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Original Article

Green Nanotechnology Approach: Comparative Evaluation of Silver Nanoparticles from Two Plant Species and Three Solvent Systems

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Abstract

Introduction: Green synthesis using plant extracts provides an eco-friendly alternative to conventional nanoparticle production, reducing the use of toxic reagents while stabilizing particle surfaces. This study aimed to synthesize silver nanoparticles (AgNPs) using two plant species extracts and evaluate their antioxidant and antimicrobial potentials.

Methods: The plant was extracted with 70% methanol, 70% ethanol, and distilled water. Extracts were mixed with 1 mM $AgNO_3$ solution at 25 °C for 24 h to synthesize AgNPs. The total phenolic content was measured using the Folin–Ciocalteu method, expressed as gallic acid equivalents. Antimicrobial activity was assessed against seven bacterial and three yeast strains using agar well diffusion, and minimum inhibitory, bactericidal, and fungicidal concentrations were determined by microdilution following CLSI standards.

Results: The findings revealed that solvent type significantly influenced both the total phenolic content of the extracts and the physicochemical properties of the synthesized nanoparticles. Higher phenolic content was associated with enhanced stability and improved antimicrobial efficacy. Notably, AgNPs synthesized with ethanol extracts demonstrated stronger antibacterial activity, whereas those produced with aqueous extracts showed relatively lower bioactivity.

Conclusions: The plant extract can act as a natural reducing and capping agent for green synthesis of silver nanoparticles. Although free extracts lacked antimicrobial activity, AgNP-enriched extracts displayed broad-spectrum antibacterial and antifungal effects while retaining measurable antioxidant capacity. These findings suggest that plant-based AgNPs offer an environmentally friendly and biocompatible approach for enhancing the biological efficacy of phytochemicals and hold promise for biomedical applications.

Keywords: Silver nanoparticles, *Capparis spinosa* L., *Prunus laurocerasus* L., antioxidant activity, green synthesis

1. Introduction

Nanotechnology is a burgeoning discipline that has garnered global interest among researchers, with nanoparticles extensively utilised in scientific methodologies owing to their distinctive attributes, including diminutive size, extensive surface area, and targeted mechanisms of action. (1).

Oxidative stress, caused by an imbalance between free radicals and antioxidant defenses, plays a role in the pathogenesis of numerous chronic diseases, including aging and conditions such as cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions. Therefore, nanoparticles with strong radical scavenging abilities can function as protective agents by reducing oxidative damage (2).

Nanoparticles (NPs) are versatile molecules widely used in the biomedical field due to their biocompatibility, stability, and lack of toxicity (3). It has potential applications in medicine, such as drug delivery, antimicrobial, antioxidant, and other biological activities, as well as disease diagnosis (4). They can be easily functionalized for targeted drug delivery (3). The synthesis of metal nanoparticles (MNPs) is considered a progressive field attracting significant scientific research, holding importance in imaging and drug delivery. The small size of MNPs often allows them to leak through biological or physiological membranes that are generally impermeable to other macromolecules (5). Metal nanoparticles (platinum (Pt), copper (Cu), gold (Au), silver (Ag), zinc (Zn)) exhibit extensive antibacterial efficacy against many pathogens, including Gram-negative and Gram-positive bacteria as well as fungi, and are regarded as a viable alternative to antibiotics (6).

Extensive research has been conducted on the use of naturally occurring resources for synthesizing MNPs (7). The biological systems involved in the green synthesis of MNPs are microorganisms such as plants and their derivatives, bacteria, fungi, algae, and yeast (4). Plant extracts serve as reducing and stabilising agents in biosynthesis. Bioreduction entails the conversion of metal ions or metal oxides into zero-valent metal nanoparticles utilising phytochemicals, including tannins, polyphenolic compounds, amino acids, polysaccharides, and vitamins (7). Among metallic nanoparticles, silver nanoparticles (AgNPs) have become a focus of interest due to their special biological properties (8). The antibacterial efficacy of AgNPs against multidrug-resistant pathogens is significant and remarkable. The pursuit of novel antibacterial agents persists in response to the rising prevalence of infectious diseases caused by microorganisms and the scarcity of effective antimicrobial agents and antibiotics. Consequently, the pharmaceutical industry and research sectors are concentrating on the development of new drugs to address antimicrobial resistance effectively (9). When synthesized using plant-mediated methods, these antimicrobial effects can be further enhanced by the synergistic activity of bioactive plant metabolites. Traditional methods for producing AgNPs are expensive, toxic, and not environmentally friendly. To overcome these problems, researchers have found naturally occurring sources and their products that can be used for the synthesis of NPs.

