Biogenic synthesis of silver nanoparticles from *Lonicera caprifolium* extract: characterization and evaluation of antibacterial properties against *Pectobactrrium carotovorum* subsp *carotovorum (Pcc)* (the causal agent of bacterial soft rot in vegetables)

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Abstract:

Nanoscience enables the manipulation of matter at the nanoscale, with biologically synthesized nanoparticles offering applications in medicine, optics, and agriculture. In this study, the antibacterial (phytopathogenic) effect of silver nano particles (AgNPs) produced using the aqueous extract of the aerial parts (flowers and leaves) of the *Lonicera caprifolium* was investigated using the Agar well diffusion method. UV-Vis spectroscopy (300–700 nm) confirmed AgNP synthesis, while SEM/TEM revealed spherical nanoparticles sized 10–50 nm (flower extract) and 10–80 nm (leaf extract). FTIR identified organic compounds involved in reduction. Based on the results of this research, the nanoparticles produced by the aqueous extract of the *Lonicera caprifolium* have effective antimicrobial activity against *Pectobactrrium carotovorum* subsp *carotovorum (Pcc)*. In this way, the aerial parts (flowers and leaves) of *Lonicera caprifolium* can be used as a useful biological resource for the synthesis of AgNPs on an industrial scale at a very low cost. This research aims to contribute valuable insights into the potential use of plant-mediated silver nanoparticles as effective antibacterial agents for combating phytopathogens and addressing challenges in agricultural disease management.

Keywords: nanosilver, *Lonicera caprifolium*, antibacterial, *Pectobactrrium carotovorum* subsp *carotovorum,* phytopathogen.

2. Introduction

Nanotechnology is a field of applied knowledge and technology that includes a wide range of sciences such as pharmacy, drug design, and biology [1].In nanotechnology, materials with dimensions less than one micrometer, usually 1 to 100 nanometers, are used [2]. To synthesize nanoparticles, there are different physical and chemical methods such as chemical reduction, lithography, electrochemical, laser, and microwave waves[3]. One of the disadvantages of chemical methods that play the role of rejuvenating and stabilizing agents is that they remain undegraded and eventually cause chemical pollution of the environment [4]. Other disadvantages of these methods are low production and the use of high pressure, temperature, and energy during the reaction process[5]. Recently, the biosynthesis of silver nanoparticles by natural and biological agents such as bacteria, fungi, and plants has attracted the attention of researchers. One of the biological methods is the green synthesis method, in which metal ions are converted into silver nanoparticles using plant compounds without the need for surfactants and other stabilizing compounds during a one-step reaction [6]. *Lonicera caprifolium* is one of the native plants of Iran. It is a climbing and twisting shrub native to East Asia (China, Taiwan, Japan, and Korea). *Lonicera caprifolium* flower has a high medicinal value in traditional Chinese medicine. It is used to treat fever, influenza, headache, cough, thirst, and sore throat. The essential oil of *Lonicera caprifolium* L. has shown strong analgesic activity in chemical and thermal pain. In addition, the oil had a strong wound healing activity, superior to that of the standard povidone-iodine ointment [7]. Methanolic extract of Lonicera caprifolium has potential cytotoxic and antiangiogenic effects on C6 rat glioma cells [8]. One of the most important applications of silver nanoparticles is their use in destroying cancer cells[9][10]. In recent years, due to the increasing prevalence of deaths caused by cancers and the failure of chemotherapy and radiotherapy methods in advanced forms of cancer, the need to find new methods to control cancer is felt, and one of these methods is the use of nanoparticles, especially silver nanoparticles [11].Anticancer treatments that employ nanotechnology utilize nanoparticles to improve therapeutic effectiveness while reducing side effects. These nanoparticles can be engineered to precisely target and deliver drugs directly to cancer cells, thereby sparing healthy cells[12]. In recent years, various plant extracts have been used to synthesize silver nanoparticles [13]. Excessive use of antibiotics to destroy bacteria has caused them to become resistant to antibiotics and spread infectious diseases [14]. *Pectobacterium carotovorum* subsp carotovorum (formerly known as Erwinia carotovorum) is a Gram-negative bacterium that infects plants. It causes soft rot in many plants and blackleg in potatoes by breaking down the plant cell wall. These bacteria live between plant cells and use a type III secretion system to inject harmful molecules into the plant. The EC1 strain of *P. carotovorum* has a special flagellum (a tail-like structure) that helps it move. When grown in a medium with fructose at 26°C, the bacteria become very motile and develop many flagella. This strain has the most densely packed flagellar genes of any species[15]. Using chemical bactericides to control these bacteria is not ideal because they are not long-lasting, can be toxic, are expensive, and bacteria can develop resistance to them[16].Therefore, it is very necessary to search for new substances with antibacterial properties (new generation of antibacterial drugs) to prevent the growth of bacteria. Silver, gold, and platinum nanoparticles show remarkable antibacterial activity. This feature is due to the very small size and high surface-to-volume ratio of these particles. Considering the cost-effectiveness and absence of environmental toxicity effects of plant extracts for the synthesis of silver nanoparticles and also having antimicrobial effects of silver nanoparticles, the synthesis of these nanoparticles is very important for scientists. Therefore, due to the high antibacterial activity of nanoparticles, they can be used to increase the safety level in food packaging and also in the production of a new generation of antibacterial drugs [17].There are several reports regarding the use of biosynthesis of silver nanoparticles and their antimicrobial activity. For example, Chahardoli and Khodadadi (2014) used silver nanoparticles obtained from oak fruit extract against bacteria causing hospital infections [18].

Also, Khatami et al. (2015) reported the antimicrobial effect of silver nanoparticles synthesized by exuding the seeds of the rattle weed [19]. Also, in 2024, Patel et al. investigated the antimicrobial properties of silver nanoparticles obtained from Elettaria cardamomum extract on several Gram-positive and Gram-negative bacteria [20]. In another study, Rezaei et al. (2019) synthesized silver nanoparticles from the Extract of *Lonicera Nummulariifolia* and investigated the antioxidant, antibacterial, and anticancer effects on lung cancer cells [21]. As well as CuS NPs showed excellent antibacterial activity against the plant pathogenic bacteria *Pectobacterium carotovorum* subsp. carotovorum, with low acute toxicity to soil organisms and low cytotoxicity to normal human cells [22]. A bioprospecting study identified 50 fungal isolates from potato-cultivated soils, and five isolates with high nitrate reductase activity were able to biosynthesize silver nanoparticles that showed antibacterial activity against the phytopathogenic bacterium *Pectobacterium carotovorum*[23].

This study aims to explore the biological synthesis of silver nanoparticles using *Lonicera caprifolium* extract, marking the first instance of such synthesis, and evaluate the antimicrobial (phytopathogenic) effects of the produced nanoparticles. The findings from this investigation can inform decision-making regarding the potential use of silver nanoparticles in the treatment of microbial infections, specifically, *Pectobactrrium carotovorum* subsp carotovorum (phytopathogen).

2. Material and methods

All materials, chemicals, and reagents used in this study were of analytical grade and used without further purification unless otherwise stated. The following materials and reagents were used: Silver nitrate ($AgNO_3$): Purchased from Sigma-Aldrich (99.9% purity, catalog number 209139).

Lonicera caprifolium plant material: Fresh aerial parts (flowers and leaves) of *Lonicera caprifolium* were collected from Bojnord,North Khorasan Province,Iran in July, 2023. The plant material was identified and authenticated by Dr.Nadaf.

2.1. Extract Preparation

10 grams of *Lonicera caprifolium* aerial parts (Figure 1) after drying, including flowers and leaves, were weighed individually and washed thoroughly with distilled water. The parts were then finely chopped into smaller pieces. The finely chopped flowers and leaves were mixed with 100 ml of distilled water and boiled for 15 minutes. After boiling, the solutions were cooled and filtered using Whatman No.1 filter paper. The filtrates obtained were stored at 4°C for future experimentation.

2.2. AgNPs Biosynthesis and Characterization

For the biosynthesis of silver nanoparticles (AgNPs), 5 mL of the prepared *Lonicera caprifolium* extract was slowly added to 5 mL of a 0.001 M silver nitrate ($AgNO_3$) solution, maintaining a 1:1 ratio of plant extract to silver nitrate solution. The mixture was kept in a dark environment to prevent photodegradation and allowed to react for 24 hours at room temperature. Following this, the solution was sonicated for 15 minutes at 30°C to ensure uniform dispersion of the nanoparticles. The resulting colloidal solution was centrifuged at 12,000 rpm for 15 minutes to separate the AgNPs from the reaction mixture. The supernatant was discarded, and the pellet was washed three times with deionized water to remove any unreacted components and impurities. After each centrifugation step, the supernatant was carefully removed, and deionized water was added to the precipitate to redisperse the nanoparticles. Finally, the purified nanoparticle suspension was transferred to a petri dish and dried at room temperature to obtain the synthesized AgNPs in powder form.[24],[25].The confirmation of silver nanoparticle production was accomplished using a spectrophotometer

device (Thermo Evolution 201/220, UV-Vis), operating within the wavelength range of 300 to 700 nm. Additionally, the size and shape of the nanoparticles were evaluated using a scanning electron microscope (SEM) and a transmission electron microscope (TEM). Furthermore, an FTIR device (Thermo Nicolet IS10) was utilized to investigate any potential organic compounds within the *Lonicera caprifolium* extract that may have contributed to the nanoparticle synthesis.

2.3. Investigating the effect of silver nanoparticles on *Pectobactrrium carotovorum* subsp *carotovorum* (Phytopathogenic bacterial)

The Agar well diffusion method is commonly used to assess the antimicrobial activity of plant extracts. Mueller Hilton Agar culture medium is utilized in this method. The bacteria studied in this experiment was *Pectobactrrium carotovorum* subsp *carotovorum* (*Pcc*), known for causing tuber rot (e.g., in potatoes) during storage or stem rot in the field[26]. A microbial suspension with a concentration of 0.5 McFarland is evenly spread on the agar plate surface using a sterile swab. Six wells are created using a sterile pipette (size 5). Various dilutions of nanoparticles (1, 0.5, 0.25, 0.125, and 0.0625 mg/ml) are then added to each well, with the final well containing 50 microliters of DMSO solvent as a negative control. Additionally, three antibiotic discs (Oxoid, UK) - 10µg of gentamicin (GM10), 30µg of chloramphenicol (C30), and 10U of penicillin G (P10) - are placed in the center of the plate as positive controls. The agar plates are then incubated at 37°C for 24 hours under suitable conditions. The diameter of the inhibition growth zones around each well and antibiotic disc is measured in millimeters after 24 hours[27]. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are determined using the following method. One milliliter of the nanoparticle extract was added to the initial tube, followed by a dilution test from the first tube to the second and last tubes. Subsequently, a 0.5 McFarland concentration of bacteria was prepared, and 100 microliters were inoculated into all culture media. The test tubes were then incubated at 37°C for 24 hours. Following this incubation period, the lowest concentration without turbidity was identified as the MIC. For the MBC test, all tubes without turbidity were cultured on Mueller Hilton Agar culture medium. The minimum concentration without bacterial growth was determined as the MBC. This dilution process is carried out for bacteria sensitive to the extract, with the zone of non-growth being visible in the culture medium[28],[29].

