including silver nitrate (AgNO_3), acetonitrile, sulfuric acid (H_2SO_4), copper(II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), ethanol, methanol, dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), sodium phosphate, ammonium molybdate, Folin–Ciocalteu phenol reagent, Trolox (T), ascorbic acid (AA), gallic acid (GA), quercetin (Q), ferric chloride (FeCl_3), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), and hydrochloric acid (HCl) were obtained from Merck (Darmstadt, Germany). Additional reagents including sodium carbonate (Na_2CO_3), aluminum chloride (AlCl_3), formic acid, sodium nitrite (NaNO_2), potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), iron(II) sulfate (FeSO_4), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ampicillin, Mueller–Hinton Broth (MHB), Mueller–Hinton Agar (MHA), and ammonium formate were procured from Sigma-Aldrich (St. Louis,

MO, USA). Also, patulin solution (100 µg/mL, 34127) was purchased from Merck (Darmstadt, Germany), and HMF ($\geq 99\%$, FG) was supplied by Sigma-Aldrich (St. Louis, MO, USA). For the evaluation of antibacterial activity, bacterial strains comprising gram-positive species (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923) and gram-negative species (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) were employed. All strains were sourced from the Food Engineering Department laboratories at Gumushane University.

Preparation of Plant Extract

Pointed fruits of *Prunus laurocerasus* (Fig. 1a) were collected in July 2023 from wild trees located in Zeytinlik, Çarşıbaşı, Trabzon, Turkey (41°03'29.3"N, 39°22'38.9"E). Species identification was confirmed by Dr. Mutlu Gültepe, and a voucher specimen (Herbarium No: Gültepe 725) was deposited at the Herbarium of the Department of Biology, Karadeniz Technical University. After removing the stems, the fruits were washed with deionized water to eliminate dust and debris, then air-dried overnight at room temperature in the laboratory (Fig. 1b). Cleaned fruits were deseeded and homogenized using a laboratory blender (Blender 8011ES, Waring, CT, USA) (Fig. 1c).

For aqueous extraction, 1 kg of the prepared fruit was boiled in 2 L of ultrapure water at 90–95 °C for 1.5 h. After cooling, the extract was filtered through Whatman No. 1 filter paper. To increase the dry matter concentration, 250 mL of the filtrate was concentrated using a rotary evaporator (Hei-VAP Advantage, Heidolph, Schwabach, Germany) at 60 °C under 250 mbar pressure. The total dry matter content of the extract was determined as 7.64% using a digital refractometer (Hanna HI96801, Hanna Instruments, Woonsocket, RI, USA). The prepared extract was stored in amber-colored bottles at 4 °C to prevent photodegradation for use in subsequent experiments. The extract obtained from pointed *Prunus laurocerasus* fruits was designated as PF-E.

Biosynthesis of AgNPs and CuNPs from Aqueous Extract of PF-E

The biosynthesis (green synthesis) of AgNPs was conducted based on the method applied by Karpuz et al. (2025), with some modifications. A 0.1 M AgNO₃ solution (100 mL) was added dropwise to the PF-E extract solution at a volumetric ratio of 1:1 (v:v), at a rate of approximately 1–2 drops per second. The synthesis was carried out at room temperature using a magnetic stirrer (MSH-20D, Daihan Scientific, Wonju, South Korea) at 500 rpm. The synthesis of AgNPs was terminated once a dark brown color was observed in the solution. The AgNP-containing solution was then subjected to purification by centrifugation at 10,000 rpm for 15 min using a centrifuge (Allegra X-30R, Beckman Coulter, Krefeld, Germany). The AgNPs obtained in the pellet were washed three times with deionized water and ethanol to remove impurities by repeated centrifugation. After the final centrifugation, the supernatant in the tubes was discarded, and the pellets were collected. The collected pellets were dried overnight at 50 °C in an oven (Daihan Scientific, Thermo Stable IG-105, Wonju, Korea) and then ground using a grinder (Waring, Blender 8011ES, Connecticut, USA) for further analyses.

The production of CuNPs followed the green synthesis approach outlined by Ingle et al. (2022), with certain adjustments applied. A 0.25 M CuSO₄·5H₂O solution (250 mL) was added dropwise to the PF-E extract solution at a volumetric ratio of 1:1 (v:v), at a rate of approximately 1–2 drops per second. The process was carried out under dark conditions at 40 °C with shaking at 500 rpm and continued overnight. Following the reaction, the purification steps, including centrifugation, washing, pellet collection, drying, and grinding, were performed as described for AgNP synthesis. The synthesized NPs were coded as PFCuNP and PFAgNP.

Physicochemical Properties of the Biosynthesized PFAgNP and PFCuNPs

Before proceeding with the characterization of the synthesized NPs, the verification of localized surface plasmon

Fig. 1 a. Fruit samples of the *Prunus laurocerasus* species, b. separation and cleaning of the fruit samples from their stems, and c. Grounding the fruit samples after separating them from their seeds



resonance (LSPR) was performed spectroscopically using a UV–Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). For spectral analysis, 100 mg of each NP type (PFAgNP and PFCuNP) was dispersed in 5 mL of distilled water. To ensure homogeneous distribution, the suspensions were subjected to sonication in an ultrasonic bath at a frequency of 40 kHz for 10 min, followed by centrifugation at 4000 rpm to remove large aggregates and unreacted components. The resulting clear supernatant was analyzed spectroscopically using distilled water as a blank. Absorption spectra were recorded in the wavelength range of 260–800 nm and used to confirm the formation of NPs (Agustina et al., 2021).

To elucidate the functional groups responsible for reduction and stabilization in PFAgNPs and PFCuNPs, FT-IR spectroscopy (Perkin-Elmer, Waltham, MA, USA) was performed in the range of 400–4300 cm^{-1} . Crystallographic properties were investigated using XRD with a PANalytical Empyrean diffractometer (Almelo, Netherlands), operating at 45 kV and 40 mA, with a scan speed of 0.5° per second over a 2 θ range of 0°–90°. Morphological attributes and particle size distributions were determined using TEM (FEI TALOS F200S, Oregon, USA) and SEM (JEOL JSM-6610, Tokyo, Japan). The elemental composition of the NPs was further characterized via EDX (2SDD EDX system, TALOS F200S, FEI, Oregon, USA) integrated with the TEM system. Particle size analysis was carried out using ImageJ software (National Institutes of Health, Bethesda, MD, USA) based on TEM images.

Quantification of Total Phenolic Content (TPC)

TPC was determined using a modified Folin–Ciocalteu method adapted from Ahmed et al. (2015). For the assay, 300 μL of each test sample (PF-E and NPs) and GA standard was first mixed in test tubes. Subsequently, 1 mL of methanol, 3.16 mL of deionized water, and 200 μL of Folin–Ciocalteu reagent (diluted 1:10 with water) were added sequentially to each tube. After a 10-min incubation, 600 μL of 10% Na_2CO_3 solution was introduced, and the mixtures were incubated in the dark for 2 h. Following incubation, aliquots from each tube were transferred into wells of a 96-well microplate, and absorbance was measured at 760 nm using a microplate reader (Multiskan EX, Thermo Scientific, Helsinki, Finland). Methanol was used as a blank by replacing the sample volume accordingly. The TPC values were calculated using a GA standard calibration curve ranging from 20 to 160 $\mu\text{g}/\text{mL}$, defined by the equation $y = 0.0005x + 0.0085$ with an R^2 of 0.9992. The findings are presented as mg of GA equivalents per gram of the dry extract (mg GAE/g).

Quantification of Total Flavonoid Content (TFC)

TFC was quantified through a colorimetric assay based on the formation of an aluminum chloride complex, adapted with modifications from Ahmed et al. (2015). For the analysis, 500 μL aliquots of both the test samples and the standard solution were pipetted into test tubes. Then, 3.20 mL of 30% (v/v) methanol, 150 μL of 0.5 M NaNO_2 , and 150 μL of 0.3 M AlCl_3 were sequentially added to each tube. Following a 5-min incubation, 1 mL of 1 M NaOH was introduced. The reaction mixtures were subsequently kept in darkness for 10 min. Upon completion, absorbance readings were taken at 506 nm using a microplate reader. A blank was prepared by substituting the test samples with an equivalent volume of methanol. The TFC values were calculated based on a quercetin Q standard calibration curve ranging from 25 to 400 $\mu\text{g}/\text{mL}$, defined by the equation $y = 0.0005x + 0.0085$ with an R^2 of 0.9992. The findings are presented as mg of Q equivalents per gram of the dry extract (mg QE/g).

Antioxidant Activity of PFAgNP and PFCuNPs

Determination of Total Antioxidant Capacity (TAC)

TAC of the samples was determined using a modified method based on Kasangana et al. (2015), adapted to the 96-well microplate format. For the assay, 300 μL of each test sample and standard solution were placed into capped test tubes, then 2.7 mL of phosphomolybdenum reagent containing 28 mM sodium phosphate and 4 mM ammonium molybdate dissolved in 0.6 M H_2SO_4 was added. The mixtures were vortexed thoroughly and incubated at 95 °C in a water bath for 90 min. After incubation, absorbance was measured at 695 nm with a microplate reader, using methanol as the blank by replacing the sample volume. AA served as the standard. TAC was calculated using the AA standard calibration curve prepared within the range of 25 to 800 $\mu\text{g}/\text{mL}$, defined by the equation $y = 0.0034x - 0.1472$, with an R^2 of 0.9981. The findings are presented as mg of AA equivalents per gram of the dry extract (mg AAE/g).

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the samples was quantified following a modified protocol adapted from Ahmed et al. (2015). Initially, a stock DPPH solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol, then diluted to achieve an absorbance value of 0.98 ± 0.02 at 517 nm. For the assay, 100 μL aliquots of each sample and standard were introduced into test tubes, to which 3 mL of the DPPH solution was subsequently added. The mixtures were carefully vortexed and incubated under dark conditions at ambient temperature for

30 min before absorbance measurement. Absorbance was recorded at 517 nm using a microplate reader. A blank was prepared by replacing the sample with methanol. AA was used as the standard, and the DPPH radical scavenging activities of the samples were calculated using the AA standard calibration curve within the range of 25–250 µg/mL ($y = -0.004x + 0.9238$, $R^2 = 0.9991$). The percentage of DPPH radical scavenging activity was determined using Eq. 1:

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (1)$$

where, A_0 denotes the absorbance of the control, which includes all reagents except the test samples, while A_1 corresponds to the absorbance of the samples. The DPPH radical scavenging activities of the tested samples were evaluated against the AA standard, and the results are reported as mg AAE/g of dry extract.

ABTS Radical Scavenging Activity

ABTS radical scavenging activity was determined using a modified microplate-based method, adapted from the procedure described by Ahmed et al. (2015). The ABTS working solution was generated by combining equal volumes of 7 mM ABTS and 2.45 mM $K_2S_2O_8$, followed by incubation in the dark for 12 to 16 h to allow radical formation. After incubation, the ABTS stock solution was diluted with 0.1 M potassium phosphate buffer (pH 7.4) to obtain an absorbance of 0.700 ± 0.020 at 734 nm. For the assay, 150 µL of each sample and standard solution was transferred into test tubes, and 2850 µL of the freshly prepared ABTS solution was added. The reaction mixtures were then incubated in darkness for 10 min at room temperature. After incubation, absorbance was measured at 734 nm using a microplate reader. Methanol was used as the control in place of the test samples. T served as the reference antioxidant. The ABTS scavenging capacity of each sample was calculated using a T standard curve within the range of 2 to 100 µg/mL, defined by the equation $y = -0.0083x + 0.641$ ($R^2 = 0.9998$). The percentage of radical scavenging was determined according to Eq. 1. The findings are presented as mg of T equivalents per gram of the dry extract (mg TE/g).

FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP of the samples was measured using a slightly modified version of the protocol reported by Sethi et al. (2020). The FRAP reagent was formulated by combining 300 mM acetate buffer (pH 3.6), 20 mM $FeCl_3$ solution, and 10 mM TPTZ reagent prepared in 40 mM HCl at a volumetric ratio of 10:1:1 (v/v/v). Aliquots of 250 µL from each test

sample and standard solution were mixed with 2750 µL of the FRAP reagent. The mixtures were subsequently incubated in the dark for 30 min to allow the reaction to proceed. Following the incubation period, absorbance was recorded at 593 nm using a microplate reader, with blanks prepared by substituting the test samples with deionized water. The FRAP values of the samples were quantified utilizing an $FeSO_4$ standard calibration curve constructed over a concentration range of 10–200 µg/mL ($y = 0.009x - 0.0381$, $R^2 = 0.9996$). The findings are presented as mg of $FeSO_4$ equivalents per gram of the dry extract (mg $FeSO_4$ /g).

Antibacterial Activity of PFAgNP and PFCuNPs

The antibacterial activities of test samples were evaluated against two Gram-negative (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) and two Gram-positive (*E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923) human pathogens using the agar well diffusion method (Yıldırım et al., 2025) with modifications. Bacterial strains were revived and cultured in MHB for 18 h to reach logarithmic growth phase. The suspensions were adjusted to 0.5 McFarland turbidity (1.5×10^8 cells/mL) using a densitometer (Densichek Plus, Biomerieux, USA). Bacterial strains were prepared on MHA plates via swab inoculation, and 6-mm wells were punched. Test samples (PF-E (10 mg/mL) and PFCuNPs/PFAgNPs (4000, 2000, 1000 µg/mL) in DMSO) were added (50 µL/well) and allowed to diffuse at 25 °C for 2 h. Plates were incubated at 37 °C for 24 h, and inhibition zone diameters were measured digitally. Results were expressed as mean $\pm$ SD, with ampicillin (50 µg/mL) as positive control.

Patulin and HMF Adsorption Studies

In this study, the adsorption efficiencies of the synthesized NPs were evaluated for both patulin and HMF under model (standard aqueous solutions) and real food matrix (apple juice) conditions. NP suspensions with concentrations of 2.5 and 5.0 g/L were prepared and aliquoted into test tubes. Subsequently, 250 µL of patulin (102 mg/L) or HMF (100 mg/L) stock solutions were added to the tubes, followed by deionized water to reach a total volume of 10 mL (9750 µL). For real sample experiments, commercially available apple juice was used as the adsorption medium instead of deionized water to simulate food-relevant conditions and evaluate the performance of NPs under a complex matrix. The mixtures were incubated at room temperature on a horizontal shaker set at 50 rpm for 60, 120, and 240 min. After incubation, the tubes were centrifuged at 7000 rpm for 10 min, and the obtained supernatants were filtered through 0.45 µm membrane filters. The filtrates were then analyzed using a high-performance liquid chromatography with UV detection (HPLC–UV, Thermo Finnigan, San Jose, CA, USA) system

to quantify the residual concentrations of patulin and HMF. The adsorption efficiency was calculated based on the initial toxin concentrations using Eq. (2):

$$\text{Adsorption (\%)} = \left(\frac{C_0 - C_t}{C_0} \right) \times 100 \quad (2)$$

Here, C_0 represents the initial concentration of patulin, and C_t is the concentration of patulin in the supernatant after incubation at the corresponding time point. This unified approach ensured consistent experimental conditions for both analytes, enabling a direct comparison of adsorption behaviors between patulin and HMF under identical parameters.

HPLC–UV Analysis of Patulin and HMF

For HMF adsorption studies, commercially available solid HMF was dissolved in distilled water to prepare a stock solution at a concentration of 100 mg/L. Patulin, on the other hand, had previously been isolated from natural food sources by our research group and was used in this study as a standard. To verify its purity, a commercially obtained patulin standard was used as a reference. A 250 μ L aliquot of the naturally derived patulin solution was transferred into a test tube, and the ethyl acetate solvent was removed by nitrogen-assisted evaporation. The resulting residue was reconstituted in distilled water adjusted to pH 4.0, and the final volume was brought to 10 mL. The concentration of the solution was determined to be 102 mg/L using an HPLC–UV system with reference to the commercial patulin standard. The prepared solution was subsequently used in patulin adsorption experiments. Quantitative analyses of patulin and HMF were performed using HPLC–UV, equipped with a C18 column (4.5 $\times$ 250 mm; 5 μ m particle size, Phenomenex, Aschaffenburg, Germany). The system included a binary pump (G1312A), degasser (G1379A), autosampler (G1329B), and UV detector (Thermo Finnigan, San Jose, CA, USA). The mobile phase consisted of ultrapure water (mobile phase A) and acetonitrile (mobile phase B). The analysis was performed under isocratic conditions with a 90:10 (v/v) mixture of ultrapure water and acetonitrile. Detection was carried out at 276 nm using a UV detector. The flow rate was maintained at 1 mL/min throughout the run. For the determination of HMF content, analysis was performed at 285 nm under isocratic conditions using a mobile phase composed of 50:50 (v/v) ultrapure water (mobile phase A) and methanol (mobile phase B). The flow rate was set at 1 mL/min. All standards and samples were filtered through 0.45 μ m membrane filters and injected into the HPLC–UV system at a volume of 50 μ L. To determine the extent to which the NPs adsorbed HMF and patulin, calibration curves were constructed using an HPLC–UV system. Standard solutions

were prepared at concentrations of 1, 2, 4, 8, and 12 mg/mL for HMF, and 50, 100, 150, 200, 250, and 300 μ g/L for patulin, respectively. The calibration curve obtained for patulin was $y = 451.76x - 1143.6$, with an R^2 of 0.9996, while for HMF, the calibration curve was $y = 251628x - 3379.2$, with an R^2 value of 0.9980. Following treatment with the NPs, the concentrations of unadsorbed (residual) patulin and HMF in the solution were calculated using these calibration equations (Dağ, 2020; Oğuz et al., 2023).

Kinetics through Pseudo First Order (PFO) and Pseudo Second Order (PSO) Models

The adsorption kinetics of HMF and patulin onto PFCuNP and PFAgNP were evaluated using the pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetic models for both aqueous solutions and real samples. Time-dependent adsorption data were fitted to the linearized forms of the PFO and PSO equations to determine the corresponding rate constants (k_1 and k_2) and theoretical adsorption capacities (Q_e), which were subsequently compared with experimentally obtained Q_e values. The suitability of each kinetic model was assessed based on the correlation coefficients (R^2) and the agreement between calculated and experimental adsorption capacities. This approach enabled the differentiation of diffusion-dominated and surface-reaction-controlled adsorption behavior in the removal of HMF and patulin by PFCuNP and PFAgNP, while also allowing evaluation of the influence of solution matrix complexity on kinetic performance under identical experimental conditions (Karpuz et al., 2025).

