

Enhanced Antibacterial Activity of Silver Nanoparticles Biosynthesized with Gamma-Irradiated Iranian Propolis

Farimehr Ghanizadeh Geraili^{1*}, Seyed Mahmoud Reza Aghamiri², Marzieh Heidarieh³, and Najmeh Sheikhzadeh⁴

¹Farimehr Ghanizadeh Geraili, Department of Medical Radiation Engineering, Shahid Beheshti University, Tehran, Iran, E-mail: ghanifarmehr@gmail.com, ORCID: 0000-0002-6210-9891

²Seyed Mahmoud Reza Aghamiri, Department of Medical Radiation Engineering, Shahid Beheshti University, Tehran, Iran, E-mail: smr-aghamiri@sbu.ac.ir; ORCID: 0000-0002-5080-0482

³Marzieh Heidarieh, Nuclear Science and Technology Research Institute, Tehran, Iran, E-mail: mheidarieh@aeoi.org.ir, ORCID: 0000-0003-2097-960X

⁴Najmeh Sheikhzadeh, Department of Food Hygiene and Aquatic Animals, University of Tabriz, Tabriz, Iran, E-mail: nsheikh@tabrizu.ac.ir, ORCID: 0000-0002-0909-7270

Abstract

Introduction: Owing to their pronounced antimicrobial potential against a wide array of microorganisms, silver nanoparticles (AgNPs) have arisen as materials of considerable interest for the development of pharmaceutical and biomedical technologies.

Materials and Methods: The current work details the green biosynthesis of AgNPs utilizing ethanolic extracts of Iranian propolis (EEP), employing gamma irradiation as a pre-treatment strategy for a portion of the extract. The antibacterial performance of the resulting nanoparticles was rigorously evaluated against *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Bacillus subtilis* (*B. subtilis*) utilizing Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) determinations.

Results: Notably, AgNPs synthesized with gamma-treated propolis demonstrated overall enhanced antimicrobial activity compared with those synthesized with untreated propolis. MIC and MBC values for AgNPs derived from gamma-treated EEP ranged from 7.8 to 31.25 $\mu\text{g/mL}$ against the investigated bacterial strains, whereas AgNPs from untreated EEP showed higher values of 7.8 to 62.5 $\mu\text{g/mL}$. These findings suggest that gamma irradiation effectively improves the reducing potential of propolis, leading to the fabrication of more bioactive nanoparticles with improved antibacterial properties, particularly against *B. subtilis*, where both MBC and MIC were recorded as 7.8 $\mu\text{g/mL}$.

Conclusion: Considering their high efficacy, inherent safety profile, and environmentally benign synthesis process, these AgNPs present considerable promise as alternative antimicrobial agents, particularly in the context of escalating antibiotic resistance.

Keywords: propolis, gamma irradiation, silver nanoparticles, antimicrobial activity, green synthesis

1. Introduction

Nanotechnology has emerged as a powerful platform driving innovation in diverse scientific fields, including materials engineering, medicine, and environmental science. This revolution is largely attributed to the distinctive physicochemical properties inherent in nanomaterials [1]. Among these, nanoparticles, defined by their nanoscale dimensions (1–100 nm), present novel functionalities stemming from the unusually high proportion of exposed surface associated with their small dimensions [2, 3]. Silver nanoparticles (AgNPs) have received widespread scientific acclaim for their potent antimicrobial properties against numerous microbial species, as well as

their antiviral, antifungal, and anti-inflammatory properties. This multifaceted bioactivity positions AgNPs as compelling candidates for diverse biomedical fields, including the development of antimicrobial surfaces for healthcare equipment, controlled drug delivery, and strategies aimed at improving wound repair [4].

While conventional chemical and physical synthesis techniques for AgNPs are well-established, they often entail the use of energy-intensive processes and toxic chemicals, raising biocompatibility and environmental concerns. Conversely, green synthesis techniques utilizing renewable biological resources like propolis, fungi, bacteria, and plant extracts offer an eco-friendlier and more biocompatible alternative for nanoparticle fabrication [5, 6].

Propolis, a complex resinous matter meticulously accumulated by honeybees from various plant sources, is a rich reservoir of bioactive mixtures, containing terpenoids, phenolics, and flavonoids [7]. These naturally occurring molecules display a wide array of therapeutic benefits, encompassing anti-inflammatory, antimicrobial, and antioxidant activities. In addition, they serve dual functions during nanoparticle biosynthesis, acting as agents responsible for both reduction and stabilization processes [8].

