OPEN ACCESS

Kashf Journal of Multidisciplinary Research
Vol: 02 - Issue 2 (2025)
P-ISSN: 3007-1992 E-ISSN: 3007-200X
https://kjmr.com.pk

GREEN SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SILVER NANOPARTICLES FROM MEDICINALLY IMPORTANT PLANT ALLIUM URSINUM

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DOI: https://doi.org/10.71146/kjmr277

Article Info

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Abstract

This study reports a straightforward, cost-effective and environmentally friendly approach for synthesizing silver nanoparticles (AgNPs) utilizing Allium ursinum (wild garlic) leaves as both reducing and stabilizing agent. The green synthesis method is advantageous as it eliminates the needs for toxic chemical reducing agents, making it a sustainable and eco-friendly alternative The formation of (AgNPs) was initially validated by the distinct color change of silver nitrate solution from colorless to deep brown. Comprehensive characterization was performed using ultraviolet-visible (UV- vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, Scanning electron microscopy, transmission electron microscopy (TEM) and X- ray diffraction. These analysis revealed the formation of spherical, crystalline nanoparticles with an average diameter of about 24nm. The FTIR analysis further revealed the presence of biomolecules responsible for caping and stabilizing the nanoparticles. Furthermore, the biosynthesized AgNPs exhibited significant antibacterial activity against pathogens such as staphylococcus aureus, vibrio cholerae, and salmonella typhi, as well as antifungal activity against various candida species. The promising antimicrobial properties of these green synthesized AgNPs underscore their potential application in pharmaceutical and biomedical fields and antimicrobial coating industries.

Keywords:
silver nanoparticles, Allium ursinum, green synthesis, antimicrobial activity UV, SEM, TEM, FTIR, XRD

Introduction

Nanoparticles have attracted considerable interest due to their unique size and shape dependent properties, which are applicable in areas such as optical and chemical sensing, electronics, catalysis, and biomedicine[1]. Noble metal nanoparticles have been extensively reached, primarily due to their strong optical absorption in the visible spectrum, which arises from the collective excitation of free electrons[2]. Silver nanoparticles (AgNPs) have garnered considerable attention because of their diverse of applications, including nonlinear optics[3], selective coating for solar energy absorption[4], optical receptors[5], chemical catalysts, and antibacterial agent[6]. Silver is widely recognized for its inhibitory effects on various bacterial strains and microorganisms commonly found in medical and industrial settings[7]. The common approach for synthesizing silver nanoparticles through chemical reduction involves forming colloidal dispersion in water or organic solvents[8]. Conventional chemical and physical synthesis routes often involve toxic reagents, high energy consumption, and low yield, motivating the development of sustainable alternatives[9]. The green synthesis approaches employs nontoxic reagents, environmentally friendly solvents, and sustainable materials[10]. Various biological methods for the green synthesis of silver nanoparticles have been documented, utilizing plant leaf extracts from species such as Alternanthera sessilis[11], Decaschistia tribolata[12], youngia japonica[13], and Ocimum gratissmum[14]. These methods capitalize on the natural phytochemicals present in plants, which act as reducing, stabilizing, and caping agents during nanoparticle formation[15]. Allium ursinum is perennial herbaceous plant belong to the Alliaceae family[16].Allium ursinum, known for its rich phytochemicals such as sulfur compounds, polyphenols, flavonoids, phenolic acids, fatty acids and terpenoids[17], has been used traditionally for medicinal purposes and is a promising candidate for nanoparticles synthesis[18]. In this study we utilizing Allium ursinum leaves for the biosynthesis of AgNPs and investigate their antimicrobial efficacy.

General Experimental Condition

2.1 Plant Materials

Allium ursinum leaves (10 kg) were collected during the flowering season from District Shangla, Khyber Pakhtunkhwa, Pakistan, and authenticated by the University of Swat. The leaves were shade-dried for two weeks, and subsequently ground into a fine powder. The powder was macerated in methanol, an eco-friendly solvent, for 15 days in at room temperature with daily agitation. The resulting methanolic extract was then concentrated using a rotary evaporater set at 80ºC(80-95RPM) to obtain a thick gummy crude extract.