3. Results and Discussion

3.1. Characterization of silver nanoparticles

3.1.1. UV- vis: During the synthesis process of silver nanoparticles, Ag+ ions are exposed to the reducing compounds of the extract, initiating the reduction of silver nitrate salt. The complete reduction of Ag+ ions to silver nanoparticles was achieved, leading to a noticeable color change in the medium and spectrometry analysis[21]. The solution transitioned from colorless to brown within 120 minutes upon the addition of plant extract to the silver nitrate solution, indicating the successful reduction of silver nitrate and the formation of silver nanoparticles in the solution[27]. Furthermore, the presence of peaks at 452 and 425 nm wavelengths was confirmed for silver nanoparticles obtained from leaf and flower extracts, respectively, using the Vis-UV spectrometer (Figure 2, 3).

3.1.2. X-ray diffraction (XRD):

The XRD peak patterns of nanoparticles derived from *Lonicera caprifolium* flower and leaf extracts were compared with the standard pattern (Figure 4c)[30]. The conformity of these patterns with the standard face-centered cubic (fcc) structure was confirmed (Figure 4a, b).

3.1.3. EDX analysis

The chemical composition characteristics and location of silver nanoparticles on the cell surface were analyzed using X-ray-enhanced SEM (EDX). EDX analysis was conducted to verify the synthesized silver nanoparticles by the aerial parts (flowers and leaves) of *Lonicera caprifolium*. Figure 5-6 displays the results of EDX analysis in point profile mode. In this map, silver is highlighted in green (Fig 5c, 6c), oxygen in red (Fig 5b, 6a), and carbon in blue (Fig 5a, 6b). As shown in Figure 5d and Figure 6d, the EDX analysis confirms the presence of Ag in the synthesized nanosilver, primarily identified by its highest and most transparent appearance in the XRD image. The sharp diffraction patterns of the XRD spectrum reveal the structure of pure crystalline silver, consistent with a previous report[31].

The number of x-rays counted in the peak of AgNPs synthesized by the flower and leaf extract of *Lonicera caprifolium* in Table 1 is shown. For the flower, it exhibited a 85.77% concentration of silver (Ag), 40.4% of carbon (C), and 0.03% of oxygen (O) mass concentration. For the leaf, it displayed an 89.20% concentration of silver (Ag), 10.77% of carbon (C), and 0.03% of oxygen (O) mass concentration respectively.

3.1.4. Infrared spectroscopy (FTIR)

FTIR was utilized to identify the functional groups on the surface of silver nanoparticles produced by the flower and leaf extract of *Lonicera caprifolium*. In Figures 7-8, four distinct peaks at 3335, 2114-2115, 1635-1636, and 667-668 cm^{-1} were observed in the FTIR spectrum of the nanoparticles under study. The wide and intense peak at 3335 cm^{-1} is attributed to the stretching vibration of hydroxyl groups (-OH) from phenols and carboxylic acids. The peak around 1635-1636 cm^{-1} corresponds to the amide I group (-CO-NH2) of peptide bonds. The peak at 2114-2115 cm^{-1} is likely related to alkynyl C≡C stretch, while the broad peak atapproximately 667-668 cm^{-1} may be associated with the C-H bond of aromatic compounds[32]. These findings suggest the presence of flavanones or terpenoids on the surface of AgNPs[33].

The FTIR spectra results indicate the participation of various functional groups of phytochemical compounds from the extract, serving as reducing and coating agents in the silver nanoparticles' synthesis, particularly plant phenols and flavonoids[34].

3.1.5. The SEM and TEM electron microscope

The SEM and TEM electron microscope results revealed that the nanoparticles produced from the flower and leaf parts of *Lonicera caprifolium* exhibit a spherical structure, with sizes ranging from 10-50 nm for the flower extract and 10-80 nm for the leaf extract (Figure 9a, 9b, 10a, and 10b). Additionally, the particle distribution was assessed using image j software, showing that the average size of AgNPs synthesized from the flower extract and leaf extract were 21.08 and 26.94 nm, respectively (Figure 9c, 10c).

3.2. Antimicrobial activity

In vitro antimicrobial activity of AgNPs synthesized by leaf and flower extracts of *Lonicera caprifolium* against *Pectobacterium carotovorum* subsp *carotovorum* was examined using a well diffusion method (Figure 11). The diameter of the inhibitory zones (mm) around each well containing silver nanoparticle solutions is presented in Table 2.

Also, the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) for both types of silver nanoparticles (synthesized with flower and leaf extracts) were determined at concentrations of 0.5 and 0.25 mg/mL, respectively, and are detailed in Table 3.

According to Table 2, the largest inhibition diameter was seen for the 1 mg/ml concentration of silver nanoparticles produced by flower and leaf extracts (18.57mm and 14.81mm, respectively). The Inhibitory level is explained as follows: + = Moderate inhibition zone (6-9 mm); ++ = Strong inhibition zone (10-14mm); +++ = Very strong inhibition zone (15-18mm); - = No inhibition zone[35].

Also, the antibacterial effect of AgNPs synthesized with flower extract has shown superior antibacterial properties compared to antibiotics used as a positive control. The diameter of inhibition is even greater than that of the antibiotic Chloramphenicol (30 µg) and shows little difference with Gentamicin (10 µg). Additionally, the antibacterial activity of AgNPs derived from leaves is lower than those from flowers, possibly due to the compounds present in the flowers, particularly the more abundant monoterpenes such as linanool[7],[28]. Monoterpene compounds like linalool, a key component in the essential oil and flower extract of this plant, exhibit strong antibacterial properties[36],[37],[38].

4. Conclusion

This study successfully demonstrated the biogenic synthesis of silver nanoparticles (AgNPs) using the flower and leaf extracts of *Lonicera caprifolium*, marking the first reported use of this plant for nanoparticle production. The synthesized AgNPs were thoroughly characterized using advanced techniques, including Fourier-transform infrared spectroscopy (FTIR), Ultraviolet-visible (UV-vis) spectroscopy, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Energy-Dispersive X-ray spectroscopy (EDX). The nanoparticles exhibited a spherical morphology, with average sizes of 21.08 nm (flower extract) and 26.94 nm (leaf extract), confirming their nanoscale dimensions and uniformity.

The antibacterial activity of the AgNPs against *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), a major phytopathogen responsible for bacterial soft rot in vegetables, was evaluated in vitro. The results revealed significant antibacterial efficacy, with the flower-derived AgNPs exhibiting superior activity compared to conventional antibiotics, such as Gentamicin and Chloramphenicol, at a concentration of 1 mg/mL. This highlights the potential of *Lonicera caprifolium*-mediated AgNPs as a sustainable and cost-effective alternative to chemical antibiotics, which are often associated with high costs, environmental toxicity, and health risks.

The findings of this study underscore the potential of *Lonicera caprifolium* as a valuable biological resource for the large-scale synthesis of AgNPs. These nanoparticles offer a promising solution for combating phytopathogens and addressing challenges in agricultural disease management. Future research should focus on scaling up production, conducting field trials, and exploring the broader applications of these nanoparticles in agriculture and other fields. This work contributes to the growing body of knowledge on green nanotechnology and its potential to promote sustainable and eco-friendly solutions for global challenges.

Conflict of interest:

No conflict of interest was declared.

References:

1. Patra, J. K., Das, G., Fraceto, L. F et al., "Nano based drug delivery systems: Recent developments and future prospects", *Journal of Nanobiotechnology*, **16**(1), p. 71 (2018), doi: 10.1186/s12951-018-0392-8.

2. Graciano, R. C. D., Ribeiro, J. A. T., Macêdo, A. K. S et al., "Recent Patents Applications in Red Biotechnology: A Mini-Review", *Recent Patents on Biotechnology*, **13**(3), pp. 170–186 (2019), doi: 10.2174/1872208313666190114150511.

3. Sharma, G., Sharma, A. R., Lee, S.-S et al., "Advances in nanocarriers enabled brain targeted drug delivery across blood brain barrier", *International Journal of Pharmaceutics*, **559**, pp. 360–372 (2019), doi: 10.1016/j.ijpharm.2019.01.056.

4. Zhang, X.-F., Liu, Z.-G., Shen, W et al., "Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches", *International Journal of Molecular Sciences*, **17**(9), p. 1534 (2016) , doi: 10.3390/ijms17091534.

5. Jorge de Souza, T. A., Rosa Souza, L. R., and Franchi, L. P., "Silver nanoparticles: An integrated view of green synthesis methods, transformation in the environment, and toxicity", *Ecotoxicology and Environmental Safety*, **171**, pp. 691–700 (2019), doi: 10.1016/j.ecoenv.2018.12.095.

6. Jha, M. and Shimpi, N. G., "Green synthesis of zero valent colloidal nanosilver targeting A549 lung cancer cell: In vitro cytotoxicity", *Journal of Genetic Engineering and Biotechnology*, **16**(1), pp. 115–124 (2018), doi: 10.1016/j.jgeb.2017.12.001.

7. Gavit, A. A., Gagrani, M. B., Gurav, S. S et al., "Chemical composition and biological activities of Lonicera caprifolium L. (Caprifoliaceae) essential oil", *Natural Product Research*, **38**(5), pp. 719–726 (2024), doi: 10.1080/14786419.2023.2193745.

8. Turgut, N. H., Altun, A., Kara, H et al., "Anticancer and antiangiogenic effects of methanol extracts of Lonicera caprifolium L. on C6 rat glioma cells", *Cumhuriyet Medical Journal*, **38**(1), p. 6 (2016), doi: 10.7197/cmj.v38i1.5000165142.

9. de Groot, P. M., Wu, C. C., Carter, B. W et al., "The epidemiology of lung cancer", *Translational Lung Cancer Research*, **7**(3), pp. 220–233 (2018), doi: 10.21037/tlcr.2018.05.06.

10. Qian, C., Dong, X., Hussain, S. A et al., "The application of silver nanoparticles green-formulated by Origanum majorana leaf extract as a hydrazine sensor and treatment of human breast cancer by following the P53 and STAT3 signaling pathways", *Journal of Science: Advanced Materials and Devices*, **9**(2), p. 100691 (2024), doi: 10.1016/j.jsamd.2024.100691.

11. Wang, P. and Aguirre, A., "New Strategies and In Vivo Monitoring Methods for Stem Cell-Based Anticancer Therapies", *Stem Cells International*, **2018**, pp. 1–9 (2018), doi: 10.1155/2018/7315218.

12. Madaniyah, L., Fiddaroini, S., Hayati, E. K et al., "Biosynthesis, characterization, and in-vitro anticancer effect of plant-mediated silver nanoparticles using Acalypha indica Linn: In-silico approach", *OpenNano*, **21**, p. 100220 (2025), doi: 10.1016/j.onano.2024.100220.

13. Thomas, S., Gonsalves, R. A., Jose, J et al., "Plant-based synthesis, characterization approaches, applications and toxicity of silver nanoparticles: A comprehensive review", *Journal of Biotechnology*, **394**, pp. 135–149 (2024), doi: 10.1016/j.jbiotec.2024.08.009.

14. Morones, J. R., Elechiguerra, J. L., Camacho, A et al ., "The bactericidal effect of silver nanoparticles", *Nanotechnology*, **16**(10), pp. 2346–2353 (2005), doi: 10.1088/0957-

4484/16/10/059.

15. Aizawa, S.-I., “Chapter 18 - Pectobacterium carotovorum — Subpolar Hyper-Flagellation”, S.-I. B. T.-T. F. W. Aizawa, Ed., Academic Press, pp. 58–59 (2014), doi: 10.1016/B978-0-12-417234-0.00018-9

16. Abd-El-Khair, H., Abdel-Gaied, T. G., Mikhail, M. S et al., “Biological control of Pectobacterium carotovorum subsp. carotovorum, the causal agent of bacterial soft rot in vegetables, in vitro and in vivo tests”, *Bulletin of the National Research Centre*, **45**(1), p. 37 (2021), doi: 10.1186/s42269-021-00491-4.