Phytochemical Analysis of Plant Extract

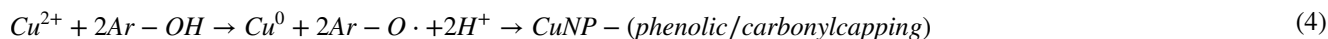
The phytochemical content of the PF-E was quantitatively analyzed using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–ESI–MS/MS). The analyses were conducted on a Shimadzu Nexera UHPLC system (Shimadzu, Kyoto, Japan), comprising a column oven (CTO-10Asvp), an autosampler (SIL-30AC), a binary pump (LC-30AD), and a degassing unit (DGU-20A3R). Optimization of chromatographic conditions was carried out to maximize the separation efficiency of the targeted phytochemicals. Various reversed-phase columns, including Agilent Poroshell 120 EC-C18, different mobile phase solvents (e.g., acetonitrile and methanol), and several additives such as formic acid, acetic acid ammonium formate, and ammonium acetate were evaluated. Phytochemical separation in the analysis was carried out using an Agilent Poroshell 120 EC-C18 column (150 mm $\times$ 2.1 mm, 2.7 μ m), with the column temperature maintained at 40 °C. The mobile phase system consisted of Eluent A (ultrapure water containing 5 mM ammonium formate and 0.1% formic

acid) and Eluent B (methanol containing 5 mM ammonium formate and 0.1% formic acid). The gradient elution started with a mobile phase composed of 20% Eluent B and 80% Eluent A, which was linearly increased to 100% Eluent B over the first 25 min. This composition was maintained for the following 10 min, after which the system was returned to the initial condition of 20% Eluent B and 80% Eluent A over the final 10 min. The flow rate was set at 0.5 mL/min, and the injection volume was 5 µL.

Mass spectrometry was carried out using a Shimadzu LCMS-8040 system equipped with an electrospray ionization source, operated in both positive and negative modes. Data were collected and analyzed through LabSolutions software. Identification and quantification of phytochemicals were conducted in multiple reaction monitoring mode. Ion transitions and collision energies were specifically optimized for each compound to ensure high sensitivity and selectivity. The drying gas was maintained at a flow rate of 15 L/min, while the nebulizing gas was supplied at 3 L/min. Instrumental temperatures were set to 250 °C for the desolvation line, 400 °C for the heat block, and 350 °C for the interface, ensuring optimal ionization and signal stability during analysis (Yilmaz, 2020).

Statistical Analysis

Each experimental analysis was repeated three times to ensure reproducibility. Results are reported as the average values accompanied by their standard deviations (SD). Statistical evaluations were carried out using IBM SPSS Statistics software (version 21). To determine whether there were significant differences between groups, a one-way ANOVA test was applied. When significant variation was detected, Duncan's multiple range test was employed for pairwise group comparisons. Statistical significance was defined as $p < 0.05$.



In the second stage of green synthesis, biologically active phytochemicals—particularly phenolics and flavonoids—adsorb onto the NP surface, forming a stable organic capping layer (phenolic/carboxyl capping) that prevents agglomeration and enhances colloidal stability (Eqs. 3 and 4). This bioactive coating not only stabilizes the NPs but also imparts antioxidant, antimicrobial, and anti-inflammatory properties, thereby improving their suitability for biomedical and environmental applications (Al-darwesh et al., 2025; Li

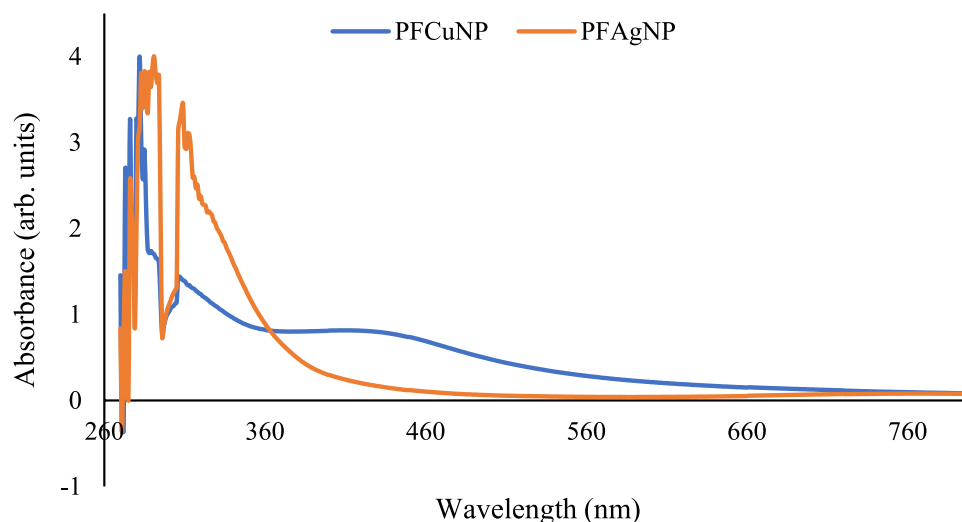
Results and Discussion

Characterization of Biosynthesized NPs

The green synthesis of AgNPs and CuNPs using plant extracts has gained prominence due to their unique physicochemical properties, including high surface area, catalytic activity, and antimicrobial potential (Chakravarty et al., 2022; Crisan et al., 2021). In this study, PF-E was employed as a sustainable reducing and stabilizing agent for the green synthesis of PFAgNPs and PFCuNPs. The green synthesis process carried out using PF-E extract proceeds in two main stages: the reduction of metal ions and the coating of NP surfaces with phytochemical compounds. The phenolic acids, flavonoids, and carbonyl-containing compounds present in the PF-E extract act both as reducing agents and as stabilizing capping materials in this process (Eker et al., 2025).

In the initial stage of green synthesis, the hydroxyl (–OH) and carbonyl (–C=O) functional groups present in plant extracts act as reducing agents by transferring electrons to the metal ions (Ag^+ or Cu^{2+}) in the solution, thereby reducing them to their zero-valent metallic forms (Ag^0 , Cu^0). This reduction process primarily occurs through the oxidation of phenolic compounds. Upon donating electrons, the phenolic structures are converted into phenoxyl radicals ($\text{Ar}-\text{O}\cdot$) or quinone derivatives (Zuhrotun et al., 2023). In this context, $\text{Ar}-\text{OH}$ represents a phenolic compound, where 'Ar' denotes an aromatic ring (such as a benzene derivative). Therefore, $\text{Ar}-\text{OH}$ symbolizes a phenol molecule, such as gallic acid or quercetin. The protons (H^+) released during the reaction help maintain the chemical equilibrium of the medium. The neutral metal atoms formed (Ag^0 , Cu^0) subsequently collide and aggregate randomly to create nucleation centers, initiating the formation of NPs (AgNPs and CuNPs) (Eqs. 3 and 4) (Nguyen et al., 2022).

et al., 2023b; Sysak et al., 2023). Consequently, the green synthesis approach based on PF-E extract is considered an effective, eco-friendly, economical, and biocompatible strategy for NP production without the use of toxic chemicals (Ashokkumar et al., 2024; Daphedar et al., 2024). Based on these findings, the successful formation and stabilization of these NPs were confirmed through comprehensive characterization techniques, including UV–Vis spectroscopy, FT-IR, XRD, SEM–EDX, and TEM.

Fig. 2 UV–Vis absorbance spectra for green synthesis of PFAgNP and PFCuNP**Table 1** UV–Vis spectral parameters of synthesized NPs

Samples	λ_{max} (nm)	A_{max}	FWHM (nm)	Spectral Feature
PFAgNP	309	3.452	32.1	Distinct UV band (phytochemical and AgNP overlap)
PFCuNP	306	1.442	153.8	Broad band; Cu ₂ O/organic absorption overlap

UV–Vis Spectroscopy Analysis of PFAgNPs and PFCuNPs

The optical properties of the biosynthesized NPs were examined using UV–Vis spectroscopy in the wavelength range of 260–800 nm (Fig. 2). The obtained spectra exhibited distinct absorption characteristics, confirming the successful formation of phytogenic metallic NPs. From the recorded data, relevant spectroscopic parameters such as the maximum absorption wavelength (λ_{max}), the corresponding absorbance intensity (A_{max}), and the full width at half maximum (FWHM) were calculated and summarized in Table 1. The FWHM value represents the spectral width at half of the peak height and provides insight into the particle size distribution and surface homogeneity of the synthesized NPs.

The PFAgNP sample exhibited a distinct absorption band with a maximum at 309 nm ($A_{\text{max}}=3.452$; FWHM ≈ 32 nm), whereas PFCuNP displayed a broader and less intense feature centered around 306 nm ($A_{\text{max}}=1.442$; FWHM ≈ 154 nm). Although classical LSPR bands for Ag and CuNPs are typically observed at approximately 400–440 nm and 560–600 nm, respectively, the dominance of sub-340 nm absorbance in the present spectra is attributed to the strong optical activity of phytochemicals (mainly polyphenols and flavonoids) remaining on the NP surfaces. These organic

moieties act as natural capping and stabilizing agents, inducing intense UV absorption and light scattering that can mask or attenuate the visible-range plasmon resonance signal.

Recent studies have also confirmed that the abundance of plant-derived phenolic compounds significantly influences the optical response of green-synthesized NPs by modifying their surface dielectric environment and plasmon coupling behavior (Kaliappan et al., 2025; Khan et al., 2025; Rama et al., 2023). Similar spectral shifts and band broadening effects have been reported in green-synthesized AgNPs prepared from various phytochemical-rich extracts, where polyphenols and flavonoids were shown to act as both reducing and capping agents. Such phytochemical interactions enhance NP stability but suppress the visibility of the LSPR band in the visible region, leading to strong UV absorption dominance in the spectra.

The relatively broad absorption band observed for PFCuNP ($\lambda_{\text{max}} \approx 306$ nm) can be attributed to a combination of dielectric damping and partial surface oxidation (formation of Cu₂O/CuO phases), phenomena that are commonly encountered in green-synthesized CuNPs. Such effects arise from the strong interaction between the NP surface and the residual phytochemicals, which modify the local dielectric environment and can suppress or shift the LSPR toward the UV region. Similar observations have been widely reported in recent studies, where broad and weak CuNP bands were associated with surface oxidation and organic capping layers (Alshammari et al., 2023; Baral et al., 2025; Dhanalakshmi & Losetty, 2024; Ssekatawa et al., 2022). These reports also emphasized that high phytochemical concentrations not only stabilize the NPs but also alter their optical response, leading to diminished LSPR visibility in the visible region.

Despite these spectral shifts, the observed optical features confirm the formation of nanoscale Ag and Cu systems, as further supported by TEM and SEM analyses

showing average particle sizes of 7.37 nm (PFAgNP) and 3.69 nm (PFCuNP). The combined evidence indicates that both NP types possess sufficient surface plasmonic and functionalized characteristics for adsorption and antioxidant applications.

FT-IR Analysis of PFAgNPs and PFCuNPs

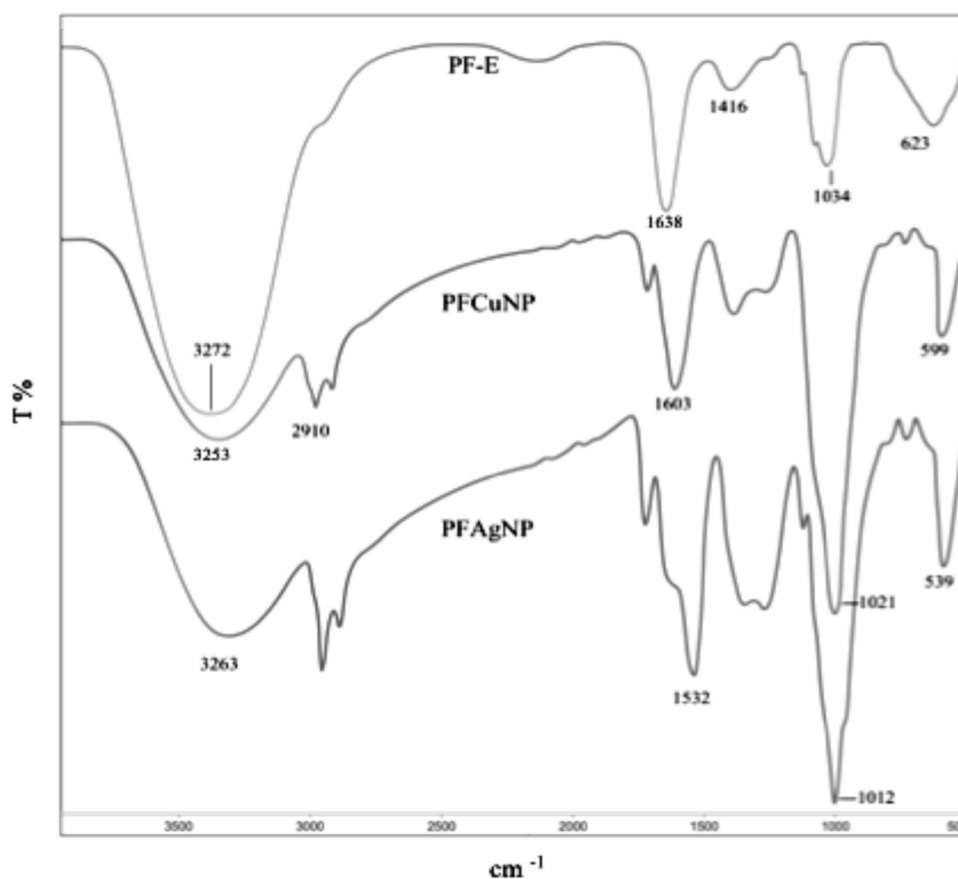
The functional groups present in the structures of PFAgNP and PFCuNP synthesized using PF-E extract were examined through FT-IR spectral analysis in comparison with the PF-E extract itself (Fig. 3). The FT-IR spectrum of the PF-E extract exhibited characteristic absorption bands at 3272.34, 1638.78, 1416.04, 1034.30, and 623.62 cm^{-1} (Fig. 3). The broad band observed at 3272.34 cm^{-1} is attributed to the stretching vibrations of O–H and N–H groups, typically associated with polyphenolic compounds. This observation confirms the presence of various functional groups. The absorption band detected at 1638.78 cm^{-1} may correspond to stretching vibrations of C=C bonds typically found in alkenes. The signal at 1416.04 cm^{-1} can be attributed to either C=O stretching or C–H bending vibrations. The band observed at 1034.30 cm^{-1} is likely associated with C–O–C stretching vibrations commonly present in polysaccharide structures. Finally, the peak at 623.62 cm^{-1} suggests

an out-of-plane bending vibration of C–H bonds, which is characteristic of alkynes (Karan & Erenler, 2023; Yigit et al., 2023).

The FT-IR spectral profile of PFAgNPs exhibited distinct absorption bands at 3263.44, 1532.20, 1375.79, 1012.17, and 539.29 cm^{-1} (Fig. 3). The broad absorption band at 3263.44 cm^{-1} is attributed to the O–H stretching vibrations of phenolic compounds and/or N–H stretching of plant-derived amines, indicating the presence of hydrogen-bonding functional groups originating from the extract. The peak at 1532.20 cm^{-1} is likely associated with aromatic C=C stretching vibrations, possibly coupled with C=O stretching from carbonyl groups. The band at 1375.79 cm^{-1} may correspond to C–N stretching or –CH₃ bending modes (Tian et al., 2022). Additionally, the absorption at 1012.17 cm^{-1} is characteristic of C–O–C or C–O stretching vibrations arising from alcohols and esters (Alemu & Zeleke, 2024). Notably, the band observed at 539.29 cm^{-1} has been previously reported as indicative of Ag–O bond vibrations, confirming the formation of Ag–O interactions on the NP surface (Di Fraia et al., 2025).

In the case of PFCuNPs, the FT-IR spectrum displayed absorption bands at 3253.09, 2910.76, 1603.02, 1021.08, and 599.05 cm^{-1} (Fig. 3). The broad band at 3253.09 cm^{-1} likewise corresponds to O–H and/or N–H stretching

Fig. 3 FT-IR spectra of PF-E extract, PFAgNP and PFCuNP



vibrations. The peak at 2910.76 cm^{-1} is associated with aliphatic C–H stretching (Alsubaie, 2022), while the absorption at 1603.02 cm^{-1} is indicative of C=O stretching, suggesting the attachment of carbonyl-containing biomolecules to the NP surface (Sumaila et al., 2024). The band at 1021.08 cm^{-1} can be assigned to C–O stretching, and the pronounced band at 599.05 cm^{-1} is ascribed to Cu–O stretching vibrations, confirming the presence of Cu–O bonds (Jassim & Salman, 2022; Sumaila et al., 2024).

A comparative analysis of the FT-IR spectra of the PF-E extract, PFAgNPs, and PFCuNPs reveals notable shifts in peak positions and variations in band intensities within the major functional group regions. Specifically, the –OH/–NH stretching band, which appears at 3267.50 cm^{-1} in the PF-E extract, shifts to 3263.44 and 3253.09 cm^{-1} in PFAgNPs and PFCuNPs, respectively. This shift is indicative of hydrogen bonding and coordination interactions occurring at the NP surfaces. Likewise, the characteristic carbonyl (C=O) stretching band at 1638.78 cm^{-1} in PF-E is observed at 1532.20 cm^{-1} in PFAgNPs and 1603.02 cm^{-1} in PFCuNPs, suggesting the active involvement of carbonyl groups in metal ion reduction and NP surface stabilization. These spectral modifications strongly confirm that phenolic, carbonyl, and aliphatic moieties originating from the extract are bound to the NP surfaces, functioning simultaneously as reducing and capping agents. Furthermore, the reappearance of PF-E-derived bands at shifted wavenumbers in the NP spectra substantiates the surface modification processes, while the persistence of similar functional groups across both nanostructures implies that plant-derived biomolecules remain anchored on the surface, thereby imparting biofunctional characteristics to the green-synthesized NPs (Altınsoy et al., 2024; Ramzan et al., 2024).