2.2 Fractionation of crude Alkaloids

A portion of methanolic extract was dissolved in 1.5 L of 0.5N H2SO4 and shaken for one hour to obtain an acidic aqueous fraction (PH 1-2) this fraction was extracted three times with 2L portion of chloroform to remove non-alkaloidal components. The chloroform layer was concentrated by rotary evaporator to yield a non-alkaloid acidic fraction, which tested negative with Dragendorff's reagent. The remaining aqueous phase was basified to PH 8-10 using a 10% KOH solution and repeatedly extracted with chloroform until the extract become clear. The combine organic layer was concentrated to produce the basic alkaloidal fraction, which was confirmed by a positive Dragendorff's test [19]. Finally, the residual aqueous phase was neutralized to PH 7 using 0.5N H2SO4 and stored for further use.

2.3. Synthesis of Nanoparticles (AgNPs)

2.3.1. Preparation of Stock Solution

- **Silver nitrate solution:** dissolve 215mg of AgNO3 in 500ml distilled water (PH 5.3) and stored at room temperature.
- **Crude alkaloid solution:** dissolve 200mg of allium ursinum crude alkaloid in 500ml of distilled water (7.8) and mixed thoroughly using a shaker.

2.3.2 Nanoparticle synthesis procedure

for the synthesis of AgNPs, stock solutions were mixed in varying ratios. In a typical synthesis, 1ml of AgNO3 solution was added to 9ml of a crude alkaloid solution in a 100ml round-bottom flask. The mixture was maintained at 70ºC on a magnetic hotplate under constant stirring for one hour. The reaction's progress was monitored by observing the color change of the solution from colorless to deep brown indicating the formation of AgNPs.

2.4 characterization techniques

UV-Vis spectroscopy: The formation and stability of silver nanoparticles were monitored by measuring the absorbance between 400 and 500nm

Fourier transform infrared (FTIR) spectroscopy: FTIR analysis was used to identify the functional groups involved in the reduction and stabilization of AgNPs.

X-Ray Diffraction (XRD): XRD was employed to determine the crystalline structure and estimate the average particle size of the AgNPs.

Scanning and transmission electron microscopy (SEM and TEM): SEM and TEM analysis provide insight into the morphology and distribution of the nanoparticles.

2.5 Antimicrobial Evaluation

The antimicrobial activity of the synthesized AgNPs was evaluated using the minimum inhibitory concentration (MIC) method against bacteria strains including Staphylococcus aureus, Vibrio cholerae, Salmonella typhi, and Escherichia coli. Additionally antifungal activity was evaluated against various candida species. zone of Inhibition were measured and compared to standard antibiotic chloramphenicol and antifungal agent like fluconazole.

3.Results and Discussion

3.1. Visual Confirmation of AgNPs

the synthesis process was visually confirmed by a change in the reaction mixture color from colorless, which is characteristic of AgNP formation due to surface plasmon resonance effect.

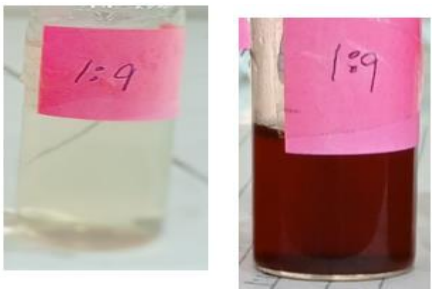


Figure 1. Color change from colorless to deep brown coloration

3.2UV–Vis spectroscopy analysis

UV-Vis spectroscopy revealed a distinct absorption peak between 400 and 500nm, the maximum absorbance observed at the optimized mixing ratio (1ml AgNO3 to 9ml crude alkaloid solution) this peak is associated with the surface plasmon resonance of silver nanoparticles, confirming their formation.

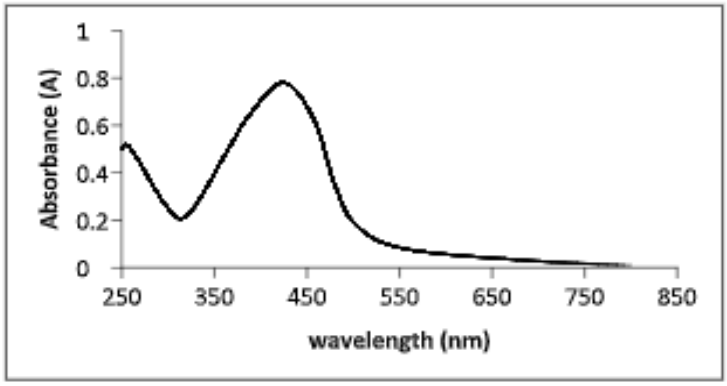


Figure 2. Absorption spectra of synthesized AgNPs in UV-Vis range

3.3 Stability studies

Stability tests indicated that the AgNPs remained most stable within a temperature range 25ºC to 45ºC. Additionally, the nanoparticles exhibited enhanced stability under basic PH conditions compared to neutral. PH as illustrated in figure 3.