17. Rai, M., Yadav, A., and Gade, A., “Silver nanoparticles as a new generation of antimicrobials”, *Biotechnology Advances*, **27**(1), pp. 76–83 (2009), doi: 10.1016/j.biotechadv.2008.09.002.

18. chahardoli, m and Khodadadi, E., “The Biosynthesis of Silver Nanoparticles using OAK fruit extract and the investigation of their Anti-microbial Activities against nosocomial infection agents TT”, *sjimu*, **22**(4), pp. 27–33 (2014), [Online]. Available: http://sjimu.medilam.ac.ir/article-1-1437-fa.html

19. Khatami, M., Porseyedi, S., Khatami, M et al., “Antimicrobial effect of silver nanoparticles synthesized using weed seed exudates against of Bacillus cereus and Acinetobacter baumannii”, *Journal of Microbial World*, **8**(1), pp. 18–25 (2015),[Online]. Available: https://www.ijnnonline.net/article_17014.html

20. Patel, N. D., Patel, D. A., and Shrimali, G. D., “Green Synthesis Of silver Nanoparticles Using Elettaria cardamomum: Characterization and Antimicrobial Potential”, *Current Trends in Biotechnology and Pharmacy*, **18**(2), pp. 1680–1687 (2024), doi: 10.5530/ctbp.2024.2.16.

21. Rezaie, H., HAmdi, S. M. M., and Mirzaie, A., “Green Synthesis of Silver Nanoparticles Using the Extract of Lonicera Nummulariifolia and Investigating Its Antioxidant, Antimicrobial and Anticancer Effects Against Lung Cancer Cell Line A549 TT”, *Babol-Jbums*, **21**(1), pp. 207–214 (2019), doi: 10.22088/jbums.21.1.207.

22. Yan, W., Fu, X., Gao, Y et al., “Synthesis, antibacterial evaluation, and safety assessment of CuS NPs against Pectobacterium carotovorum subsp.carotovorum.”, *Pest Management Science*, **78**(2), pp. 733–742 (2022), doi: 10.1002/ps.6686.

23. Pineda, M. E. B., Lizarazo Forero, L. M., and Sierra Avila, C. A., “Antibacterial activity of biosynthesized silver nanoparticles (AgNps) against Pectobacterium carotovorum”, *Brazilian Journal of Microbiology*, **53**(3), pp. 1175–1186 (2022), doi: 10.1007/s42770-022-00757-7.

24. Halimi, M., Nasrabadi, M., Soleamani, N et al., “Green synthesis of nanosilver particles from extract of Dracocephalum Lindbergii”, *Asian Journal of Nanoscience and Materials*, **1**(1), pp. 19–24 (2018), doi: 10.26655/ajnanomat.2018.1.3.

25. Halimi Khalil Abad, M., Nadaf, M., and Taghavizadeh Yazdi, M. E., “Biosynthesis of $ZnO.Ag_2O_3$ using aqueous extract of Haplophyllum obtusifolium : Characterization and cell toxicity activity against liver carcinoma cells”, *Micro & Nano Letters*, **18**(6) (2023), doi: 10.1049/mna2.12170.

26. Pérombelon, M. C. M., “Potato diseases caused by soft rot erwinias: an overview of pathogenesis”, *Plant Pathology*, **51**(1), pp. 1–12 (2002), doi: 10.1046/j.0032-0862.2001.Shorttitle.doc.x.

27. Logeswari, P., Silambarasan, S., and Abraham, J., “Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties”, *Scientia Iranica*, **20**(3), pp. 1049–1054 (2013), doi: 10.1016/j.scient.2013.05.016.

28. Rahmati, M., Eghbali, S., Mokaber-Esfahani, M et al., "Volatile oil constituents, antioxidant and antibacterial activities of Lonicera caprifolium L. in different areas of Iran", *Natural Product Research*, pp. 1–7 (2024),doi: 10.1080/14786419.2024.2369229.

29. Robatsarpooshi, M., Haji Hosseini, R., Halimi Khalilabd, M et al., "Synthesis of Silver Nanoparticles by Green Method from Different Parts of Saffron and Their Effect on Hospital Antibiotic Resistant Bacteria (Acinetobacter baumannii)", *Experimental animal Biology*, **11**(3), pp. 33–41 (2023), doi: 10.30473/eab.2023.59216.1833.

30. Vanaja, M. and Gurusamy, A., "Coleus aromaticus leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity", *Applied Nanoscience*, **3** (2012), doi: 10.1007/s13204-012-0121-9.

31. Saif, S., Tahir, A., and Chen, Y., "Green Synthesis of Iron Nanoparticles and Their Environmental Applications and Implications", *Nanomaterials*, **6**(11), p. 209 (2016), doi: 10.3390/nano6110209.

32. Reddy, N., Li, H., Hou, T et al., "Phytosynthesis of Silver Nanoparticles Using Perilla frutescens Leaf Extract: Characterization and Evaluation of Antibacterial, Antioxidant, and Anticancer Activities", *International Journal of Nanomedicine*, **Volume 16**, pp. 15–29 (2021), doi: 10.2147/IJN.S265003.

33. Logaranjan, K., Raiza, A. J., Gopinath, S. C. B et al., "Shape- and Size-Controlled Synthesis of Silver Nanoparticles Using Aloe vera Plant Extract and Their Antimicrobial Activity", *Nanoscale Research Letters*, **11**(1), p. 520 (2016), doi: 10.1186/s11671-016-1725-x.

34. Masum, M. M. I., Siddiqa, M. M., Ali, K. A., Zhang, Y et al., "Biogenic Synthesis of Silver Nanoparticles Using Phyllanthus emblica Fruit Extract and Its Inhibitory Action Against the Pathogen Acidovorax oryzae Strain RS-2 of Rice Bacterial Brown Stripe", *Frontiers in Microbiology*, **10** (2019), doi: 10.3389/fmicb.2019.00820.

35. Liasi, S. A., Ibrahim, T., Azmi, T et al., "Antimicrobial activity and antibiotic sensitivity of three isolates of lactic acid bacteria from fermented fish product, Budu.", *Malaysian Journal of Microbiology*, **5**(1), pp. 33–37 (2009), doi: 10.21161/mjm.15008

36. Li, Y., Ren, F., Chen, D et al.,"Antibacterial Mechanism of Linalool against Pseudomonas fragi: A Transcriptomic Study", *Foods*, **11**(14), p. 2058 (2022), doi: 10.3390/foods11142058.

37. Park, S. N., Lim, Y. K., Freire, M. O et al.,"Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria", *Anaerobe* (2012), doi: 10.1016/j.anaerobe.2012.04.001.

38. Guo, F., Chen, Q., Liang, Q et al., "Antimicrobial Activity and Proposed Action Mechanism of Linalool Against Pseudomonas fluorescens", *Frontiers in Microbiology*, **12** (2021), doi: 10.3389/fmicb.2021.562094.

Figure1: Lonicera caprifolium

Figure 2: UV-vis spectroscopy of silver nanoparticles synthesized using flower extract.

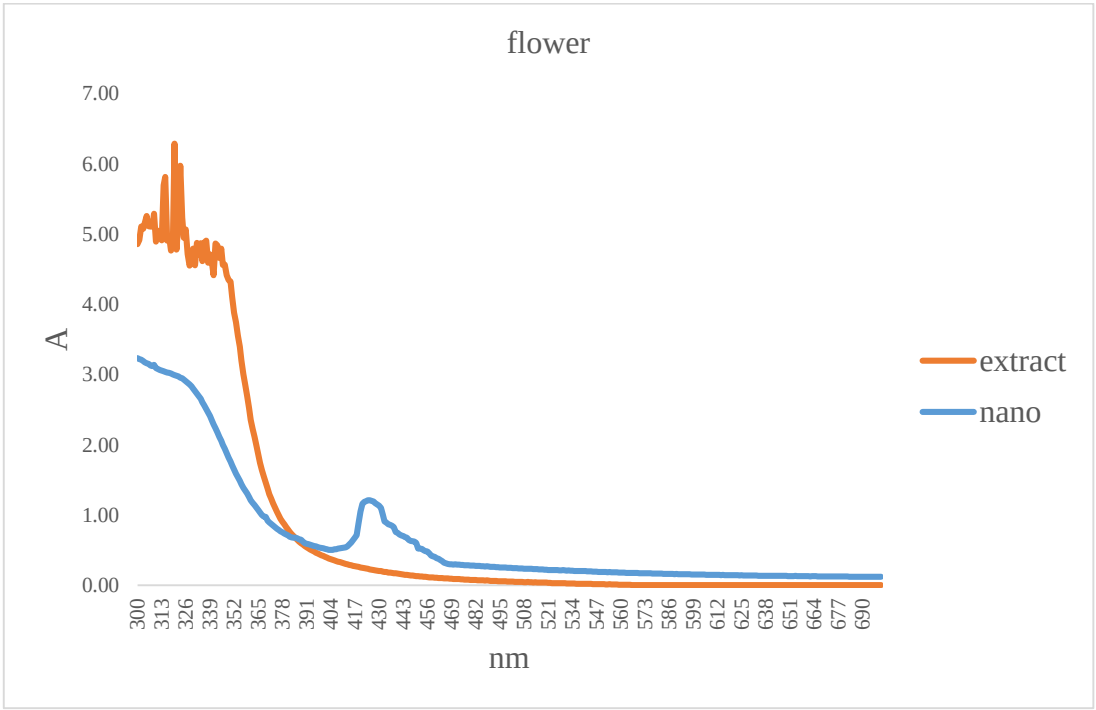


Figure 3: UV-vis spectroscopy of silver nanoparticles synthesized using leaf extract.