XRD Analyses of PFCuNPs and PFAgNPs

The crystal structure of the PFAgNPs synthesized using PF-E extract was characterized by XRD (Fig. 4A). The diffraction peaks match metallic silver with a face-centered cubic (FCC) structure (ICDD PDF 04–0783). Bragg reflections at $2\theta = 38.18^\circ$, 44.20° , 64.94° , and 77.41° index to the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes of Ag^0 , respectively, confirming crystalline Ag formation via phytochemical reduction of Ag^+ by PF-E constituents (Labulo et al., 2022; Lakhan et al., 2020; Parvathalu et al., 2023; Trak & Arslan, 2023). A weak feature near 81.6° (2θ , Cu K α), consistent with the (2 2 2) plane, further supports the FCC Ag phase (ICDD PDF 04–0783). The lattice parameter estimated from the (1 1 1) reflection is a $\approx 4.09\text{ \AA}$, very close to the bulk value of metallic silver ($a = 4.086\text{ \AA}$; ICDD PDF 04–0783), indicating retention of the FCC geometry without measurable lattice distortion or substitution (Abdoon et al., 2023). The slight deviation can be ascribed to microstrain

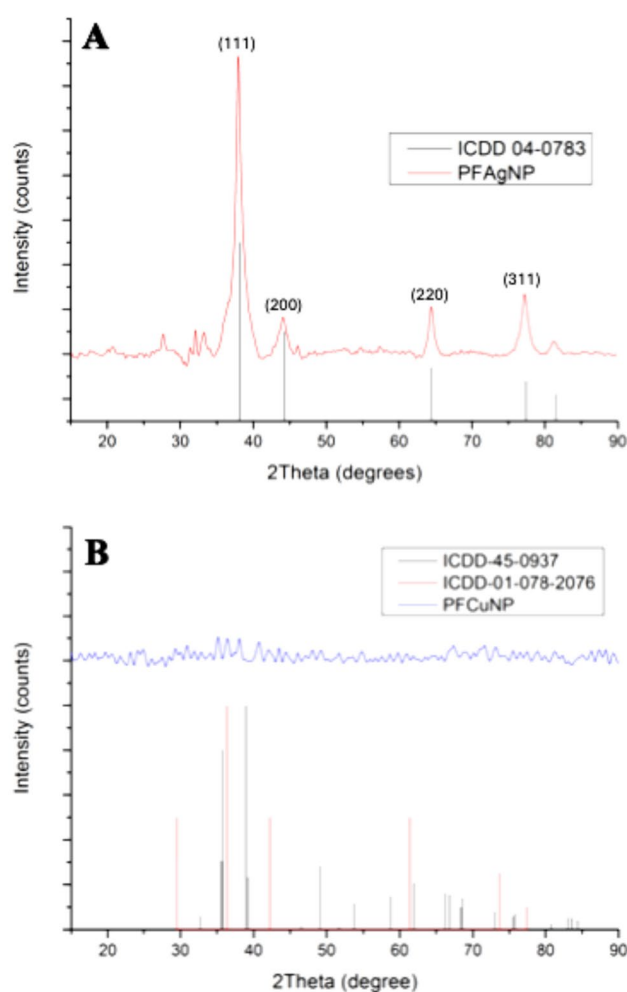


Fig. 4 XRD graphs of **A.** PFAgNP and **B.** PFCuNP

and finite-size effects typical of nanoscale crystallites produced via green synthesis. Scherrer analysis of the (1 1 1) peak yields an average crystallite size of $\sim 6\text{ nm}$, corroborating the nanocrystalline nature of the PFAgNPs (Ashraf et al., 2025). In agreement, the average particle diameter of PFAgNPs was 7.37 nm (Fig. 11), close to the XRD crystallite size and consistent with largely single-crystalline domains (Sherpa et al., 2024). No additional crystalline phases are evident within the detection limit, indicating high phase purity and effective reduction during synthesis.

In stark contrast to PFAgNPs, the XRD pattern of PFCuNPs (Fig. 4B) shows no discernible Bragg reflections attributable to crystalline Cu_2O (ICDD-01-078-2076), or CuO (ICDD-45-0937). The horizontal baseline, devoid of characteristic peaks, further corroborates the absence of crystalline phases. Instead, the diffractogram is dominated by a diffuse background (broad scattering in the $\sim 15\text{--}35^\circ$ region) with no peak maxima rising appreciably above the baseline—consistent with an amorphous or highly disordered product. Such featureless

profiles are recurrently documented for plant-mediated CuNPs, where strong phytochemical capping, sometimes ultrasmall coherent domains ($\approx 3\text{--}4\text{ nm}$), and thin amorphous oxide/hydroxide shells suppress long-range order and broaden diffraction beyond recognition (Ayadi Hassan et al., 2022; Das et al., 2020; Mahmoud et al., 2023; Millavithanachchi et al., 2025; Siddiqui et al., 2022). In agreement, TEM analysis yielded an average particle diameter of 3.69 nm for PFCuNPs (Fig. 12), a size regime where line broadening approaches instrument resolution; accordingly, Scherrer-type sizing is not applicable. This amorphous/disordered character can benefit surface-driven applications (e.g., adsorption, ion exchange), although it may limit catalytic selectivity and turnover relative to well-crystallized copper phases. Consistent

with this picture, the agglomeration observed by electron microscopy is typical of organically capped, structurally disordered Cu materials formed via green synthesis routes (Das et al., 2020).

SEM Analyses of PFAgNPs and PFCuNPs

High-resolution SEM imaging (Figs. 5A–B, 6A–B) revealed the surface morphology that both PFAgNPs and PFCuNPs form agglomerated architectures typical of biogenic syntheses (Ashokkumar et al., 2024; Chakraborty et al., 2022; Phang et al., 2021). The agglomeration is attributed to a combination of hydrogen bonding among surface phenolic/amine groups, hydrophobic association of organic capping layers, $\pi\text{--}\pi$ interactions between aromatic moieties, van der

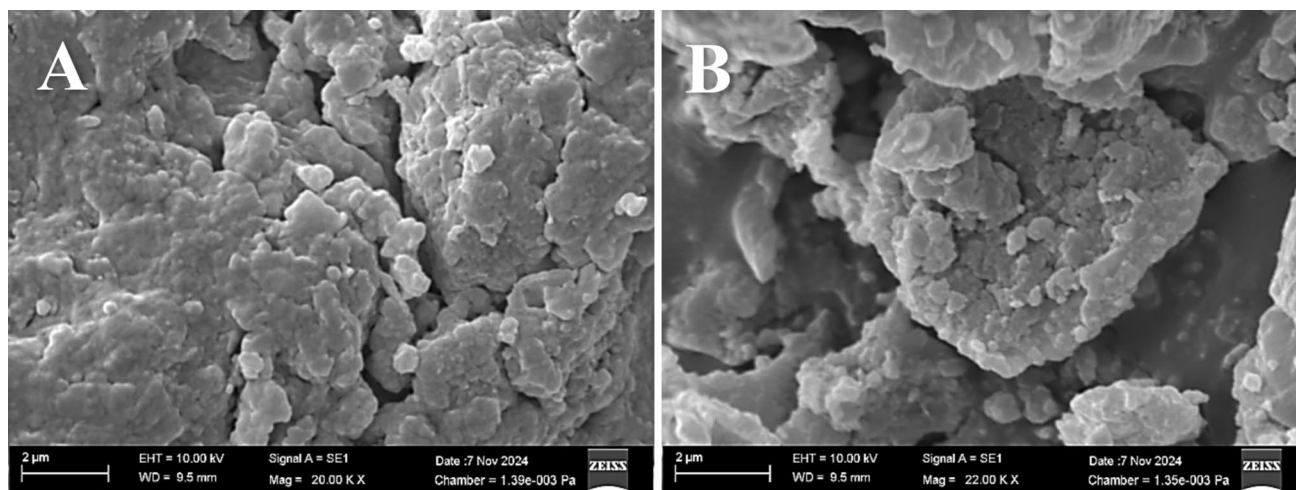


Fig. 5 SEM images of PFAgNPs **A**, Magnification: 20.00 K, **B**, Magnification: 22.00 K

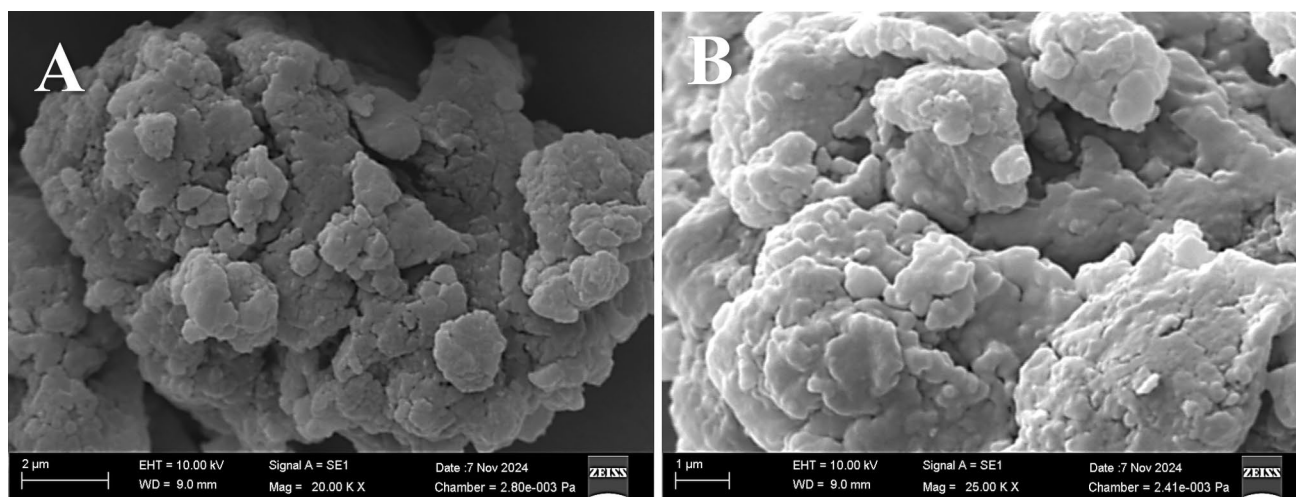


Fig. 6 SEM images of PFCuNPs **A**, Magnification: 20.00 K, **B**, Magnification: 25.00 K

Waals forces, and metal–ligand coordination at the particle interface. Consistent with this interpretation, FT-IR evidence (Fig. 3) confirms phenolic/amine and carbonyl groups bound to the NP surfaces together with metal–oxygen signatures. More compact aggregates are typically observed for AgNPs—consistent with weaker Lewis acidity and predominant Ag–O/Ag–N coordination—whereas more extensive, network-like assemblies are observed for CuNPs, plausibly owing to stronger Cu–O coordination and partial surface oxidation/hydroxylation that promotes interparticle bridging. Such aggregation increases hydrodynamic size but does not preclude functional performance, as evidenced by the retained bioresponses (Karpuz et al., 2025).

TEM, EDX and Size Distribution Examinations of PFCuNPs and PFAgNPs

The nanostructural characteristics of PFAgNP and PFCuNP synthesized using the PF-E extract were examined by TEM, and the corresponding images are presented in Figs. 7A–B and 8A–D, respectively. As shown in Figs. 7A–B, PFCuNP exhibited a predominantly spherical morphology, with particle diameters typically below 10 nm. These particles were uniformly distributed without notable agglomeration, demonstrating the strong capping and stabilization effect of the biomolecules present in the extract. Similarly, PFAgNP displayed well-defined, uniformly spherical nanostructures throughout the examined field (Figs. 8A–B), indicating a homogeneous dispersion and narrow size distribution.

At higher magnification (Fig. 8C), the high-resolution TEM image revealed distinct lattice fringes with an interplanar spacing of $d = 0.24$ nm, corresponding to the (1 1 1) crystallographic plane of FCC metallic silver (ICDD No. 04–0783). The clarity of these lattice fringes confirms the crystalline nature of the AgNPs. The corresponding selected-area electron diffraction (SAED) pattern (Fig. 8D) exhibited well-defined concentric diffraction rings, further

validating the polycrystalline character of the nanoparticles. The observed diffraction rings in SAED patterns correspond to the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes of FCC Ag, in agreement with the XRD results. These observations align well with previous reports on plant-mediated synthesis of noble metal NPs, in which spherical and uniformly dispersed morphologies are frequently observed (Al Qhtani et al., 2020; Alsubki et al., 2021; Jiang et al., 2023; Sherpa et al., 2024). The fine distribution and strong crystallinity of PFAgNPs thus indicate that the phenolic and flavonoid constituents of PF-E acted efficiently as both reducing and stabilizing agents during nucleation and growth.

In contrast, no SAED pattern or lattice-resolved image could be obtained for the PFCuNP sample. This limitation is attributed to several well-known factors: the extremely small primary particle size (a few nanometers), the presence of an amorphous phytochemical capping layer, and the inherent electron-beam sensitivity of metallic Cu and sub-stoichiometric Cu-oxide domains. These factors collectively attenuate and broaden diffraction intensities, making ring patterns difficult to resolve under conventional SAED conditions (Bijelić et al., 2024; Li et al., 2023a). Moreover, the thermionic SEM system employed for complementary imaging provides lower probe brightness and surface sensitivity than field-emission instruments, rendering isolated sub-10 nm primaries unresolved in SEM micrographs (Egerton, 2022). Consequently, PFCuNPs appear as agglomerated clusters in SEM, whereas TEM—acquired on thin supports under low mass-thickness conditions—reveals homogeneously dispersed primaries. This contrast between SEM and TEM observations has been widely reported for plant-mediated CuNPs, where strong phytochemical capping and partial oxidation promote secondary clustering in the dried state (Okpara et al., 2021; Priya et al., 2023).

EDX was also performed to confirm the elemental composition of the synthesized NPs (Figs. 9 and 10). EDX is a rapid technique for elemental identification based on the

Fig. 7 HRTEM images **A.** PFCuNP, scale bar: 50 nm, **B.** PFCuNP, scale bar: 100 nm

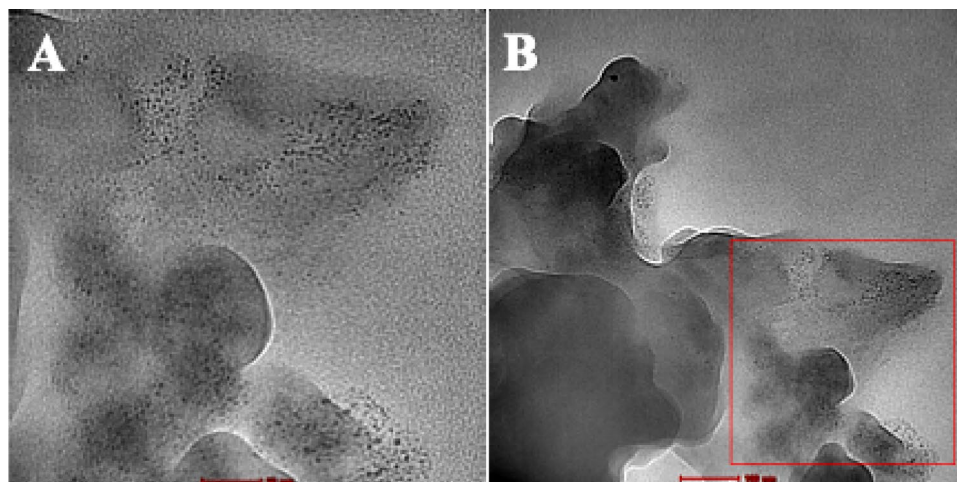
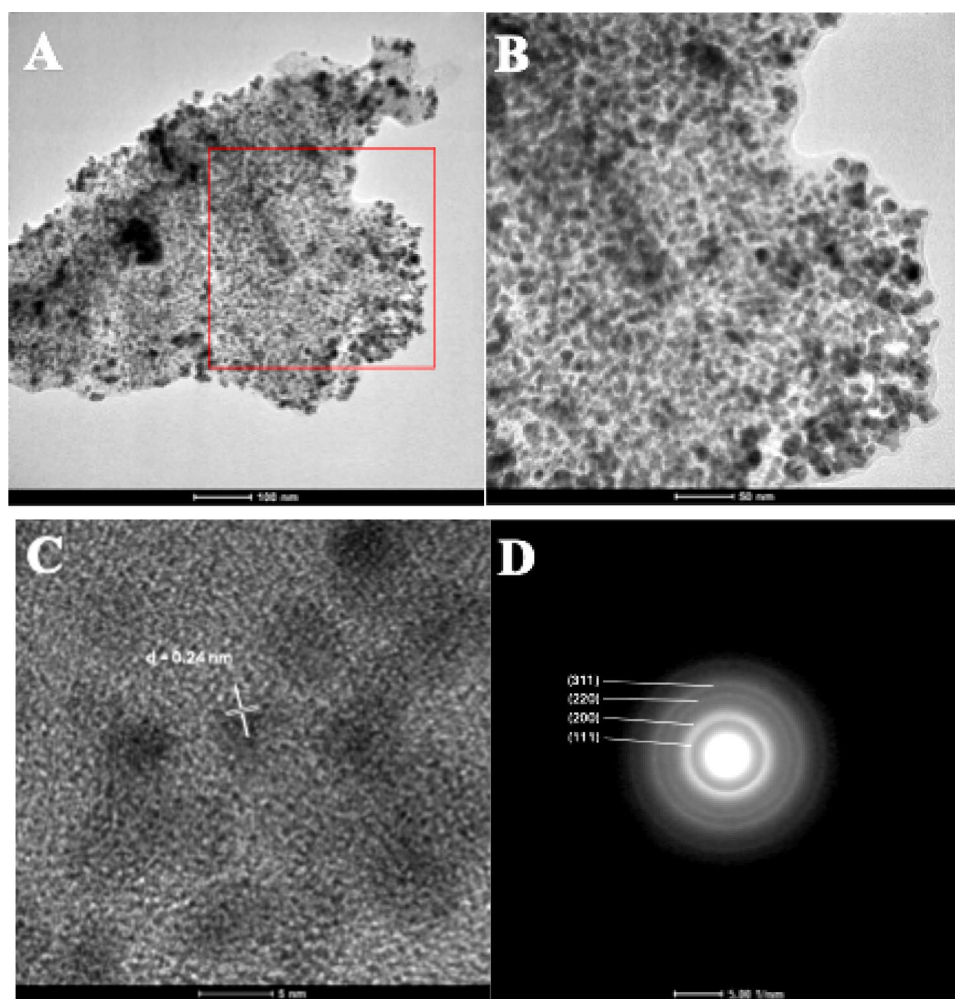


Fig. 8 HRTEM images of PF_{Ag}NP **A.** Scale bar: 50 nm, **B.** Scale bar: 100 nm **C.** Scale bar: 5 nm **D.** SAED pattern