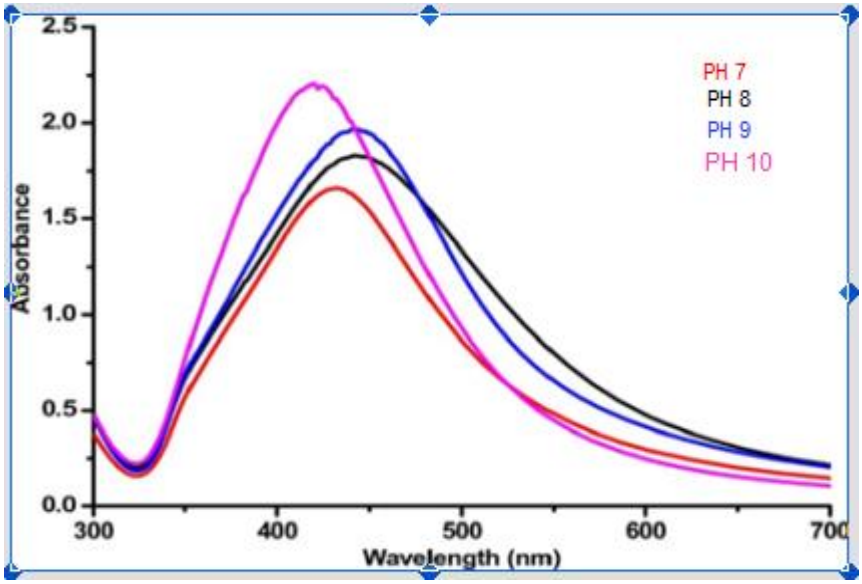


Figure 3. The change in PH from neutral to basic

Structural and Morphology Characterization

- **XRD Analysis:** the XRD pattern confirmed the crystalline nature of the AgNPs, with distinct diffraction peaks corresponding to t (110), (200), (221) and (310) planes. The average particle size was calculated to be approximately 24nm as illustrated in figure 4.

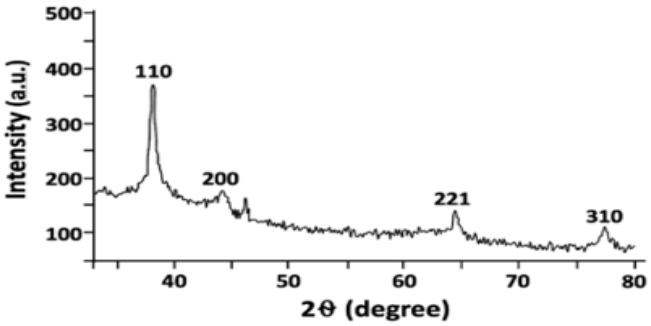


Figure 4. The XRD pattern of synthesized AgNPs using Allium ursinum leaves extract

- **FTIR Analysis:** FTIR spectra (as shown in the figure) showed broad peaks at 3375cm-1 (indicative of O-H stretching from surface absorbed water) and other peaks at 1410cm-1 and near 610cm-1 which are attributed to methanol and C-H stretching vibrations[20], respectively. Thes results confirm the involvement of phenolic compounds, aromatic molecules, and proteins in the nanoparticle synthesis[21].

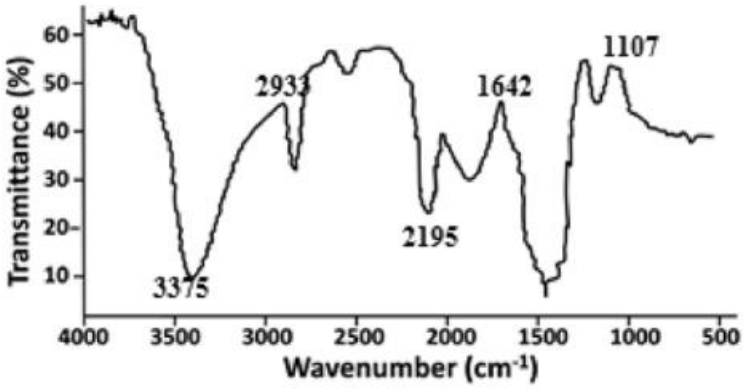


Figure 5. FTIR pattern of synthesized AgNPs

- **SEM and TEM observations:** both SEM and TEM images revealed that the AgNPs are predominantly spherical with a size distribution ranging from 15 to 28nm, demonstrating good dispersion and uniformity