The green synthesis of NPs is a newly emerging branch of nanotechnology. Green synthesis does not require high temperatures, energy, pressure, or harmful chemicals (10). Studies have not only identified the ability of natural extracts and microorganisms to form AgNPs but also their excellent antioxidant activities, which are higher compared to the substrates. It is believed that this activity stems from the preferential absorption of extract components on the surface of nanoparticles. Various studies have found that AgNPs obtained from plant extracts exhibit antitumor and antimicrobial effects (11). Csakvari et al. have demonstrated the usefulness of *Cannabis sativa* leaf extracts in mediating the green synthesis of AgNPs and their antibacterial activities against various human pathogens (12). Ali et al. elucidated the anti-candidal properties of AgNPs synthesised via the aqueous leaf extract of *Calotropis gigantea* (13)

Prunus laurocerasus L. (*P. laurocerasus*) , also known as cherry laurel, is an evergreen shrub from the Rosaceae family, native to the Black Sea region and widely found in Europe and Asia. Traditionally,

the fruits, leaves, and seeds have been used in folk medicine to treat ailments such as digestive disorders, coughs, and inflammation (14-16). The caper bush (*Capparis spinosa* L.) is a perennial plant belonging to the Capparidaceae family. The flower buds, fruits, and root bark of the plant are utilised in traditional medicine for their analgesic, cell-regenerative, wound-healing, diuretic, and tonic properties (17). Phytochemical studies on these plants have revealed high concentrations of phenolic compounds, flavonoids, anthocyanins, and other secondary metabolites with strong antioxidant and antimicrobial effects (14-16, 18).

In this study, the biosynthesis of AgNPs was synthesized using three different extracts of *P. laurocerasus* L. fruit and *C. spinosa* L. seed. The phytochemical potential of these extracts was evaluated, particularly their total phenolic content (TPC), role in nanoparticle formation, and bioactivity. The antimicrobial activity of the obtained AgNPs was evaluated against selected pathogenic microorganisms, while their solubility in dimethyl sulfoxide (DMSO) was examined to assess their potential applicability in biomedical formulations.

2. Methods

2.1. Plant material

The *C. spinosa* L. samples were obtained from Caper Research and Development Food Production Center (Burdur). *P. laurocerasus* L. dried fruit was supplied by a local herbalist. The seeds of *C. spinosa* L. were dried at room temperature and then ground into powder using a grinder.

2.2. Preparation of plant extract

The extraction procedure was described by Alkaya et al.2019 (19). For the extraction process, 2 g of ground fruit sample was mixed with 25 mL of extraction solvent (70% methanol (Me), 70% ethanol (Et), and distilled water (w)). As part of optimization studies in biosynthesis, extraction conditions were carried out using 70% methanol, 70% ethanol, and water as solvents, at 60°C, and with a sequential extraction method lasting 1 hour and three consecutive extractions. The obtained extracts were filtered through Whatman blue band filter paper. Until the analysis stage, it was stored at – 20°C (19).

2.3. Synthesis of AgNPs

For the synthesis of silver nanoparticles, a 500 mL aqueous solution with a concentration of 1 mM was prepared from solid $AgNO_3$. 40 mL of this solution was taken and used in an incubator to mix with 40 mL of plant extract (70% methanol (AgNPMe), 70% ethanol (AgNPEt), and distilled water (AgNPw) in a 250 mL Erlenmeyer flask. It was left to react at room temperature for 24 hours in a thermal shaker at 37 °C (BIOSAN TS-100). Then, the samples were centrifuged at 5000 rpm for 20 minutes. The supernatant was discarded,and the pellet was washed once with distilled water. At the end of the process, the obtained AgNPs were transferred to beakers and dried in an oven at 80°C for 24 hours. The dried AgNPs were scraped and transferred to sterile tubes, and stored covered with aluminum foil to prevent light exposure (20).

2.4. UV-visible spectral analysis

The absorbance spectrum of the green synthesized AgNPs was analyzed in the 300−600 nm range using UV-vis spectroscopy (Shimadzu, UV-1601 spectrophotometer, Japan) (21).

2.5. Determination of TPC in extracts and AgNPs

TPC of extracts and AgNPs was determined with Folin-Ciocalteu modified method, using gallic acid (GA) as a standard phenolic compound (19). 100 µL of each sample was mixed with 4 mL of distilled water and 100 µL of Folin-Ciocalteu reagent. Then, 100 µL of 6% sodium carbonate solution was added. After the mixtures were incubated for 30 minutes, their absorbance values were measured using a spectrophotometer (Shimadzu UV-1601 spectrophotometer, Japan) within the 685–760 nm wavelength range (19). The calibration curve was prepared using a gallic acid standard in the concentration range of 62.5–1000 µM ($y = 0.0026x – 0.0563$, $R^2 = 0.9978$), and the results were expressed as gallic acid equivalents (GAE/mL) (Fig 1). All measurements were taken three times.

