 DOI: 10.31276/VJSTE.2024.0055

Investigation of factors affecting the green synthesis of silver nanoparticles from guava leaf extract

Dong Duc Tran[1], Van Hoang Bui[1], Quang Nguyen Dinh[1], Duc Cuong Nguyen[1], Cong Trinh Bui[2], Thao Vu Thi[1*]

[1]University of Engineering and Technology, Vietnam National University - Hanoi, 144 Xuan Thuy Street, Dich Vong Hau Ward, Cau Giay District, Hanoi, Vietnam

[2]Institute for Technology of Radioactive and Rare Elements, 48 Lang Ha Street, Lang Ha Ward, Dong Da District, Hanoi, Vietnam

Received 26 May 2024; revised 3 June 2024; accepted 16 July 2024

Abstract:

Green-synthesised silver nanoparticles offer substantial advantages for biomedical applications due to reduced chemical impacts, straightforward methods, and environmental friendliness. However, the outcome of nanoparticle synthesis is heavily influenced by reaction conditions, affecting their formation, size, and properties. It is, therefore, crucial to establish precise synthesis protocols and optimise conditions pertaining to reducing agents, silver salt concentration, and other influential factors. This study identifies optimal conditions for the green synthesis of silver nanoparticles using guava leaf extract. Various analytical techniques such as Ultraviolet-visible spectroscopy (UV-Vis), Dynamic light scattering (DLS), X-ray diffraction (XRD), Field-emission scanning electron microscopy (FE-SEM), Fourier-transform infrared spectroscopy (FT-IR), and Energy-dispersive X-ray spectroscopy (EDX) were employed to characterise the material properties. The synthesised silver nanoparticles exhibited absorption at 430 nm wavelength, demonstrated a face-centred cubic crystalline structure with high crystallinity, and showed an average particle size ranging from 30-40 nm, predominantly spherical and uniformly distributed. Flavonoids present in the guava leaf extract were identified as the primary compounds responsible for the reduction of silver ions. To minimise residual extract and enhance the applicability of silver nanomaterials, a washing and filtration process was implemented. A thorough investigation into influencing factors and the resolution of issues related to the green synthesis of silver nanoparticles can pave the way for future research and boost the commercial potential of green nanomaterial products for medical and environmental applications.

Keywords: **green synthesis, guava leaf extract, silver nanoparticles.**

Classification numbers: **2.1, 2.2, 3.5**

1. Introduction

Nanomaterials, defined as materials with at least one dimension ranging from 1 to 100 nm, can exist in various forms such as particles, fibres, tubes, or thin films [1]. They play a crucial role in different fields such as information technology [2, 3], materials science, and biotechnology [4]. Metallic nanoparticles, in particular, are extensively studied and developed due to their unique properties encompassing morphology, size, composition, crystallinity, and structure [5]. Among metallic nanoparticles, silver nanoparticles are renowned in fields such as pharmaceutical sciences [6-8], cosmetics [9, 10], and antimicrobial coatings [11, 12]. Owing to their exceptional antibacterial properties, silver nanoparticles are highly effective against multidrug-resistant microorganisms [13-15]. The properties of nanomaterials, including particle size, morphology, and uniformity, are pivotal factors in biomedical applications [16, 17]. Therefore, there is a need for research and development of synthesis methods to control the size of silver nanoparticles. Silver nanoparticles have been synthesised using various techniques to meet commercial development demands, primarily adopting top-down and bottom-up approaches (Fig. 1). However, chemical synthesis methods can lead to the accumulation of harmful chemicals, causing adverse effects in biological applications, while physical synthesis methods suffer from low stability, high production costs, significant energy consumption, and complex production processes [18]. Consequently, the trend toward green, environmentally friendly production methods for silver nanoparticles is gaining considerable research attention.

*Corresponding author: Email: vtthao@vnu.edu.vn

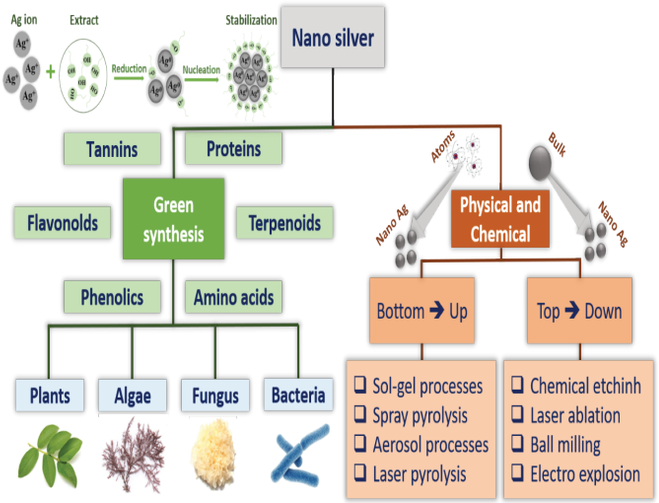

Fig. 1. Schematic diagram of silver nanoparticle synthesis.