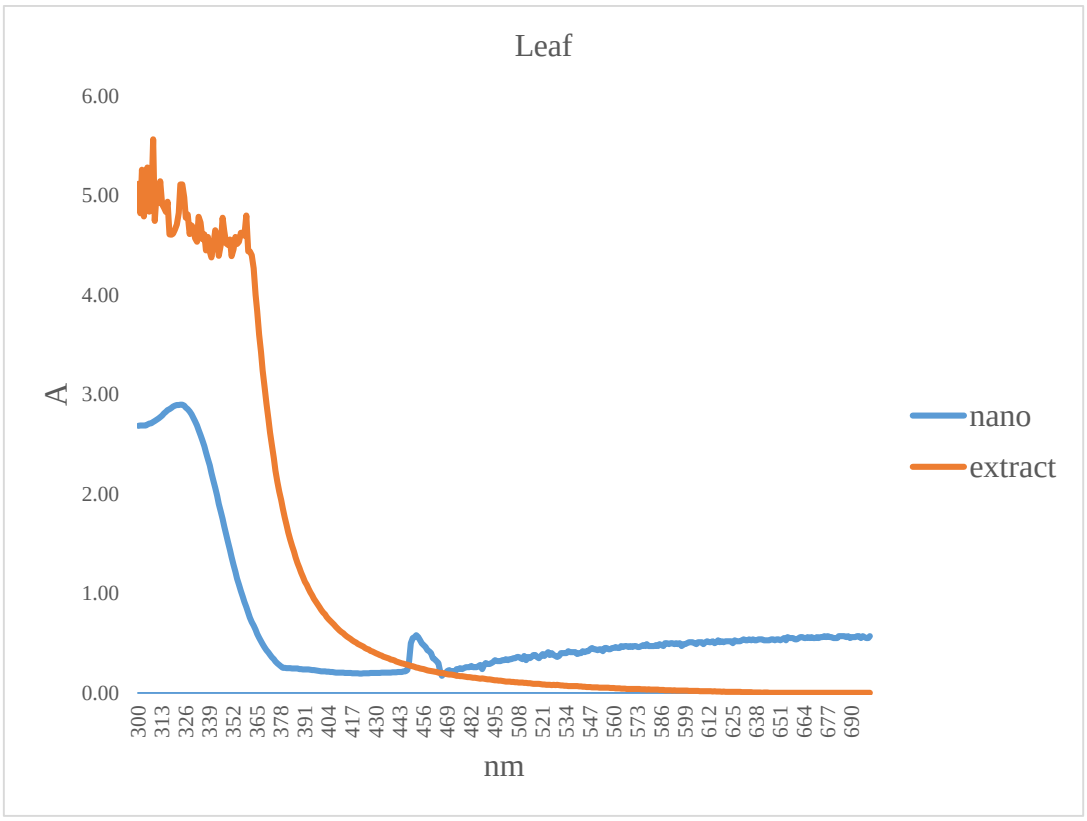
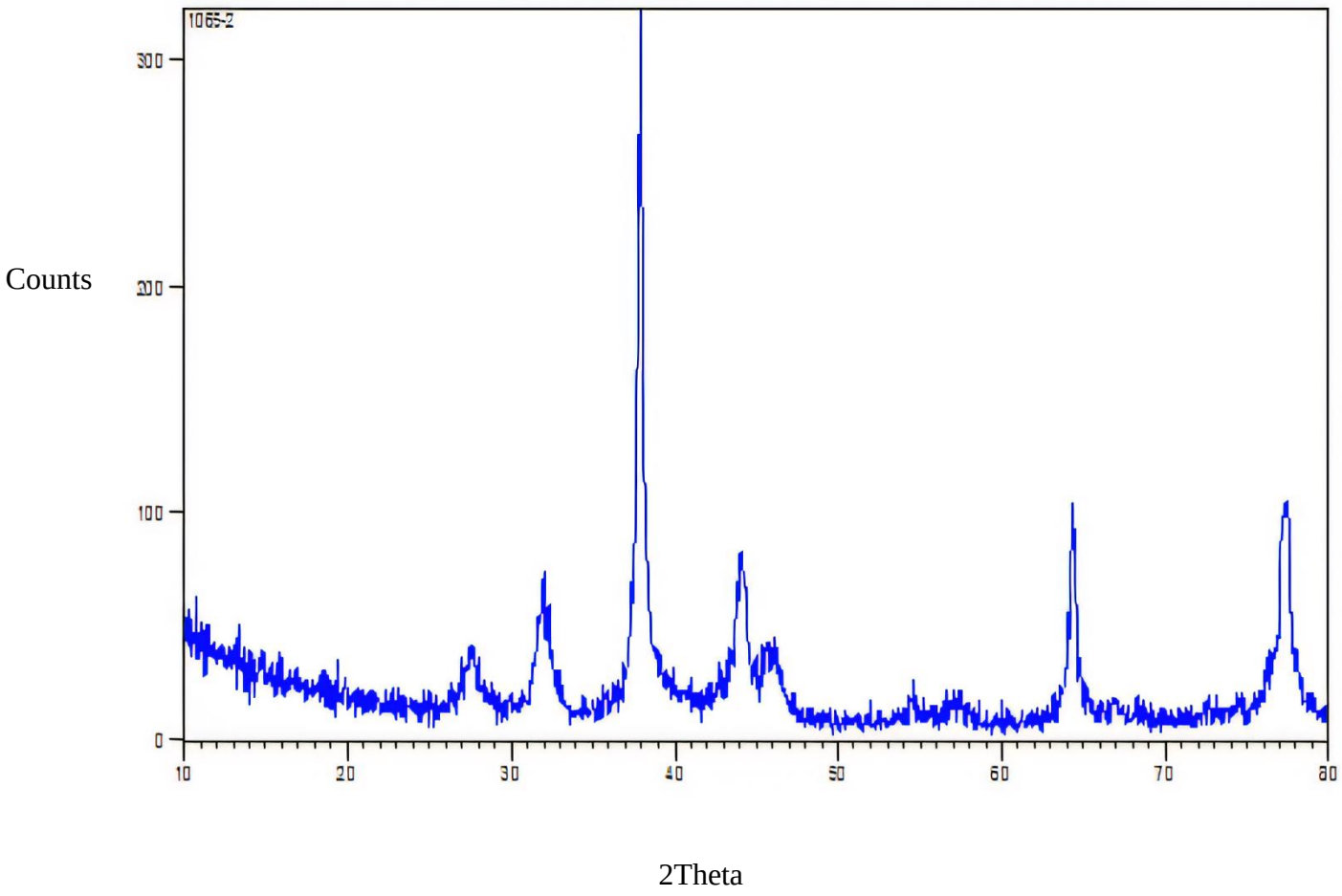
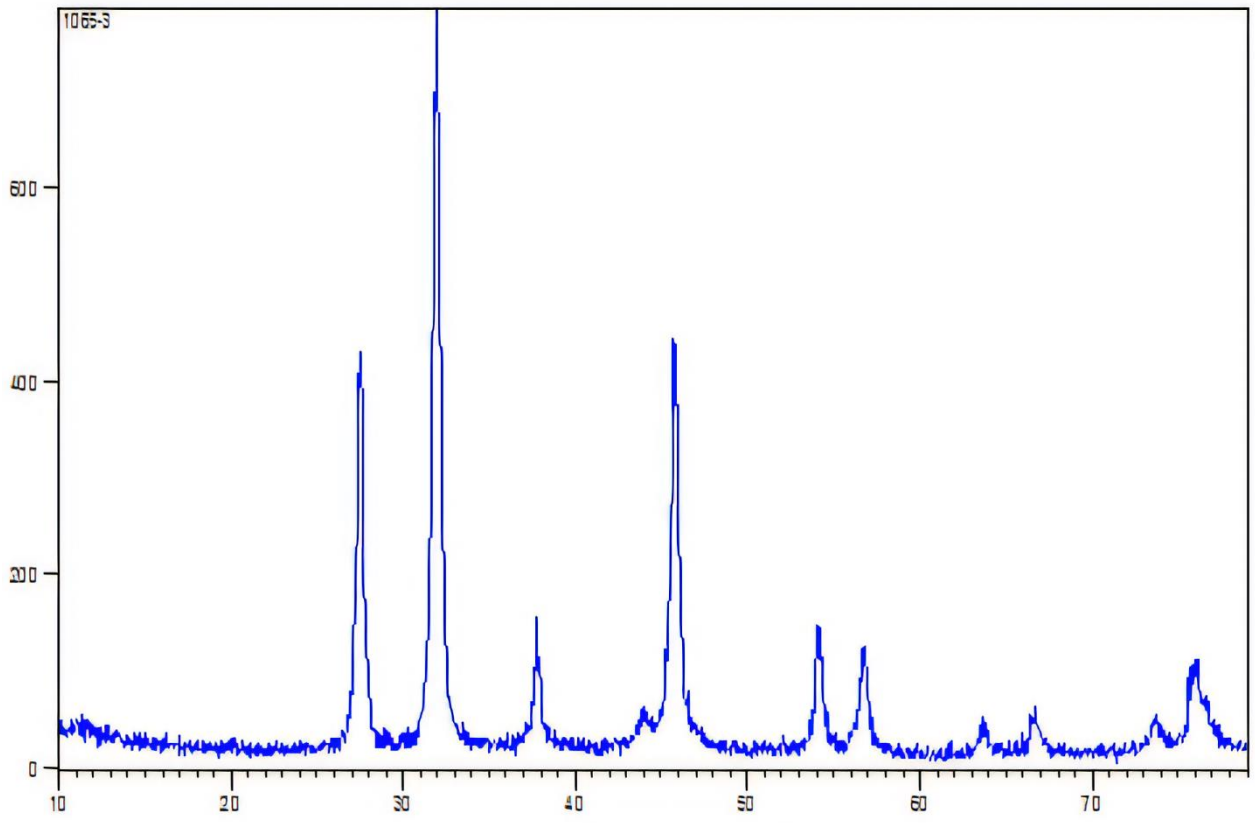


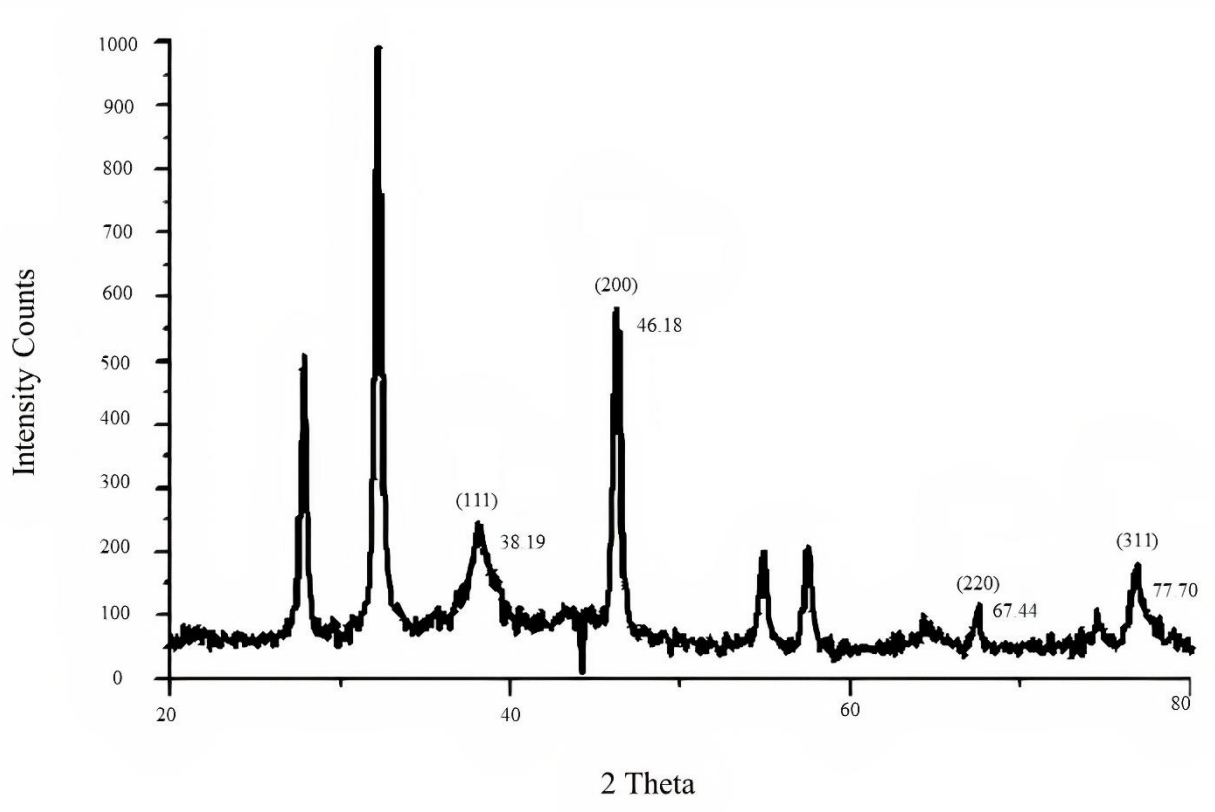
Figure 4: a: XRD pattern of silver nanoparticles synthesized from flower extract, b: XRD pattern of silver nanoparticles synthesized from leaf extract, and c: Standard sample spectrum of silver nanocrystals[30].