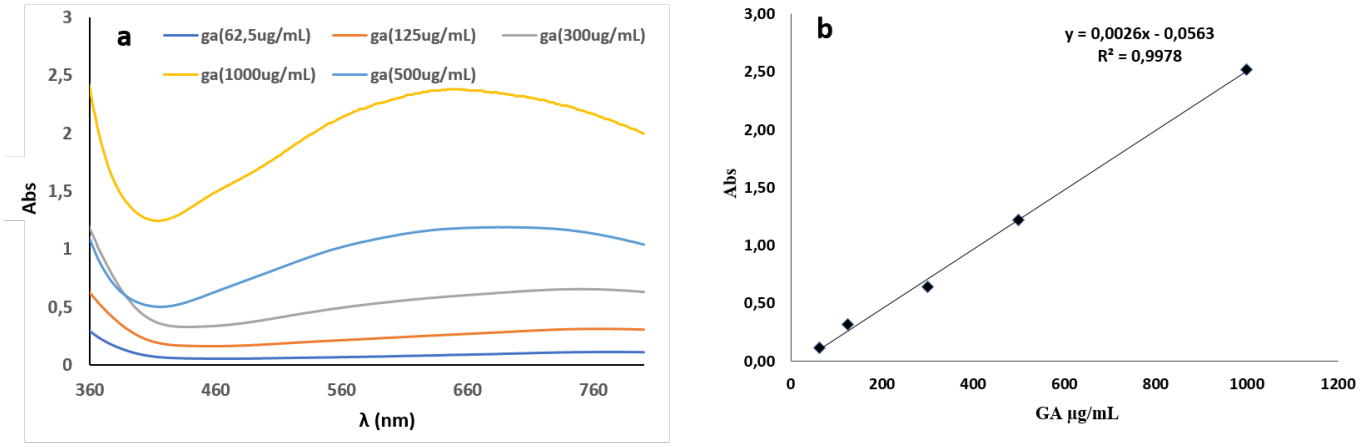

Figure 1. (a) Absorption spectra of GA at different concentrations and (b) GA calibration curve

2.6. Antimicrobial activity

The potential of plant extracts for antimicrobial activity was determined by using the agar well diffusion method. Additionally, minimum inhibitory concentrations (MICs) have been determined for the extracts. Subsequently, the extracts' minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values were also determined.

2.6.1. Agar Well Diffusion Test

In our study, the following bacterial strains were used: *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, and *Klebsiella pneumoniae* ATCC 4352; and the following yeast strains were: *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 90018, and *Candida tropicalis* KUEN 1021.

Bacteria were cultured on Mueller-Hinton agar (MHA) (Merck), and Yeasts on Sabouraud Dextrose Agar (SDA) (Merck), and incubated at 37°C for 24 hours. After incubation, microorganism suspensions were prepared from colonies formed in a 0.85% NaCl physiological saline (PSS) solution. Bacterial suspensions were adjusted to 108 CFU/mL, and yeast suspensions were adjusted to 10^6 CFU/mL based on a McFarland 0.5 standard turbidity value. Microorganism suspensions were spread onto the surface of MHA for bacteria and SDA for yeasts under aseptic conditions using sterile swab sticks, followed by the creation of 5 mm diameter wells on the surface of the medium using sterile punches. 50 µL (50 mg/mL) of extract dissolved in suitable solvents was added to the wells. Additionally, meropenem (10 µg/well) was used for bacteria, and amphotericin B (100 µg/well) as a positive control for yeast, DMSO as a solvent, and FTS as a negative control. The inoculated petri dishes were incubated at 37°C for 18-24 hours for bacteria and at 35°C for 24-48 hours for yeast, and the inhibition zones were measured at the end of the incubation period. The experiments were conducted in triplicate, and the average values were taken (22-24).

2.6.2. Determination of MIC for bacteria

MIC determination for bacteria was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) standards. Cation-adjusted Mueller-Hinton Broth (CAMHB) was used as the culture medium. A bacterial suspension was prepared from colonies in an overnight bacterial culture according to the McFarland 0.5 turbidity standard and then diluted to a final inoculum concentration of 5×10^5 CFU/mL. 100 µL of CAMHB was distributed into sterile U-bottom microplates. Soluble extracts were placed in the first wells at 100 µL, and serial dilutions were made accordingly. Subsequently, 5 µL of bacterial suspension was added to the wells containing the extract, and the plates were incubated at 37°C for 24 hours (25).

2.6.3. Determination of the MIC for yeasts

The dilutions of the tested extracts were prepared in RPMI-1640 medium in U-bottom microdilution plates. The tested yeasts were inoculated onto SDA medium and incubated at 37°C for 24 hours. Subsequently, suspensions were prepared from the cultures in RPMI to a McFarland 0.5 turbidity. 100

µL of each suspension was taken and placed into the relevant wells. The prepared plates were incubated at 37°C for 24-48 hours. After a 24-hour incubation period, the plates were evaluated, re-incubated, and then re-evaluated at 48 hours.

