Original Article
DOI: https://doi.org/10.70749/ijbr.v3i1.477

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Eco-friendly Fabrication of Silver Nanoparticles from Propolis Extract and Their Antimicrobial and Antioxidant Efficacy

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ARTICLE INFO

Keywords

Silver Nanoparticles (AgNPs) , Propolis Extract , Green Synthesis , Antioxidant Activity , Antimicrobial Efficacy, Biogenic Nanoparticles, Sustainable Nanotechnology, Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) X-Ray, Diffraction (XRD) , Phenolic Compounds.

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Declaration

Author's Contributions: All authors equally contributed to the study and approved the final manuscript.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History
Received: 20-10-2024
Revised: 18-12-2024
Accepted: 01-01-2025

ABSTRACT

Chemical method synthesis nanoparticles have adverse environmental and health implications because they are toxic. Consequently, intensified efforts have been put into developing green synthesis methods for nanomaterials using plant extract for the synthesis of nanoparticles. This approach can be seen as a cheaper method and more environmentally friendly than traditional nanoparticle synthesis methods. Among the number of plant-derived materials available, propolis a by-product of honey, has been found to have potential for its use as a green reducing agent in the synthesis of nanoparticles. Propolis contains many bioactive compounds, especially flavonoids, which makes propolis a suitable medium for the green synthesis of AgNPs. In the present study, propolis extract is used as the reducing agent to prepare silver nanoparticles as propolis contains antioxidants and antimicrobial properties. The preparation of the propolis extract involves the use of an extraction process that is employed to get the highest yield activity. Physic chemical techniques and NIR spectroscopy are used to deduce the chemical constitution and functional group present in the extract. After that, the synthesized silver nanoparticles are characterized using FT-IR, SEM, and XRD with the purpose of structural, morphological, and compositional analysis. These analytical tools offer useful structural details about the synthesized nanoparticles such as size, morphology, and crystallinity to assess their suitability in applications including; medicine and the environment. Thus the present study presents an efficient green method for the synthesis of nanoparticles that is credible and which also employs the use of a waste product in the process.

INTRODUCTION

The remarkable physicochemical features of silver nanoparticles (AgNPs), which include distinctive optical, catalytic, and antibacterial behaviors, have attracted considerable interest. The outstanding characteristics of AgNPs have rendered them extremely beneficial in several fields including medicine, pharmacology, environmental science, and material engineering. The antibacterial characteristics of silver nanoparticles (AgNPs) have established them as a crucial instrument in opposing infections and tackling issues in healthcare and biomedical research(Mumtaz et al., 2024). Typically, conventional techniques for producing AgNPs require the use of chemical reducing agents that may provide risks to the environment and human health. As a result, there is an increasing fascination with green synthesis methods that utilize

natural extracts, providing a sustainable approach with lower toxicity(Kumar et al., 2021). In accordance with the principles of green chemistry, green synthesis of nanoparticles focuses on using plant-based or natural extracts as reducing and stabilizing agents, therefore minimizing the use of hazardous chemicals and lowering environmental effect(Ahmad et al., 2024). Propolis, a resinous material obtained by honeybees from plant exudates, has become a highly promising option for biosynthesis of nanoparticles among other natural sources. Propolis is abundant in bioactive substances such as flavonoids, polyphenols, terpenoids, aromatic acids, and essential oils. These components not only enhance its widely recognized medicinal qualities but also aid in the conversion of metal ions into nanoparticles under reduced conditions.

Propolis has been widely employed in traditional medicine because of its potent antifungal, antibacterial, antiviral, and anti-inflammatory properties(Sulieman, Al-Anaizy, Al-Anaizy, Abdulhakeem, & Snoussi, 2023). The chemical makeup of propolis is varied according on the geographical area, plant origins, and environmental circumstances(Stojanović, Najman, Popov, & Najman, 2020). However, its fundamental bioactive components demonstrate continued efficacy in the reduction of metal ions. In the green production of AgNPs, the phenolic compounds and flavonoids found in propolis serve as natural reducing agents that transform silver ions (Ag^{+}) into metallic silver (Ag^{0}). Moreover, these chemicals function as stabilizers that inhibit the aggregation of nanoparticles, therefore improving the stability and effectiveness of the produced nanoparticles(Alghoraibi et al., 2020).

The antibacterial characteristics of silver nanoparticles (AgNPs) are well acknowledged, since they have strong efficacy against a wide range of pathogens, particularly bacteria, fungi, and viruses. Incorporating propolis into the manufacture of silver nanoparticles (AgNPs) can further augment the antibacterial activity by leveraging the intrinsic bioactive characteristics of propolis components(Garibo et al., 2020; Rahman et al., 2022). This synergistic impact is especially advantageous in biomedical applications, including wound healing, drug administration, and the enhancement of antibacterial coatings for medical equipment(Shahmoradi, Amini Nogorani, Mansouri, & Zarei, 2023). The synergistic advantages of silver nanoparticles (AgNPs) and propolis offer a comprehensive strategy to tackle microbial resistance and create technologically enhanced antibacterial materials(Garibo et al., 2020; Hamad & Salman, 2023).