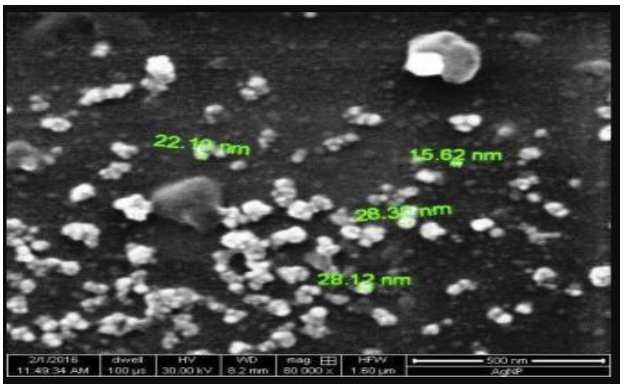


Figure 6. SEM image of AgNPs of Allium ursinum leaves extract

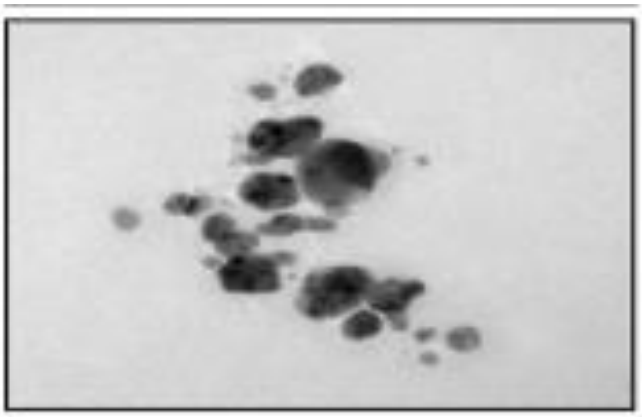
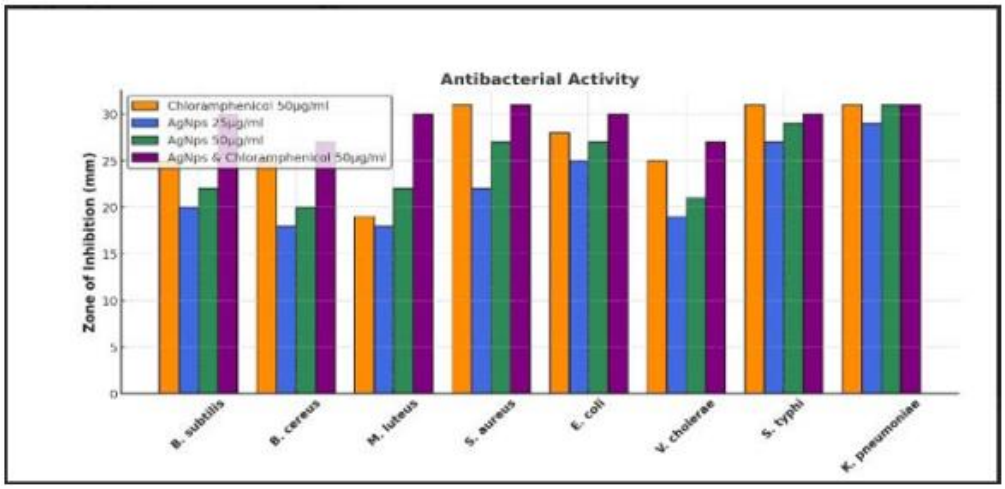
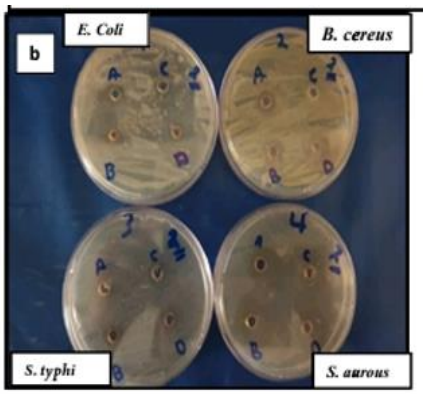


Figure 7. and Figure 6. TEM image of AgNPs using Allium ursinum leaves extract

Antimicrobial Activity

The antimicrobial evaluation demonstrated the AgNPs possess significant antibacterial activity. For instance, at concentration of 50µg/ml, AgNPs produce inhibition zones of 26mm against S. aureus, which was further enhanced when used in combination with chloramphenicol. Similarly synergistic effects were observed against V. cholerea and S. typhi. Although E. coli exhibited a consistent inhibition zone of 20nm, the overall antibacterial performance of AgNPs was comparable to or better than that of standard antibiotics. The findings align with those obtained by Yuet et al [22].