Green methods for synthesising silver nanoparticles employ biological agents such as microorganisms [19], plant extracts [20], and natural polymers [20]. Among these, the use of silver nitrate salts and plant extracts is advantageous due to their high bioactivity, low cost, rapid synthesis time, and environmental safety. This method utilises chemical compounds in plant extracts (such as flavonoids, amino acids, phenolics, etc.) to reduce silver salts while also acting as capping and stabilising agents for the nanoparticles in the solution [20]. Extracts from different plants yield silver nanoparticles with varying sizes and bioactivities. In previous studies, the green synthesis methods for silver nanoparticles have primarily focused on biomedical applications, including antibacterial and biofilm inhibitory activities [21]. Beyond their antibacterial properties, silver nanoparticles have also demonstrated significant anticoagulant capabilities at a concentration of 1 mg/ml, which suggests that these materials have the potential to become a biopharmaceutical agent in the near future [22]. To advance their applications in the biomedical field, extracts that are beneficial to human health are actively under study and development. Guava leaf extract, known in traditional medicine for treating stomach pain, diabetes, and diarrhoea, contains compounds such as gallic acid, quercetin, morin, catechin, and rutin-flavonoids suitable for synthesising silver nanoparticles. Additionally, its high biocompatibility with the human body and large, readily available supply make guava leaf extract a popular choice for synthesising silver nanoparticles in many countries [23]. Silver nanoparticles synthesised from guava leaf extract typically range from 5-90 nm in size, with maximum UV-Vis absorption between 400-500 nm due to surface plasmon resonance [24]. These nanoparticles are primarily used in biomedical fields, demonstrating antibacterial activity against strains such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, as well as in biocoatings and mosquito larvicides [23]. Despite previous reports on the green synthesis of silver nanoparticles from plant extracts, the precision and reproducibility remain inconsistent, particularly across different climatic regions. In this study, evaluating the influencing factors and determining the optimal ratios for synthesising silver nanoparticles using guava leaf extract is essential, considering the climate and conditions in Vietnam. This research will serve as a foundation for subsequent studies aimed at large-scale silver nanoparticle production in Vietnam.

2. Materials and methods

2.1. Materials

Guava (*Psidium guajava* L.) leaves were sourced from Bac Giang province, Vietnam. The leaves were fresh, healthy, disease-free, and measured 6-8 cm in length and 3-4 cm in width. Silver nitrate ($AgNO_3$) with a purity of 99.8% was obtained from Xilong, China. Distilled water (99%) was produced using the FST-UV device at the Laboratory of the Faculty of Engineering Physics and Nanotechnology, University of Engineering and Technology, Vietnam National University - Hanoi.

2.2. Synthesis of silver nanoparticles

Guava leaf extract primarily contains polyphenols (flavonoids, tannins, meroterpenoids), saponins, and essential oils (limonene, menthol, caryophyllene). The principle of the green synthesis method for silver nanoparticles is based on the reduction of silver ions (Ag^+) to elemental silver (Ag^0) in plant extracts, as detailed in the previous report [25]. According to this report, the -OH groups in flavonoids play a crucial role in the reduction of silver ions to silver nanoparticles, as well as in capping and stabilising the nanoparticles. The transformation of flavonoids from the enol form to the keto form releases a hydrogen atom, facilitating the reduction of metal ions to form nanoparticles (Fig. 2).

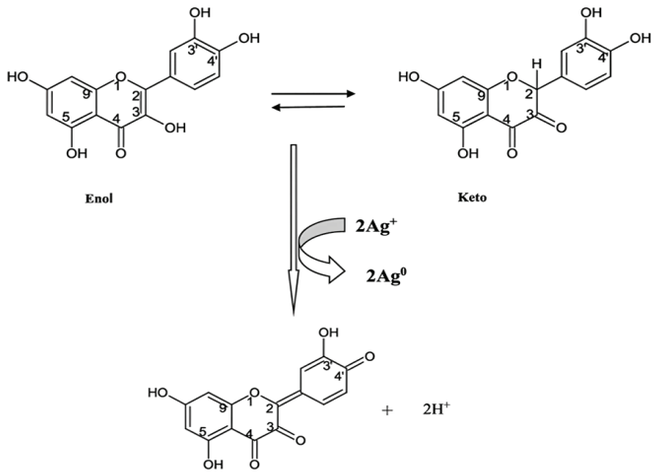

Fig. 2. Mechanism of reduction and stabilisation of silver nanoparticles by flavonoids in guava leaf extract [26].

To optimise the synthesis process of silver nanoparticles using Vietnamese guava leaves, various parameters related to the reaction conditions were modified. For extract preparation, guava leaves were thoroughly washed with distilled water, air-dried, and finely ground. Deionised water was then added at ratios of 1, 2, 5, 10, and 15 g of leaves per 100 ml of water, and the mixture was boiled for 10 minutes. The mixture was filtered multiple times to remove the leaf debris, resulting in extracts of different concentrations. The guava leaf extract was then centrifuged at 10,000 rpm for 10 minutes to remove any remaining sediment. The extract was stored in a refrigerator at a low temperature until further use. To investigate the formation process of silver nanoparticles, $AgNO_3$ solution was gradually added to the extraction flask containing the guava leaf extract (Fig. 3). The synthesis process involved varying parameters such as the concentration of the extract, the concentration of silver nitrate, the extract/silver nitrate ratio, and reaction conditions like temperature and time. The resulting silver nanoparticle solution was sonicated for 10 minutes before further analysis and application.