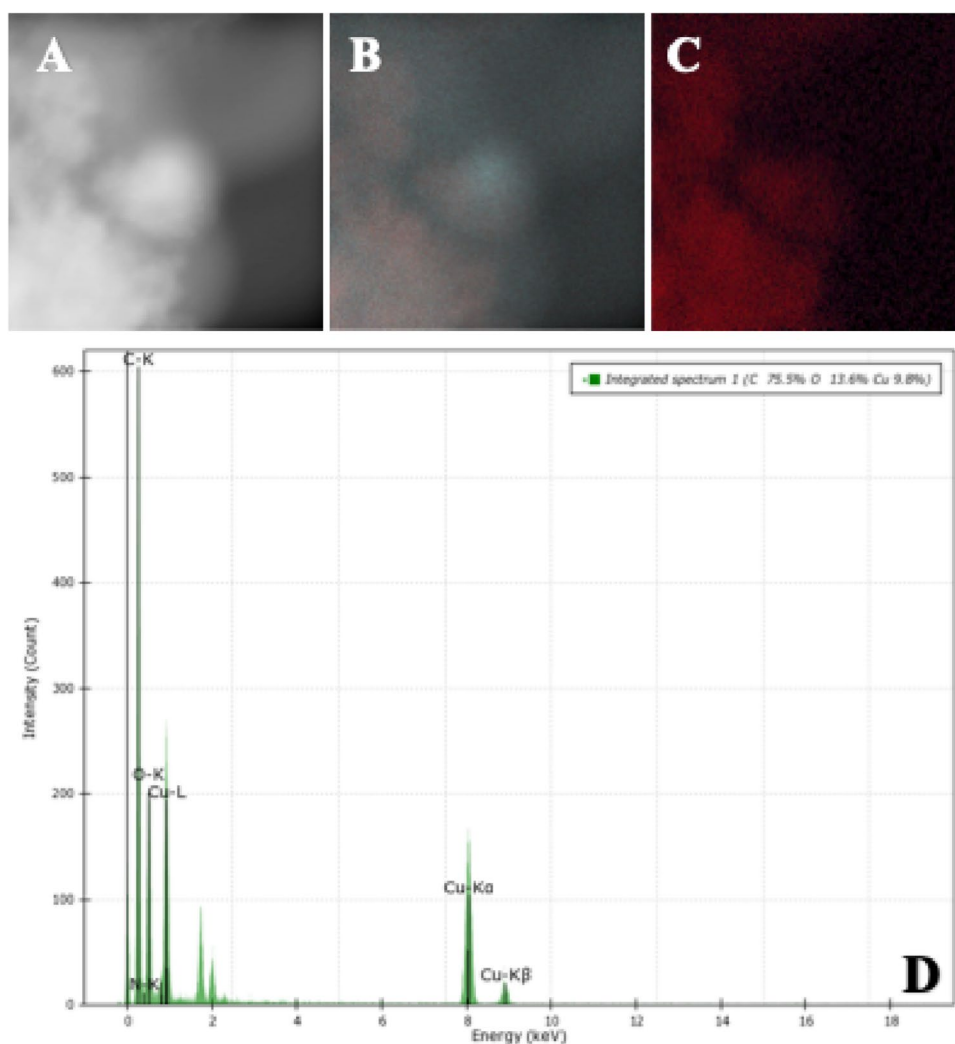


detection of characteristic X-rays emitted during electron-beam interaction (Shweqa et al., 2024). Figures 9A and 10A show high-angle annular dark-field (HAADF) images of PFCuNPs and PF_{Ag}NPs, respectively, while Figs. 9B and 10B depict the corresponding EDX elemental maps, illustrating the uniform spatial distribution of Cu and Ag within the selected regions. The spectra presented in Figs. 9C and 10C confirm the presence of Cu and Ag as the principal metallic constituents. Quantitative analysis (Figs. 9D and 10D) indicated Cu and Ag mass fractions of 9.8% and 12.1%, respectively. The co-localization of these signals with the HAADF regions provides direct evidence of successful NP formation and corroborates the reductive and stabilizing role of the PF-E extract during synthesis. EDX analysis confirmed the presence of Cu and Ag signals co-localized with nanoparticle regions, with 9.8 wt% Cu and 12.1 wt% Ag detected. These values are typical for plant-mediated NPs, where the phytochemical capping matrix—rich in polyphenols, proteins, and sugars—introduces substantial carbon and oxygen within the electron interaction volume, thereby lowering the apparent metal

fraction. Comparable results have been reported in the literature: Naidoo et al. (2023) observed 8.8–37.0 wt% Ag in *Tabernaemontana ventricosa*-derived AgNPs, while Tsilo et al. (2023) reported 6.8 wt% Cu for biogenic CuNPs stabilized by a yeast-derived biofloculant. These findings are consistent with EDX's surface-weighted and semi-quantitative nature, which underestimates metallic content when particles are coated or embedded in organic matrices (Mourdikoudis et al., 2018; Scimeca et al., 2018). Together, the TEM and EDX analyses conclusively demonstrate that the PF-E extract efficiently facilitated the nucleation and stabilization of metallic NPs with well-defined spherical morphology, nanoscale dimensions, and high compositional purity.

The particle size distribution of the NPs was characterized using the polydispersity index (PDI), which reflects both nanoscale dimensions and uniformity. A PDI value ranging from 0.1 to 0.25 indicates a narrow size distribution, whereas values exceeding 0.5 suggest a broader distribution. Particle size and PDI are considered two fundamental parameters in the evaluation of NPs, as they are influenced by various factors, such as the composition and temperature

Fig. 9 EDX spectrum and elemental composition analysis of PFCuNPs, including **A.** TEM-HAADF image, **B.** elemental mapping showing carbon (blue), oxygen (orange), copper (red), and nitrogen (purple), **C.** spatial distribution of copper (red), and **D.** EDX spectrum



conditions applied during synthesis (Hoseini et al., 2023). For instance, in many applications, including drug delivery systems, a PDI value between 0.1 and 0.3 is generally regarded as acceptable (Kushwaha et al., 2019). In this context, the particle size distribution analysis was carried out using ImageJ software based on the NPs synthesized with PF-E extract, whose morphology was also observed in the TEM analysis. The average particle diameters of PFAgNP and PFCuNP were calculated as 7.37 nm and 3.69 nm, respectively, as illustrated in Figs. 11 and 12. The calculated PDI values for PFAgNP and PFCuNP were found to be 0.150 and 0.023, respectively. As presented in Figs. 11 and 12, both NP types exhibited favorable size distributions based on their PDI metrics. The exceptionally low PDI value of PFCuNP is considered a desirable characteristic, particularly in the context of nanoadsorbent fabrication. This outcome aligns well with the intended objectives of the current study.

Table 2 summarizes several studies in which AgNPs and CuNPs were synthesized via green methods using various

fruit extracts, along with their reported characteristics. Among these investigations, spherical NP morphology has been frequently observed, particularly for both AgNPs and CuNPs. It is also evident that the particle sizes reported in earlier studies involving fruit-derived extracts are largely comparable to those obtained in the present work, as shown in Table 2.

Results of TPC, TFC, and TAC

TPC refers to the total phenolic content in a substance, representing compounds known for their antioxidant properties in plants (Zeb, 2020). TFC denotes the quantitative determination of flavonoids in plant extracts, compounds recognized for their antioxidant activity and associated health benefits (Nicolescu et al., 2025). The TFC values of metal oxides may vary depending on their chemical characteristics. TAC analysis, on the other hand, assesses the overall antioxidant capacity of a sample by estimating its potential to neutralize free radicals (Sangaonkar & Pawar, 2018). Based on

Fig. 10 EDX spectrum and elemental composition analysis of PFaAgNPs, including **A.** TEM-HAADF image, **B.** elemental mapping showing carbon (blue), oxygen (orange), silver (red), and nitrogen (purple), **C.** spatial distribution of silver (red), and **D.** EDX spectrum

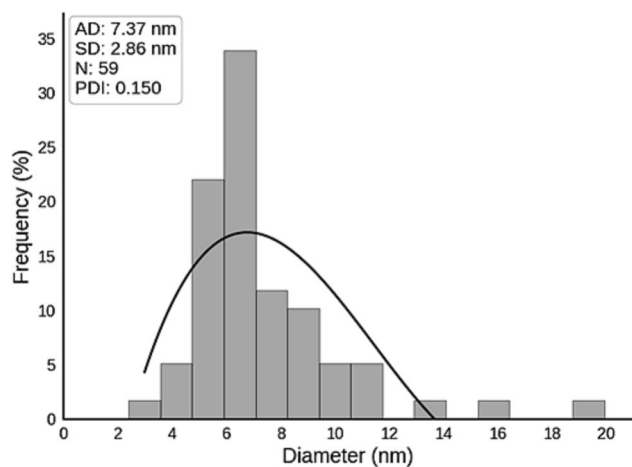
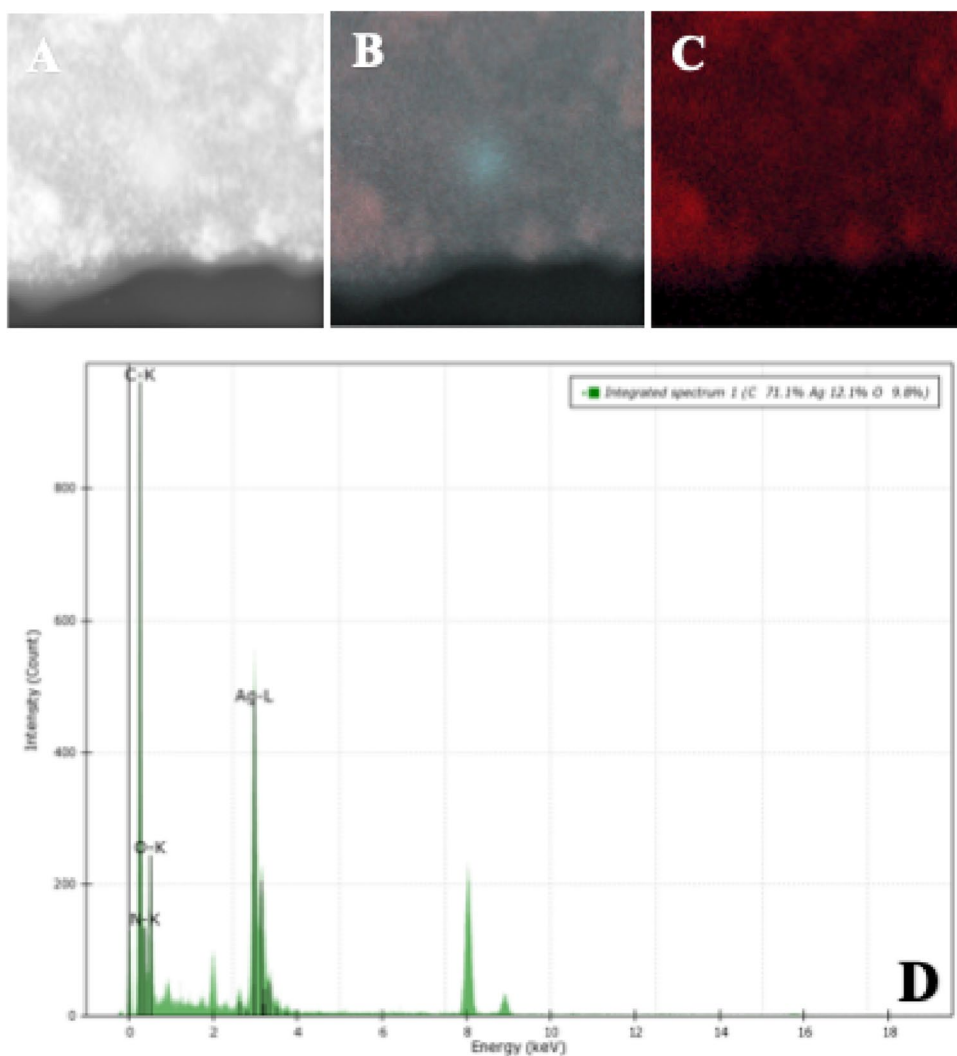


Fig. 11 Size distribution of PFaAgNPs (AD: Average diameter, SD: Standard deviation, N: Number of measured NPs, PDI: Polydispersity Index)

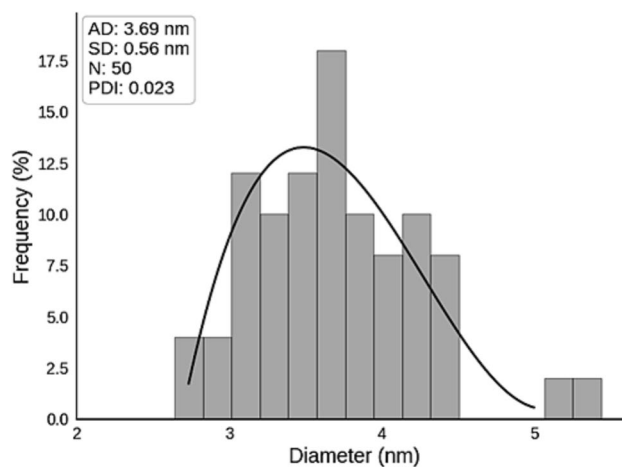


Fig. 12 Size distribution of PFCuNPs (AD: Average diameter, SD: Standard deviation, N: Number of measured NPs, PDI: Polydispersity Index)

Table 2 Green-synthesized NPs from fruit extracts: particle size and morphology

Extract and NP type	Size	Morphology	References
Cherry laurel – AgNP	80.39 nm	Spherical	Yigit et al., 2023
Cornelian cherry – AgNP	50.86 nm	Spherical	Özeşer & Karagözlü, 2024
Vitis vinifera – AgNP	30–65 nm	Mostly Spherical	Hashim et al., 2020
Clammy cherry (<i>Cordia obliqua</i> Willd) – AgNP	7.13 nm	Spherical	Saidu et al., 2019
Capuli (<i>Prunus serotina</i> Ehrh. Var. Capuli) cherry – AgNP	20–100 nm	Spherical	Kumar et al., 2016
Capuli (<i>Prunus serotina</i> Ehrh. Var. Capuli) Cherry – CuNP	30–55 nm	Spherical	Kumar et al., 2021
<i>Prunus mahaleb</i> L. – CuNP	20–30 nm	Spherical	Dashtizadeh et al., 2021
Andean blackberry (<i>Rubus glaucus</i> Benth.)– CuNP	45–53 nm	Spherical	Kumar et al., 2017
Barberry fruit extract – CuNP	35 nm	Spherical	Nasrollahzadeh et al., 2015
Malpighia glabra – CuNP	22.5 nm	Spherical	Nguyen et al., 2024
Sea Buckthorn berry – AgNP	27.3 nm	Spherical	Wei et al., 2020
Red currant (<i>Ribes rubrum</i>) – AgNP	4–59 nm	Roughly spherical	Rizwana et al., 2022
Bilberry (<i>Vaccinium myrtillus</i>) waste – CuNP	2–10 nm	Tiny globular	Benassai et al., 2021
<i>Prunus laurocerasus</i> L. – CuNP	3.69 nm	Spherical	This study
<i>Prunus laurocerasus</i> L. – AgNP	7.37 nm	Spherical	This study

Table 3 TPC, TFC and TAC values of PF-E, PFAgNP and PFCuNP*

Samples	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg AAE/g)
PF-E	3.32 ± 0.18 ^a	17.58 ± 1.70 ^a	7.91 ± 1.17 ^a
PFAgNP	143.90 ± 1.80 ^b	90.66 ± 9.24 ^c	74.51 ± 6.94 ^c
PFCuNP	192.10 ± 8.50 ^c	75.70 ± 7.57 ^b	54.37 ± 1.87 ^b

*The data were the mean of three replicate samples ($n=3$) ± SD. Samples marked with distinct lowercase letters (a–c) were found to differ significantly from one another based on Duncan's multiple range test, with statistical significance considered at $p < 0.05$

this framework, the TPC, TFC, and TAC results of the NPs synthesized via green synthesis using the PF-E extract are presented in Table 3. As shown in Table 3, each sample is marked with distinct letters within the same column, indicating that the mean values differ significantly from one another according to Duncan's multiple range test ($p < 0.05$). The TPC, TFC, and TAC values of the PF-E extract were determined to be 3.32 ± 0.18 mg GAE/g, 17.58 ± 1.70 mg QE/g, and 7.91 ± 1.17 mg AAE/g, respectively. In comparison, the TPC values of PFAgNP and PFCuNP were found to be 143.90 ± 1.80 and 192.10 ± 8.50 mg GAE/g, respectively, while their TFC contents were 90.66 ± 9.2 and 75.70 ± 5.57 mg QE/g. The TAC measurements revealed values of 74.51 ± 1.17 mg AAE/g for PFAgNP and 54.37 ± 1.87 mg AAE/g for PFCuNP. These findings indicate that both synthesized NPs exhibit higher TPC, TFC, and TAC contents compared to the PF-E extract. Although metallic NPs such as AgNPs and CuNPs are inorganic in nature, those synthesized through green routes using plant extracts possess organic surface layers derived from phytochemicals that act as reducing, stabilizing, and capping

agents. During biosynthesis, secondary metabolites such as phenolic acids, flavonoids, terpenoids, and proteins from the plant extract adsorb or covalently attach to the NP surface, forming a bio-organic corona (as seen in Eqs. 3 and 4) (Aboyewa et al., 2021; Bhardwaj & Singh, 2024; Villagrán et al., 2024). This coating not only stabilizes the NPs but also imparts functional properties originating from these biomolecules. Therefore, the high TPC, TFC, and TAC values observed in Table 3, as well as the strong antioxidant capacities in Table 4, do not indicate that the metallic core itself is phenolic or antioxidant. Rather, these results arise from the surface-bound phytochemicals that remain strongly associated with the NPs following synthesis. Because the surface-to-volume ratio of NPs is extremely large, the concentration of these bioactive surface groups per gram of material can be several-fold higher than in the original crude extract. This explains why green-synthesized AgNPs and CuNPs often show higher measured phenolic and antioxidant values than their parent extracts, as also supported by recent reports (Bhardwaj & Singh, 2024; Villagrán et al., 2024; Balčiūnaitienė et al., 2022). Furthermore, when the NPs are evaluated in comparison to each other, PFAgNP shows statistically significant higher TPC and TAC values than PFCuNP. The observed variations in TPC, TFC, and TAC measurements may be attributed to multiple factors, including extraction conditions, types of solvents used, NP synthesis methods, and particle size differences (Atak & Uslu, 2018; Dey et al., 2016).

No prior research has, to the best of our knowledge, investigated the TPC, TFC, and TAC contents of PFAgNP and PFCuNP synthesized using PF-E extract. However, AgNPs and CuNPs have been synthesized using various plant

extracts. For instance, in one such study, *Morus nigra* leaf extracts (BM) were employed to synthesize BM-AgNPs, and their TPC and TFC contents were quantified (Jeon et al., 2024). In that study, the TPC values of the BM extract and BM-AgNPs were reported as 24.57 ± 0.17 mg and 148.42 ± 2.33 mg GAE/g, respectively, while the TFC values were determined as 8.07 ± 0.04 mg QE/g for the extract and 12.80 ± 0.65 mg QE/g for the BM-AgNPs. It is seen that the TPC and TFC contents of the synthesized BM-AgNPs are higher than those of the BM extract. These findings are in alignment with the results obtained in the present study. Moreover, existing literature reports that when different plant secondary metabolites are employed as reducing and capping agents, the resulting NPs often exhibit significantly higher TPC and TFC levels compared to their respective extracts (Nazir et al., 2023; Sharifi-Rad et al., 2021; Sivakumar, 2021).