a

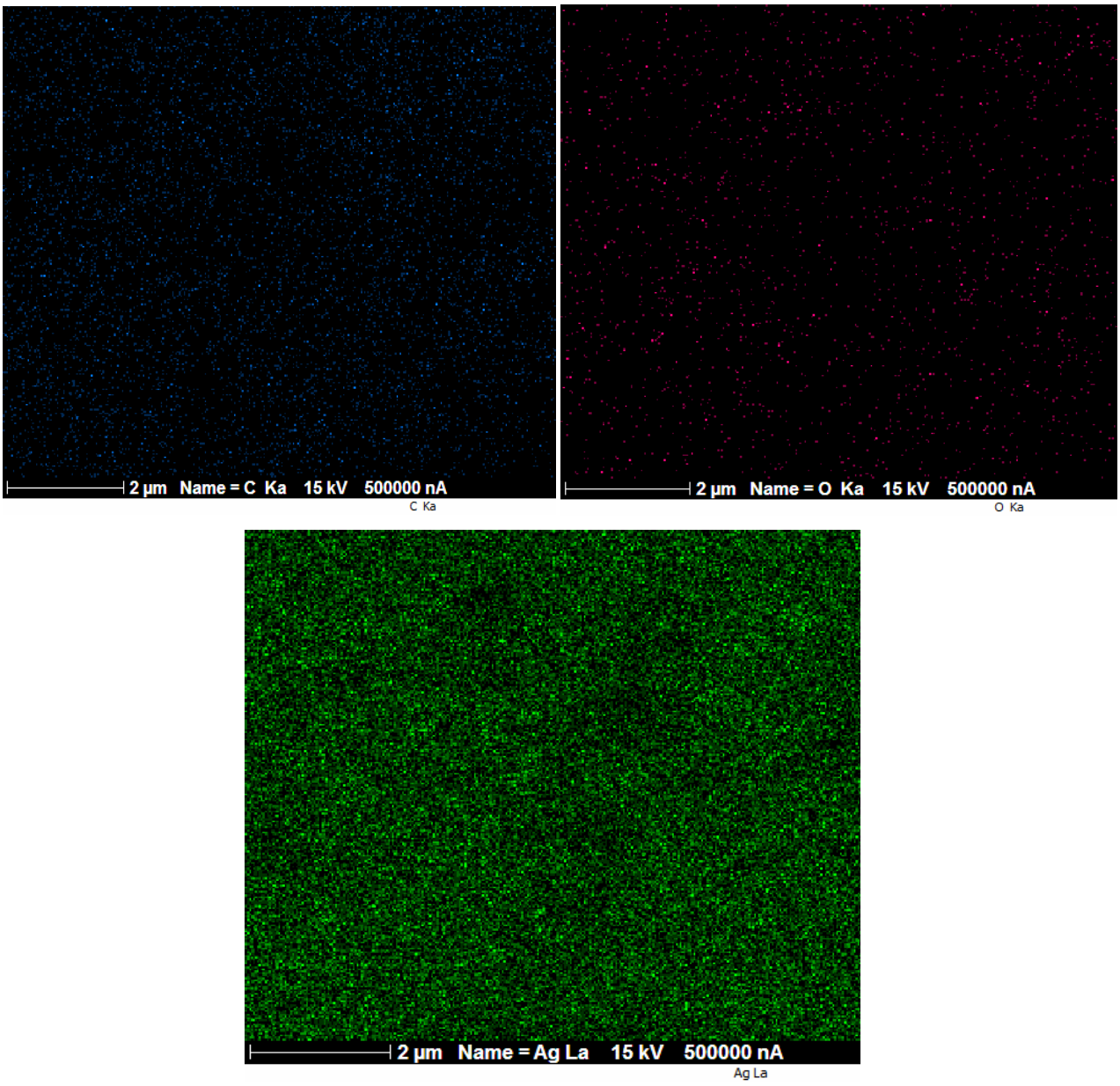

1065-3
Counts
2Theta

b

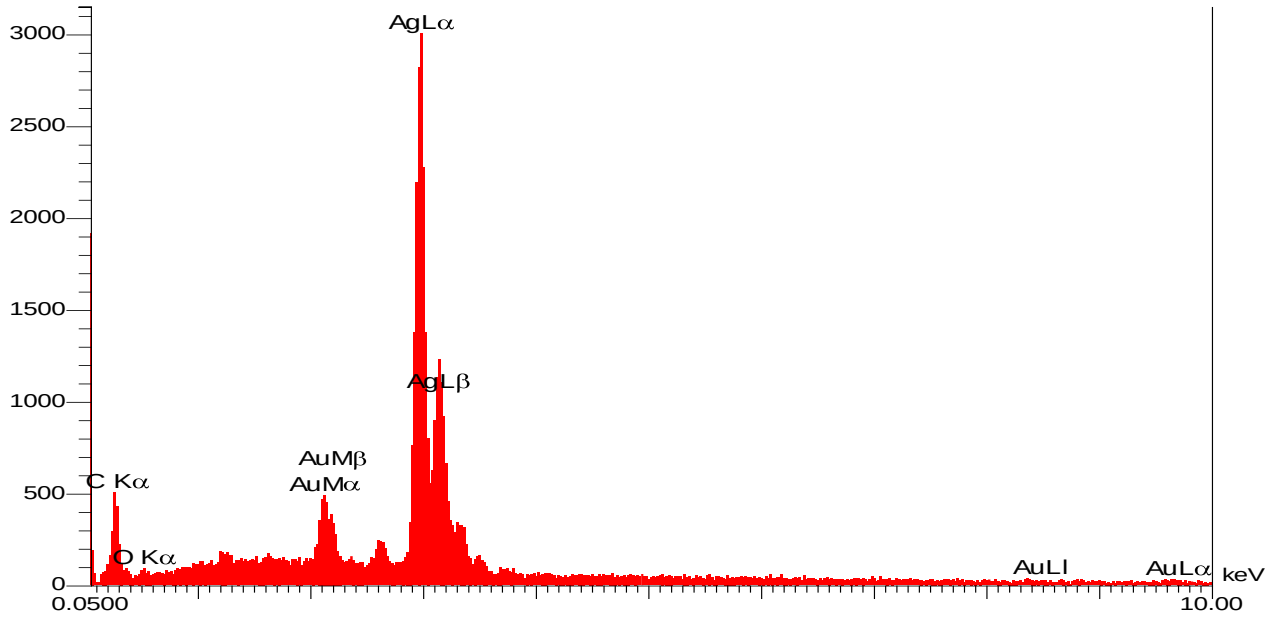

Intensity Counts
(200)
46.18
(111)
38.19
(220)
67.44
(311)
77.70
2 Theta

c

Figure 5: EDX mapping of AgNPs of flower extract (d), elemental maps of C (a), O (b) and Ag(c).

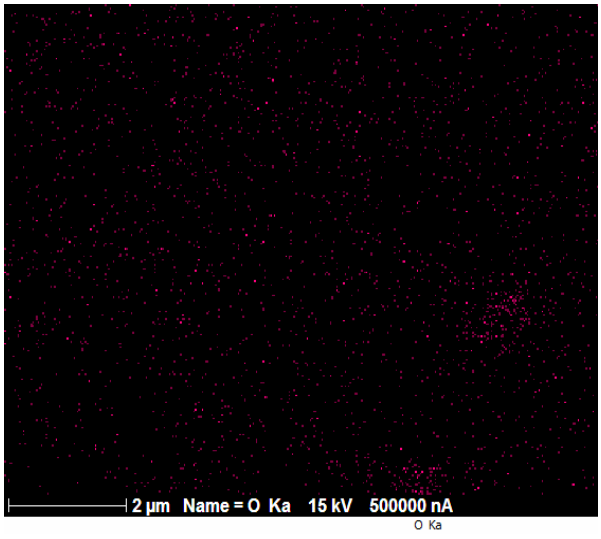


c

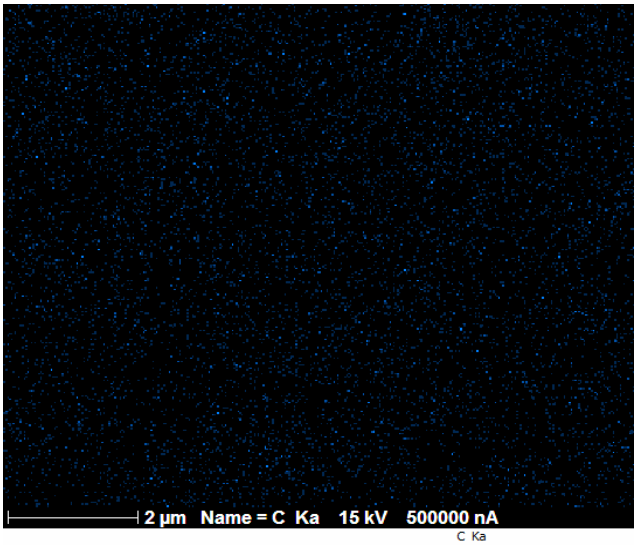


d

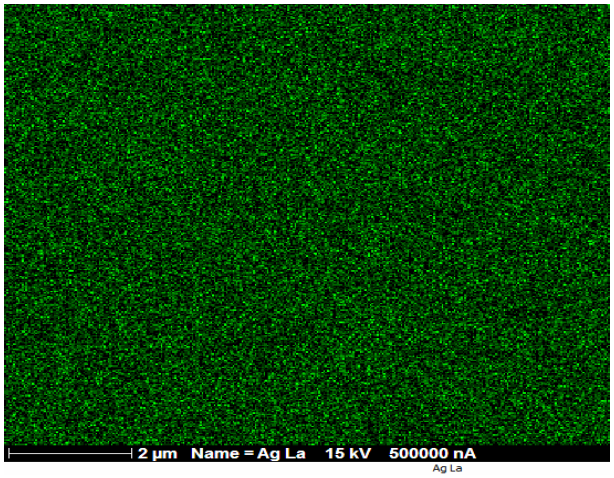
Figure 6: EDX mapping of AgNPs of leaf extract (d), elemental maps of O (a), C (b) and Ag(c).