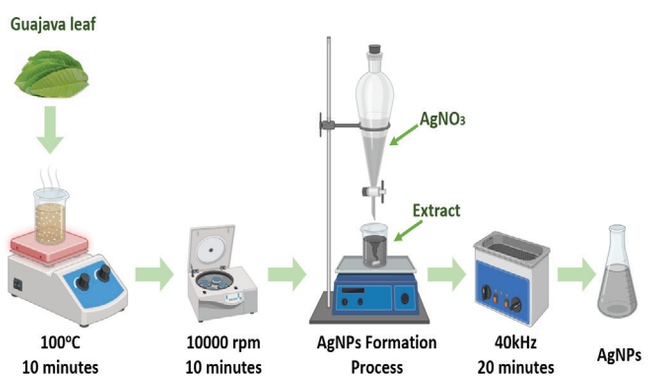

Fig. 3. Silver nanoparticles synthesis process.

2.3. Characterisation methods

2.3.1. Ultraviolet - Visible absorption characteristics of silver nanoparticles

The UV-Vis absorption characteristics of the silver nanoparticles were measured using a Hitachi UH-5300 dual-beam spectrophotometer in the Laboratory of the Faculty of Engineering Physics and Nanotechnology, University of Engineering and Technology, Vietnam National University - Hanoi. The samples were measured in the wavelength range of 250 to 650 nm, with the sample solution prepared in distilled water.

2.3.2. Particle size distribution by dynamic light scattering

Particle size distribution was measured using DLS on a Horiba LB-550 device in the laboratory of the Faculty of Engineering Physics and Nanotechnology, University of Engineering and Technology, Vietnam National University - Hanoi. The samples were measured using a quartz cuvette in distilled water.

2.3.3. Fourier transform-infrared spectroscopy

The silver nanoparticle powder sample, prepared as described above, was examined using a Nicolet™ iS50 FT-IR spectrometer. The spectrum was recorded over the wavenumber range from 4000 to 500 cm^{-1}, including 16 scans of the sample at a resolution of 4 cm^{-1}.

2.3.4. X-ray diffraction

The silver nanoparticle solution was centrifuged at high speed to remove water, and the silver nanoparticles were dispersed in acetone and dried to obtain silver nanoparticle powder. The material sample was analysed using XRD (λ=0.15406 nm) on an LA-6490 diffractometer at the Vietnam Academy of Science and Technology.

2.3.5. Field emission-scanning electron microscopy

The silver nanoparticle solution was coated onto a glass substrate using the dip-coating method on a heated glass substrate. The morphology of the silver nanoparticles was examined using a Hitachi S-4800 FE-SEM at an accelerating voltage of 3.00 kV at the University of Science and Technology of Hanoi.

2.3.6. Energy dispersive X-ray spectroscopy

The silver nanoparticle sample was prepared according to the method used for FE-SEM examination. The elemental composition analysis of the silver nanoparticle sample was conducted using an EDX/Oxford 2022 system integrated with the Hitachi S-4800 FE-SEM.

3. Results and discussion

3.1. Investigation of the influence of guava leaf extract and silver nitrate solution

3.1.1. Guava leaf extract concentration

The concentration of the extract significantly influences the formation of silver nanoparticles. Guava leaf extract contains various components, primarily flavonoids, amino acids, and phenolics, which play roles in capping and stabilising the silver nanoparticles. The mass ratio of guava leaves to DI water is crucial for adjusting the concentration of the reducing agents. In this experiment, the effect of extract concentration on the formation of silver nanoparticles was investigated (Fig. 4). Extract samples with ratios of 1, 2, 5, 10, and 15 g per 100 ml showed UV-Vis spectra with two peaks: one major peak at 285 nm and a smaller peak at 365 nm, identified as peaks of flavonoid compounds [26]. The absorption intensity increased with the guava leaf-to-water ratio (Fig. 5A).

Fig. 4. The colour of guava leaf extract at different concentrations.

The extract was then used to synthesise silver nanoparticles at room temperature with a 5 mM $AgNO_3$ concentration, an extract-to-$AgNO_3$ volume ratio of 4/45, and a reaction time of 90 minutes. UV-Vis spectra of silver nanoparticle samples with varying extract concentrations were also examined (Fig. 5B). For the extract concentration of 5 g/100 ml, the absorption peak of silver nanoparticles was observed at 435-440 nm with the highest absorption intensity (0.9 Cd). Other ratios showed the silver nanoparticle absorption peak, albeit with lower intensity. Additionally, a slight shift in the absorption peak of the silver nanoparticles was noted due to surface plasmon resonance, influenced by factors such as particle size, shape, and aggregation [27].

Insufficient quantities of guava leaf result in an inadequate supply of reducing agents necessary for the reduction of silver salts. Conversely, an excessive amount of guava leaves in the extract introduces surplus non-beneficial substances, which promote nanoparticle aggregation, subsequently causing sedimentation and a decrease in optical density. Therefore, a concentration of 5 g/100 ml is optimal for facilitating the effective reduction of silver nanoparticles.