At the end of the incubation period for bacteria and yeasts, the lowest extract concentrations at which no visible growth was observed were determined as MIC. Similarly, CAMHB, DMSO, and RPMI were used as negative controls, while meropenem and amphotericin B were used as positive controls (25-27).

2.6.4. Determination of minimum bactericidal and fungicidal concentrations

To determine the MBC and MFC values of plant extracts, 5 µL from each well of the microplate was seeded onto MHA for bacteria and SDA for yeast.. The Petri dishes were then incubated at 37°C for 24 hours for bacteria and 48 hours for yeasts. Finally, after incubation, the lowest dose of bacteria where no growth occurred was evaluated as MBC and in yeasts as MFC (28).

3. Results

3.1. TPC of extracts and AgNPs

The TPC of *P. laurocerasus* L. and *C. spinosa* L. extracts and their corresponding silver nanoparticles (AgNPs) was determined using the Folin–Ciocalteu method, and the results are presented in Table 1.

Table 1. TPC of plant extracts and AgNPs by the Folin-Ciocalteau method

Sample	Total phenolic content*	Sample	Total phenolic content*
P. laurocerasus-Me	181 ± 1.22	*C. spinosa* – Me	399 ± 2.01
P. laurocerasus-Et	113 ± 0.97	*C. spinosa* – Et	320 ± 1.29
P. laurocerasus-w	124 ± 1.11	*C. spinosa*-w	273 ±1.86
P. Me-AgNP	29 ± 0.88	*C.* Me-AgNP	340 ± 1.63
P. Et-AgNP	26 ± 0.93	*C.* Et-AgNP	123 ± 1.56
P. W-AgNP	24 ± 1.03	*C.* W-AgNP	77 ± 1.27

* mg gallic acid equivalent/ g dry plant±SD (n = 3); P. laurocerasus-Me and C. spinosa – Me: methanol extract; P. laurocerasus-Et and C. spinosa – Et: ethanol extract; P. laurocerasus-w and C. spinosa-w: water extract; P. Me-AgNP and C. Me-AgNP: AgNP obtained from methanol extract; P. Et-AgNP and C. Et-AgNP: AgNP obtained from ethanol extract; P. w-AgNP and C. w-AgNP: AgNP obtained from water extract.

The analysis revealed that *C. spinosa* exhibited significantly higher phenolic content in all solvent extracts compared to *P. laurocerasus*. The methanol extract of *C. spinosa* showed the highest TPC, followed by the ethanol and water extracts. In contrast, *P. laurocerasus* displayed considerably lower values, with 181, 113, and 124 mg GAE/g for methanol, ethanol, and water extracts, respectively. The choice of solvent also influenced extraction efficiency. For both plants, methanol proved to be the most effective solvent for phenolic recovery, followed by ethanol, with water being the least efficient. This observation is consistent with previous reports that methanol, due to its polarity, facilitates the extraction of a broader spectrum of phenolic compounds compared to other solvents.

Following the biosynthesis of AgNPs using plant extracts, a marked reduction in phenolic content was observed. For *P. laurocerasus*-derived nanoparticles, the TPC values decreased sharply. This substantial decrease suggests that a majority of the phenolic compounds were consumed during nanoparticle synthesis, likely acting as reducing and stabilizing agents. Methanol, ethanol, and water mediated AgNPs of *C. spinosa* and *P. laurocerasus* showed lower but still phenolic content. These results indicate that while phenolics participate in nanoparticle formation, *C. spinosa* and *P. laurocerasus* possess a capacity to transfer and preserve phenolic compounds within the nanoparticle system.

3.2. UV-visible spectral analysis

UV-Vis absorption spectra of AgNPs synthesized with *C. spinosa* and *P. laurocerasus* extracts demonstrate their behavior in dimethyl sulfoxide (DMSO), a widely used polar aprotic solvent (Fig 2). The presence of a distinct surface plasmon resonance (SPR) band in the 400–450 nm range – typical for AgNPs – and the absence of broad secondary peaks suggest that the particles remain largely dispersed and stable in DMSO rather than precipitating or aggregating. In the figure, the main band appears only slightly shifted, which implies that the phenolic compounds from the *C. spinosa* extract are still attached and stabilizing the particles in DMSO (29).