The primary objective of this work is to synthesize silver nanoparticles (AgNPs) by utilizing propolis extract as an environmentally friendly and long-lasting reducing agent. The objective of this study is to streamline the synthesis parameters, analyze the nanoparticles using UV-Vis spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD), and assess their antibacterial activity. This work makes a valuable contribution to the field of green nanotechnology by combining the well-documented therapeutic advantages of propolis with the advanced characteristics of AgNPs. It emphasizes the potential of biogenic synthesis protocols in creating functional nanomaterials that have improved biological activity.

METHODOLOGY

Apparatus and Instruments

The study utilized the following apparatus and instruments: Soxhlet extractor, rotary evaporator, UV-Vis spectrophotometer, centrifuge, mortar and pestle, cellulose thimble, round bottom flask, and oven.

Chemicals

The chemicals used were ethanol (analytical grade), n-hexane, sodium carbonate (Na_2CO_3), Folin-Ciocalteu reagent, gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), silver nitrate ($AgNO_3$), guar gum, sodium alginate, Tween-20, and deionized water.

Sample Collection

The propolis sample was manually collected from a honey bee hive in District Mandi Bahauddin, Punjab, Pakistan, with coordinates 32.5742° N and 73.4828° E. The collected sample was stored at 4°C prior to analysis.

Table 1

(Parameters of Propolis sample)

Parameters	Propolis
Area of collection	Pindi Rawan
Plant source	Bamboo
Collection time	October 2021
Colour	Yellowish brown
Weight	56 g

Propolis Extraction

Propolis was ground using a mortar and pestle and dewaxed with n-hexane. The residue was placed in a cellulose thimble, and ethanol (300 mL) was added to the Soxhlet extractor. Extraction was conducted at a temperature maintained below 50°C for 3 hours. The ethanol was then evaporated using a rotary evaporator, yielding a yellowish sticky propolis extract with a yield of 12%(Pant et al., 2021).

Analysis of Propolis Extract

The extract was subjected to various assays to determine its phenolic content and antioxidant properties.

Total Phenolic Content (TPC)

To measure the total phenolic content, 20 µL of propolis extract was mixed with 1 mL of Folin-Ciocalteu reagent and diluted with 1.6 mL of distilled water. After a 10-minute incubation at room temperature, 3 mL of Na_2CO_3 solution was added, and the mixture was incubated at

40°C for 30 minutes. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer and expressed as gallic acid equivalents (mg/g)(Dudonne, Vitrac, Coutiere, Woillez, & Mérillon, 2009).

DPPH Assay

The antioxidant activity was evaluated using the DPPH assay by the proptocl used by (Dudonne et al., 2009). Propolis extract (0.5 mL) was mixed with 2.5 mL of 0.1 mM DPPH solution and incubated at 30°C for 30 minutes. The absorbance was measured at 517 nm, and antioxidant inhibition (% AI) was calculated using the formula:

$$\% \text{ AI} = \frac{Ao - A1}{Ao} \times 100$$

Where A_0 is reaction solution without sampleand A1 is reaction solution with sample.

Synthesis of AgNPs

Silver nanoparticles were synthesized using modified protocol of (Balavandy, Shameli, & Abidin, 2015)Silver nitrate solutions of varying concentrations (1 mM, 3 mM, 5 mM, and 10 mM) were prepared. Propolis extract (20 µL) was diluted with 10 mL of distilled water, and pH was adjusted to 10.62 using 1 M NaOH. The silver nitrate solution (1 mL) was added to the propolis extract, resulting in the reduction of silver ions (Ag^{+}) to silver nanoparticles (Ag^{0}), indicated by a color change from light yellow to dark brown (Figure 7). This reduction was confirmed via UV-Vis spectroscopy. The reactions were carried out at temperatures of 30, 33, 36, and 37°C. The resulting nanoparticles were centrifuged at 6000 rpm for 20 minutes, air-dried, and stored in Eppendorf tubes for further analysis.

Preparation of Polymeric Blends with AgNPs

Polymeric blends were prepared, and AgNPs were loaded onto these blends.

Preparation of AIN-01

A solution of guar gum (0.8 g) was prepared in deionized water at 45-50°C and mixed with 0.2 g of Tween-20. The mixture was homogenized for 5 hours at 50°C. Propolis-mediated AgNPs (1 mg/5 mL) were dissolved in ethanol and slowly loaded onto the solution with continuous stirring for an hour. The loaded mixture was cast into a petri dish and oven-dried at 50°C(Ilk, Tan, Emül, & Sağlam, 2020)

Preparation of AIN-02

Guar gum (0.6 g) and sodium alginate (0.2 g) solutions were prepared separately in deionized water, combined, and mixed with 0.2 g of Tween-20. The mixture was homogenized for 5 hours at 50°C). AgNPs were dissolved in ethanol and slowly loaded onto the polymer blend. The mixture was cast into petri dishes and dried at 50°C(Ilk et al., 2020).