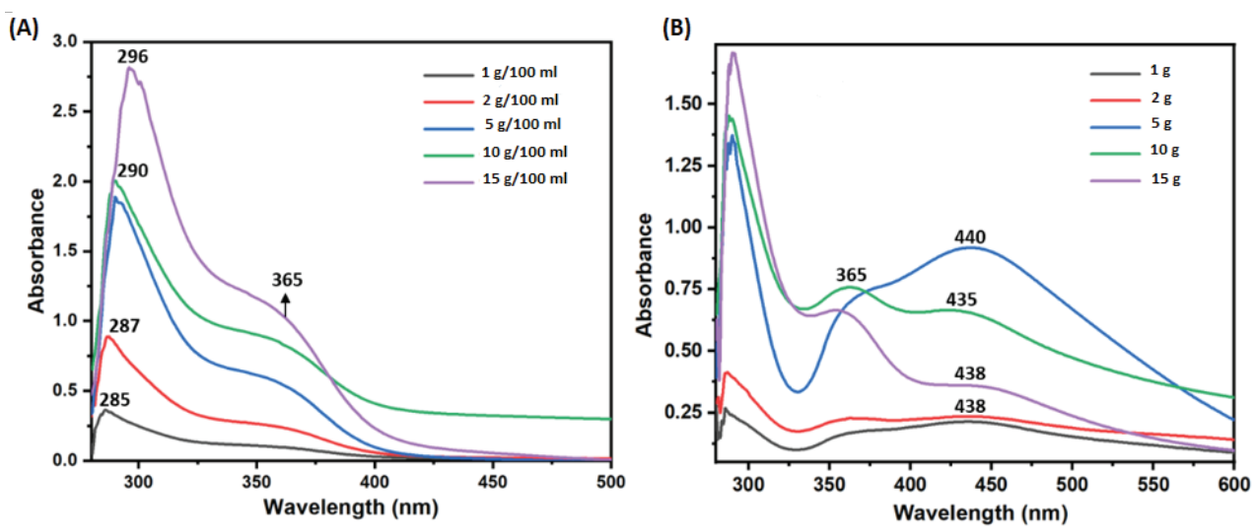

Fig. 5. Ultraviolet - Visible absorption spectra. (**A**) Guava leaf extract samples; (**B**) Silver nanoparticle samples with varying extract concentrations.

3.1.2. $AgNO_3$ concentration

Previous studies have demonstrated that an increase in the concentration of $AgNO_3$ results in the enhanced crystal size and aggregation of silver nanoparticles [28]. However, employing an insufficient amount of $AgNO_3$ leads to a low yield of silver nanoparticles, often significantly lower than the quantities of other components present in the extract. In this study, we examined silver nanoparticle samples using $AgNO_3$ precursor solutions with concentrations of 1, 2, 5, 7, and 10 mM (Fig. 6). At low concentrations of $AgNO_3$, the formation of silver nanoparticles is minimal, leaving an excess of plant extract. Increasing the concentration of silver salt results in a higher yield of silver nanoparticles in the solution. Nevertheless, when the $AgNO_3$ concentration exceeds 7 mM, the absorption intensity tends to decrease. This phenomenon is attributed to an excess of silver ions, which complicates the control of the reaction process, leading to the formation of undesirable silver nanoparticle clusters and a reduction in optical density. Therefore, a concentration of 5 mM silver salt is optimal for controlling particle size and balancing synthesis efficiency with the concentration of the silver nanoparticle solution.

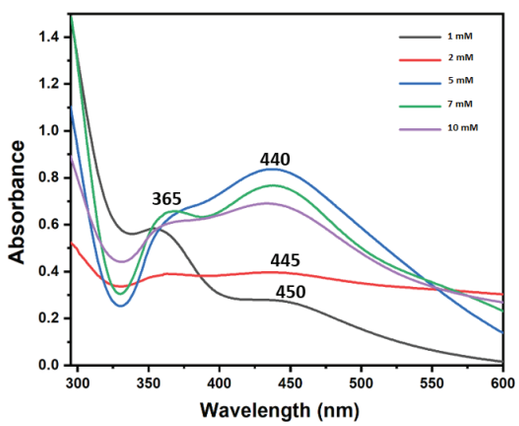

Fig. 6. Ultraviolet - Visible absorption spectra of silver nanoparticles with varying $AgNO_3$ concentration.

3.1.3. Extract/silver nitrate solution

The investigation into the optimal ratio between the extract, which serves as a reducing agent for Ag^+ to Ag^0, and $AgNO_3$ was conducted at room temperature. This study utilised 45 ml of $AgNO_3$ solution at a concentration of 5 mM, with a reaction time of 90 minutes. The UV-Vis spectra of the samples, prepared with varying extract volumes (3, 3.5, 4, 4.5, and 5 ml), are presented in Fig. 7. The formation of silver nanoparticles was evidenced by a characteristic peak in the 440-450 nm range. As the extract volume increased from 3 to 4 ml, the optical density correspondingly increased, indicating a higher formation rate of silver nanoparticles and increased absorption intensity. However, further increases in extract volume beyond 4 ml resulted in a decline in absorption intensity, likely due to the increased size of the silver nanoparticles, which reduced optical density. Consequently, the optimal extract-to-silver salt volume ratio was determined to be 4/45.