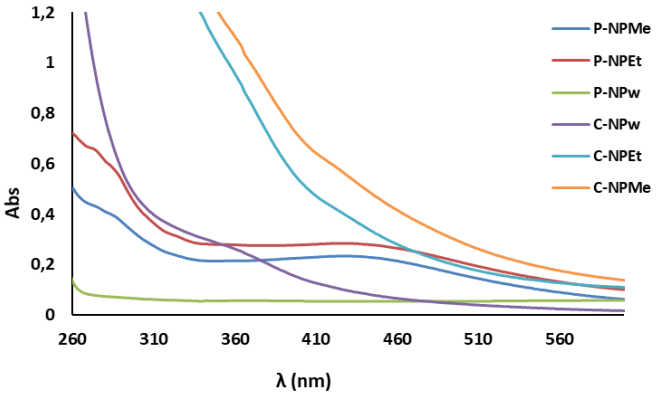

Figure 2. Spectra of AgNPs synthesized from *C. Spinosa* and *P. laurocerasus* extracts in DMSO

3.3. Antimicrobial activity

The results of the agar well diffusion assay to determine the antimicrobial effects of *C. spinosa* L. and *P. laurocerasus* extracts are presented in Tables 2 and 3. *C. spinosa* extracts and their AgNPs showed stronger antimicrobial effects across most tested microorganisms than those of *P. laurocerasus*. This trend is consistent with the higher total phenolic content previously observed in *C. spinosa*, which may contribute synergistically to antimicrobial activity. AgNPs obtained from both plant extracts exhibited inhibitory activity against the tested microorganisms, but the degree of activity varied depending on the plant species and the solvent used. In general, *C. spinosa* extracts exhibited broader and more potent antimicrobial activity than *P. laurocerasus* extracts. The AgNPs showed activity against both Gram-positive and Gram-negative bacteria, as well as fungi, although the degree varied. Generally, Gram-negative bacteria (with an additional outer membrane) were slightly more resistant than Gram-positive strains, but AgNPs still exhibited significant inhibitory effects.

Table 2. The agar well diffusion assay to determine the antimicrobial effects of *C. spinosa* L. extracts and AgNPs

Microorganisms	Inhibition Zone Diameter (mm)					
	C. spinosa-Me extract	*C. spinosa* Et-extract	*C. spinosa* Et-extract AgNPs-DMSO	*C. spinosa* Me-extract AgNPs-DMSO	Meropenem	Amphotericin B
***S. aureus* ATCC 29213**	0	0	12.61±0.43	11.81±0.28	34.68±0.29	-
***S. epidermidis* ATCC 12228**	0	0	10.48±0.05	10.34±0.05	51.25±0.27	-
***E. faecalis* ATCC 29212**	0	0	12.03±0.08	10.59±0.17	19.87±0.44	-
***E. coli* ATCC 25922**	0	0	11.74±0.07	11.79±0.24	35.06±0.15	-
***P. aeruginosa* ATCC 27853**	0	0	11.68±0.06	13.02±0.31	32.47±0.19	-
***A. baumannii* ATCC 19606**	0	0	11.00±0.09	12.05±0.17	32.50±0.24	-
***K. pneumoniae* ATCC 4352**	0	0	12.24±0.11	11.74±0.39	33.24±0.13	-
***C. albicans* ATCC 10231**	0	0	8.37±0.07	9.17±0.06	-	26.97±0.18
***C. parapsilosis* ATCC 90018**	0	0	10.33±0.11	12.48±0.31	-	24.26±0.13
***C. tropicalis* KUEN 1021**	0	0	8.15±0.07	8.94±0.47	-	19.58±0.21

Table 3. The agar well diffusion assay to determine the antimicrobial effects of *P. laurocerasus* extracts and AgNPs

Microorganisms	Inhibition Zone Diameter (mm)					
	***P. laurocerasus* – Me extract**	***P. laurocerasus-Et* extract**	***P. laurocerasus-Et* extract AgNPs-DMSO**	***P. laurocerasus* –Me extract AgNPs-DMSO**	**Meropenem**	**Amfoterisin B**
***S. aureus* ATCC 29213**	0	0	10.17±0.09	9.49±0.26	36.71±0.57	-
***S. epidermidis* ATCC 12228**	0	0	10.65±0.44	17.14±0.03	50.17±0.08	-
***E. faecalis* ATCC 29212**	0	0	13.85±0.58	9.96±0.48	18.97±0.63	-
***E. coli* ATCC 25922**	0	0	10.61±0.37	10.32±0.16	34.81±0.24	-
***P. aeruginosa* ATCC 27853**	0	0	13.02±0.04	13.06±0.02	33.29±0.18	-
***A. baumannii* ATCC 19606**	0	0	9.91±0.63	9.61±0.27	32.96±0.34	-
***K. pneumoniae* ATCC 4352**	0	0	10.84±0.15	9.76±0.38	33.07±0.03	-
***C. albicans* ATCC 10231**	9.66±0.18	8.17±0.06	11.93±0.39	11.01±0.03	-	27.43±0.32
***C. parapsilosis* ATCC 90018**	0	0	10.35±0.28	10.32±0.17	-	24.31±0.26
***C. tropicalis* KUEN 1021**	0	0	8.71±0.59	8.92±0.45	-	19.37±0.21

The antimicrobial potency of *C. spinosa* L. and *P. laurocerasus* L. extracts and their AgNPs was evaluated through the determination of MIC and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC). The results are summarized in Tables 4 and 5.