Studies investigating TPC and TFC levels in different cherry laurel (*Prunus laurocerasus* L.) varieties are available in the literature. Some of these studies reported low TFC levels (Alasalvar et al., 2005; Capanoglu et al., 2011; Karahalil & Şahin, 2011; Ozturk et al., 2017), while others indicated notably higher values (İslam et al., 2020; Ozturk et al., 2019). For instance, İslam et al. (2020) determined TFC levels ranging from 101.00 to 611.27 mg QE/g across seven different cherry laurel cultivars. In contrast, Karahalil and Şahin (2011) reported a considerably low TFC value of 0.080 mg QE/g for the same species.

There are notable discrepancies in the literature regarding the TPC content of cherry laurel, expressed as GAE. In a study by İslam et al. (2020), TPC values across seven different cherry laurel cultivars were reported to range between 275.80 mg and 830.17 mg GAE/100 g, depending on the variety. In contrast, Karahalil and Şahin (2011) reported a significantly lower TPC value of 1.094 mg GAE/100 g for cherry laurel. Celep et al. (2012), in their investigation of the antioxidant potential of three edible CLFs, determined the TPC and TFC values to be 23.64 and 16.87 mg QE/g, respectively. In a separate study exploring how various extraction methods affect both the phytochemical profile and antioxidant potential of cherry laurel leaf and fruit extracts, TPC values were found to range between 36.2 and 46.3 mg GAE/g, whereas TFC levels varied from 12.9 to 13.7 mg QE/g (Karabegovic et al., 2014). In a separate study conducted by Koc and Karabulut (2023), the TPC contents of four cherry laurel samples collected from different altitudes in the Trabzon and Rize regions were reported to range between 16.84 and 23.32 mg GAE/100 g. In comparison, the TPC value of the PF-E extract in the present study was determined to be 3.32 mg GAE/g. This variation may be attributed not only to geographical differences but also to factors such as extraction conditions, the type of solvent used, environmental influences, plant variety, and tree

age, as well as post-harvest handling and storage practices (Kolayli et al., 2003). The phenolic content in fruits is influenced by several factors, including temperature, cultivation site, light intensity, altitude, and the age of the tree (Coklar, 2017; Karabegovic et al., 2014). Consequently, it is evident from the literature that the amounts of TFC, TPC, and TAC vary depending on the cherry laurel variety, geographical origin, and applied processing methods, such as extraction and drying.

Results of Antioxidant Activity

Reactive nitrogen species (RNS), reactive oxygen species (ROS), and reactive sulfur species (RSS) are toxic free radicals that arise as byproducts of cellular metabolism and contribute to oxidative stress, ultimately leading to cellular damage (Baruah et al., 2024). The harmful effects of these radicals can be mitigated through the use of NPs with antioxidant properties, as NPs can enhance radical scavenging capacity and reduce oxidative stress (Sangaonkar & Pawar, 2018). The DPPH assay evaluates a compound's ability to neutralize free radicals, while the ABTS assay assesses antioxidant activity through the neutralization of ABTS radical cations (Mahdavi et al., 2020). Additionally, the FRAP assay determines antioxidant potential based on the reduction of Fe^{3+} ions to Fe^{2+} , providing insight into the antioxidant strength of compounds in food or biological samples (Sağlam, 2021). These methods are widely employed to assess the efficacy of compounds in combating oxidative stress.

In line with these considerations, the antioxidant capacities of the NPs synthesized using PF-E extract were evaluated in the present study using widely adopted in vitro chemical assays, including DPPH, ABTS, and FRAP tests. It should be noted, however, that the results of these assays may be influenced by factors such as bioavailability, metabolism, and interactions with other biomolecules, and thus may not directly reflect their behavior in real food systems or biological environments. The scavenging activities against DPPH and ABTS radicals were assessed using two different antioxidant standards (AA and T) (Table 4). Strong antioxidant activity (ABTS, DPPH) is generally correlated with high reducing power, as measured by the FRAP assay. Accordingly, the FRAP test was included as a complementary method to determine the total reducing capacity of the antioxidants. The antioxidant capacities of PF-AgNP and PFCuNP, measured by DPPH, ABTS, and FRAP assays, are presented in Table 4. For the PF-E extract, the DPPH and ABTS values were determined as 4.21 ± 0.19 mg AAE/g and 6.91 ± 0.25 mg TE/g, respectively, while the FRAP value was recorded as 8.62 ± 0.81 mg $\text{FeSO}_4\text{E/g}$. In a study conducted on seven different *Prunus laurocerasus* genotypes, the DPPH antioxidant activity of the samples ranged from

1.07 to 12.19 mmol TE/g (İslam et al., 2020). This study also assessed antioxidant capacities using FRAP and DPPH assays. Based on FRAP analysis, antioxidant activities of the genotypes ranged from 4.96 to 25.37 mmol TE/g, while DPPH activities ranged from 1.07 to 12.19 mmol TE/g (İslam et al., 2020). In another study conducted on cherry laurel, the antioxidant activity measured by the DPPH assay was reported as 3.49% (Gürdal, 2021). As mentioned above, the findings of some studies on the DPPH values of cherry laurel extracts show similarity to the results of our study. However, other studies report differing results, with DPPH values lower than those observed in our research.

As shown in Table 4, both PFAgNP and PFCuNP exhibit statistically significant higher DPPH, ABTS, and FRAP values compared to the PF-E extract. The superior antioxidant performance observed in the NPs compared to the extract itself is likely linked to bioactive constituents in the PF-E extract that function as both reducing and stabilizing agents, enabling their interaction with the NPs surface and contributing to the overall antioxidant capacity (Siakavella et al., 2020). When comparing the NPs among themselves, PFCuNPs were found to exhibit higher antioxidant activity than PFAgNPs (Table 4). A similar trend was observed in the DPPH % inhibition results. Overall, PFCuNPs demonstrated a stronger capacity to neutralize free radicals. This enhanced activity may be attributed to their smaller particle size and consequently larger specific surface area, which allows more effective interaction with free radicals.

To the best of our knowledge, there is no study in the literature reporting the synthesis of AgNPs and CuNPs using PF-E extract and evaluating their antioxidant properties. However, several studies have investigated the antioxidant activities of NPs synthesized using different plant extracts. For instance, in studies examining the DPPH values of AgNPs synthesized from various plants and fruits, the reported DPPH values ranged between 6.54 and 20.94 mg TE/g (Abibullayeva, 2023; Adıgüzel, 2023). The DPPH value range reported in these studies (6.54–20.94 mg TE/g) is considerably lower than the values obtained in the present study. In another study, AgONPs were synthesized using *Artemisia dracunculus* (tarragon) extract, and the DPPH assay was applied to assess their antioxidant capacity. It was

observed that doubling the amount of synthesized AgONPs led to approximately a 37% increase in antioxidant activity (Valizadeh et al., 2024). Similarly, Velsankar et al. (2020) synthesized CuNPs using *Allium sativum* L. (garlic) extracts and evaluated their antioxidant potential using the DPPH method. The results demonstrated that the CuNPs exhibited high antioxidant activity. At increasing concentrations of 20, 40, 60, and 80 µg/mL, the radical scavenging activities were reported to be 29%, 48%, 74%, and 96%, respectively (Velsankar et al., 2020).

Based on the existing literature, there are no studies in the literature that investigate the ABTS and FRAP values of AgNPs synthesized from different cherry laurel varieties. However, studies involving AgNPs synthesized from various other plant and fruit sources have reported ABTS values of 25.66 mg TEQ/g (Adıgüzel, 2023) and 37.82 mg TEQ/g (Abibullayeva, 2023). When compared to these previously reported values, the ABTS results obtained in the present study are evidently higher, indicating a potentially greater antioxidant capacity of the synthesized NPs. In another study, the antioxidant activity of AgNPs synthesized from *Thymus sipyleus* Boiss. Subsp. Rosulans (Borbás) Jelas was investigated, and their FRAP value was reported as 35.91 mg TE/g (Abibullayeva, 2023). Similarly, in a separate study, AgONPs synthesized from avocado leaf extract exhibited a FRAP activity of 41.99 mg TE/g (Adıgüzel, 2023). Furthermore, AgNPs synthesized from *Daphne oleoides* SCHREBER subsp. Oleoides SCHREBER were evaluated for their antioxidant properties, yielding a FRAP value of 47.61 mg TE/g (Eraslan, 2020). Overall, the FRAP results obtained in the present study appear to indicate a generally higher antioxidant capacity compared to NPs synthesized using other plant materials and sources reported in the literature.

Results of Antibacterial Assay

Pathogenic resistance to conventional antibiotics has led to a major global health crisis and is currently considered one of the most serious threats to human health. Certain bacterial strains have developed resistance to nearly all available antibiotics. Consequently, there is a growing demand for novel antibacterial agents to combat these resistant pathogens

Table 4 Antioxidant capacity of PF-E, PFAgNP and PFCuNP *

Samples	DPPH AAE (mg/g)	DPPH Inhibition (%)	ABTS TE (mg/g)	FRAP FeSO ₄ E (mg/g)
PF-E	4.21 ± 0.19 ^a	36.42 ± 1.57 ^a	6.91 ± 0.25 ^a	8.62 ± 0.81 ^a
PFAgNP	84.99 ± 2.56 ^b	71.70 ± 1.86 ^b	56.89 ± 2.12 ^b	80.89 ± 7.47 ^b
PFCuNP	93.86 ± 2.72 ^c	78.15 ± 1.98 ^c	69.19 ± 2.17 ^c	105.55 ± 4.61 ^c

*The values were the mean of three replicate samples ($n=3$) ± SD. Samples marked with distinct lowercase letters (a–c) were found to differ significantly from one another based on Duncan's multiple range test, with statistical significance considered at $p < 0.05$

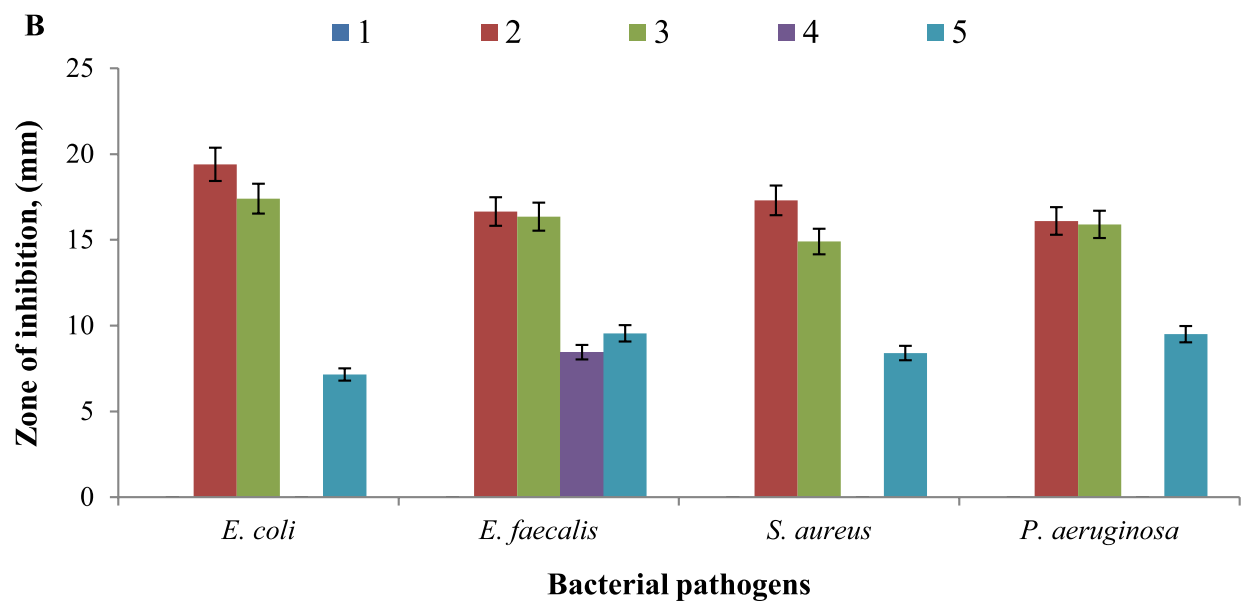
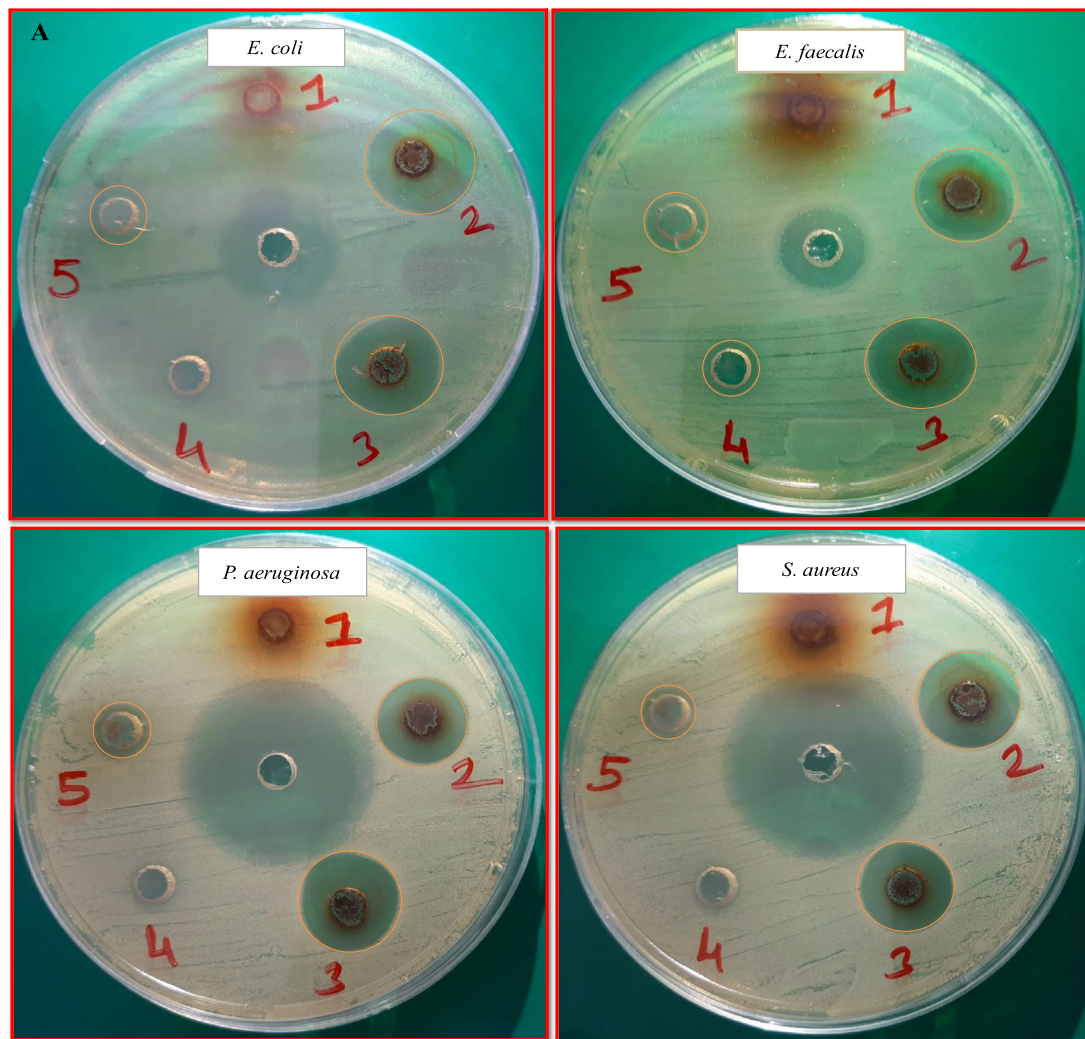


Fig. 13 **A.** Petri plates showing the antibacterial activity, **B.** Inhibition zones (Zone of inhibition, mm) of antibacterial activity of samples against Gram-positive and Gram-negative bacteria (1: PF-E (10 mg/mL), 2: PFAgNP (2 mg/mL), 3: PFAgNP (1 mg/mL), 4: PFCuNP (1 mg/mL), 5: PFCuNP (2 mg/mL)). The values were the mean of three replicate samples ($n=3$) $\pm$ SD ($p<0.05$)

(Ahmad et al., 2024). Recent studies have made mention of metal-based NPs due to their remarkable antibacterial properties. In particular, metal NPs such as AgNPs and CuNPs exhibit broad-spectrum antibacterial activity against a wide range of microorganisms (Erdoğan et al., 2019). In this study, the antibacterial properties of green-synthesized NPs were evaluated using the agar well diffusion method. The antibacterial activity was tested against two Gram-negative (*E. coli* and *P. aeruginosa*) and two Gram-positive (*E. faecalis* and *S. aureus*) pathogenic bacterial strains. These strains were selected due to their significant threat to human health and the increasing demand for alternative antibiotics (Breijyeh et al., 2020). The images of the petri dishes obtained from the experiments are presented in Fig. 13A, while the corresponding inhibition zone diameters are shown in Fig. 13B.