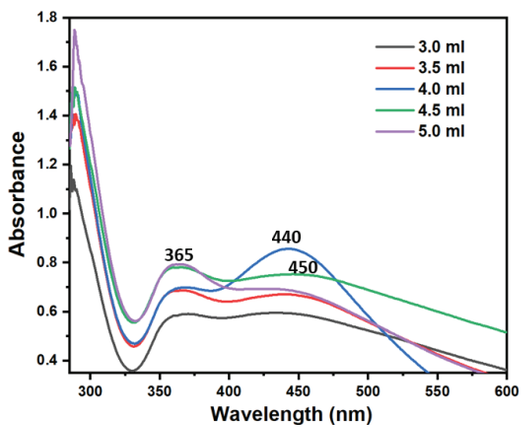

Fig. 7. Ultraviolet - Visible silver nanoparticles as a function of guava leaf extract/$AgNO_3$ solution.

3.2. External factors influencing silver nanoparticle formation

3.2.1. Reaction time

The synthesis process of silver nanoparticles using plant extract adheres to a bottom-up mechanism, as previously described (Fig. 2). In this mechanism, reaction time is a critical factor influencing the formation and stabilisation of nanomaterials. Silver nanoparticles require a specific duration to complete their growth and stabilisation phases. This study employed 4 ml of extract and 45 ml of silver nitrate (5 mM), with varying reaction times of 0, 30, 60, 90, and 120 minutes (Fig. 8). The UV-Vis spectra of the silver nanoparticle samples displayed a peak at 435-450 nm, with absorption intensity progressively increasing as the reaction time extended from 0 to 90 minutes. However, at 120 minutes, the characteristic peak of silver nanoparticles was no longer evident. This observation suggests that the growth and development of silver nanoparticles predominantly occur within the first 90 minutes, with the majority of nanoparticles forming by this time. Beyond this period, the silver nanoparticles tend to continue growing, leading to

larger particle sizes, aggregation, reduced optical density, and sizes exceeding the nanoscale range. Therefore, the optimal reaction time for synthesising silver nanoparticles in this experiment is determined to be approximately 90 minutes.

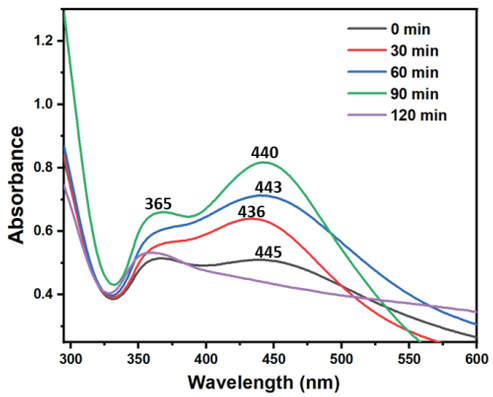

Fig. 8. Ultraviolet - Visible absorption spectra of silver nanoparticles as a function of reaction time.

3.2.2. Temperature

For a chemical reaction, increasing the temperature accelerates the reaction and vice versa. Therefore, the reaction temperature directly affects the formation of silver nanoparticles. In this experiment, temperature was varied at 5, 27 (room temperature), 45, 60, and 75°C, with 4 ml of extract and 45 ml of silver nitrate (5 mM), reacting for 90 minutes (Fig. 9). At 5°C, the reaction rate is slower, but the characteristic peak of silver nanoparticles still appears, although with low optical density. At room temperature, the

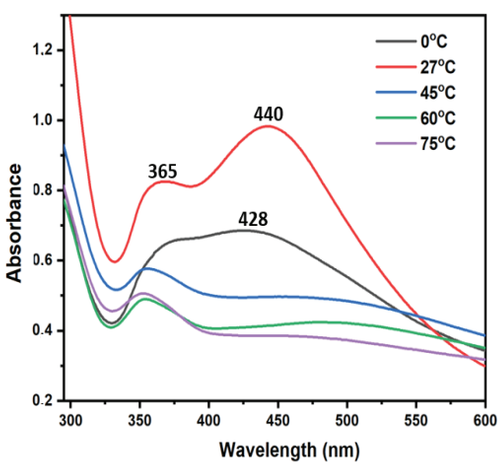

Fig. 9. Ultraviolet - Visible absorption spectra of silver nanoparticles as a function of reaction temperature.

silver nanoparticle peak shows high absorption. However, as the temperature increases further, the reaction rate also increases, and the formation of silver nanoparticles continues during the 90-minute reaction time, leading to larger silver particle sizes exceeding the nanoscale. Therefore, with increasing temperature, the reaction time needs to be reduced to achieve corresponding nanoscale particle sizes. For the conditions used in this experiment (90 minutes reaction time), room temperature is determined to be the optimal temperature for the reaction.

3.3. Formation of silver nanoparticles by reducing agents

To detect functional groups on the surface of silver nanoparticles, FT-IR spectroscopy was employed to identify the elements responsible for the reduction of Ag^+ ions, capping, and stabilisation of silver nanoparticles (Fig. 10). The transmission peak around 3400 cm^{-1} was attributed to the stretching vibrations of -OH groups (alcohol and phenol). A minor peak at 2932 cm^{-1} corresponded to the stretching vibrations of -C-H bonds in alkanes. Additionally, a small vibrational band appearing in the range of 1600-1500 cm^{-1} was associated with C-H bonds and highly conjugated carboxylic functional groups [29].