Gamma irradiation is increasingly considered an effective approach for enhancing the bioactivity of natural extracts without compromising their inherent structural integrity. Notably, the investigation by Heidarieh et al. [9] illustrated that gamma irradiation at 10 kGy significantly increases the total phenolic content of ethanolic extract of Iranian propolis (EEP), while higher doses (20–50 kGy) do not provide additional benefit and may induce partial degradation of bioactive compounds. Therefore, 10 kGy was selected as the optimal dose to maximize phenolic enhancement while preserving extract integrity, improving its suitability for nanoparticle synthesis.

As far as current literature indicates, the influence of gamma irradiation pre-treatment of Iranian propolis on the antibacterial performance of propolis-mediated AgNPs has not been systematically investigated. Therefore, this work compares the antibacterial property of AgNPs biosynthesized using gamma-treated and untreated propolis extracts against selected bacterial strains. Antimicrobial performance of the prepared AgNPs was examined against three bacterial strains, including *Staphylococcus aureus* (S. aureus), *Escherichia coli* (E. coli), and *Bacillus subtilis* (B. subtilis). The results demonstrate that gamma treatment prior to synthesis may enhance the antibacterial properties of propolis-derived AgNPs.

2. Materials and Methods

A schematic flowchart illustrating the experimental workflow, including propolis extraction and gamma treatment, green fabrication of AgNPs from gamma-treated and untreated EEP, along with antibacterial assessment through MBC and MIC analyses, is presented in Figure 1.

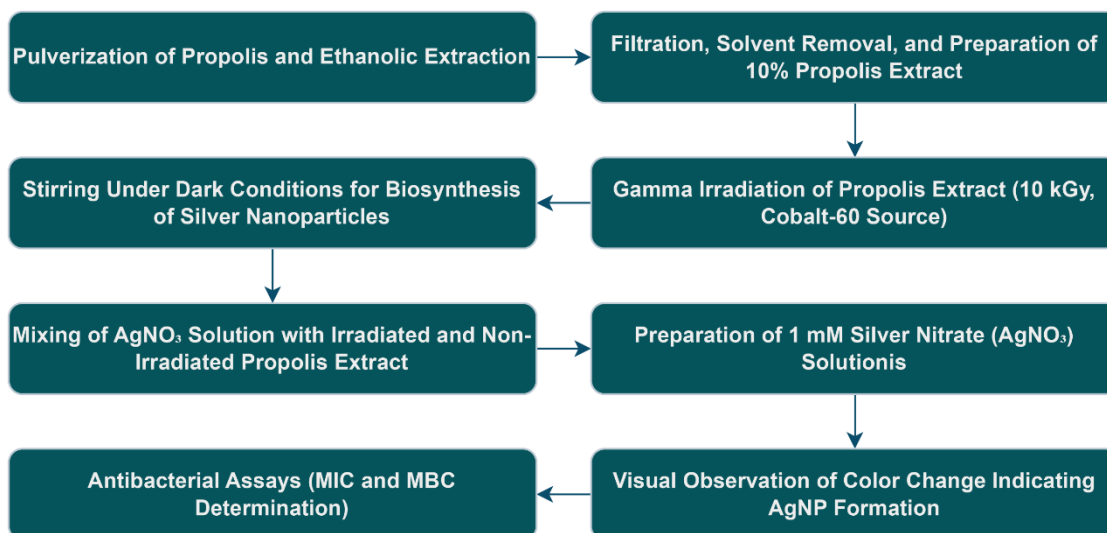


Figure 1. Overview of the workflow followed in this investigation, illustrating propolis extract preparation and gamma treatment, biosynthesis of AgNPs using gamma-treated and untreated EEP, and antibacterial assays (MBC and MIC)

2.1. Preparation and Irradiation of Propolis Extract

Propolis was initially pulverized using liquid nitrogen [10]. The preparation of the propolis extract involved an ethanol-based extraction procedure in which 25 g of pulverized propolis was introduced into 250 mL of 80% ethanol. The extraction medium was kept under mechanical mixing (150 rpm) over a time span of 48 h at ambient temperature to promote the transfer of bioactive constituents. The obtained ethanolic suspension was subsequently clarified by two sequential passages through grade 4 Whatman filter paper. Concentration of the filtrate was achieved by vacuum-assisted rotary evaporation, resulting in a condensed extract. Finally, the extract was brought to a 10% (w/v) concentration using 80% ethanol and preserved at 4 °C under dark conditions until analysis. Based on the optimal dose reported by Heidari et al. [9], a portion of this extract was subjected to gamma treatment at 10 kGy utilizing a Cobalt-60 γ -ray source (Gamma cell model 220, dose rate 1.04 Gy/s) at the Nuclear Science and Technology Research Institute in Tehran, Iran.