Antioxidant and Antimicrobial Assays

The antioxidant activities of the AgNPs and the loaded blends were assessed using TPC and DPPH assays.

Total Phenolic Content

For TPC, 20 µL of each sample was mixed with 1 mL Folin-Ciocalteu reagent, diluted, incubated, and absorbance was measured at 765 nm(Dudonne et al., 2009).

DPPH Assay

For the DPPH assay, samples (0.5 mL) were mixed with 2.5 mL of 0.1 mM DPPH solution, incubated, and absorbance was recorded at 517 nm. The antioxidant inhibition was calculated(Dudonne et al., 2009).

Antimicrobial Assay

The antimicrobial activity of propolis extract was determined using the agar well diffusion method against Gram-positive and Gram-negative bacteria.Nutrient broth was prepared by dissolving 1.5 g in 100 mL of distilled water and autoclaved at 121°C for 1 hour. Nutrient agar (2.8 g) was prepared similarly. After autoclaving, the media were cooled and used for bacterial growth(Ilk et al., 2020).Gram-positive (*Clostridium botulinum*, *Bacillus subtilis*) and Gram-negative (*Agrobacterium tumefaciens*, *Proteus mirabilis*) bacteria were used for screening. Wells were formed in the agar plates, and samples were loaded for observation after 24-48 hours.

RESULTS AND DISCUSSION

Traditionally, propolis has been used to alleviate the symptoms of certain illnesses. It functions as a hepatoprotective, anticonvulsant, antibacterial, anti-inflammatory, and antifungal agent. The extract contains a wide range of pharmacologically active substances such as flavonoids, resin, waxes, water, essential oils, and phenolic compounds. Propolis extract yields silver nanoparticles (AgNPs) that exhibit enhanced stability over time and find utility in diverse applications(Šuran et al., 2021).

Silver nanoparticles (AgNPs) are widely prevalent and very significant due to their shown broad spectrum of activities, including antibacterial effects against a diverse array of human diseases(Khan et al., 2023; Mumtaz et al., 2024). Furthermore, they have been employed in diverse medicinal applications such as anticancer, radiation, medication administration, antibacterial, antifungal, and gene delivery. The primary prerequisites for the production of a silver nanoparticle include the choice of solvent medium, the presence of a reducing agent, and the use of a non-toxic nanostructure stabilizer. Due to their significant effectiveness against numerous bacteria, silver nanoparticles have attracted considerable interest(Genc, 2021).

The present study involved the preparation of propolis extract using the Soxhlet extraction mode.

Propolis silver nanoparticles (AgNPs) were engineered and incorporated into hydrogel formulations made from Guar Gum, Sodium Alginate, and Tween-20. The unloaded and AgNP-loaded hydrogels underwent antioxidant screening using DPPH and evaluation of total phenolic contents.

The ethanolic extract of propolis was prepared by Soxhlet extraction method. Raw propolis sample was washed to remove impurities and stored in freezer

Analysis of Propolis Extract

Prepared extract (Fig 1) was subjected to chemical test to build chemical profile.

Total phenolic content (TPC)

Determination of total level of phenolic compounds is based on colour oxidation-reduction reaction using Follin Ciocalteu reagent under alkaline condition and performed at 765nm wavelength using UV spectrophotometer. Total amount of phenolic compounds is expressed as Gallic acid equivalents (GAE) mg/g in table 4.1 using standard curve of Gallic acid.

Figure 1

(Propolis Extract)

Table 2

(Anti-oxidant Potential of propolis extract with Follin Ciocalteau reagent at different concentration)

Serial No	Concentration (µg/mL)	GAE mg/g
1	A-25	480
2	A-50	450
3	A-100	250
4	A-200	220

Figure 2

(Results of Total phenolic Content)