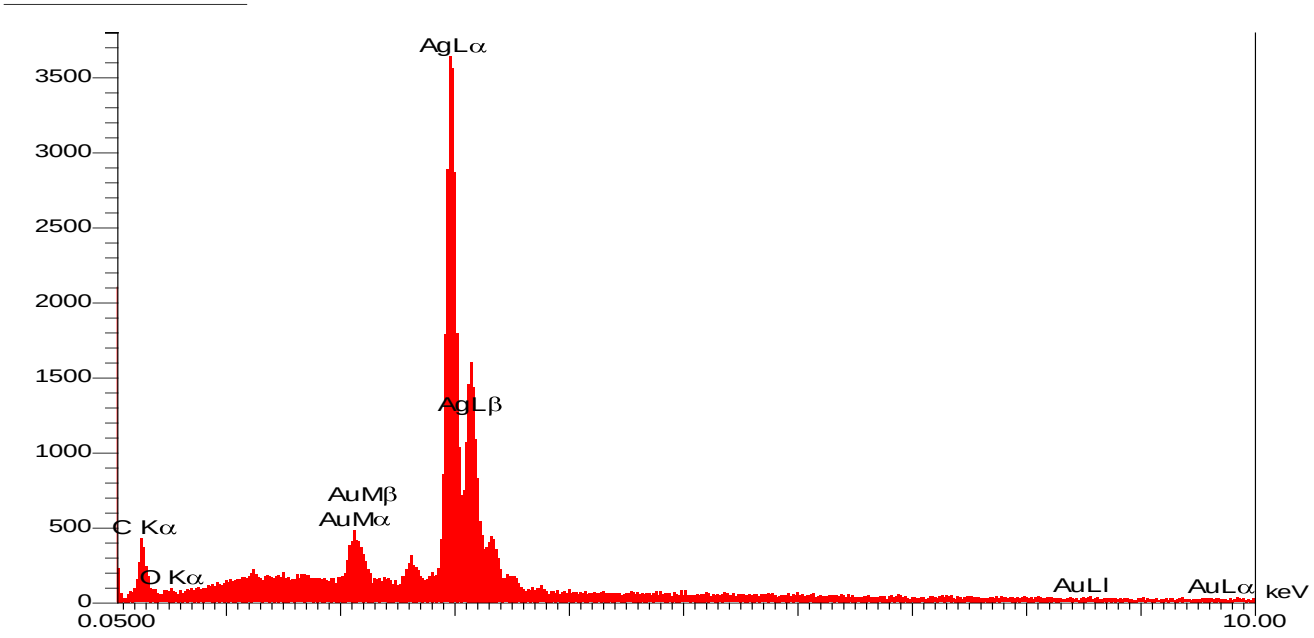
a



b


2 µm Name = Ag La 15 kV 500000 nA
Ag La

c


AgLα
3500
3000
2500
2000
1500
1000
500
0
AgLβ
AuMβ
AuMα
C Kα
O Kα
AuLl
AuLα
keV
0.0500
10.00

d

Table 1: Quantitative results for Carbone, oxygen, and silver in an AgNPs sample (Flower, Leaf)

flower										
Elt	Line	Int	K	Kr	W%	A%	ZAF	Pk/Bg	LConf	HConf
C	Ka	40.4	0.0937	0.0840	14.19	59.72	0.5920	7.75	13.38	15.01
O	Ka	0.0	0.0001	0.0001	0.03	0.10	0.1600	9.47	-0.09	0.15
Ag	La	645.1	0.9063	0.8129	85.77	40.18	0.9477	26.50	84.54	87.01
			1.0000	0.8970	100.00	100.00				
leaf										
C	Ka	35.9	0.0685	0.0630	10.77	51.97	0.5850	5.69	10.11	11.43
O	Ka	0.0	0.0000	0.0000	0.03	0.10	0.1604	8.32	-0.07	0.13
Ag	La	806.0	0.9314	0.8561	89.20	47.93	0.9597	26.91	88.06	90.35
			1.0000	0.9192	100.00	100.00				

Figure 7: Infrared spectroscopy (FTIR) of silver nanoparticles synthesized using of leaf extract of *Lonicera caprifolium*

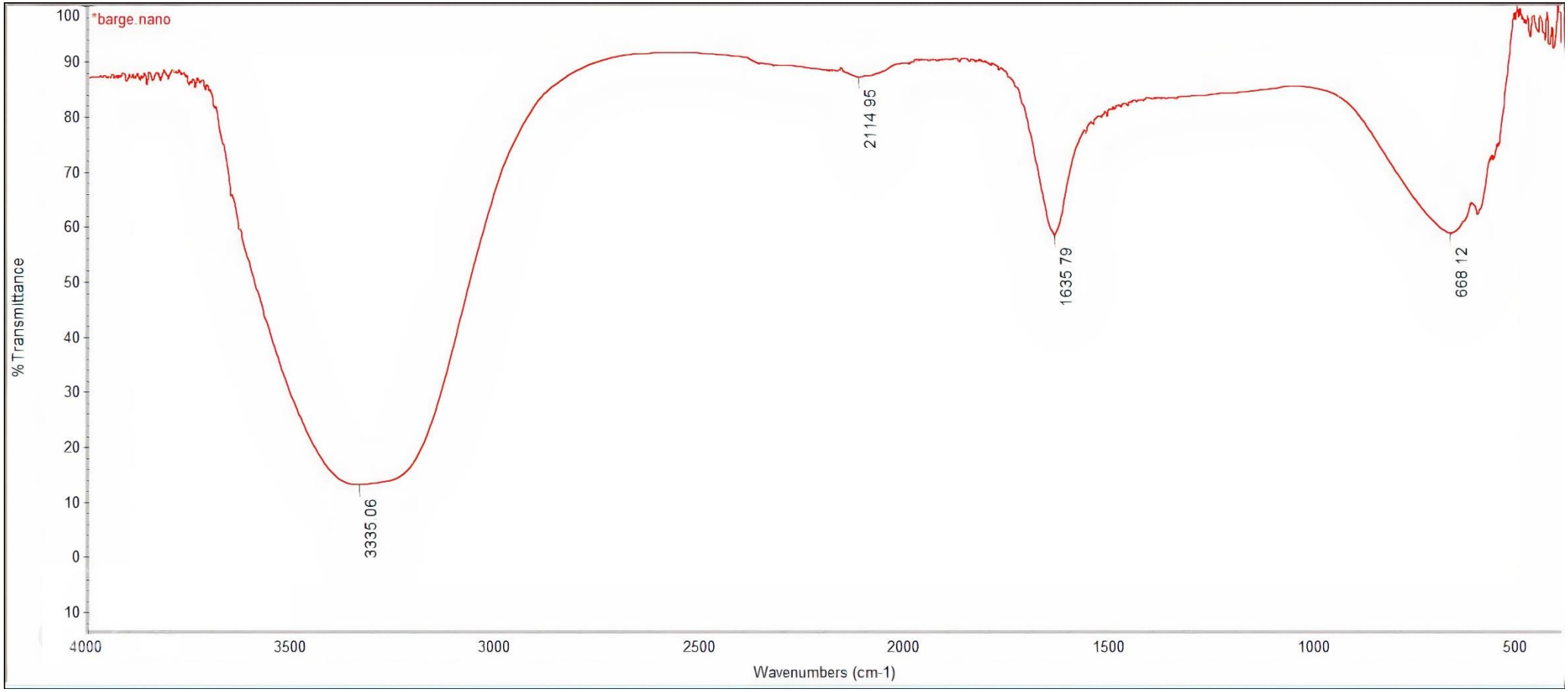


Figure 8: Infrared spectroscopy (FTIR) of silver nanoparticles synthesized using of flower extract of *Lonicera caprifolium*

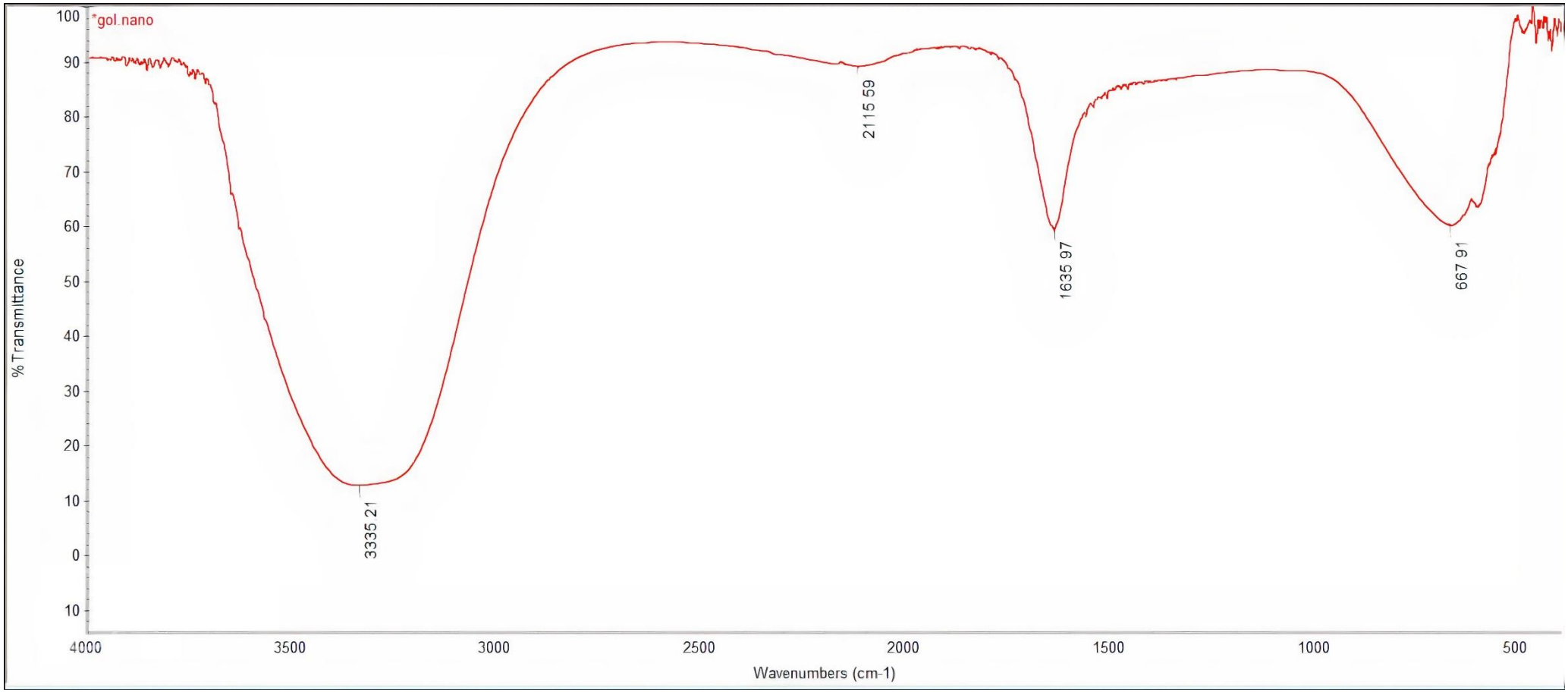
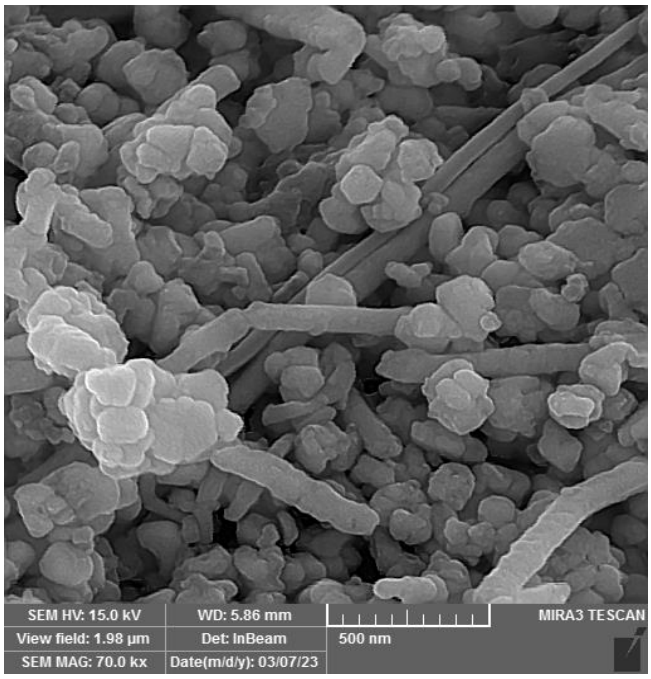
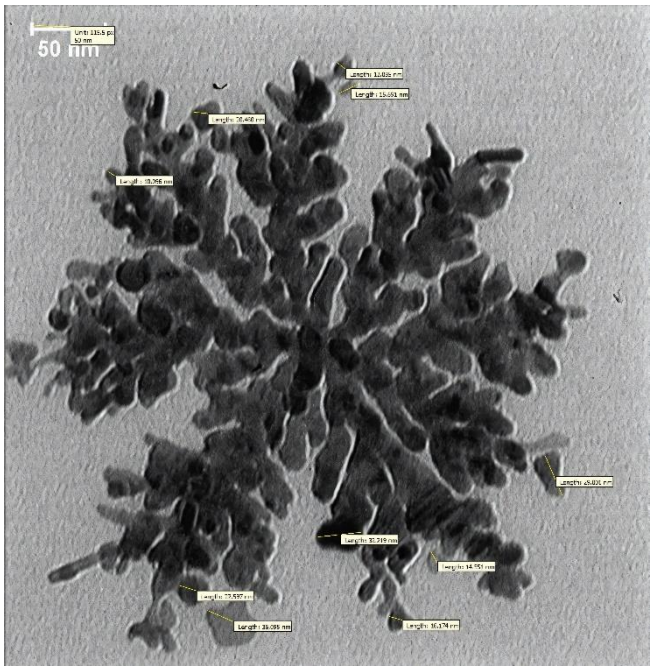