2.2. Biosynthesis of AgNPs

2.2.1. Preparation of Silver Precursor Solution

Aqueous silver nitrate ($\text{AgNO}_3 > 99.9\%$; MAY & BAKER Ltd., Dagenham, UK) was applied in this work. The precursor solution was generated by introducing 0.17 g of AgNO_3 into 1 L of deionized water, resulting in a final molar strength of 1 mM. The solution was stored in amber-colored glassware prior to use to prevent photoreduction of silver ions [11].

2.2.2. Biosynthesis Utilizing EEP

Biosynthesis of AgNPs was conducted utilizing a straightforward and environmentally friendly green synthesis approach, employing the EEP in both gamma-treated and untreated forms. Propolis

samples were collected from a beekeeping apiary located in Marvdasht, Fars Province, Iran. In a typical synthesis reaction, equal volumes (5 mL each) of the prepared 1 mM AgNO₃ solution and either gamma-treated or untreated EEP were thoroughly mixed in separate sterile containers. The reactions were carried out under dark conditions to prevent photoreduction of silver ions, which could result in uncontrolled nanoparticle formation. Each mixture was maintained under constant agitation for 60 min at 25 °C under controlled mixing conditions. The successful production of AgNPs were visually illustrated by a progressive color change in the colloidal solutions, transitioning between pale yellow or colorless and a characteristic yellowish-brown or dark brown appearance. This characteristic color development is associated with the surface plasmon resonance phenomenon of the synthesized nanoparticles, indicating the conversion of silver ions from Ag⁺ to elemental silver atoms, which results in the fabrication of stable AgNPs [12, 13].

2.3. Antibacterial Assay (MBC and MIC)

The antimicrobial performance of the prepared AgNPs and EEP was determined using the standard tube dilution method against three bacterial strains obtained from the Iranian Research Organization for Science and Technology, including *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), and *Staphylococcus aureus* (ATCC 6538) based on the methodology outlined by Cavassin et al. [14]. The tested samples were serially diluted in Mueller-Hinton Broth using a two-fold dilution scheme, followed by inoculation with bacterial suspensions adjusted to a turbidity equivalent to 0.5 McFarland standard. The tested concentrations covered a range of 1000 to 7.8 µg/mL, and MIC values were determined based on growth inhibition. After incubation at 37 °C for 16–20 h, the inhibition of the growth of bacteria was evaluated by visual examination of culture turbidity, and the MIC was identified as the lowest concentration without detectable growth. For MBC assessment, aliquots collected from tubes showing no visible growth of bacteria

were transferred onto Mueller-Hinton agar plates, where incubation was carried out at 37 °C for a time span of 48–72 h. The minimum concentration that resulted in the absence of colony formation on the agar medium was considered the MBC. Figure 2 provides a schematic summary of the experimental workflow, including propolis extraction, gamma treatment, and subsequent antibacterial evaluations. All antibacterial determinations were performed in triplicate, and statistical analysis of the obtained results was performed as described in Section 2.4.