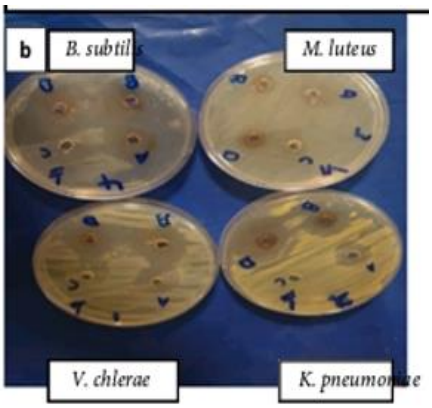


Figure 8. The graphical values of inhibition zone(mm) and the antibacterial activity of produce AgNPS against the selected bacterial strains.

Table1. zone of inhibition produced by AgNPs, reference antibiotic chloramphenicol and AgNPs with chloramphenicol

Bacterial strains	Zone of inhibition(mm)			
	Chloramphenicol 50µg/ml	AgNPs 25µg/ml	AgNPs 50µg/ml	AgNPs & Chloramphenicol 50µg/ml
B. subtilis MTCC1133	20	20	22	23
B. cereus ATCC10987	30	25	26	26
M. luteus ATCC4698	28	21	21	30
S. aureus MTCC96	19	23	26	28
E. coli MTCC118	23	20	20	20
V. cholerae ATCC14035	21	25	27	30
S. typhi MTCC733	18	25	29	31
K. pneumoniae MTCC109	31	29	31	31
Fungal strains	**Zone of inhibition(mm)**			
	Fluconazole 50µg/ml	AgNPs 25µg/ml	AgNPs 50µg/ml	AgNPs& Fluconazole 50µg/ml
C. parapsilosis MTCC 2509	-	18	20	20
C. tropicalis MTCC 184	27	27	29	30
C. albicans MTCC 183	-	19	25	25

Antifungal activity

The antifungal potential of AgNPs was evaluated against several candida strains. while fluconazole showed limited efficacy against certain strains, the AgNPs at concentration 25 and 50µg/ml demonstrated measurable inhibition zones. In some cases, combining AgNPs with fluconazole resulted in enhanced antifungal activity, suggesting a strain dependent synergistic effect.

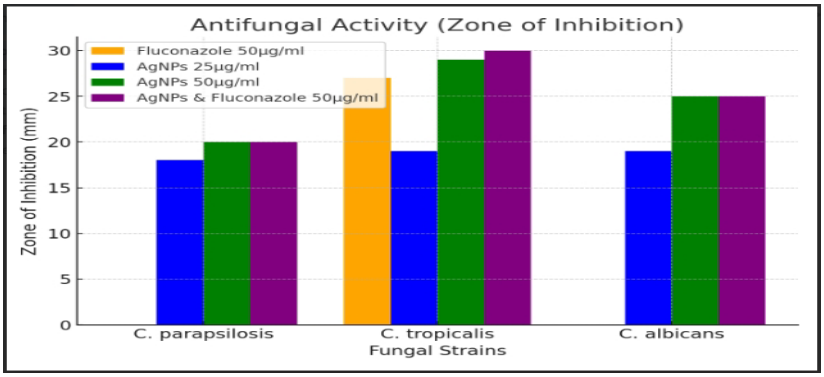


Figure 9. Antifungal activity of biosynthesized AgNPs

6. Conclusion

This work demonstrates that Allium ursinum leaves extract is effective and environmentally friendly agent for the ecofriendly synthesis of silver nanoparticles. The biosynthesized silver nanoparticles were confirmed by a series of analytical techniques and displayed a crystalline, spherical morphology having an average diameter of around 24nm. Importantly, the silver nanoparticles exhibited significant antibacterial and antifungal activities, highlighting their potential for use in antimicrobial therapies and pharmaceutical applications. The green synthesis approach presented here provides a sustainable alternative to conventional nanoparticle production methods.

References

1. Ray, P.C., Size and shape dependent second order nonlinear optical properties of nanomaterials and their application in biological and chemical sensing. Chemical reviews, 2010. 110(9): p. 5332-5365.