The formation of silver nanoparticles was determined through the discrepancy between the two spectral peaks and transmission intensities (Fig. 10). In the silver nanoparticle spectrum, the transmission intensities of the regions at 3400 and 2932 cm^{-1} increased compared to the guava leaf extract graph, indicating that characteristic polyphenolic acid molecules in the extract, which carry -OH and -C-H functional groups, interacted with the surface of the silver nanoparticles, causing a decrease in transmission intensity. These two functional groups are considered the main reducing agents in the formation of silver nanoparticles. Additionally, at the wavenumber of 3400 cm^{-1}, a shift in peak position between the two graphs indicated the attachment of organic molecules to the silver nanoparticle surface. The peak shift is due to the interaction of -OH functional groups with the metallic nanoparticle surface, causing the transmission peak to shift slightly towards lower wavelengths.

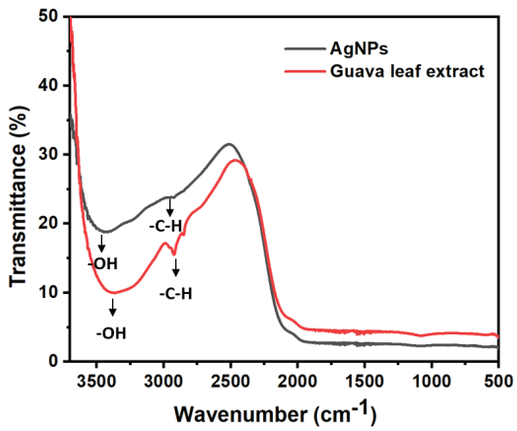

Fig. 10. Fourier-transform infrared spectroscopy spectra of silver nanoparticles and aqueous extract of guava leaves.

contribute to stabilising the structure within polyphenols [30]. The elements Al and K were identified as external agents from the plant growth environment; however, potassium showed a notably higher measurement error compared to the other elements in the extract. The formation of silver nanoparticles reduced the elemental content in the guava leaf extract (Fig. 11A), indicating that the elements C, O, Ca, and Mg in the polyphenolic group had been reduced and deposited onto the surface of the silver nanoparticles. The spherical silver nanoparticles exhibit surface plasmon resonance characteristics and have a distinctive optical absorption in the range of 2.8-3.2 keV. In Fig. 11B, the EDX spectrum reveals the presence of Ag elements, indicating the formation of silver nanoparticles following the reduction

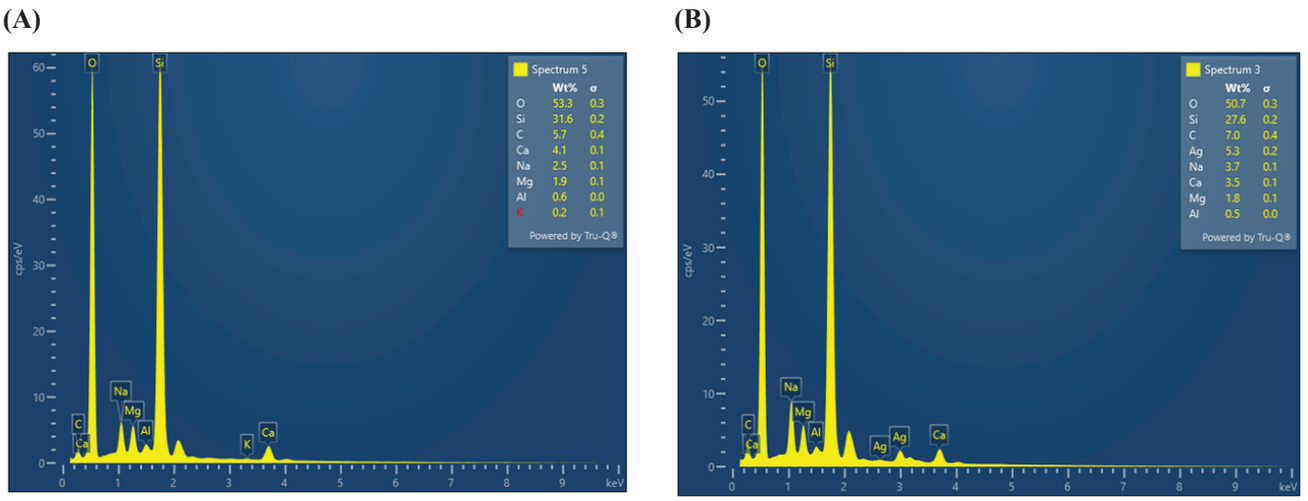

Fig. 11. Energy dispersive X-ray spectrum analysing the elemental composition of the sample. (**A**) Guava leaf extract; (**B**) Silver nanoparticles.

To better understand the components present in the extract and on the surface of silver nanoparticles, EDX analysis was utilised (Fig. 11). Initially, the EDX spectrum of the extract sample revealed numerous elements, with oxygen and silicon being the predominant components. Based on the elemental composition indicated in the EDX spectrum, the elements C, O, Ca, and Mg are characteristic of polyphenolic compounds. The surveyed samples were deposited on silicon substrates, hence the high content of Si and O in the samples. The presence of carbon indicates a significant amount of C=C bonds, hydroxyl (-OH) groups, and carboxyl (COOH) groups with high oxygen content in the extract. Additionally, the metal ion elements Ca and Mg process from the guava leaf extract. Overall, the surface of the silver nanoparticles predominantly contains compounds from the reducing agents in the extract, suggesting that the silver nanoparticle possesses high purity with minimal impurities.