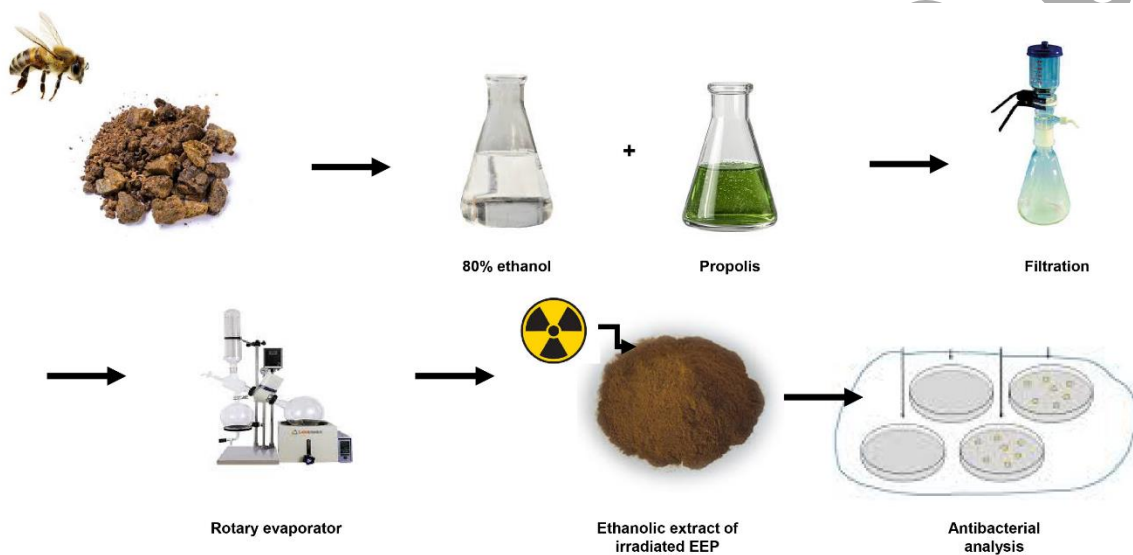


Figure 2. Overview of the experimental design illustrating the sequential processes of propolis extract preparation, gamma irradiation, and antibacterial evaluation

2.4. Statistical Analysis

The experimental dataset underwent statistical treatment through SPSS software (version 19). The effects of different experimental conditions were assessed through one-way analysis of variance (ANOVA), and significant differences among individual groups were further identified utilizing

Tukey's multiple comparison test. A significance level of $p < 0.05$ was applied for the interpretation of statistical differences.

3. Results

The antimicrobial activity of AgNPs biosynthesized utilizing both gamma-treated and untreated EEP was evaluated against three bacterial strains: *B. subtilis* (ATCC 6633), *E. coli* (ATCC 25922), and *S. aureus* (ATCC 6538). The MIC and MBC levels for each condition are illustrated in Table 1. **Table 1.** MIC and MBC determinations ($\mu\text{g/mL}$) of EEP and AgNPs synthesized with gamma-treated and untreated EEP

Pathogenic bacteria	Based on various dilutions utilized for AgNPs and EEP ($\mu\text{g/mL}$)							
	A		A'		B		B'	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacillus subtilis (Gram-positive) ATCC 6633	7.8	15.62	7.8	7.8	250	250	125	250
Staphylococcus aureus (Gram-positive) ATCC 6538	31.25	31.25	15.62	31.25	500	1000	250	500
Escherichia coli (Gram-negative) ATCC 25922	62.5	62.5	31.25	31.25	1000	1000	500	500

Note: **A** refers to AgNPs biosynthesized by employing untreated EEP, **A'** refers to AgNPs biosynthesized by the gamma-treated EEP, **B** refers to untreated EEP, and **B'** refers to gamma-treated EEP

The results demonstrate that both gamma-treated and untreated AgNPs exhibit significant antibacterial potential across all tested bacterial strains. The MBC and MIC values indicate a marked difference in antibacterial efficacy between AgNPs synthesized using gamma-treated and untreated EEP.

B. subtilis exhibited a high degree of susceptibility toward the AgNPs. AgNPs obtained from gamma-treated EEP showed improved antibacterial efficacy, achieving both MBC and MIC values of 7.8 µg/mL, in comparison with AgNPs from untreated EEP, which showed an MBC of 15.62 µg/mL and an MIC of 7.8 µg/mL.

S. aureus showed a similar trend. The gamma-treated EEP-derived AgNPs exhibited an MBC of 31.25 µg/mL and an MIC of 15.62 µg/mL, whereas the untreated AgNPs displayed identical MBC and MIC readings of 31.25 µg/mL. This highlights the increased antibacterial potency of AgNPs synthesized with gamma-treated EEP, particularly toward *E. coli* and *S. aureus*. Interestingly, the gamma-treated EEP alone showed some antimicrobial effects, but its activity was significantly weaker in comparison with the AgNPs ($p < 0.05$).

For *E. coli*, the AgNPs produced using gamma-treated EEP demonstrated equal MBC and MIC values of 31.25 µg/mL, significantly better than those synthesized from untreated EEP, for which both MBC and MIC were observed at 62.5 µg/mL. Similarly, gamma-treated EEP alone exhibited weaker antibacterial activity, with both MIC and MBC values reaching 500 µg/mL, compared to its nanoparticle form.

When comparing the activity of AgNPs with the raw EEP extracts (both gamma-treated and untreated), it is clear that AgNPs were far more effective. For instance, while EEP alone showed weaker antibacterial effects against all three bacterial strains, the synthesized AgNPs demonstrated significantly lower MIC and MBC values.