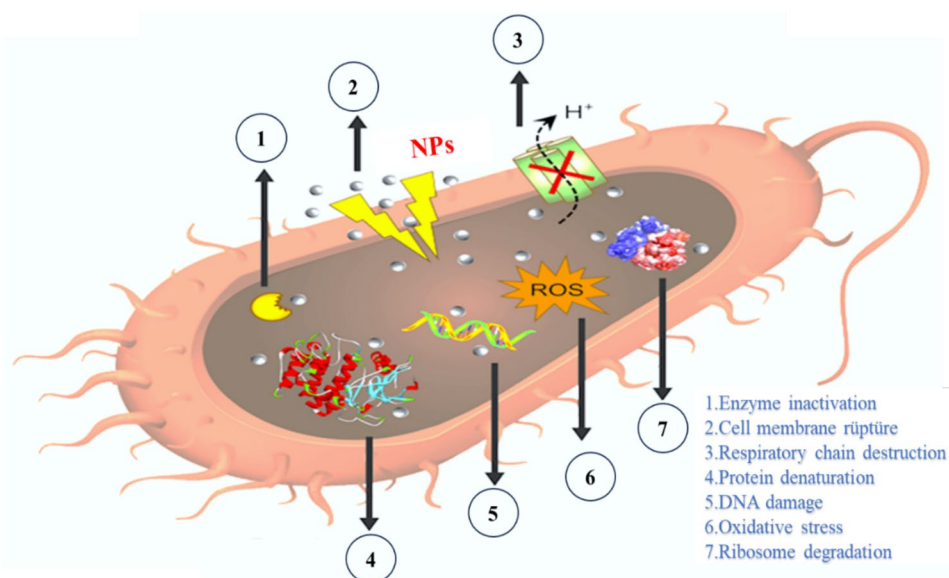
The antibacterial activity of NPs is known to be a concentration- and size-dependent phenomenon (Razavi et al., 2020). Therefore, in the present study, preliminary trials were conducted to determine the lowest effective NP concentrations. Based on these findings, the ability of the NPs to inhibit bacterial growth was assessed by evaluating the inhibition zones. As shown in Fig. 13A, PFAgNPs exhibited strong antibacterial activity against all tested bacterial strains. In contrast, the antibacterial effect of PFCuNPs varied depending on the applied concentration. At higher concentrations (2 mg/mL), PFCuNPs were effective against all tested strains, whereas at lower concentrations (1 mg/mL), they showed activity only against the *E. faecalis* strain. This difference may be attributed to the smaller particle size of PFAgNPs compared to PFCuNPs. Various researchers have reported that the antibacterial efficacy of NPs is influenced by their particle size as well as the rate of ion release, which is facilitated by easier cellular uptake and larger surface area (Dong et al., 2019). In this study, PFAgNPs exhibited the highest antibacterial activity against *E. coli* with an inhibition zone of 19.4 ± 0.3 mm, followed by *S. aureus* at 17.3 ± 0.2 mm, and *E. faecalis* at 16.65 ± 0.1 mm. Conversely, PFCuNPs demonstrated their greatest effect against *E. faecalis* (9.6 ± 0.2 mm) and the lowest against *E. coli* (7.15 ± 0.1 mm). No antibacterial activity was detected for the PF-E extract against any bacterial strain. The antibiotic ampicillin, used as a positive control in the study, exhibited antibacterial effects against all tested strains (Fig. 13A). In this study, PFAgNPs exhibited greater antibacterial

effectiveness against gram-negative strains compared to gram-positive ones.

The inhibitory mechanisms by which NPs suppress bacterial growth have not yet been fully clarified. However, various studies have shown that NPs, especially AgNPs and some other metal-based NPs, demonstrate strong antibacterial effects against both gram-positive and gram-negative bacteria (Fernandez et al., 2021; Vasiliev et al., 2023; Wahab et al., 2021). In addition to damaging bacterial cell membranes and structures, NPs also cause intracellular damage, as illustrated in Fig. 14. Initially, NPs penetrate the cell membrane and enter the cytoplasm, leading to multiple intracellular injuries. They bind to sulfur-containing proteins, causing denaturation of functional proteins and enzyme inactivation. Furthermore, NPs affect ribosomal subunits, resulting in premature termination of protein synthesis (Abdalla et al., 2020; Salleh et al., 2020). Importantly, NPs induce intracellular oxidative stress by generating large amounts of ROS (Ferreira Maillard et al., 2024). Excessive ROS can trigger harmful outcomes such as DNA damage, disruption of the respiratory chain, and ultimately bacterial cell death (Franco et al., 2022; Yu et al., 2020).

As far as we know, there are no reported studies investigating the antibacterial activities of PFAgNP and PFCuNP biosynthesized using PF-E extract. Therefore, our study represents the first report examining the antibacterial efficacy of PFAgNP and PFCuNP. Additionally, most antibacterial research in the literature has primarily focused on AgNPs synthesized from various plants and plant parts. In one study, Trak and Arslan (2023) synthesized AgNPs using dried *M. nigra* fruit extract. The antibacterial activities of these AgNPs were evaluated by the agar diffusion method against both Gram-positive bacteria (*E. faecalis* ATCC 29212, *Listeria monocytogenes* ASKK472, *B. cereus* ATCC 11778, *S. aureus* ATCC 43300) and Gram-negative bacteria (*Salmonella Typhimurium* ATCC 14028, *Enterobacter aerogenes* ATCC 13048, *K. pneumoniae*, *E. coli* O157:H7, *Salmonella enterica*, *E. coli* O157:H7 ATCC 43895, *E. coli* ATCC 35150). The highest antibacterial activity was observed against *Salmonella Typhimurium* and *E. coli* O157:H7 ATCC 43895 with inhibition zones of 14 mm, followed by *E. coli* O157:H7 with 13.5 mm. The lowest inhibition zone was recorded against *Enterobacter aerogenes* ATCC 13048 at 11 mm (Trak & Arslan, 2023). While these results support the findings of our study, the PFAgNPs synthesized in our work demonstrated greater antibacterial efficacy. In a separate study, AgONPs synthesized using *Moringa oleifera* Lam. Extract exhibited inhibition zones of 15, 22, and 25 mm against *E. coli* at concentrations of 20%, 50%, and 80%, respectively (Ugwoke et al., 2020). These results are comparable to those obtained in the present study, where inhibition zones of 19.40 mm against *E.*

Fig. 14 A schematic representation of the potential antibacterial mechanisms of NPs (bacterial model adapted from “Vecteezy.com”) includes: **1.** Generation of ROS, **2.** Formation of pits on the bacterial cell wall and plasma membrane, **3.** Binding to ribosomes causing disassembly of ribosomal subunits and inhibition of translation, **4.** Interaction with DNA leading to damage and inhibition of replication, **5.** Denaturation of proteins upon NP binding, and **6.** Attachment to ATP synthase systems resulting in inhibition of ATP production



coli and 17.40 mm against *S. aureus* were recorded. In another study evaluating the antibacterial effect of AgO-NPs against *E. coli*, the inhibition zone was reported to be 10 mm (Singh et al., 2015). Furthermore, AgNPs synthesized from *Camellia sinensis* were found to inhibit bacterial growth by approximately 80%, significantly reducing bacterial counts and exhibiting strong antibacterial activity, particularly against gram-negative bacteria (Göl et al., 2020). Collectively, these findings confirm that plant-based AgNPs are effective against *E. coli* and support the antibacterial performance observed in the current study. In one study, CuONPs synthesized using aqueous extracts of the *Protoparmeliopsis muralis* (Schreb.) Rabenh. Were evaluated for their antibacterial activity against *E. coli* at varying metal salt concentrations (0.1, 0.01, and 0.001 M). The resulting inhibition zones were measured as 14.33, 14.00, and 7.66 mm, respectively (Alavi et al., 2019). In another study, CuONPs synthesized from *Artemisia haussknechtii* Boiss. Exhibited antibacterial activity against *E. coli*, producing an inhibition zone of 34 mm (Alavi & Karimi, 2018).

In a separate study conducted by Celik et al. (2020), the antibacterial activity of powders derived from CLFs dried using various methods was tested using both methanolic and aqueous extracts. However, none of the extracts exhibited any antibacterial activity against *E. coli* BL21, *S. aureus* NCTC 8530, and *L. monocytogenes* ATCC 7644. It has been emphasized that antibacterial activity is often species- and even strain-specific. Additionally, the extraction technique and solvent type play a crucial role in determining the inhibitory effects of plant extracts (Celik et al., 2020). These findings support the results obtained in the present study regarding PF-E.

The significant outcomes of this study are particularly important in the context of increasing antibiotic-resistant strains, as they contribute to the development of effective antibacterial agents and the protection of public health.

Phenolic Constituents Identified and Quantified by LC–MS/MS

In this study, LC–MS/MS analysis was employed to identify the phytochemicals present in the PF-E extract. The developed analytical method underwent full validation, including parameters such as linearity, accuracy (recovery), intra-day and inter-day precision (repeatability and reproducibility), limits of detection and quantification (LOD/LOQ), and relative standard uncertainty (within a 95% confidence interval, $k=2$). Among 56 standard phenolic compounds analyzed by LC–MS/MS, six phytochemical compounds were identified. The quantitative results of the identified compounds are summarized in Table 5,

Table 5 Quantitative profile of phenolic compounds identified in PF-E (mg/g extract)

No	Components	PF-E (mg/g extract)*
1	Quinic acid	63.42 ± 2.85
2	Protocatechuic acid	0.07 ± 0.01
3	Chlorogenic acid	2.38 ± 0.80
4	Protocatechuic aldehyde	0.04 ± 0.01
5	Hesperidin	0.03 ± 0.01
6	Naringenin	0.02 ± 0.01

*The values were the mean of three replicate samples ($n=3$) ± SD ($p < 0.05$)

while those present at concentrations below the established LOD and LOQ thresholds were excluded. In this context, the LOD is defined as the minimum analyte concentration that can be distinguished with confidence from baseline noise, whereas the LOQ represents the lowest level at which the analyte can be quantified with acceptable precision and accuracy under the experimental conditions employed. As shown in Table 5, the most abundant phenolic acids in PF-E were quinic acid, chlorogenic acid, and protocatechuic acid, respectively. The dominant flavonoids were determined to be hesperidin and naringenin. Concentrations of these compounds were also quantified: quinic acid at 63.43 mg/g, chlorogenic acid at 2.38 mg/g, protocatechuic acid at 0.07 mg/g, protocatechuic aldehyde

at 0.04 mg/g, hesperidin at 0.03 mg/g, and naringenin at 0.02 mg/g. Among them, quinic acid was found to be significantly more abundant compared to other phenolic constituents. The molecular structures of the identified phenolic compounds are shown in Fig. 15. Additionally, the base peak chromatogram of the PF-E extract obtained by the LC–MS/MS method is provided in Fig. 16.

According to previous literature, the phytochemical composition of cherry laurel varieties significantly varies depending on their geographical origin. In cherry laurel species, a diverse range of phenolic acids has been reported, with considerable variation in their concentrations. Among these, caffeic acid has been detected in the range of 64.6–74.8 mg/100 g fresh weight (FW), while chlorogenic

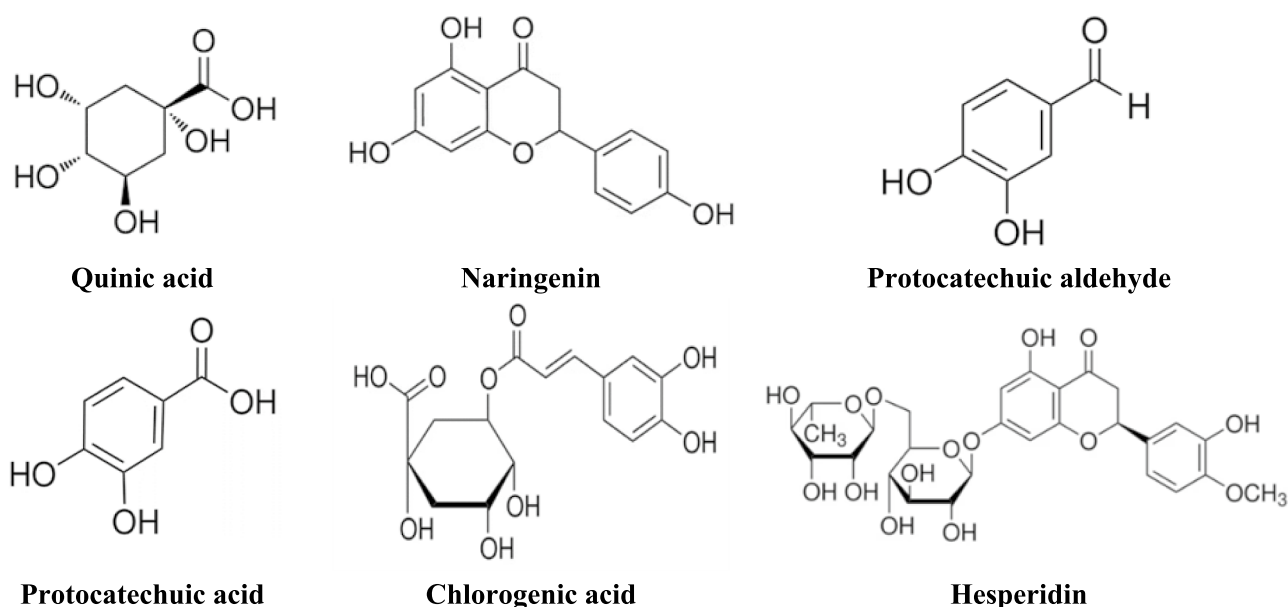
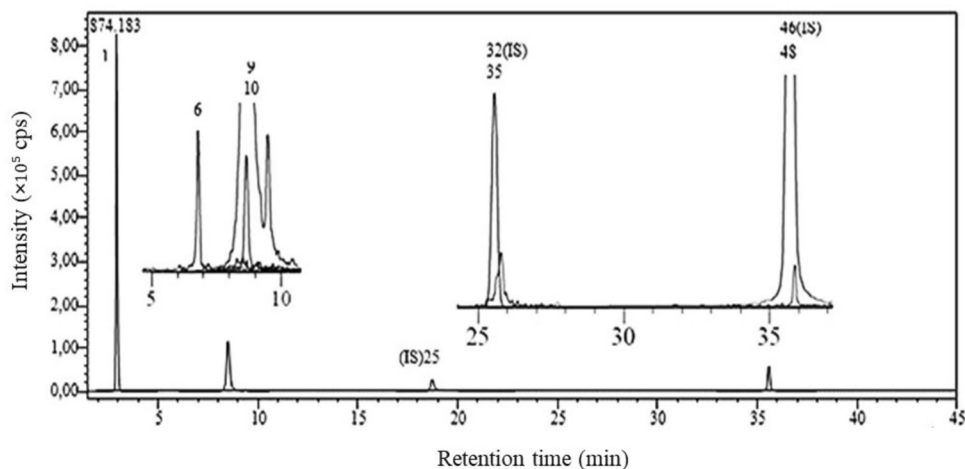


Fig. 15 Structures of bioactive compounds detected via LC–MS/MS

Fig. 16 LC–MS/MS chromatogram of the PF-E extract depicting the detected bioactive constituents. The numbered peaks correspond to the following compounds: (1) quinic acid, (6) protocatechuic acid, (9) chlorogenic acid, (10) protocatechuic aldehyde, (25 IS) ferulic acid-D3 as the internal standard, (32 IS) rutin-D3 as the internal standard, (35) hesperidin, (46 IS) quercetin-D3 as the internal standard, and (48) naringenin



acid represents one of the most abundant phenolics, varying between 103 and 160 mg/100 g FW. Other hydroxycinnamic acids include p-coumaric acid (11.5–49.7 mg/100 g FW) and o-coumaric acid (not detected (ND)–2.24 mg/100 g FW). Ferulic acid has been reported at levels up to 6.2 mg/100 g FW. In addition, gallic acid shows wide fluctuations from 0.2 to 37.6 mg/100 g FW, whereas p-hydroxybenzoic acid occurs in the range of 2.3–3.0 mg/100 g FW. Protocatechuic acid concentrations range from ND–1.12 mg/100 g FW, while its ethyl ester derivative has been measured at 0.04–19.0 mg/100 g FW. Syringic acid has been quantified between 1.0 and 56.2 mg/100 g FW, and vanillic acid between 1.5 and 12.4 mg/100 g FW. These findings, as reported in earlier studies (Alasalvar et al., 2005; Çapanoglu et al., 2011; Karahalil & Şahin, 2011; Karabegovic et al., 2014; Ozturk et al., 2017; Bayrambaş et al., 2019), indicate that cherry laurel is a rich source of phenolic acids with considerable variability depending on cultivar, growing conditions, and analytical methods employed. Another study identified vanillic acid as the major phenolic acid across multiple cultivated and wild CLF varieties, also verifying the presence of p-hydroxybenzoic, protocatechuic, caffeic, vanillic, and p-coumaric acids (Alasalvar & Chang, 2020).

The results obtained in this study are consistent with previous research reporting chlorogenic acid as one of the major phenolic acids present in PF-E extracts (Alasalvar et al., 2005; Karahalil & Şahin, 2011). In addition to chlorogenic acid, other free phenolic acids such as protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, and p-coumaric acid have been identified and quantified in fruit extracts (Alasalvar et al., 2005). Differences observed between this study and prior findings confirm that factors including cultivation season, species variety, environmental and climatic conditions, plant diseases, soil type, geographic location, and maturity stage can significantly influence the concentration and variability of phenolic compounds.

Effect of Contact Time, Type of NP, and Concentration on Patulin Adsorption

In this study, the effectiveness of PF_{Ag}NP and PF_{Cu}NP, which were synthesized using PF-E, was investigated in the adsorption of patulin. To achieve maximum removal efficiency of patulin from food matrices, adsorption experiments were conducted under varying conditions, including contact time, NP type, and concentration. After each adsorption process, the residual amount of patulin in the samples was quantified using HPLC based on a calibration curve, and the adsorption percentage of the NPs was calculated. The selected NP concentrations (2.5 and 5.0 g/L) and contact times (60, 120, and 240 min) were determined through preliminary experiments aimed at identifying effective adsorption ranges, in accordance with similar studies in the literature. The failure to reach the desired adsorption levels at shorter durations and lower concentrations was a key factor in selecting these parameters. In addition, an apple juice matrix was included in the study to enable a realistic evaluation of NP adsorption efficiency under food-relevant conditions, where competitive adsorption effects may occur. Previous studies and the present findings confirm that higher concentrations and longer contact times generally enhance adsorption efficiency. Therefore, these experimental parameters were considered suitable for evaluating optimal adsorption efficiency in both aqueous and real food systems.