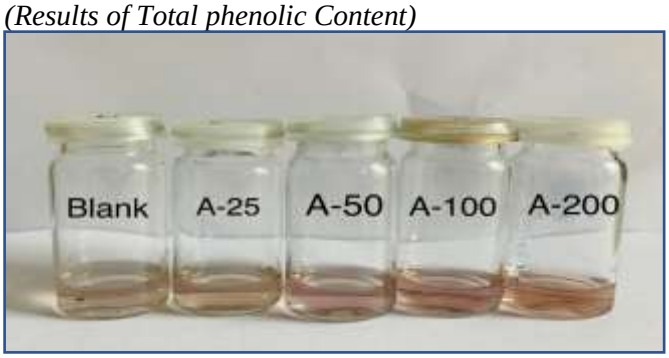
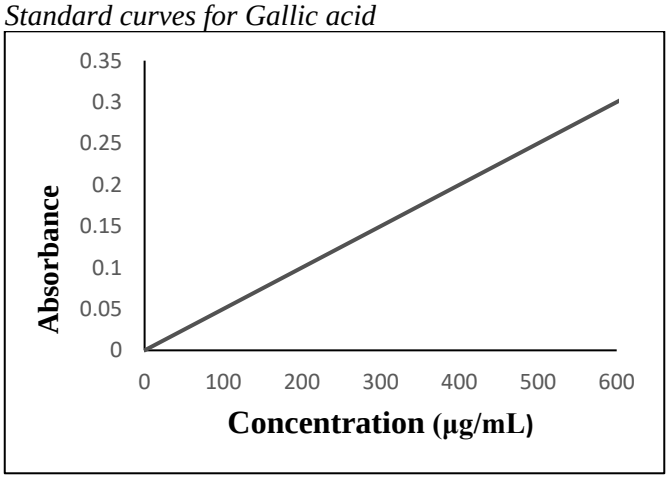


Figure 2

Standard curves for Gallic acid



DPPH Free Radical Scavenging Assay

A violet solution is produced in ethanol by the antioxidant assay known as the DPPH (2,2-diphenyl-1-picrylhydrazylhydrate) free radical assay. This method is based on electron transfer. the presence of an antioxidant molecule, this free radical which is stable at room temperature is reduced resulting in the formation of an ethanol solution that is colourless. It is possible to access many items at once due to the use of the DPPH assay, which offers a quick and simple method for evaluating antioxidants. Antioxidant capacity of different concentration of sample has been evaluated by DPPH radical scavenging activity as shown in **Fig.4**

Figure 4

(Reaction mixture containing sample solution and DPPH)

Table 3

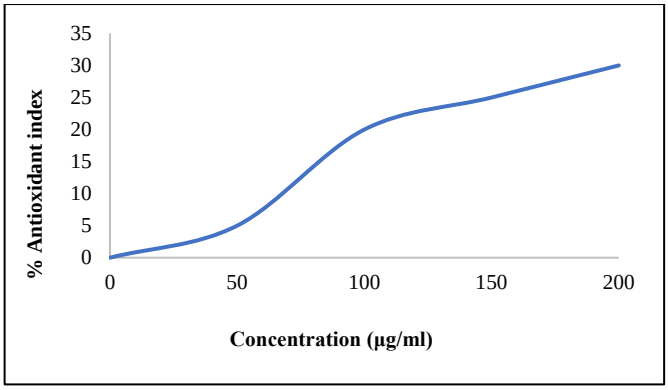
Antioxidant potential of propolis extract with DPPH radical scavenging assay at different concentration

Serial No	Concentration (µg/mL)	%Antioxidant Index
1	B-25	7
2	B-50	28
3	B-100	32
4	B-200	36

The antioxidant index of propolis extract was accessed for its capability to scavenge free radicals by the reaction of DPPH with extract and demonstrated as percentage antioxidant index as listed in **table.**Antioxidant activity of silver nanoparticle swere also assessed by (Keshari, Srivastava, Singh, Yadav, & Nath, 2020).

Figure 3

Anti-oxidant activity of propolis extract with DPPH at various concentrations

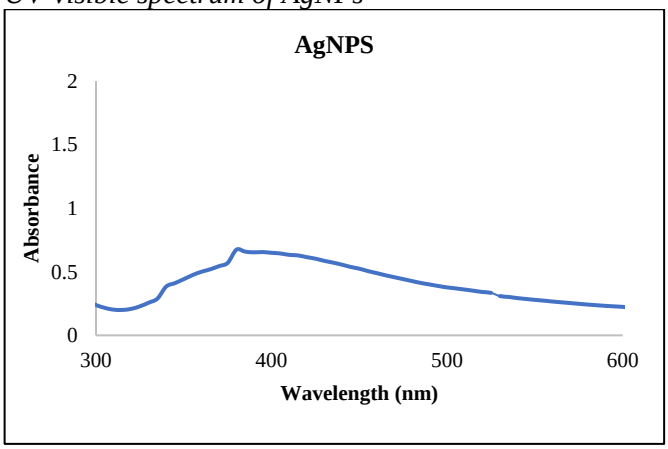


UV-visible spectroscopy

By the addition of AgNO3 and propolis extract, the reaction colour changed from yellow to dark brown, confirmed visually. Metal nanoparticles in the conduction band exhibit the phenomenon known as surface plasmon resonance (SPR), which is simply observed by UV-visible spectroscopy and involves the interaction of electrons with electromagnetic radiation. By measuring absorbance in a UV-visible spectrometer, the reduction of silver ion to AgNPs may be easily observed. Major SPR bands were shown between 400 and 500 nm. Similar results were also observed by (Sarkar, Kumbhakar, & Mitra, 2010).