4. Discussion

This enhanced antibacterial activity observed against *B. subtilis* suggests that gamma treatment might improve the bioactive properties of the EEP, which results in the production of smaller, more

effective nanoparticles. These smaller particles are likely more bioactive and thus increase their antibacterial efficacy [9].

Similarly, the greater effectiveness of gamma-treated AgNPs against *S. aureus* highlights the enhanced antibacterial potency achieved through the use of gamma-treated EEP. The modest activity observed from gamma-treated EEP alone, although significantly weaker, reinforces the superior effectiveness of the nanoparticles in this study.

For *E. coli*, the considerably lower MIC and MBC values of AgNPs synthesized from gamma-treated EEP, compared to both untreated AgNPs and raw extracts, reflect the ability of gamma irradiation to alter the phenolic profile of EEP. This alteration likely enhances its ability to reduce silver ions and form more potent nanoparticles [15].

The increased efficacy of AgNPs relative to their corresponding EEP extracts further supports the role of nanoparticle formation in enhancing antimicrobial potential of propolis. While EEP alone displayed some antibacterial activity, it was substantially less effective than when formulated into AgNPs.

This overall enhancement in activity can be attributed to modifications of the phenolic compounds in propolis during gamma irradiation. This process may break down larger molecular structures into smaller, more reactive bioactive molecules, while also inducing the formation of free radicals and carbonyl-containing compounds, which together may enhance the transformation of silver ions into AgNPs. The increased reducing capacity of gamma-treated propolis likely contributes to the observed antimicrobial improvement [9].

The strong antibacterial effects of AgNPs against all tested strains are consistent with known mechanisms, including their capability to disrupt bacterial membranes, generate oxidative stress, and interact with intracellular components [16]. The smaller size and larger surface area of AgNPs

synthesized from gamma-treated EEP enhance these interactions, resulting in stronger antimicrobial activity.

These observations are consistent with earlier reports demonstrating the superior antimicrobial performance of AgNPs generated from plant-derived or irradiated biological materials. Enhanced bacterial cell penetration and increased bactericidal potential of these nanoparticles may explain their notable effectiveness [15].

The potent antibacterial activity demonstrated by the synthesized AgNPs suggests potential applications in various biomedical fields. These nanoparticles may be considered for incorporation into wound dressings, antimicrobial coatings for medical devices, topical formulations, and other infection-control strategies. Their environmentally friendly synthesis route further enhances their attractiveness for the advancement of alternative antimicrobial agents aimed at combating antibiotic-resistant bacterial infections.

Although the findings of this work indicate notable antibacterial potential, certain aspects require further investigation. The current work was limited to in vitro experiments, which may not completely represent the complex interactions occurring within biological environments. Moreover, important considerations, including the toxicity profile, distribution within biological systems, and long-term safety of the synthesized AgNPs, were beyond the scope of this study. Consequently, additional in vivo investigations are necessary to establish the therapeutic applicability and safety profile of these nanoparticles prior to their potential use in biomedical settings.

5. Conclusion

This study confirms that AgNPs synthesized using gamma-treated propolis exhibit enhanced antibacterial activity compared with those prepared using untreated extracts. Gamma irradiation had a beneficial effect on propolis-mediated AgNPs synthesis, resulting in nanoparticles with improved antibacterial performance as evidenced by lower MBC and MIC values. The process is eco-friendly, avoids toxic chemicals, and produces biocompatible AgNPs suitable for potential medical applications. The nanoparticles demonstrated bactericidal activity against *S. aureus* and *B. subtilis*, while propolis extract alone demonstrated only inhibitory effects against *E. coli*. Further in vivo investigations are recommended to evaluate pharmacokinetics, safety, and therapeutic potential.