*gol.nano
% Transmittance
100
90
80
70
60
50
40
30
20
10
0
10
3335 21
2115 59
1635 97
667 91
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

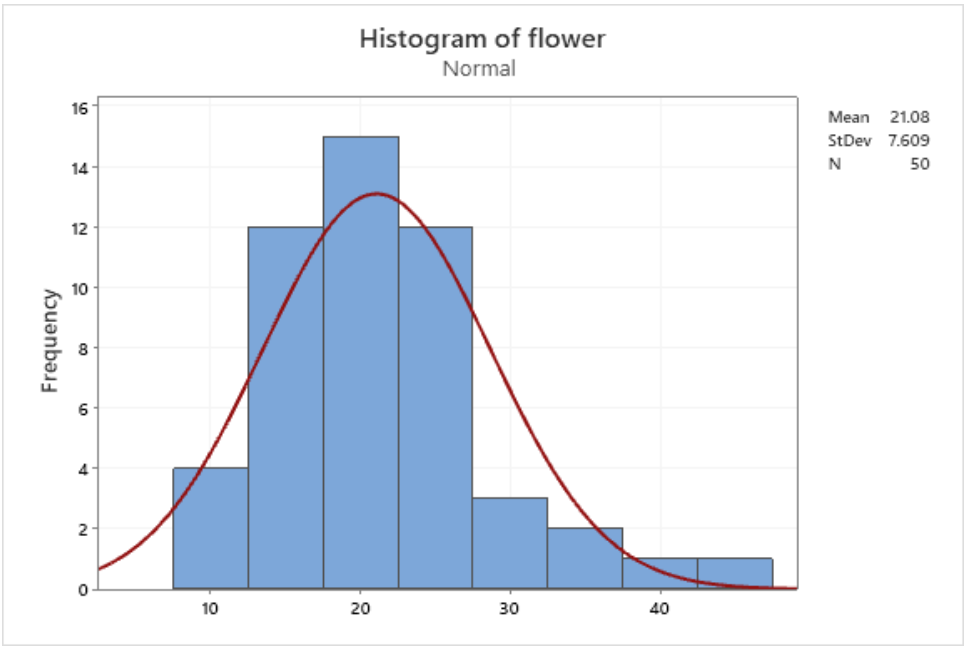
Figure 9: SEM (a), TEM (b), and Nanoparticle size distribution diagram(c) of AgNPs synthesis of flower extract.



a

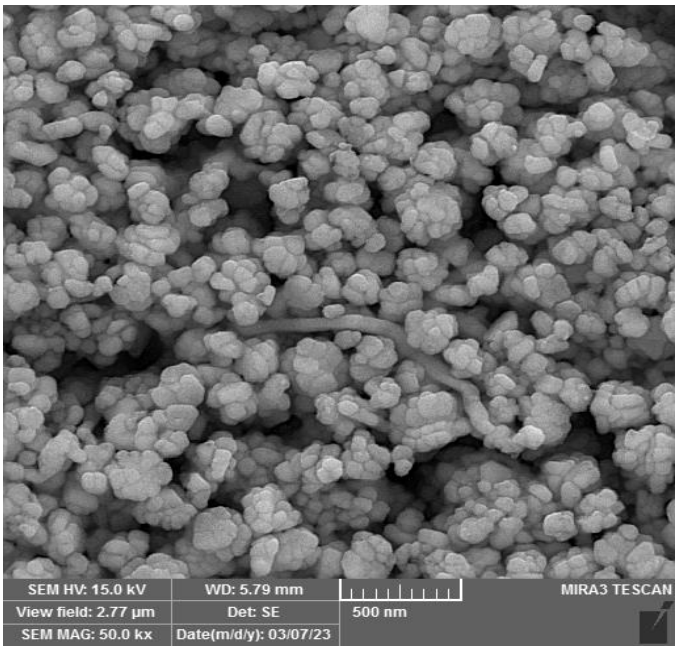


b

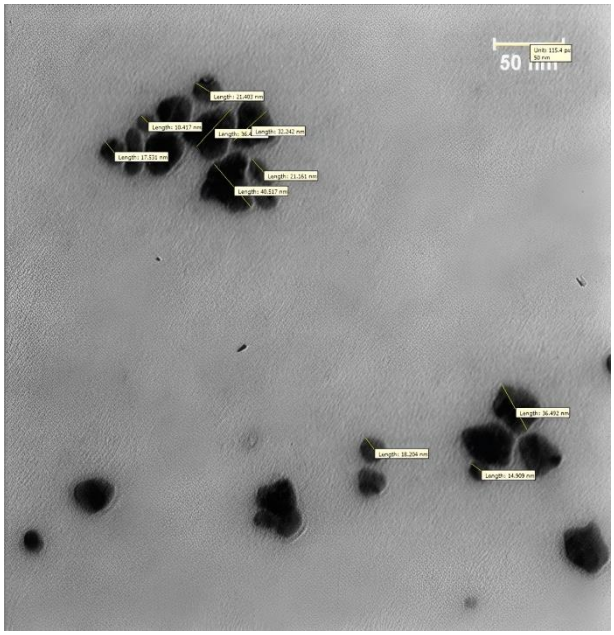


c

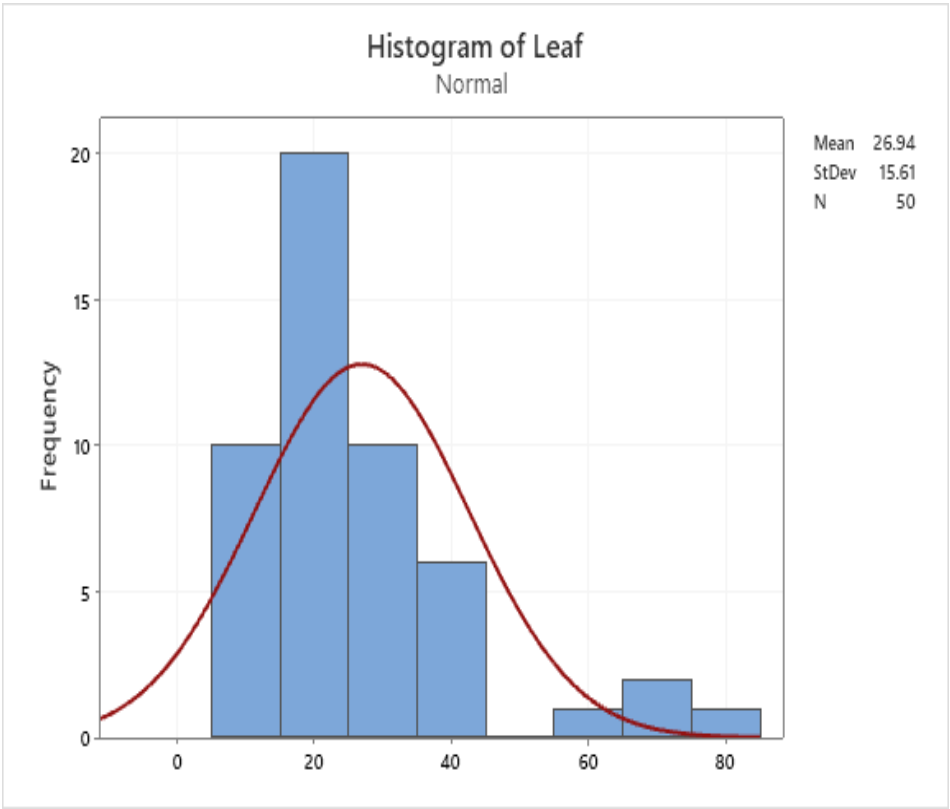
Figure10: SEM (A), TEM (B), and Nanoparticle size distribution diagram(C) of AgNPs synthesis of leaf extract.



a

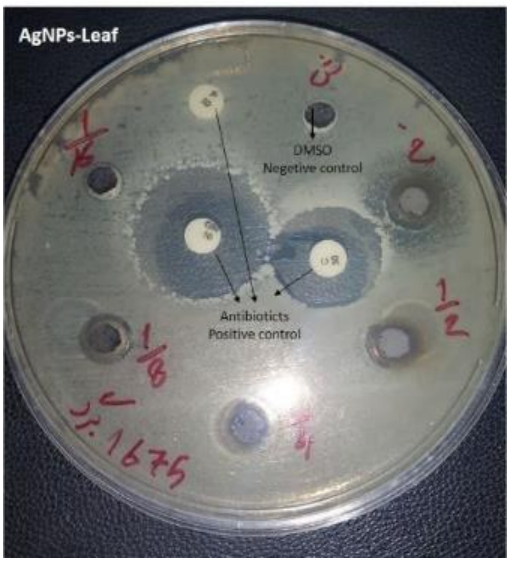


b



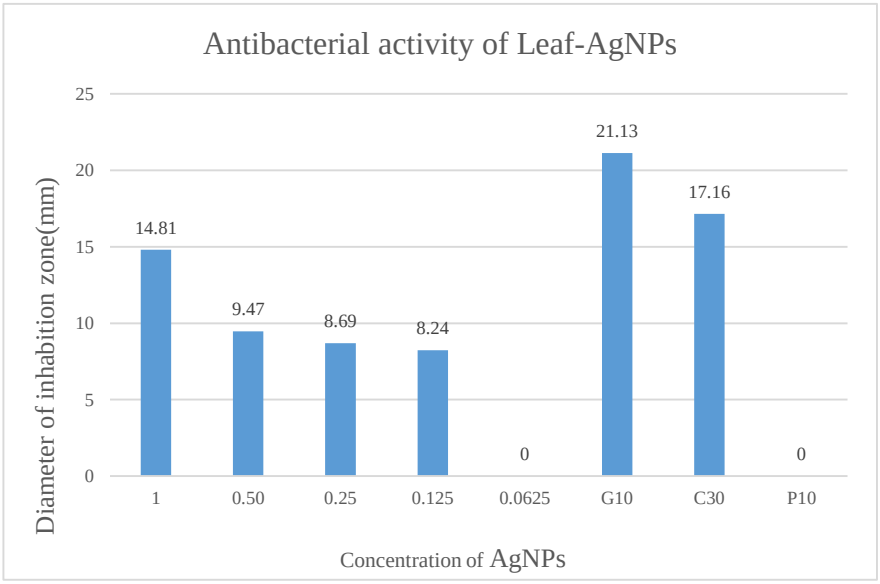
c

Figure 11: **a** and **b**: In vitro inhibitory effect of silver nanoparticles (AgNPs) synthesized by *Lonicera caprifolium* flower and leaf extract against *P. carotovorum.* **c and d**: Diameter of bacterial growth inhibition (mm) caused by different concentration of Leaf-AgNPs and Flower-AgNPs at 1, 0.5, 0.25,0.125, and 0.0625 mg/ml and G10, C30 and P10 as positive control.