Figure 4

UV-visible spectrum of AgNPs



Effects of Experimental Parameters on AgNPs synthesis

The synthesis of AgNPs is affected by several factors. In the current study, many of these factors, including salt concentration, duration, and temperature were examined to determine the reaction's optimum conditions**.**

Concentration of $AgNO_3$

AgNO3 was examined at different concentrations of 1mL, 3mL, 5mL, and 10mL. (Fig.4.7). The absorption peak was quite low at lower concentrations of AgNO3 (1mL) The peak intensity progressively raised to the maximum value at the increasing concentration of $AgNO_3$i-e., 3mL, 5mL, and 10mL. Moreover, the greater the concentration of $AgNO_3$, the darker the colour of the AgNP solution. The study showed that the 5 mL solution had the highest absorbance.

Figure 7

AgNPs synthesis using different AgNO3

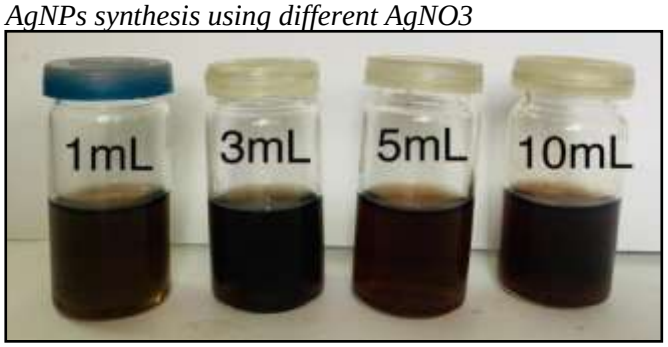
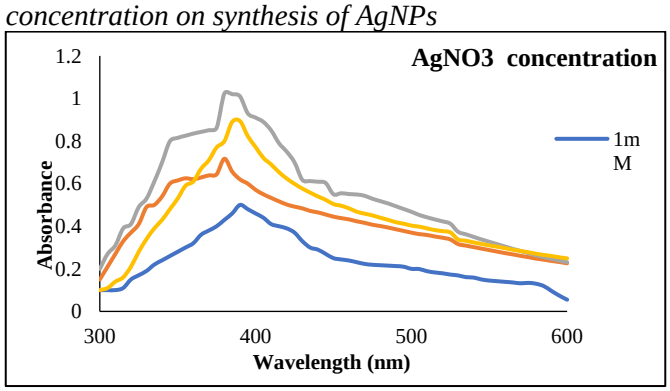


Figure 5

UV-visible spectrum showing the different AgNO3 concentration on synthesis of AgNPs

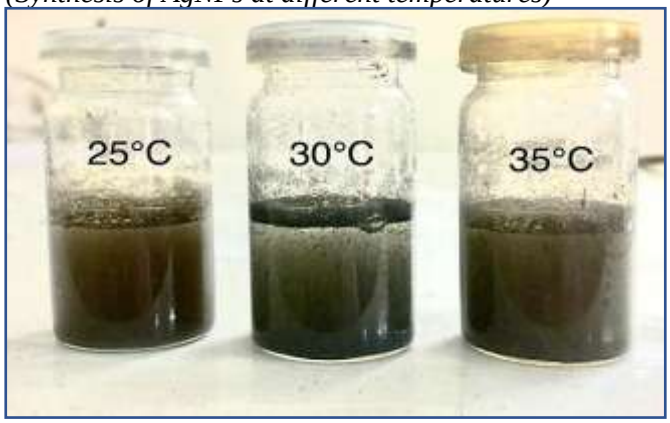


Temperature

Another factor that greatly affects the production of Ag-nanoparticles is temperature. The influence of temperature on the reduction reaction had been reported by several studies on the synthesis of nanoparticles. The rate of nanoparticle formation is directly proportional to temperature due to Increases in reaction kinetics. Temperature fluctuations those caused a rise in the absorbance peak in the UV-visible spectra at 25°C, 30°C, and 35°C, respectively, had a significant impact on reaction rate. (Fig. 9).

Figure 6

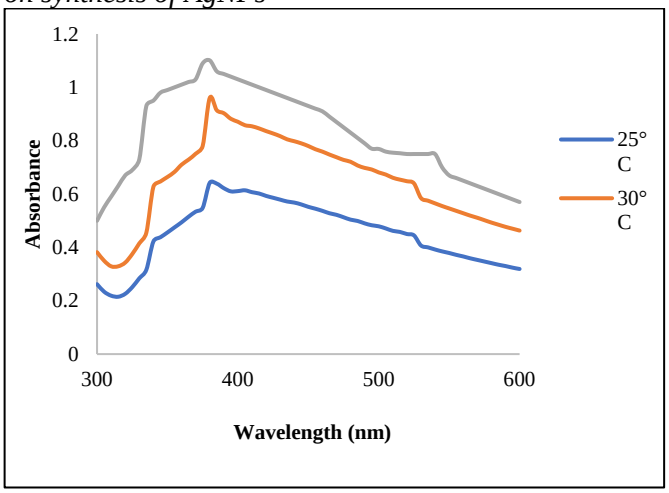
(Synthesis of AgNPs at different temperatures)