3.4. Pure silver nanoparticles and sample concentration determination

The green synthesis method of silver nanoparticles significantly reduces the amount of hazardous chemicals and solvents, enhancing their safety for biomedical applications [31]. However, plant extracts still pose challenges in controlling stability and may contain certain toxic precursors that can affect the efficacy of

silver nanoparticles [31]. To apply silver nanoparticles in medical or biological contexts, it is crucial to eliminate by-products of the synthesis process. To obtain pure silver nanoparticles, we separated the nanoparticles from the crude suspension using high-speed centrifugation. Specifically, the synthesised silver nanoparticle solution was centrifuged at 12,000 rpm for 30 minutes to sediment the silver nanoparticles, followed by multiple centrifugation cycles in DI water to remove residual compounds. The UV-Vis spectra of the samples before (silver nanoparticle suspension) and after purification (pure silver nanoparticles) (Fig. 12) indicated that the pure silver nanoparticle samples no longer exhibited the absorption peak of the extract at 365 nm. Additionally, the residual extract's absorption bands were also separated from the initial sample, demonstrating that the sedimentation centrifugation method effectively purified the silver nanoparticles. The silver nanoparticle sample with conditions of 4 ml plant extract/45 ml $AgNO_3$ (5 mM), reacting for 90 minutes at 27°C, was determined to have a concentration of 510 ppm using the centrifugation sedimentation method and subsequently dried to obtain powdered silver nanoparticles.

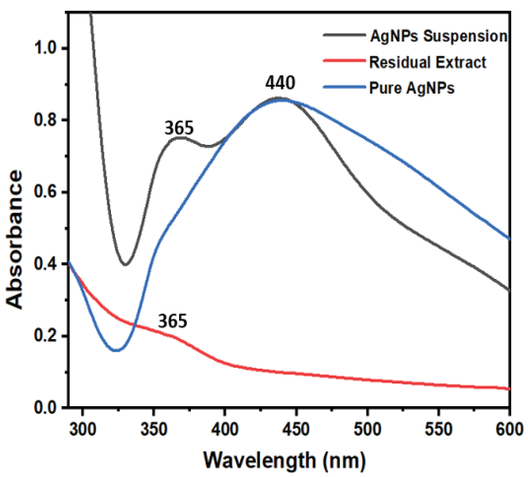

Fig. 12. Ultraviolet - Visible absorption spectra of the silver nanoparticles sample before and after excess extract treatment.

3.5. Crystal properties of silver nanoparticles

The structure and properties of the silver nanoparticles were analysed using XRD with reflections at various angles (Fig. 13). The silver nanoparticles synthesised from guava leaf extract exhibited diffraction peaks at positions 27.76°, 32.31°, 38.22°, 44.28°, 46.37°, 54.88°, 57.54°, 64.49°, and 77.45° corresponding to the crystal planes (210), (122), (111), (200), (231), (142), (241), (220), and (311). The peaks with strong radiation intensity at the planes (111), (200), (220), and (311) are characteristic of silver nanoparticles (matching JCPDS No. 03-0921), indicating that the silver nanoparticles possess a face-centred cubic (FCC) crystal structure [32]. The parameters such as full width at half maximum (FWHM), interplanar spacing, crystal size, and lattice constant were processed using Origin software and are presented in Table 1. The FWHM values were obtained from software analysis, interplanar spacing d (Å) was calculated based on Bragg's law, crystallite size D was determined using the Debye-Scherrer equation, and the lattice constant a (Å) was derived from the d values [29]. The green-synthesised silver nanoparticle sample using guava leaf extract under optimal conditions exhibited an average crystallite size of 9.13 nm and a crystallinity of 70.09%. Additionally, the presence of weaker peaks compared to those of silver nanoparticles indicates the existence of organic compounds on the surface of the silver nanoparticles, resulting from the reduction process using the guava leaf extract [29].

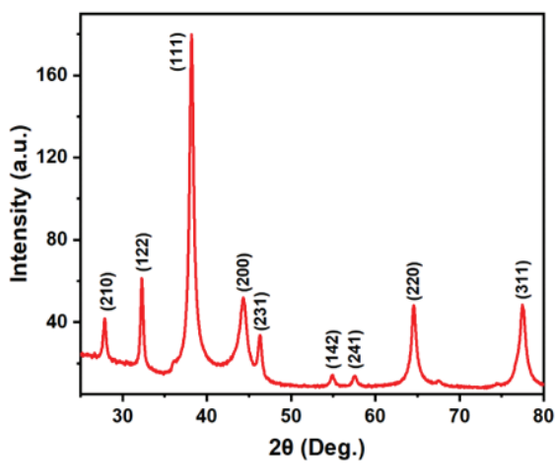

Fig. 13. X-ray diffraction pattern of silver nanoparticles.

Table 1. Structural parameters of silver nanoparticles synthesised from guava leaf extract.