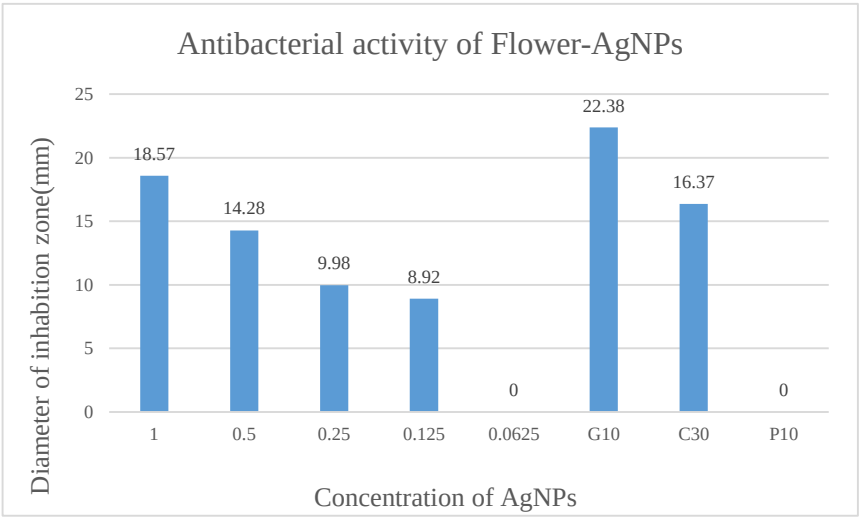


a

b



c



d

Table 2: Diameter of the inhibitory zone (mm) of silver nanoparticles synthesized by leaf and flower extract of *Lonicera caprifolium* against *Pectobactrrium carotovorum subsp carotovorum.*

sample	Concentration(mg/mL)	zone of inhibition *pectobacterium Carovorum*(PTCC No: 1675)(mm)	Inhibitory level
AgNPs-Flower	1	**18.57±1.52**	**+++**
	0.5	14.28±.068	++
	0.25	9.98±1.03	++
	0.125	8.92±0.19	+
	0.0625	0	-
Positive control	Gentamicin (10µg)	22.38 ±0.20	+++
	Chloramphenicol (30 µg)	16.37 ±0.01	+++
	Penicillin G (10U)	0	-
AgNPs-Leaf(mg/mL)	1	**14. 81 ±0.73**	**++**
	0.5	9.47 ±0.30	+
	0.25	8. 69 ±0.66	+
	0.125	8.24 ±0.06	+
	0.0625	0	-
Positive control	Gentamicin (10µg)	21. 13 ± 0.46	+++
	Chloramphenicol (30 µg)	17.16 ±0.86	+++
	Penicillin G (10U)	0	-

Table 3: MIC and MBC of AgNPs-Leaf and AgNPs-Flower

Bacteria	**AgNPs**			
	Leaf		Flower	
	MBC	**MIC**	**MBC**	**MIC**
pectobacterium Carovorum (PTCC No: 1675)	0.5 mg/mL	0.25 mg/mL	0.5 mg/mL	0.25 mg/mL

Ms. Sahar Firozeh:

Born on April 3, 1988, in Bojnord, North Khorasan Province, Iran. Ms. Sahar Firozeh holds a **B.Sc. in Applied Chemistry** from *Payame Noor University, Bojnord (2007–2012)* and a **M.Sc. in Chemistry and Essential Oil Technology** from *Gonbad Kavous University (2020–2023)*. Her graduate research focused on extraction techniques for bioactive compounds from medicinal plants, laying the foundation for her ongoing work in phytochemistry. Since 2021, Ms. Firozeh has served as an educator with the **Bojnord Education Organization**, where she inspires young learners as an elementary school teacher. Parallel to her teaching career, she maintains an active research practice, specializing in:

- **Essential oil extraction** through distillation methods
- **Analysis of bioactive compounds** in medicinal plants
- **Sustainable applications** of plant-derived chemicals

Ms. Firozeh is particularly devoted to the **distillation and extraction of active ingredients** from medicinal plants. This work combines her academic training in chemistry with her deep appreciation for traditional herbal medicine. She continues to explore innovative extraction techniques that bridge modern chemistry with natural therapeutics.

Dr. Majid Halimi Khalilabad:

Born on June 25, 1977, in Bojnord, North Khorasan Province, Iran. Dr. Majid Halimi Khalilabad is a dedicated scholar and academic whose career has been defined by a deep commitment to advancing the field of chemistry, particularly in phytochemistry, nanomaterials, and green synthesis. With a strong foundation in organic chemistry, his work bridges innovative research and impactful teaching, earning him recognition in both national and international academic circles.

Dr. Halimi's academic path reflects his enduring passion for chemistry. He earned his **B.Sc.** in Pure Chemistry from Yazd University (1996–2000) before pursuing a **M.Sc.** in Organic Chemistry at Ferdowsi University of Mashhad (2000–2002). His thesis," **Synthesis of new derivatives of benzothiazepine compounds**", under Professor Mehdi Bakavoli and Professor Mohammad Rahimizadeh.

His intellectual curiosity led him to a **Ph.D.** in Organic Chemistry-Phytochemistry at Payame Noor University (PNU) in Tehran (2008–2012), His thesis," **Phytochemical studies and structural determination of alkaloids extracted from medicinal plants: *Verbascum Speciosum*,*Amygdalus Scoparia*, *Asperula oppositifolia* and *Eremostachys labiosiformis***.", under Professor Hooshang Vahedi and Professor Jalil Lari. where he honed his expertise in natural product chemistry and sustainable methodologies.

As a faculty member at Payame Noor University (2003–2021), Dr. Halimi played a pivotal role in educating future chemists while actively contributing to research. In 2021, he joined Kosar University of Bojnord as a faculty member, continuing his dual mission of teaching and cutting-edge research. His scholarly output includes publications in reputable journals, presentations at international conferences, and collaborations that highlight his focus on phytochemistry and green synthesis areas critical to developing eco-friendly scientific solutions. Beyond the laboratory, Dr. Halimi is deeply invested in mentorship, guiding students through their academic and research endeavors. His professional service extends to editorial boards and academic committees, underscoring his leadership in the scientific community.

Committed to transparency and knowledge sharing, Dr. Halimi maintains an active ORCID profile (0000-0003-4074-8681), which documents his contributions to chemistry. His work is also indexed under Scopus Author ID: 54412436900 and Researcher ID: ABE-6746-2021, reflecting his global

engagement. He embraces social media platforms like LinkedIn to foster collaboration and dialogue within the scientific community. Driven by a desire to address pressing challenges in chemistry, Dr. Halimi's research aims to merge theoretical innovation with practical applications for societal benefit. Whether through mentoring, publishing, or advocating for open science, he remains a steadfast contributor to the advancement of his field .

Dr. Akram Taleghani:

Born on September 16, 1988, in Razavi Khorasan Province, Iran, Dr. Akram Taleghani's academic journey reflects her passion for chemistry and natural products. She earned her **B.Sc**. in Chemistry from Hakim Sabzevari University (2006–2010) before specializing in Phytochemistry during her **M.Sc**. at Shahid Beheshti University (2010–2012). Her thesis,"Separation and Identification of Chemical Compounds in Salvia leriifolia n-Hexane Extract", under Professor Mehdi Moridi-Farimani, laid the groundwork for her expertise in plant-derived compounds.Her doctoral studies (**Ph.D**. in Organic Chemistry, 2012–2017) at Mashhad University of Medical Sciences further honed her skills in drug discovery. Under Professor Merdad Iranshahi, she explored "Conjugation of Parthenolide with Amine-Containing Anticancer Drugs via Aza-Michael Addition", pioneering research with implications for targeted cancer therapies. Dr. Taleghani began her career as an HPLC Laboratory Supervisor (2017–2019), analyzing and quantifying herbal and medicinal products. In 2019, she joined Gonbad Kavous University as an Assistant Professor of Chemistry, where she balances teaching, research, and mentorship. As a dedicated research leader, she has supervised 7 M.Sc. students and trained laboratory technicians. Currently, she guides 3 M.Sc. students as a primary supervisor and 5 as a co-supervisor, fostering the next generation of chemists. Her pedagogical training emphasizes hands-on learning, particularly in advanced analytical techniques like :

-HPLC, LC-MS, and GC-MS

-Metabolomics and Column Chromatography

-Natural Product Isolation, Identification, and Quantification

Dr. Taleghani's work bridges phytochemistry and medicinal chemistry, with a focus on isolating bioactive compounds and designing novel drug conjugates. Her publications and projects underscore her commitment to translating laboratory findings into therapeutic advancements .

Dr. Reza Akbari:

Born on March 21, 1978, in Fereydunkenar, Mazandaran Province, Iran. Dr. Reza Akbari's expertise in analytical chemistry is rooted in a robust academic foundation. He earned his **B.Sc. in Pure Chemistry** from the **University of Mazandaran (Babolsar, 1998–2003)**, followed by an **M.Sc. (2003–2005)** and **Ph.D. (2007–2011)** in *Analytical Chemistry* from the **University of Sistan and Baluchestan (Zahedan)**. His doctoral research laid the groundwork for his future contributions to electrochemical sensors and sustainable nanotechnology.

In 2015, Dr. Akbari joined **Gonbad Kavous University** as a faculty member in the Department of Chemistry, where he has since dedicated himself to teaching and cutting-edge research. His work spans:

- **Green Synthesis of Nanoparticles**: Developing eco-friendly nanomaterials for environmental remediation.
- **Essential Oils and Extracts**: Advanced extraction and characterization techniques for bioactive compounds.
- **Electrochemistry and Sensors**: Designing sensitive, efficient tools for analytical applications.

A proponent of interdisciplinary collaboration, Dr. Akbari's projects bridge fundamental chemistry with real-world challenges, such as pollution control and sustainable material design. His publications, indexed in **Scopus (Author ID: 56795136500)** and **Google Scholar**, reflect his commitment to impactful science.

Dr. Mohabat Nadaf:

Born on September 23, 1971, in Bojnord, North Khorasan Province, Iran.Dr. Mohabat Nadaf's passion for plant sciences began with a **B.Sc. in Plant Biology** from **Shahid Beheshti University (1990–1994)**. She further specialized with a **M.Sc. in Plant Systematics (1995–1998)** and a **Ph.D. in Plant Ecology (2012–2017)**, both from **Ferdowsi University of Mashhad**. Her doctoral research solidified her expertise in the ecological dynamics of plant species, laying the groundwork for her future contributions to botany and phytochemistry. With over two decades of academic experience, Dr. Nadaf has been a dedicated educator and researcher. She served as a **faculty member at Payame Noor University (2003–2024)**, where she balanced teaching with cutting-edge research. In **2024**, she joined **Kosar University of Bojnord** as a faculty member, continuing her mission to mentor students and advance knowledge in plant sciences.

Dr. Nadaf's work spans:

- **Botany and Plant Systematics**: Classifying and understanding plant diversity.
- **Phytochemistry**: Exploring bioactive compounds in plants for potential applications.
- **Plant Ecology**: Investigating the interactions between plants and their environments.

Her research has been published in reputable journals and presented at international conferences, reflecting her influence in the field. Committed to open science, she maintains an **ORCID profile (0000-0002-7480-9895)**, which documents her scholarly contributions and collaborations.

Leadership and Outreach

Beyond research, Dr. Nadaf is actively involved in:

- Mentoring the next generation of scientists.
- Participating in academic committees and editorial boards.
- Promoting the dissemination of scientific knowledge through platforms like **Scopus (Author ID: 53064182100)** and **ResearcherID (A-1649-2022)**.