The quick change in colour at higher temperatures also served as an indicator of AgNPs synthesis. At 25°C, maximum absorption of 0.622 was monitored at 385nm. Reaction mixture at temperature of 30°C gave absorbance peak of 0.914 at 385 nm. At 35°C, the absorbance peak of 1.101 at 474nm. The highest temperature i.e., 35°C was proved to be the optimum temperature for synthesis of AgNPs.Similar approach was also used by (Jiang, Chen, Chen, Xiong, & Yu, 2011).

Figure 7

UV-visible spectrum showing the effect of temperature on synthesis of AgNPs

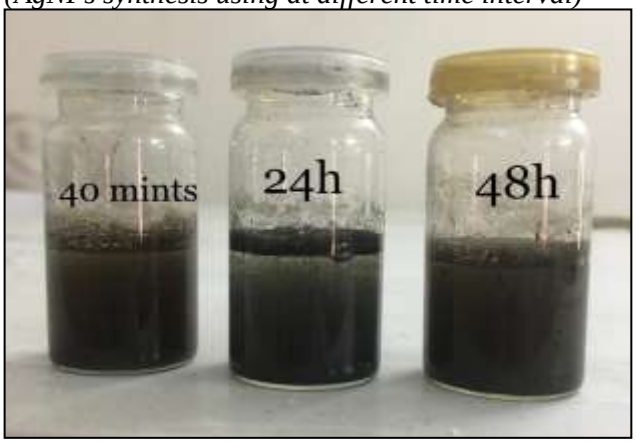


Time

The time for completion of the reaction plays an important role in the synthesis of AgNPs. The effect of the time parameter on the reaction was checked by taking absorbance at different time intervals i.e., 40min, 1440 min and 2880 by keeping all other variables uniform.

Figure 8

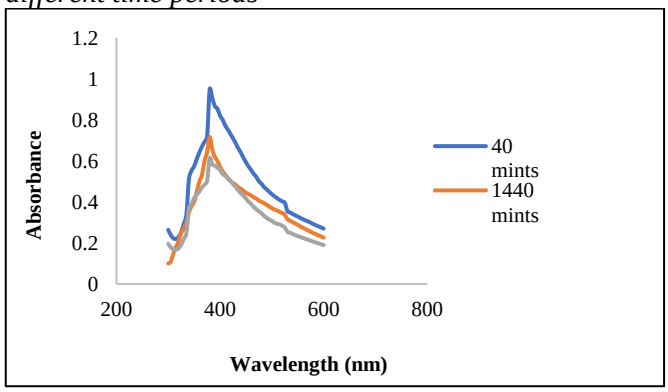
(AgNPs synthesis using at different time interval)



Extending the reaction time will allow more silver ions to interact with and be reduced by biomolecules in the extract. The study indicated that maximal absorbance occurred at 40 minutes, signifying that this duration is sufficient for the synthesis of silver nanoparticles. As reaction time increased, the peak values commenced their decline. This suggests that prolonged reaction time is detrimental to nanoparticle production.

Figure 9

UV-visible spectrum showing synthesis of AgNPs at different time periods



Preparation of AgNPs Loaded Films

Propolis biomatrices were prepared by varying concentration of Guar Gum and Alginate 0.2 to 0.8g accordingly while the concentration of propolis was kept constant 0.2. (table3)

Table 3

Composition of hydrogel films

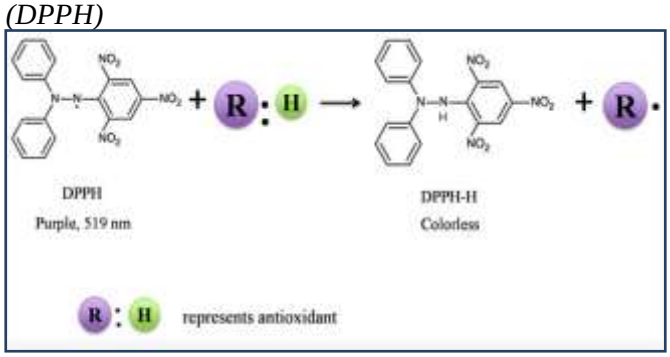
Sample	Guar Gum	Alginate (g)	Tween 20
AIN-01	O.8	0	0.2
AIN-02	0.6	0.2	0.2

Antioxidant assay of prepared films

To inspect the antioxidant potential power of AgNPs loaded films made up of Guar Gum and alginate, DPPH radical scavenging assay was employed. Antioxidant substances called "radical scavengers" that decrease the free radicals. The highest absorption of DPPH occurs at 517 nm with a purple colour. It turns yellow and reduces to a DPPH-H molecule when it interacts with an antioxidant agents.