Impact of Contact Time on Patulin Adsorption Efficiency

In this study, we examined the contact time (the duration of interaction between the adsorbent surface and the adsorbate molecules) at 60, 120, and 240 min. Each interval represents distinct stages in the interaction dynamics between the NPs and patulin. In general, adsorption efficiency increased with longer contact time for all NPs in both aqueous and real matrices (Table 6). The 60-min duration corresponds to the initial phase of interaction, during which adsorption efficiencies remained relatively low due to insufficient contact

Table 6 Effect of contact time, metal NP type, and concentration on patulin adsorption (%) *

Samples	60 min 2.5 g/L	120 min 2.5 g/L	240 min 2.5 g/L	60 min 5.0 g/L	120 min 5.0 g/L	240 min 5.0 g/L
Standard aqueous solutions¹						
PF _{Cu} NP ¹	16.54 ± 0.54 ^a	22.09 ± 0.89 ^b	23.59 ± 0.24 ^a	56.75 ± 3.78 ^a	59.02 ± 0.07 ^b	61.08 ± 0.28 ^a
PF _{Ag} NP ¹	19.09 ± 0.36 ^b	21.47 ± 0.20 ^a	24.56 ± 1.05 ^b	56.99 ± 0.12 ^a	58.59 ± 0.14 ^a	70.96 ± 0.13 ^b
Real food sample (Apple juice)²						
PF _{Cu} NP ²	16.54 ± 0.26 ^a	19.02 ± 0.29 ^a	27.78 ± 0.43 ^a	18.96 ± 0.29 ^a	23.60 ± 0.37 ^a	32.90 ± 0.52 ^a
PF _{Ag} NP ²	18.77 ± 0.29 ^b	20.85 ± 0.32 ^b	31.97 ± 0.50 ^b	20.50 ± 0.32 ^b	25.48 ± 0.39 ^b	34.24 ± 0.53 ^b

*The values were the mean of three replicate samples ($n=3$) ± SD. Samples marked with distinct lowercase letters (a–b) were found to differ significantly from one another based on Duncan's multiple range test, with statistical significance considered at $p < 0.05$

between patulin molecules and the NP surface. For example, in the water samples (standard aqueous solutions), PF_{Ag}NP at a concentration of 2.5 g/L achieved an adsorption efficiency of 19.09%, while PF_{Cu}NP showed a similar value of 20.75% under the same conditions. In the real samples (apple juice), adsorption efficiencies at 60 min were slightly lower, 18.77% for PF_{Ag}NP and 16.54% for PF_{Cu}NP, likely due to the competitive effects of sugars and organic acids occupying active surface sites on the NPs. At this initial stage, both NPs exhibited comparable and limited adsorption efficiencies, indicating that patulin requires sufficient time to diffuse and interact effectively with the adsorbent surface.

When the contact time was doubled to 120 min, a gradual increase in adsorption efficiency was observed at low NP concentrations of 2.5 g/L. In water samples, PF_{Ag}NP reached 21.47% and PF_{Cu}NP 22.09%, while in real samples, these values were 20.85% and 19.02%, respectively. The modest improvement suggests that adsorption sites become progressively saturated over time as more patulin molecules interact with the NP surfaces. These differences in adsorption efficiency between the NP types can be attributed to their distinct physicochemical properties and surface functionalities. The 240-min duration represents a critical equilibrium stage for evaluating the influence of NPs on patulin adsorption. At this point, substantial improvements were recorded, particularly at higher NP concentrations. In the water samples, at 5.0 g/L, PF_{Ag}NP achieved 70.96% adsorption, whereas PF_{Cu}NP reached 61.08%. In the real samples, the adsorption efficiencies under the same conditions were 34.24% for PF_{Ag}NP and 32.90% for PF_{Cu}NP. Despite the lower adsorption values in the complex matrix, the overall trend of increasing adsorption with time remained consistent.

The reduction in adsorption efficiency in apple juice can be attributed to the matrix complexity, as coexisting molecules such as fructose, citric acid, and polyphenolic compounds compete with patulin for available binding sites or form hydrogen bonds that hinder its direct interaction with NP surfaces. Similar observations have been reported in previous studies, where the presence of organic acids and phenolics in real juice systems significantly reduced the adsorption efficiency of nanomaterials compared to model aqueous solutions (Liang et al., 2022; Liu et al., 2019). The competitive adsorption of fructose and citric acid molecules onto the NP surface may alter the electrostatic potential and block active sites, thereby limiting patulin uptake (Hasanvand et al., 2024). Moreover, the formation of hydrogen bonds between polyphenolic compounds and NP functional groups can reduce the number of available binding regions for patulin, resulting in decreased adsorption efficiency (Safajoo et al., 2022). Despite this inhibitory effect, Ag-based NPs still maintain reasonable removal efficiency in complex matrices, consistent with previous findings that highlight their robust surface functionality and chemical stability (Paimard et al., 2021). Collectively, these

results confirm that matrix complexity is a major limiting factor in patulin removal from real food systems, emphasizing the importance of surface engineering and adsorbent selectivity for achieving high removal efficiency in fruit-based beverages.

Overall, these findings demonstrate that as contact time increases, the interactions between patulin molecules and NPs become stronger, resulting in a higher number of molecules being adsorbed onto the NP surface. Both PF_{Ag}NP and PF_{Cu}NP exhibited higher adsorption efficiency with longer exposure times, yet PF_{Ag}NP consistently showed superior removal efficiency. This suggests that PF_{Ag}NPs, owing to their enhanced surface reactivity and functional phytochemical coating derived from PF-E extract, interact more effectively with patulin molecules. Therefore, extended contact times and Ag-based nanostructures appear to be key parameters for achieving efficient removal of patulin in both aqueous and real food systems.

Impact of NP Concentration on Patulin Adsorption Efficiency

As shown in Table 6, NP concentration is one of the most critical factors affecting patulin adsorption. Higher concentrations lead to increased adsorption rates and more effective interactions between NPs and patulin molecules. This finding is important both for selecting the appropriate NP type and for designing efficient adsorption-based detoxification strategies. The study was conducted using two concentrations, 2.5 g/L and 5.0 g/L, in both aqueous and real samples.

In the water samples, the concentration of 5.0 g/L was found to be significantly superior to 2.5 g/L. For example, PF_{Cu}NP achieved an adsorption efficiency of 61.08% after 240 min at 5.0 g/L, representing a substantial increase compared to 23.59% at 2.5 g/L. Similarly, PF_{Ag}NP exhibited even higher adsorption efficiencies, reaching 70.96% at 5.0 g/L after 240 min, compared with 21.47% at 2.5 g/L. This difference can be attributed to the greater amount of adsorbent material providing a larger surface area and more available active adsorption sites, thereby enhancing the attachment of patulin molecules onto NP surfaces.

In the real samples, a similar concentration-dependent trend was observed, although the overall adsorption efficiencies were lower due to matrix complexity. For instance, at 2.5 g/L, PF_{Ag}NP and PF_{Cu}NP achieved adsorption efficiencies of 20.85% and 19.02%, respectively, after 240 min, while at 5.0 g/L, the values increased to 34.24% and 32.90%, respectively. The reduction in adsorption efficiency in the apple juice matrix is mainly attributed to the presence of coexisting organic compounds such as fructose, citric acid, and polyphenols, which can compete for available binding sites or interact with the NP surfaces through hydrogen bonding, limiting patulin accessibility. Despite this matrix-related inhibition, both NP types maintained a clear

concentration-dependent increase in adsorption efficiency, with PFAgNP consistently outperforming PFCuNP in both matrices. These results confirm that higher NP concentrations significantly enhance adsorption efficiency by providing a larger number of unsaturated sorption sites, which facilitate stronger patulin–NP interactions. Furthermore, at a concentration of 5.0 g/L, adsorption efficiency continued to rise with extended contact times (120–240 min), indicating that both factors act synergistically in maximizing patulin removal efficiency (Salleh et al., 2011). The outcomes of this study are in good agreement with previous reports emphasizing that increasing adsorbent dosage and exposure time enhances the removal of mycotoxins and other organic contaminants from complex food matrices.

Impact of NP Type on Patulin Adsorption Efficiency

NPs synthesized using different metals and green synthesis methods may exhibit distinct adsorption behaviors due to their unique physical and chemical properties. This underlines the importance of selecting the appropriate NP type based on specific application objectives. In this study, both PFAgNPs and PFCuNPs synthesized using PF-E were evaluated for their patulin adsorption capabilities in both aqueous and real systems (Table 6). In the water samples, PFCuNPs achieved an adsorption efficiency of 61.08% at a concentration of 5.0 g/L after 240 min, while PFAgNPs reached a higher value of 70.96% under the same conditions, demonstrating the superior adsorption efficiency of Ag-based NPs.

In the real samples, a similar pattern was observed, although the overall adsorption efficiencies were lower due to matrix complexity. After 240 min, PFAgNPs achieved adsorption efficiencies of 34.24% at 5.0 g/L and 20.85% at 2.5 g/L, while PFCuNPs reached 32.90% and 19.02% under the same conditions, respectively. The decrease in adsorption efficiency compared to aqueous systems can be attributed to the presence of sugars, organic acids, and polyphenolic compounds in apple juice, which compete with patulin for active binding sites and form hydrogen bonds that hinder direct patulin–NP interactions. Despite this inhibitory effect,

PFAgNPs maintained higher adsorption efficiency across all test conditions, suggesting that their surface chemistry and greater reactivity favor stronger interactions with patulin molecules. The observed differences between Ag- and Cu-based NPs are closely related to their physical and chemical characteristics, including particle structure, surface area, charge, and the nature of surface functional groups. The phytochemical residues from PF-E, particularly polyphenols and flavonoids, likely contributed to the enhanced surface functionality of PFAgNPs, promoting electron transfer and hydrogen bonding with the lactone and hydroxyl groups of patulin. Consequently, the Ag-based NPs demonstrated superior removal efficiency compared to Cu-based ones, especially at higher concentrations and longer contact times.

The present study is the first to report the use of PF-E derived PFAgNPs and PFCuNPs for the adsorption of patulin, from both aqueous and real food matrices. Previous literature provides limited examples of NP-based adsorption studies targeting mycotoxins synthesized from plant extracts. For instance, Asghar et al. (2018) investigated Fe, Cu, and AgNPs synthesized from green and black tea leaves and reported that aflatoxin B1 removal followed the trend FeNPs > CuNPs > AgNPs, with adsorption efficiency increasing with contact time. Similarly, Asghar et al. (2020) synthesized Fe, Cu, and AgNPs using *Syzygium cumini* leaf extract and observed that higher NP concentrations enhanced aflatoxin B1 removal. These findings align well with the current study, which also confirms that NP type, concentration, and exposure duration play synergistic roles in determining adsorption efficiency. Collectively, the results highlight that PFAgNPs, owing to their higher surface reactivity and bio-organic capping functionality, are promising candidates for the removal or control of patulin and similar toxic compounds in both model and real food systems.

Effect of Contact Time, Type of NP, and Concentration on HMF Adsorption

In this study, the HMF adsorption properties of biosynthesized PFAgNPs and PFCuNPs were investigated (Table 7).

Table 7 Effect of contact time, metal NP type, and concentration on HMF adsorption (%)^{*}

Samples	60 min 2.5 g/L	120 min 2.5 g/L	240 min 2.5 g/L	60 min 5.0 g/L	120 min 5.0 g/L	240 min 5.0 g/L
Standard aqueous solutions¹						
PFCuNP ¹	24.47 ± 1.16 ^a	27.87 ± 0.18 ^a	29.72 ± 0.18 ^a	26.39 ± 4.07 ^a	33.76 ± 2.33 ^a	36.38 ± 3.7 ^a
PFAgNP ¹	26.18 ± 0.12 ^b	29.46 ± 0.29 ^b	30.57 ± 0.11 ^b	42.52 ± 1.17 ^b	47.02 ± 1.01 ^b	52.22 ± 0.97 ^b
Real food sample (Apple juice)²						
PFCuNP ²	18.11 ± 1.18 ^a	20.69 ± 1.43 ^a	22.68 ± 1.11 ^a	20.52 ± 1.54 ^a	26.15 ± 1.43 ^a	31.87 ± 1.12 ^a
PFAgNP ²	19.86 ± 1.03 ^b	21.68 ± 1.01 ^b	24.36 ± 1.36 ^b	31.32 ± 1.09 ^b	36.56 ± 0.93 ^b	39.09 ± 1.21 ^b

^{*}The values were the mean of three replicate samples ($n=3$) ± SD. Samples marked with distinct lowercase letters (a–b) were found to differ significantly from one another based on Duncan's multiple range test, with statistical significance considered at $p < 0.05$

AgNPs generally exhibited higher adsorption efficiencies, and this effect became more pronounced at higher NP concentrations and longer contact times. For example, PFAgNPs achieved an adsorption rate of 52.22% at a contact time of 240 min and a concentration of 5.0 g/L in water samples. In contrast, PFCuNPs displayed lower adsorption values under the same conditions (36.38%). When real samples were evaluated, both types of NPs showed lower HMF adsorption efficiencies compared to water systems, likely due to the presence of competing matrix components such as sugars, organic acids, and phenolic compounds that can occupy active adsorption sites or alter surface charge interactions. Specifically, in the real samples, PFAgNPs exhibited an adsorption efficiency of 39.09% after 240 min at 5.0 g/L, whereas PFCuNPs reached 31.87% under the same conditions. Although these values are lower than those observed in aqueous systems, the general trend remained consistent: increasing both NP concentration and contact time enhanced HMF adsorption.

These results indicate that matrix complexity plays a crucial role in adsorption efficiency. In real food systems, the competition between HMF and other soluble constituents for NP surface sites limits total adsorption efficiency. Nevertheless, the superior adsorption behavior of PFAgNPs compared to PFCuNPs persisted across both matrices, suggesting that AgNPs possess a higher affinity for HMF, potentially due to their greater surface reactivity and the presence of functional phytochemical residues from the PF-E on their surfaces. Overall, the findings demonstrate that contact time, NP concentration, and sample matrix collectively govern HMF removal performance, with PFAgNPs emerging as a more effective adsorbent both in aqueous and complex real food systems.

Similar findings were reported by Kargar et al. (2024), who demonstrated that biosynthesized AgNPs exhibit strong interaction and catalytic transformation capacity toward HMF, attributed to surface-bound phytochemicals that promote electron transfer and carbonyl group binding. Moreover, heterogeneous Ag/Au NP systems were shown to selectively oxidize HMF due to their high surface activity and tunable electronic properties (Schade et al., 2020), supporting the observed affinity of AgNPs in this study. Overall, the findings demonstrate that contact time, NP concentration, and sample matrix collectively govern HMF removal performance, with PFAgNPs emerging as a more effective adsorbent both in aqueous and complex real food systems.

Impact of Contact Time on HMF Adsorption Efficiency

The contact time between the adsorbent and adsorbate is a key factor that significantly affects the effectiveness of NPs in HMF adsorption. As shown in Table 7, an increase in contact time leads to greater HMF adsorption by the NPs. After

240 min, almost all NP concentrations had higher adsorption efficiencies than they did after 60 and 120 min. Rates that as the contact time increases, more HMF molecules are retained on the surface of the NPs, approaching experimental saturation. For example, PFCuNPs at a concentration of 2.5 g/L achieved an adsorption rate of 24.47% after 60 min, which increased to 29.72% after 240 min. A similar trend was observed for PFAgNPs. These findings demonstrate a direct relationship between contact time and the efficiency of NPs in adsorbing HMF. The type of NP plays a decisive role in determining HMF adsorption efficiencies. As shown in Table 7, the biosynthesized PFAgNPs consistently exhibited higher adsorption efficiencies than PFCuNPs, regardless of contact time, concentration, or sample matrix. In the water samples, the maximum adsorption recorded for PFAgNPs was 52.22% at 5.0 g/L and 240 min, while PFCuNPs reached 36.38% under identical conditions. This difference is likely due to the higher surface reactivity and larger effective surface area of AgNPs, as well as the strong reducing and stabilizing phytochemicals from PF-E that may enhance HMF–Ag surface interactions through hydrogen bonding or π – π stacking mechanisms.

In the real sample, although both NPs exhibited somewhat reduced adsorption efficiency due to the interference of coexisting organic constituents, PFAgNPs still maintained a higher efficiency (39.09%) than PFCuNPs (31.87%) at 240 min and 5.0 g/L. The consistent superiority of Ag-based NPs suggests that they possess a greater affinity for HMF molecules, possibly owing to the more favorable electronic properties of Ag sites and the synergistic effect of bio-organic capping agents.

Overall, these results demonstrate that the HMF adsorption efficiency is governed by both physicochemical parameters (contact time and NP concentration) and intrinsic material characteristics (metal type and surface functionality). The superior performance of PFAgNPs highlights their potential as efficient, green-synthesized adsorbents for mitigating HMF contamination not only in aqueous systems but also in complex food matrices such as fruit juices. In real samples, coexisting solutes (e.g., sugars, organic acids, and phenolics) compete with HMF for surface sites and attenuate overall uptake, consistent with prior observations on matrix effects in HMF determination and removal (Geirola et al., 2024; Martins et al., 2022). The sustained advantage of Ag-based NPs across media suggests a higher affinity rooted in their surface reactivity and bio-organic capping layers from PF-E, which can promote interactions with the carbonyl-bearing HMF (Kargar et al., 2024). Related heterogeneous noble-metal systems likewise show strong HMF surface interactions and transformations, which emphasizes the value of metal identity and surface electronics (Schade et al., 2020). Taken together, the data align with adsorption-driven control by available site density and mass-transfer

time, while the matrix moderates the effective capacity; within this framework, PFAgNPs emerge as practical, green adsorbents for HMF mitigation in both model and real food systems.

Impact of NP Concentration on HMF Adsorption Efficiency

The concentration of the synthesized NPs is another critical factor influencing their effectiveness in HMF adsorption. As shown in Table 7, increasing NP concentration generally leads to a higher adsorption efficiency for both NP types. In water samples, experiments conducted with a 5.0 g/L NP concentration resulted in higher adsorption values than those obtained at 2.5 g/L. For instance, PFAgNPs exhibited a high HMF adsorption efficiency of approximately 52.22% after 240 min at 5.0 g/L, while significantly lower adsorption values (about 26.18%) were recorded at 2.5 g/L under the same conditions. Similarly, PFCuNPs showed an increase from 29.72% to 36.38% with the same concentration increment. This pattern indicates that at higher concentrations, HMF molecules interact more intensively with the NPs and become more strongly adsorbed onto their surfaces, owing to the greater availability of active binding sites.

When the same experiments were extended to real samples, both PFAgNPs and PFCuNPs exhibited the same concentration-dependent trend, although overall adsorption efficiency values were lower than those observed in water. At 2.5 g/L, PFAgNPs adsorbed 24.36% of HMF after 240 min, whereas PFCuNPs reached 22.68%. Increasing the concentration to 5.0 g/L raised adsorption to 39.09% and 31.87% for PFAgNPs and PFCuNPs, respectively. The lower adsorption efficiency in the real matrix can be attributed to the presence of other solutes, such as sugars, organic acids, and phenolic compounds, which interact with the NP surfaces and hinder HMF binding.

Despite this matrix effect, the general trend remained consistent across both media: adsorption efficiency increased with NP concentration and contact time, and PFAgNPs consistently outperformed PFCuNPs. The higher adsorption efficiency of PFAgNPs can be ascribed to their greater surface reactivity and the presence of bio-organic capping compounds derived from PF-E, which enhance surface functionalization and facilitate hydrogen bonding or π - π interactions with HMF molecules. These results confirm that increasing NP concentration enhances the number of accessible adsorption sites, thereby promoting more effective HMF removal in both aqueous and complex real food systems.