Table 4. Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC) and MIC of *C. spinosa* extracts and AgNPs against various control strains (mg/mL)

Microorganisms	***C.spinosa-Me* extract**		***C.spinosa* – Et-extract**		***C.spinosa*-Et extract AgNPs-DMSO**		***C.spinosa*-Me extract AgNPs-DMSO**		**Meropenem**		**Amphotericin B**	
	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC	MIC	MFC
***S. aureus* ATCC 29213**	0.11	> 6.5	0.11	> 6.5	0.05	> 3.4	0.05	> 3.4	2	4	-	-
***S. epidermidis* ATCC 12228**	0.23	> 6.5	0.43	> 6.5	0.10	> 3.4	0.10	> 3.4	0.25	0.50	-	-
***E. faecalis* ATCC 29212**	0.11	> 6.5	0.21	> 6.5	0.10	> 3.4	0.10	> 3.4	8	16	-	-
***E. coli* ATCC 25922**	0.45	> 6.5	0.85	> 6.5	0.20	> 3.4	0.20	> 3.4	0.06	0.12	-	-
***P. aeruginosa* ATCC 27853**	0.11	> 6.5	0.21	> 6.5	0.10	> 3.4	0.10	> 3.4	0.5	2	-	-
***A. baumannii* ATCC 19606**	0.23	> 6.5	0.43	> 6.5	0.10	> 3.4	0.05	> 3.4	2	4	-	-
***K. pneumoniae* ATCC 4352**	0.23	> 6.5	0.43	> 6.5	0.20	> 3.4	0.20	> 3.4	0.5	2	-	-
***C. albicans* ATCC 10231**	0.11	0.23	0.43	0.85	0.05	0.10	0.05	0.10	-	-	1	2
***C. parapsilosis* ATCC 90018**	0.23	> 6.5	0.43	> 6.5	0.03	0.10	0.05	0.10	-	-	1	2
***C. tropicalis* KUEN 1021**	0.11	0.23	0.21	0.21	0.20	0.20	0.42	0.84	-	-	1	2

MIC: Minimum Inhibitory Concentration, *MBC: Minimum Bactericidal Concentration, *MFC: Minimum Fungicidal Concentration

Table 5. Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC) and MIC of *P. laurocerasus* extracts and AgNPs against various control strains (mg/mL)

Microorganisms	*P. laurocerasus*-Me extract		*P. laurocerasus* – Et extract		*P. laurocerasus-Et* extract AgNPs-DMSO		*P. laurocerasus-Me* extract AgNPs-DMSO		Meropenem		Amfoterisin B	
	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC	MIC	MFC
***S. aureus* ATCC 29213**	0.04	>1.2	0.09	> 1.4	0.063	> 2.016	0.063	> 2.016	2	4	-	-
***S. epidermidis* ATCC 12228**	0.15	>1.2	0.35	> 1.4	0.25	> 2.016	0.25	> 2.016	0.25	0.50	-	-
***E. faecalis* ATCC 29212**	0.15	> 1.2	0.18	> 1.4	0.13	> 2.016	0.13	> 2.016	8	16	-	-
***E. coli* ATCC 25922**	0.15	> 1.2	0.70	> 1.4	0.13	> 2.016	0.13	0.25	0.06	0.12	-	-
***P. aeruginosa* ATCC 27853**	0.15	> 1.2	0.18	> 1.4	0.13	> 2.016	0.13	> 2.016	0.5	2	-	-
***A. baumannii* ATCC 19606**	0.02	> 1.2	0.02	> 1.4	0.016	> 2.016	0.031	> 2.016	2	4	-	-
***K. pneumoniae* ATCC 4352**	0.15	> 1.2	0.18	> 1.4	0.13	> 2.016	0.13	> 2.016	0.5	2	-	-
***C. albicans* ATCC 10231**	> 1.4	> 1.2	> 1.4	> 1.4	0.13	0.25	0.13	0.25	-	-	1	2
***C. parapsilosis* ATCC 90018**	0.04	> 1.2	0.04	> 1.4	0.031	> 2.016	0.031	> 2.016	-	-	1	2
***C. tropicalis* KUEN 1021**	0.04	0.08	0.09	0.18	0.25	0.50	0.25	0.50	-	-	1	2

MIC: Minimum Inhibitory Concentration, *MBC: Minimum Bactericidal Concentration, *MFC: Minimum Fungicidal Concentration