Figure 13

(Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH)



This research work was carried out to compare the DPPH radical scavenging potential of Guar Gum and alginate films with AgNPs loaded films. Results showed the significant antioxidant power of both GG and Alginate unloaded and loaded with AgNPs**(Table 4.).** It is evident that least % AI of unloaded films of GG and Alginate are

3.8%. The highest % AI of both loaded samples of GG and Alginate are 94 and 95.

Figure 10
(DPPH Antioxidant indexof unloaded and loaded samples)

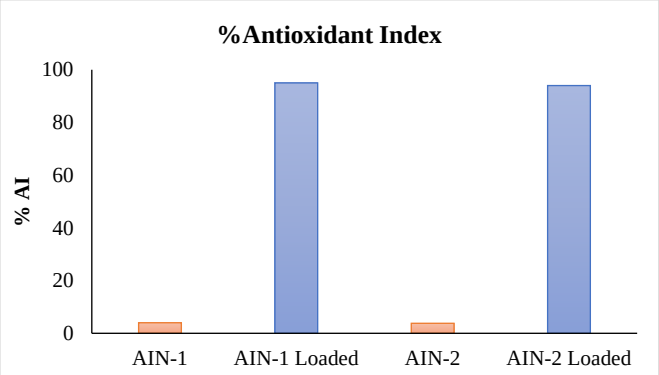


Table 4
(Prominent absorption bands in FTIR spectrum)

Bands assignments	Wavenumber (cm-1)			
	AIN-O1	AIN-02	AINL-01	AINL-02
O–H stretching	3340	3372	3370	3390
C–H stretching	2902	2919	2930	2934
O–H bending	1607	1627	1692	1699
C–H bending(–CH2)	1415	1408	1442	1460
C–O–C stretching	1021	1026	1028	1030
C–C stretching	868	877	834	849

CONCLUSION

The present work effectively validated the production of silver nanoparticles (AgNPs) by the utilization of propolis extract, so emphasizing their improved stability and notable antioxidant and antibacterial characteristics. The silver nanoparticles (AgNPs) were successfully integrated into hydrogel matrices consisting of guar gum and sodium alginate, resulting in enhanced antioxidant activity in comparison to formulations without any loading. Analyzed by UV-Vis spectroscopy, FT-IR, SEM, and XRD, synthesizedAgNPs and their loaded hydrogels were verified to have both structural and functional integrity. The findings highlight the potential of silver nanoparticles (AgNPs) facilitated by propolis in biomedical applications, providing a promising environmentally benign method for creating sophisticated therapeutic materials with strong biological effects.

REFERENCES

1) Ahmad, S., Ahmad, N., Islam, M. S., Ahmad, M. A., Ercisli, S., Ullah, R., Bari, A., & Munir, I. (2024). Rice seeds biofortification using biogenic iron oxide nanoparticles synthesized by using Glycyrrhiza glabra: A study on growth and yield improvement. *Scientific Reports*, *14*(1). https://doi.org/10.1038/s41598-024-62907-1

2) Alghoraibi, I., Soukkarieh, C., Zein, R., Alahmad, A., Walter, J., & Daghestani, M. (2020). Aqueous extract of *Eucalyptus camaldulensis* leaves as reducing and capping agent in biosynthesis of silver nanoparticles. *Inorganic and Nano-Metal Chemistry*, *50*(10), 895-902. https://doi.org/10.1080/24701556.2020.1728315

3) Balavandy, S. K., Shameli, K., & Abidin, Z. Z. (2015). Rapid and green synthesis of silver nanoparticles via sodium alginate media. *International Journal of Electrochemical Science*, *10*(1), 486-497. https://doi.org/10.1016/s1452-3981(23)05007-1

4) Dudonné, S., Vitrac, X., Coutière, P., Woillez, M., & Mérillon, J. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using dpph, abts, frap, sod, and ORAC assays. *Journal of Agricultural and Food Chemistry*, *57*(5), 1768-1774. https://doi.org/10.1021/jf803011r

5) Garibo, D., Borbón-Nuñez, H. A., De León, J. N., García Mendoza, E., Estrada, I., Toledano-Magaña, Y., Tiznado, H., Ovalle-Marroquin, M., Soto-Ramos, A. G., Blanco, A., Rodríguez, J. A., Romo, O. A., Chávez-Almazán, L. A., & Susarrey-Arce, A. (2020). Green synthesis of silver nanoparticles using Lysiloma acapulcensis exhibit high-antimicrobial activity. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-69606-7

6) Genc, N. (2020). Biosynthesis of silver nanoparticles using *Origanum onites* extract and investigation of their antioxidant activity. *Particulate Science and Technology*, *39*(5), 562-568. https://doi.org/10.1080/02726351.2020.1786868

7) Hamad, T. G., & Salman, T. A. (2023). Investigation of the Impact of Environmentally Friendly prepared Alumina Nanoparticles on Bacterial Activity. *Advancements in Life Sciences*, *10*, 95-100.