Impact of NP Type on HMF Adsorption Efficiency

As shown in Table 7, the effectiveness of the NPs in HMF adsorption varies considerably depending on the NP type.

In this study, the biosynthesized PFAgNPs and PFCuNPs exhibited distinct adsorption efficiencies, indicating that each possesses unique surface characteristics affecting their ability to capture HMF molecules. In the water samples, PFAgNPs demonstrated a more efficient adsorption behavior, particularly at higher concentrations and longer contact times. At 240 min, PFAgNPs had adsorption rates of 30.57% and 52.22% at 2.5 g/L and 5.0 g/L, respectively. PFCuNPs, on the other hand, had rates of 29.72% and 36.38% at the same concentrations. At 240 min, PFAgNPs had adsorption rates of 30.57% and 52.22% at 2.5 g/L and 5.0 g/L, respectively. PFCuNPs, on the other hand, had rates of 29.72% and 36.38% at the same concentrations.

When tested in the real sample, both NPs showed slightly lower adsorption efficiencies because HMF was competing with other solutes like sugars, organic acids, and phenolic compounds. When tested in apple juice, both NPs showed slightly lower adsorption efficiencies because HMF was competing with other solutes like sugars, organic acids, and phenolic compounds. Nevertheless, the superior performance of PFAgNPs persisted. After 240 min, PFAgNPs reached adsorption efficiencies of 24.36% and 39.09% at 2.5 g/L and 5.0 g/L, respectively, while PFCuNPs achieved 22.68% and 31.87% at the same conditions. This consistent difference highlights that Ag-based NPs possess a stronger affinity for HMF, likely due to their higher surface reactivity, greater density of active sites, and the presence of phytochemical residues from PF-E extract that provide functional groups enhancing molecular interactions. These results confirm that the adsorption efficiency is not only governed by external parameters such as concentration and contact time but also by intrinsic material properties, including metal type and surface functionality. The more reactive Ag surfaces, stabilized by bio-organic compounds, facilitate electron transfer and hydrogen bonding with the carbonyl and hydroxymethyl groups of HMF, leading to greater adsorption efficiency. Consequently, PFAgNPs emerge as a more effective and eco-friendlier adsorbent for mitigating HMF contamination in both model aqueous systems and complex real food matrices such as fruit juices.

No prior study has been identified that investigates the HMF adsorption efficiency of PFAgNPs and PFCuNPs synthesized using PF-E. In this respect, the present work stands out as the first report that explores the potential of these NPs for removing HMF, a compound known to be harmful when present above certain levels in food systems. Although research exists on the use of different NPs in HMF-related applications, AgNPs and CuNPs have not been examined in terms of their interaction or adsorption efficiency toward HMF. For example, one study conducted HMF oxidation using gold-loaded zeolite nanocapsules and achieved high FDCA yield and conversion with low NP loading, without

byproduct formation, and with satisfactory reproducibility (Saxena et al., 2021). In a separate investigation, a CuO@CF heterostructure enhanced with PtNPs was discovered to augment the adsorption characteristics of HMF and hydroxyl species, attaining a 99% FDCA yield with significant stability, even at increased HMF concentrations (Li et al., 2025). The absence of studies specifically addressing the chemical interaction between HMF and AgNPs or CuNPs, as well as their adsorption potential, underscores the originality and scientific relevance of the present research.

Kinetics through PFO and PSO Models

The kinetic results for HMF (Fig. 17 and Table 8) clearly indicate that the governing adsorption mechanism varies depending on both nanoparticle type and solution matrix. For real samples, the higher correlation coefficients obtained for the PFO model ($R^2 = 0.9788$ for PFCuNP and 0.9917 for PFAGNP), compared to the PSO model, demonstrate that the adsorption process is predominantly controlled by surface diffusion and weak physicochemical

Fig. 17 HMF adsorption kinetics on PFCuNPs and PFAGNPs: (A) Adsorption capacity as a function of time, and the corresponding linearized kinetic models for (B) PFO and (C) PSO (Experimental conditions: initial HMF concentration = 100 mg/L, adsorbent dosage = 2.5 g/L, temperature = 25 °C)

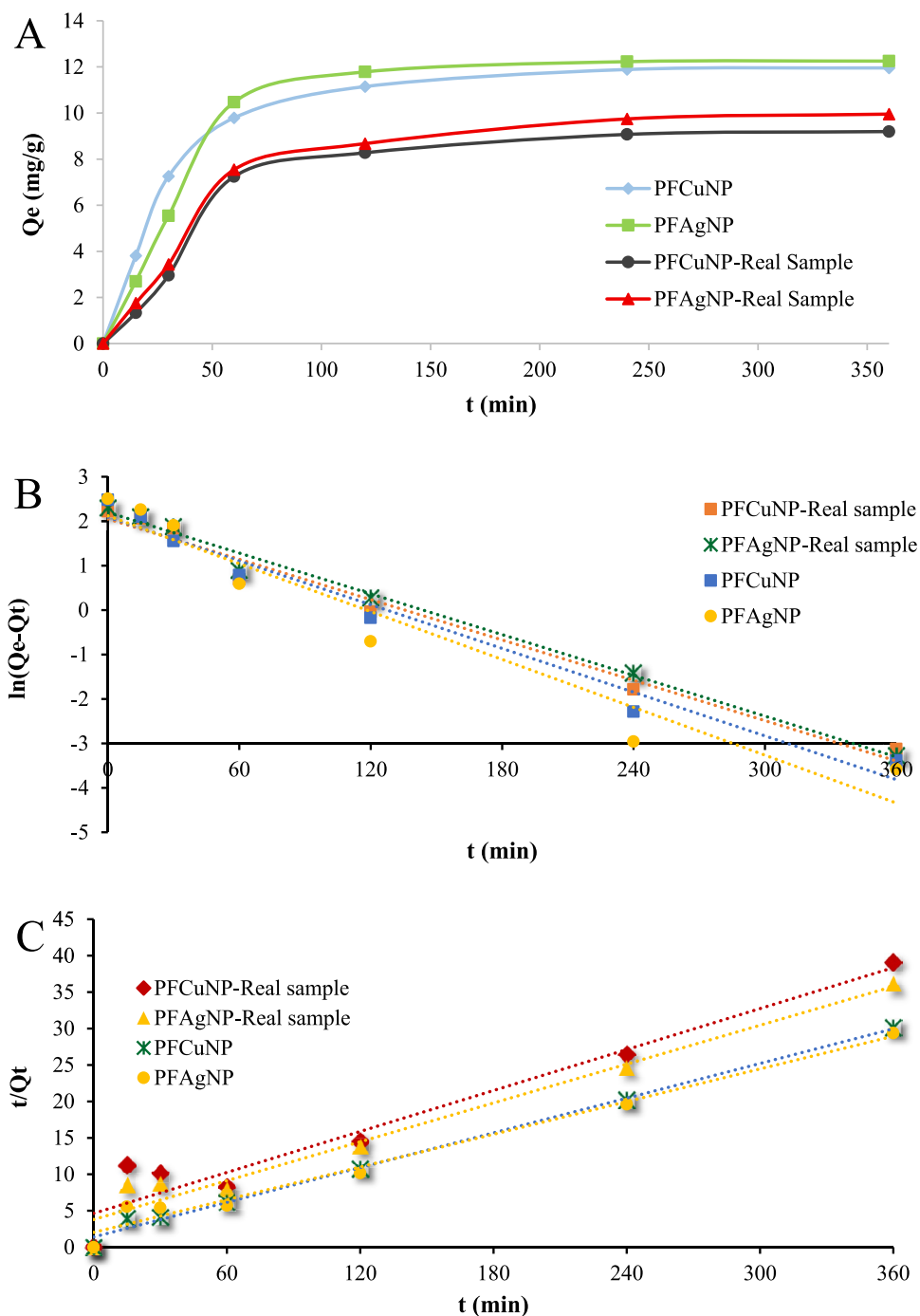


Table 8 HMF adsorption kinetics of PFCuNP ve PFAgNP (Adsorbent amount: 2.5 g/L, pollution amount: 100 mg/L)

Adsorbent	Pseudo-first-order kinetic model				Pseudo-second-order kinetic model		
	Q _e (Experimental) mg/g	Q _e (Theoretical) mg/g	k ₁ 1/min	R ²	Q _e (Theoretical) mg/g	k ₂ g/(mg.min)	R ²
PFCuNP—Real sample	9.24	7.78	0.0151	0.9788	10.68	0.0018	0.9387
PFAgNP—Real sample	9.99	9.02	0.0153	0.9917	11.26	0.0020	0.9627
PFCuNP	11.99	8.08	0.0164	0.8670	12.60	0.0043	0.9942
PFAgNP	12.28	8.33	0.0180	0.8755	13.38	0.00260	0.9794

interactions during the initial stages. This observation aligns with common understanding in adsorption kinetics that when diffusion or mass transfer limitations dominate, PFO often provides a better fit, particularly in complex matrices (Hubbe et al., 2019; Musah et al., 2022). In this case, the closer agreement between experimental Q_e and theoretical Q_e values under PFO corroborates such diffusion-controlled behavior in real samples.

In contrast, for aqueous HMF solutions, both NP systems exhibit significantly better fitting to the PSO model ($R^2 = 0.9942$ for PFCuNP and 0.9794 for PFAgNP). This shift implies that under simpler, homogeneous conditions, adsorption proceeds under a more controlled regime where surface-active sites and specific interactions play stronger roles as equilibrium is approached. Such behavior where PSO provides superior description, has repeatedly been reported for organic pollutants onto nanomaterials and carbon-based sorbents (Çiçekçi et al., 2025; Danmallam et al., 2025). Accordingly, PFAgNP shows slightly higher k₁ values, indicating a faster initial adsorption rate for HMF, while the increase of k₂ values under PSO conditions suggests that surface reactivity becomes more influential near equilibrium, especially in homogeneous synthetic systems.

Compared to HMF, patulin adsorption exhibits a more complex kinetic profile (Fig. 18 and Table 9). In real samples, both NPs demonstrate better conformity to the PFO model ($R^2 = 0.9830$ for PFCuNP and 0.9731 for PFAgNP), with theoretical Q_e values closely matching experimental data. This behavior suggests that, in complex matrices, patulin adsorption is initially governed by film diffusion and non-specific surface interactions, possibly influenced by competing organic constituents present in the real solution (Abbasi Pirouz et al., 2018; Musah et al., 2022). For aqueous patulin solutions, the PSO model provides a superior fit ($R^2 = 0.9876$ for PFCuNP and 0.9500 for PFAgNP), with theoretical Q_e values better approaching experimental measurements. This reflects a transition toward adsorption controlled by surface binding processes, where chemisorption becomes more significant

over time. Notably, although PFAgNP exhibits lower k₂ values, its higher adsorption capacity suggests stronger and more stable retention of patulin molecules on reactive surface sites — a phenomenon consistent with observations in other organic pollutant–nanomaterial systems (Awad et al., 2020; Liu et al., 2022).

Conclusions

In this study, PFAgNPs and PFCuNPs were successfully synthesized via a green synthesis route using PF-E extract and were thoroughly characterized using UV–Vis spectroscopy, FT-IR, XRD, SEM, TEM, and EDX analyses. The TPC, TFC, and TAC results indicated that the synthesized NPs exhibited higher antioxidant capacity compared to the extract alone. These findings were further supported by DPPH, ABTS, and FRAP assays, which confirmed the antioxidant potential of the NPs. One of the main objectives of the study was to assess the antibacterial activities of PFAgNPs and PFCuNPs. The antibacterial evaluations revealed that PFAgNPs were effective against all tested bacterial strains, whereas PFCuNPs exhibited lower activity. Phenolic profiling via LC–MS/MS analysis of the PF-E extract identified the presence of quinic acid (63.42 mg/g), chlorogenic acid (2.38 mg/g), protocatechuic acid (0.07 mg/g), protocatechuic aldehyde (0.04 mg/g), hesperidin (0.03 mg/g), and naringenin (0.02 mg/g). Additionally, the adsorption efficiencies of the synthesized AgNPs and CuNPs were evaluated against two foodborne contaminants, patulin and HMF, known for their toxic and carcinogenic effects, using both standard aqueous solutions and real food matrix (apple juice) samples. Both PFAgNPs and PFCuNPs demonstrated the ability to adsorb these compounds, and the adsorption efficiency was found to depend on factors such as NP type, concentration, and contact time between the adsorbent and adsorbate. Among the two, PFAgNPs exhibited superior adsorption efficiency for both patulin and HMF. The kinetic evaluation demonstrated that adsorption behavior

Fig. 18 Patulin adsorption kinetics on PFCuNPs and PFAgNPs: (A) Adsorption capacity as a function of time, and the corresponding linearized kinetic models for (B) PFO and (C) PSO (Experimental conditions: initial patulin concentration = 102 mg/L, adsorbent dosage = 2.5 g/L, temperature = 25 °C)

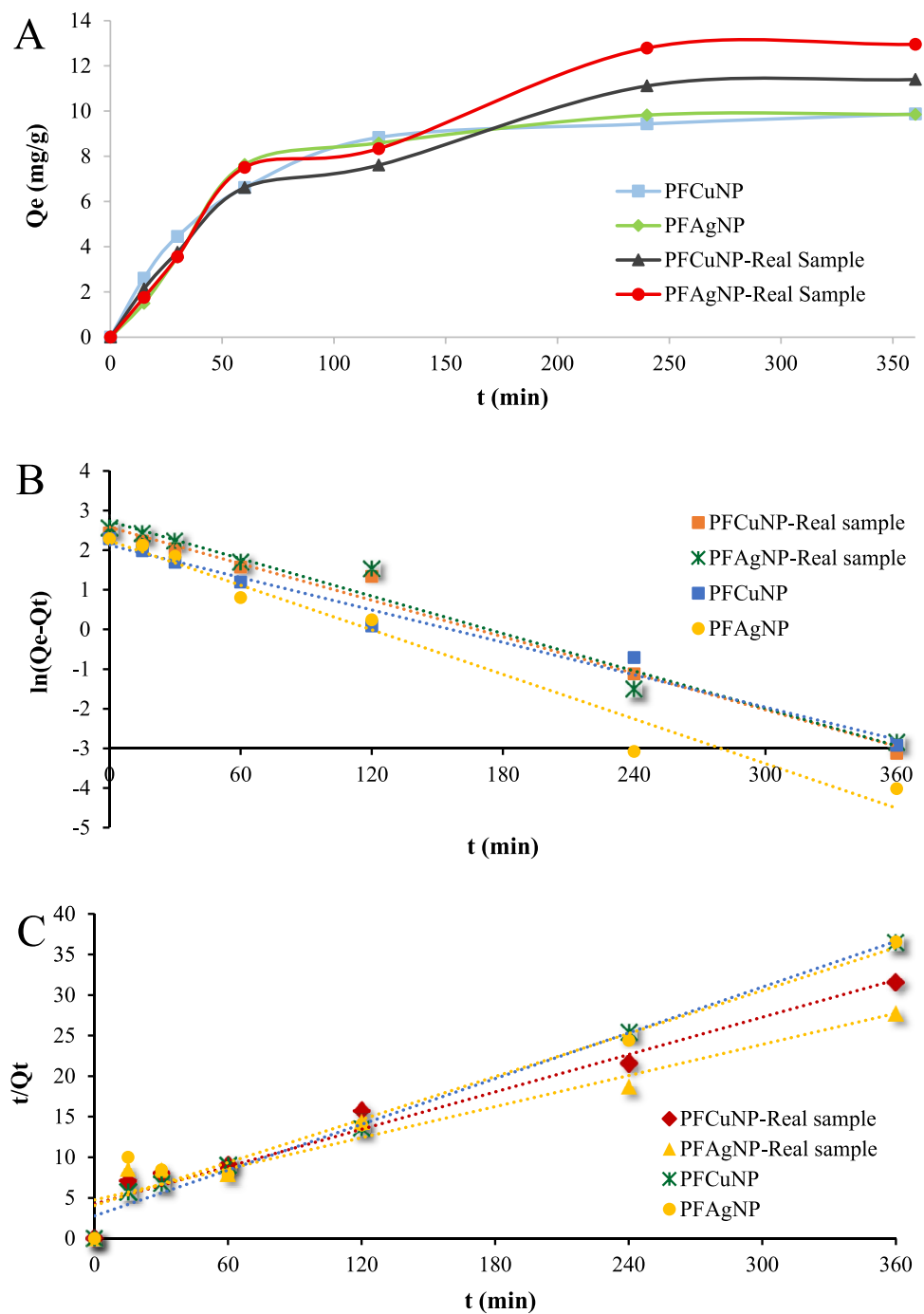


Table 9 Patulin adsorption kinetics of PFCuNP ve PFAgNP (Adsorbent amount: 2.5 g/L, pollution amount: 102 mg/L)

Adsorbent	Pseudo-first-order kinetic model				Pseudo-second-order kinetic model		
	Q_e (Experimental) mg/g	Q_e (Theoretical) mg/g	k_1 1/min	R^2	Q_e (Theoretical) mg/g	k_2 g/(mg.min)	R^2
PFCuNP—Real sample	11.44	13.20	0.0154	0.9830	13.03	0.0014	0.9563
PFAgNP—Real sample	13.01	15.34	0.0157	0.9731	15.65	0.0009	0.9182
PFCuNP	9.93	8.39	0.0136	0.8670	10.64	0.0033	0.9876
PFAgNP	9.87	9.40	0.0188	0.8755	11.32	0.0018	0.9500

varied according to both NP type and solution matrix; while the PFO model provided a better fit in real samples due to diffusion-dominated uptake, the PSO model exhibited superior agreement in aqueous solutions where surface interactions became more prominent. This matrix-dependent distinction was confirmed for both HMF and patulin through higher R^2 values and the close consistency between theoretical and experimental Q_e results, with PF-AgNP exhibiting superior performance in both media owing to its faster initial adsorption and stronger pollutant retention. The overall findings suggest that PF-E-derived NPs possess not only strong antioxidant and antibacterial properties but also significant potential for the removal of harmful food toxins, contributing to food safety strategies. Given the absence of prior studies focusing on PF-E-mediated Ag and CuNPs in this context, this study offers a novel and promising approach for multifunctional applications in both biomedical and food-related fields.

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

Declarations

Ethical Approval This article does not contain any studies with animals or human participants.

Consent to Participate The authors agreed to participate in this work.

Consent for Publication The authors agreed to publish this work.

Competing interests The authors declare no competing interests.

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