Ethical Considerations

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246 **References**

- 247 1. Joudeh N, Linke D. Nanoparticle classification, physicochemical properties,
248 characterization, and applications: a comprehensive review for biologists. *J Nanobiotechnology*.
249 2022;20(1):262. <https://doi.org/10.1186/s12951-022-01477-8>
- 250 2. Zulfiqar Z, Khan RRM, Summer M, Saeed Z, Pervaiz M, Rasheed S, et al. Plant-mediated
251 green synthesis of silver nanoparticles: Synthesis, characterization, biological applications, and
252 toxicological considerations: A review. *ISBAB*.
253 2024;57:103121. <https://doi.org/10.1016/j.bcab.2024.103121>
- 254 3. Abbas R, Luo J, Qi X, Naz A, Khan IA, Liu H, et al. Silver nanoparticles: Synthesis,
255 structure, properties and applications. *Nanomaterials*.
256 2024;14(17). <https://doi.org/10.3390/nano14171425>
- 257 4. Magdy G, Aboelkassim E, Abd Elhaleem SM, Belal F. A comprehensive review on silver
258 nanoparticles: Synthesis approaches, characterization techniques, and recent pharmaceutical,
259 environmental, and antimicrobial applications. *Microchem J*.
260 2024;196:109615. <https://doi.org/10.1016/j.microc.2023.109615>
- 261 5. Fahim M, Shahzaib A, Nishat N, Jahan A, Bhat TA, Inam A. Green synthesis of silver
262 nanoparticles: A comprehensive review of methods, influencing factors, and applications. *JCIS*
263 *Open*. 2024;16:100125. <https://doi.org/10.1016/j.jciso.2024.100125>
- 264 6. Akhter MS, Rahman MA, Ripon RK, Mubarak M, Akter M, Mahbub S, et al. A systematic
265 review on green synthesis of silver nanoparticles using plants extract and their bio-medical
266 applications. *Heliyon*. 2024;10(11). <https://doi.org/10.1016/j.heliyon.2024.e29766>
- 267 7. Abdelsattar AS, Yakoup AY, Kamel AG, El-Shibiny A. Green synthesis of silver and gold-
268 doped zinc oxide nanocomposite with propolis extract for enhanced anticancer activity. *Sci Rep*.
269 2024;14(1). <https://doi.org/10.1038/s41598-024-71758-9>
- 270 8. Barbosa VT, Souza JK, Alvino V, Meneghetti MR, Florez-Rodriguez PP, Moreira RE, et al.
271 Biogenic synthesis of silver nanoparticles using Brazilian propolis. *Biotechnol Prog*.
272 2019;35(6). <https://doi.org/10.1002/btpr.2888>
- 273 9. Heidarieh M, Chakoli AN, Shahbazi S, Shawrang P, Zhang B. Effect of gamma irradiation
274 processing on total phenol and antioxidant capacities of the Iranian extract of propolis. *RCA*.
275 2021;109(8):635-41. <https://doi.org/10.1515/ract-2021-1042>
- 276 10. Al-Waili N, Al-Ghamdi A, Ansari MJ, Al-Attal Y, Salom K. Synergistic Effects of Honey and
277 Propolis toward Drug Multi-Resistant Staphylococcus Aureus, Escherichia Coli and Candida
278 Albicans Isolates in Single and Polymicrobial Cultures. *Int J Med Sci*. 2012;9(9):793-
279 800. <https://doi.org/10.7150/ijms.4722>
- 280 11. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials.
281 *Biotechnol Adv*. 2009;27(1):76-83. <https://doi.org/10.1016/j.biotechadv.2008.09.002>
- 282 12. Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis
283 of silver nanoparticles for antimicrobial applications: a green expertise. *J Adv Res*. 2016;7(1):17-
284 28. <https://doi.org/10.1016/j.jare.2015.02.007>
- 285 13. Geraili FG, Aghamiri SMR, Heidarieh M. Comparative Green Synthesis of Silver
286 Nanoparticles Using Gamma-Irradiated and Non-Irradiated Ethanolic Extract of Iranian Propolis.
287 *BioNanoScience*. 2026;16(5):354.10.1007/s12668-026-02574-w

- 288 14. Cavassin ED, de Figueiredo LFP, Otoch JP, Seckler MM, de Oliveira RA, Franco FF, et al.
289 Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against
290 multidrug resistant bacteria. J Nanobiotechnology. 2015;13(1).[https://doi.org/10.1186/s12951-](https://doi.org/10.1186/s12951-015-0120-6)
291 [015-0120-6](https://doi.org/10.1186/s12951-015-0120-6)
- 292 15. Mohamed SO, El-Naggar K, Khalil MM. Green synthesis of silver nanoparticles using
293 egyptian propolis extract and its antimicrobial activity. Egypt J Chem. 2022;65(7):453-
294 64.<https://doi.org/10.21608/ejchem.2021.104296.4838>
- 295 16. Li W-R, Xie X-B, Shi Q-S, Zeng H-Y, Ou-Yang Y-S, Chen Y-B. Antibacterial activity and
296 mechanism of silver nanoparticles on Escherichia coli. Appl Microbiol Biotechnol.
297 2010;85(4):1115-22.<https://doi.org/10.1007/s00253-009-2159-5>