8) İLK, S., TAN, G., EMÜL, E., & SAĞLAM, N. (2020). Investigation the potential use of silver

nanoparticles synthesized by propolis extract as N-acyl-homoserine lactone-mediated quorum sensing systems inhibitor. *TURKISH JOURNAL OF MEDICAL SCIENCES*, *50*(4), 1147-1156. https://doi.org/10.3906/sag-2004-148

9) Jiang, X., Chen, W., Chen, C., Xiong, S., & Yu, A. (2010). Role of temperature in the growth of silver nanoparticles through a synergetic reduction approach. *Nanoscale Research Letters*, *6*(1). https://doi.org/10.1007/s11671-010-9780-1

10) Keshari, A. K., Srivastava, R., Singh, P., Yadav, V. B., & Nath, G. (2020). Antioxidant and antibacterial activity of silver nanoparticles synthesized by Cestrum nocturnum. *Journal of Ayurveda and Integrative Medicine*, *11*(1), 37-44. https://doi.org/10.1016/j.jaim.2017.11.003

11) Khan, R., Sohail, A., Aslam, M. U., Khan, L., Ahmad, S., & Taimur, M. (2023). Utilizing nanoparticles as innovative elicitors to enhance bioactive compounds in plants. *International Journal of Research and Advances in Agricultural Sciences*, *2*(4), 24-34.

12) Kumar, J. A., Krithiga, T., Manigandan, S., Sathish, S., Renita, A. A., Prakash, P., Prasad, B. N., Kumar, T. P., Rajasimman, M., Hosseini-Bandegharaei, A., Prabu, D., & Crispin, S. (2021). A focus to green synthesis of metal/metal based oxide nanoparticles: Various mechanisms and applications towards ecological approach. *Journal of Cleaner Production*, *324*, 129198. https://doi.org/10.1016/j.jclepro.2021.129198

13) Mumtaz, M. (2024). Possible solutions to the associated cytotoxicity of silver nanoparticles. *Journal of Survey in Fisheries Sciences*, 135-151. https://doi.org/10.53555/sfs.v11i4.2945

14) Pant, K., Thakur, M., Chopra, H., Nanda, V., Javed Ansari, M., Pietramellara, G., Pathan, S. I., Alharbi, S. A., Almoallim, H. S., & Datta, R. (2021). Characterization and discrimination of Indian propolis based on physico-chemical, techno-functional, thermal and textural properties: A multivariate approach. *Journal of King Saud University - Science*, *33*(4), 101405. https://doi.org/10.1016/j.jksus.2021.101405

15) Rahman, A., Rauf, A., Ali, B., Ullah, M., Ali, M., Ahmad, S., ... & Ahmad, M. A. (2022). Phytochemical analysis and antibacterial activity of Berberis vulgaris extract. *Advancements in Life Sciences*, *9*(3), 289-294. http://dx.doi.org/10.62940/als.v9i3.1222

16) Sarkar, R., Kumbhakar, P., & Mitra, A. K. (2010). Green synthesis of silver nanoparticles and its optical properties. *Digest Journal of Nanomaterials and Biostructures*, *5*(2), 491-496.

17) Shahmoradi, M. K., Amini Nogorani, M., Mansouri, F., & Zarei, L. (2023). Combination of zinc nanoparticles with chitosan scaffolds increased cytokine genes on wound healing of infected rats with methicillin-resistant Staphylococcus aureus (MRSA). *Advancements in Life Sciences*. https://eprints.lums.ac.ir/4258/

18) Stojanović, S., Najman, S. J., Bogdanova-Popov, B., & Najman, S. S. (2020). Propolis: Chemical composition, biological and pharmacological activity – a review. *Acta Medica Medianae*, *59*(2), 108-113. https://doi.org/10.5633/amm.2020.0215

19) Sulieman, A. M. E., Al-Anaizy, E. S., Al-Anaizy, N. A., Abdulhakeem, M. A., & Snoussi, M. (2023). Assessment of Antimicrobial and Antioxidant Activity of methanolic extract from Arnebia decumbens aerial parts growing wild in Aja Mountain. *Advancements in Life Sciences*, *10*(1), 84-92. http://dx.doi.org/10.62940/als.v10i1.1598

20) Šuran, J., Cepanec, I., Mašek, T., Radić, B., Radić, S., Tlak Gajger, I., & Vlainić, J. (2021). Propolis extract and its Bioactive compounds—From traditional to modern extraction technologies. *Molecules*, *26*(10), 2930. https://doi.org/10.3390/molecules26102930