Across all microorganisms tested, *C. spinosa*-derived AgNPs generally displayed lower MIC values compared to *P. laurocerasus*-derived AgNPs, in agreement with their higher phenolic content and stronger activity observed in agar diffusion assays. The methanol and ethanol extracts of *C. spinosa* exhibited inhibitory activity against all tested bacterial and fungal strains, with MIC values ranging from 0.11 to 0.85 mg/ml. However, their MBC/MFC values were generally greater than 6.5 mg/mL. *P. laurocerasus* extracts also showed inhibitory activity, with MIC values ranging from 0.02 to 0.70 mg/mL. Similar to *C. spinosa*, MBC/MFC values for the extracts were generally high (>1.2–1.4 mg/mL). The AgNPs synthesized from *C. spinosa* extracts demonstrated markedly improved antimicrobial activity. MIC values for AgNPs were significantly lower (0.03–0.20 mg/mL), and in several cases, fungicidal concentrations were achieved at similarly low levels. The antimicrobial performance of *P. laurocerasus*-derived AgNPs was significantly enhanced. MIC values ranged from 0.016 to 0.25 mg/mL, and several fungal strains demonstrated low MFC values (e.g., *C. albicans* 0.13/0.25 mg/mL; *C. tropicalis* 0.25/0.50 mg/mL). The results demonstrate that while plant extracts alone provide inhibitory activity, their conversion into AgNPs dramatically enhances antimicrobial potency, reducing MIC values and enabling bactericidal/fungicidal effects at lower concentrations.

4. Discussion

Plant extracts rich in bioactive compounds have recently been used in the green synthesis of NPs. The potential of biomolecules present in plant extracts to reduce metal ions to NPs is very important in the green synthesis process. Therefore, this study focused on the synthesis of AgNPs by reducing silver ions present in silver nitrate solution in the extractions of *C. spinosa* and *P. laurocerasus* with different solvents. The green method was developed for the synthesis of antioxidant and bactericidal AgNPs. For this, three solvents were used as reducing and capping agents for the synthesis of AgNPs.

AgNPs stabilized with *C. spinosa* and *P. laurocerasus* extract were synthesized with 1 mg/mL extract concentration and 1 mM $AgNO_3$ for 24 h at room temperature, and their spectra were taken. Based

on the knowledge that silver nanoparticles exhibit a yellowish-brown color in water, the reduction of silver ions to silver nanoparticles was confirmed by UV-Visible spectroscopy analysis. According to the obtained spectra, the faint SPR band in the AgNP water spectrum indicated that fewer phytochemicals could be synthesized from the NP during water extraction. These results showed that the antioxidant effect varies depending on the solvent, with the highest effect observed in ethanolic extracts, followed by methanol and aqueous extracts (ethanol>methanol>water). The enhanced biological performance of AgNPs synthesized using ethanol extracts can be attributed to the distinct phytochemical composition and extraction selectivity of ethanol as a solvent. Ethanol, with its intermediate polarity, effectively solubilizes a broad range of moderately polar phenolics, including flavonoids (e.g., quercetin, kaempferol, catechin), phenolic acids (e.g., gallic, caffeic, and ferulic acids), and tannins, which possess strong reducing and metal-chelating capacities. These compounds readily donate electrons to Ag^{+} ions, accelerating their reduction to metallic Ag^{o} nuclei, and simultaneously serve as natural capping and stabilizing agents. Consequently, the resulting nanoparticles exhibit improved surface stability, uniformity, and bioactivity. In contrast, methanol and water tend to extract either a wider range of highly polar compounds or fewer lipophilic phenolics, leading to less efficient nanoparticle nucleation and weaker biological activity (30). In this context, they indicate that the extraction solvent plays a critical role in both the yield of phenolic compounds and their binding potential to the AgNPs surface. Furthermore, it demonstrates that the phenolic compounds present in the extracts during biosynthesis not only act as reducing agents in the formation of nanoparticles but also directly affect the antioxidant properties of the synthesized particles. Beyond the contribution of residual phenolics, the antioxidant activity of the synthesized AgNPs can also be explained by intrinsic nanoparticle mechanisms. The high surface-area-to-volume ratio of AgNPs facilitates redox interactions with reactive oxygen species (ROS), enabling electron transfer and radical neutralization at the nanoparticle interface. Additionally, the adsorbed phytochemicals on the nanoparticle surface may enhance this catalytic process by forming a synergistic redox couple between the AgNP core and the organic layer. This dual mechanism — involving both phytochemical-derived surface functionalization and AgNP-mediated electron transfer — explains the persistent antioxidant effect observed even after the reduction in total phenolic content post-synthesis. Therefore, the superior performance of ethanol-derived AgNPs likely results from a combination of optimized phytochemical composition, effective surface stabilization, and enhanced catalytic redox activity (31,32).