2. Wang, L., M. Hasanzadeh Kafshgari, and M. Meunier, Optical properties and applications of plasmonic-metal nanoparticles. Advanced Functional Materials, 2020. 30(51): p. 2005400.

3. Pugazhendhi, S., R. Usha, and F.M. Anjalin, Comparative study of different plant extract mediated silver nanoparticles for nonlinear optics. Materials Today: Proceedings, 2020. 33: p. 3984-3988.

4. Hamdan, K.S., Plasmonic Silver Nanoparticles for an Improved VOPcPhO: P3HT Organic Solar Cells. 2017, University of Malaya (Malaysia).

5. Ilickas, M., A. Guobienė, and B. Abakevičienė. Photochemical synthesis of silver nanoparticles and study of their structural properties. in Open readings 2023: 66th international conference for students of physics and natural sciences, April 18-21, 2023, Vilnius, Lithuania: annual abstract book. 2023. Vilnius University press.

6. Ahmadi, F. and M. Lackner, Green synthesis of silver nanoparticles from Cannabis sativa: Properties, synthesis, mechanistic aspects, and applications. Chem Engineering, 2024. 8(4): p. 64.

7. Bruna, T., et al., Silver nanoparticles and their antibacterial applications. International journal of molecular sciences, 2021. 22(13): p. 7202.

8. Villaverde-Cantizano, G., et al., Reducing Agents in Colloidal Nanoparticle Synthesis–an Introduction. 2021.

9. Jain, K., et al., Rethinking nanoparticle synthesis: a sustainable approach vs. traditional methods. Chemistry–An Asian Journal, 2024. 19(21): p. e202400701.

10. Madani, M., et al., Green synthesis of nanoparticles for varied applications: Green renewable resources and energy-efficient synthetic routes. Nanotechnology Reviews, 2022. 11(1): p. 731-759.

11. Kumar, D., et al., Phyto-fabrication and characterization of Alternanthera sessilis leaf extract–mediated silver nanoparticles and evaluation of larvicidal potential. Biomass Conversion and Biorefinery, 2023: p. 1-16.

12. Magdum, A.B., et al., Biogenic synthesis of silver nanoparticles from leaves extract of Decaschistia trilobata an endemic shrub and its application as antioxidant, antibacterial, anti-inflammatory and dye reduction. Catalysis Communications, 2024. 187: p. 106865.

13. Arshad, F., et al., Bioinspired and green synthesis of silver nanoparticles for medical applications: a green perspective. Applied Biochemistry and Biotechnology, 2024. 196(6): p. 3636-3669.

14. Alharbi, N.S., N.S. Alsubhi, and A.I. Felimban, Green synthesis of silver nanoparticles using medicinal plants: Characterization and application. Journal of Radiation Research and Applied Sciences, 2022. 15(3): p. 109-124.

15. Bansal, S., et al., A review on green approaches utilizing phytochemicals in the synthesis of vanadium nano particles and their applications. Preparative Biochemistry & Biotechnology, 2024. 54(2): p. 127-149.

16. Krivokapic, M., et al., Phytochemical and pharmacological properties of Allium ursinum. Experimental and Applied Biomedical Research (EABR), 2018. 22(4): p. 357-362.

17. Iwar, K., et al., Alliums as Potential Antioxidants and Anticancer Agents. International Journal of Molecular Sciences, 2024. 25(15): p. 8079.

18. Stefan, G., R. Dragoja, and V. Sandra, Phytochemical characterization and antioxidant potential of Allium ursinum L. cultivated on different soil types-a preliminary study. Emirates Journal of Food and Agriculture, 2023. 4: p. 904-914.

19. Ahmad, S., et al., Green synthesis of gold and silver nanoparticles using crude extract of Aconitum violaceum and evaluation of their antibacterial, antioxidant and photocatalytic activities. Frontiers in Bioengineering and Biotechnology, 2024. 11: p. 1320739.

20. Wang, L., et al., Characterization, antioxidant and antimicrobial activities of green synthesized silver nanoparticles from Psidium guajava L. leaf aqueous extracts. Materials Science and Engineering: C, 2018. 86: p. 1-8.

21. Keshari, A.K., et al., Antioxidant and antibacterial activity of silver nanoparticles synthesized by Cestrum nocturnum. Journal of Ayurveda and integrative medicine, 2020. 11(1): p. 37-44.

22. Loo, Y.Y., et al., In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. Frontiers in microbiology, 2018. 9: p. 1555.