Agar well diffusion experiments revealed that both methanol and ethanol extracts of *C. spinosa* L. and *P. laurocerasus* L. showed no inhibitory activity against the tested bacterial strains. In contrast, AgNP-extract combinations exhibited substantial inhibition zones aganist both Gram-positive and Gram-negative bacteria (Tables 2, 3). Accordingly, it was observed that *C. spinosa* L. AgNPs prepared with Et-DMSO and Me-DMSO extracts had inhibition zones ranging from 10.34 to 13.02 mm. The greatest inhibition was observed against *S. aureus* (12.61 ± 0.43 mm) and *K. pneumoniae* (12.24 ± 0.11). Similarly, *P. laurocerasus* L. AgNP conjugates exhibited broad-spectrum antibacterial activity with inhibition zones ranging from 9.49 to 17.14 mm. Specifically, *S. epidermidis* showed the highest sensitivity (17.14 ± 0.03 mm with Me-AgNPs-DMSO). Additionally, significant antifungal activity was observed between the two plants in our study. While *C. spinosa* extracts alone showed no inhibition zone against *Candida* species, both the methanol and ethanol extracts of *P. laurocerasus* exhibited limited activity against *C. albicans* (9.66 ± 0.18 mm and 8.17 ± 0.06 mm, respectively). When combined with AgNPs, both plants showed enhanced antifungal effects. *C. spinosa* AgNP extracts also showed antifungal activity against three *Candida* species, with inhibition zones ranging from 8.15 to 12.48 mm. The highest activity was recorded against *C. parapsilosis* (12.48 ± 0.31 mm with Me-AgNPs-DMSO). *P. laurocerasus*, AgNP combinations, showed antifungal activity against all three *Candida* species with inhibition zones ranging from 8.71 to 11.93 mm, and were most effective against *C. albicans* (11.93 ± 0.39 mm with Et-AgNPs-DMSO). Although the antifungal activity of both plant-based AgNPs was lower than that of amphotericin B (19.37-27.43 mm), the observed zones of inhibition clearly indicate that AgNP conjugation enhanced the extracts' weak antifungal potential. The inclusion of AgNPs significantly improved the antimicrobial properties of both extracts, providing consistent inhibition

against Gram-positive and Gram-negative bacteria, as well as *Candida* species. Although both plant-based AgNPs generally showed stronger and more balanced antibacterial activity against bacterial strains, *P. laurocerasus* AgNPs had the highest single inhibition value (17.14 ± 0.03 mm) against *S. epidermidis*. In terms of antifungal activity, *C. spinosa* AgNPs were more effective against *C. parapsilosis*, while *P. laurocerasus* AgNPs showed slightly better activity against *C. albicans*.

The MIC and MBC values determined by the microdilution method also support these findings (Table 4,5). The MIC and MBC values of plant extracts in their methanol and ethanol forms used alone were found to be significantly higher compared to the AgNP-supported forms. This situation reveals that the amount of extract required to completely stop microbial growth and achieve a bactericidal effect is significantly reduced by AgNPs. A similar trend has also been observed in the yeast species. Both the MIC and MFC values of AgNP-enriched extract against *C. albicans* and *C. parapsilosis* were significantly lower compared to the extracts alone. For example, the MIC value for *C. albicans* is only 0.43 mg/mL for the ethanol extract alone, while this value decreased to 0.05 mg/mL in the AgNP-ethanol combination. These findings support the synergistic effect of AgNPs against fungal pathogens as well. Overall, the data obtained from both diffusion and microdilution tests clearly show that *C. spinosa L*. and *P. laurocerasus* extracts are insufficient on their own in terms of antimicrobial effect, but their efficacy against both bacteria and yeasts is significantly increased when combined with AgNPs. These results suggest that AgNPs could be an essential strategy for enhancing the biological activity of plant compounds.

These findings clearly demonstrate that *C. spinosa* and *P. laurocerasus* extracts alone are largely ineffective against tested bacterial and yeast strains. However, their conjugation with AgNPs results in a significant improvement of antimicrobial activity. The observed enhancement can be attributed to the synergistic interaction between silver nanoparticles and phytochemicals adsorbed on their surface, which facilitates binding and improves antimicrobial efficacy.

5. Conclusion

This study demonstrated that the extract of *C. spinosa* and *P. laurocerasus* significantly enhances the antioxidant and antibacterial potential of AgNPs, making it a suitable and biocompatible medium for their synthesis. AgNPs produced with *C.spinosa* and *P. laurocerasus* extract exhibited strong antibacterial effects against all tested bacterial and yeast strains. Moreover, the biosynthesized AgNPs displayed notable antioxidant activity at concentrations effective against bacterial growth. These findings emphasize the promise of eco-friendly AgNPs synthesized in the presence of *C. spinosa* extract for diverse biomedical applications.

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