2θ (degree)	FWHM (degree)	Interplanar spacing, d (Å)	(hkl)	Crystallite size D (nm)	Lattice constant, a (Å)
38.194	0.733	2.354	(111)	11.466	4.078
44.279	1.392	2.044	(200)	6.164	4.088
64.571	0.923	1.442	(220)	10.185	4.079
77.460	1.168	1.231	(311)	8.720	4.083

(A) (B)

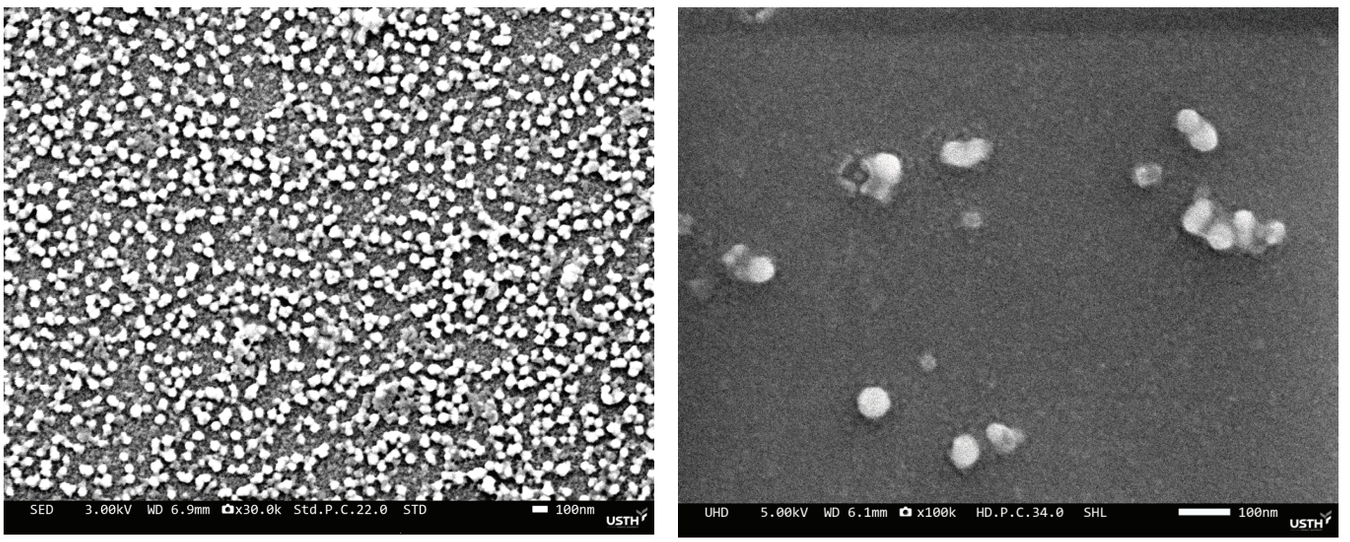

Fig. 14. Field emission-scanning electron microscopy images of silver nanoparticles. (**A**) Magnification 30.0 k; (**B**) Magnification 100.0 k.

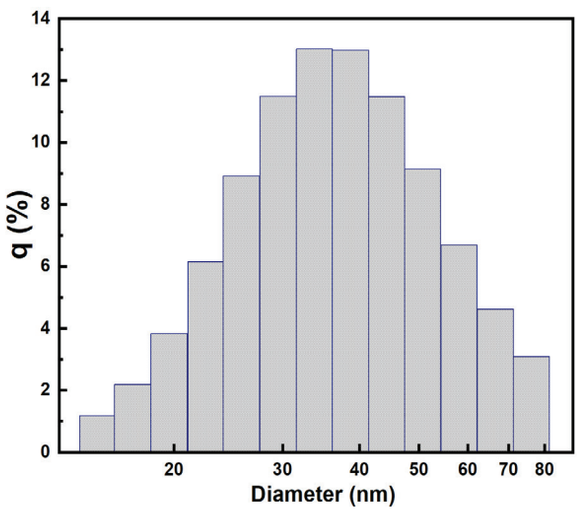

Fig. 15. Particle size distribution of silver nanoparticle determined by dynamic light scattering method.

3.6. Morphology and size distribution of silver nanoparticles

The surface morphology and size of the silver nanoparticles were analysed using FE-SEM (Fig. 14A). The FE-SEM images revealed that the silver nanoparticles were predominantly spherical, with sizes ranging from 30 to 50 nm. Following the washing and filtration process to remove residual extract, the silver nanoparticles exhibited high purity, devoid of agglomerates of $AgNO_3$ and guava leaf extract (Fig. 14B). The particle size distribution, determined by DLS (Fig. 15), indicated that the sizes of the silver nanoparticles ranged from 15 to 80 nm, with the majority falling within the 30-40 nm range. This size distribution is consistent with the sizes observed in the FE-SEM images (Fig. 14).

4. Conclusions

The green synthesis of silver nanoparticles using fresh guava leaf extract was optimised under the conditions of an extract concentration of 5 g leaves/100 ml DI water, a silver salt ($AgNO_3$) solution concentration of 5 mM, an extract/silver salt volume ratio of 4/45, and a reaction temperature of room temperature for 90 minutes. Accordingly, the reduction process of silver ions from $AgNO_3$ to Ag^0 was primarily due to the polyphenol flavonoid compounds present in the guava leaf extract. With the optimal synthesis conditions identified in this study, we successfully synthesised Ag nanoparticles with high purity and achieved a yield of up to 21.64%. The silver nanoparticles exhibited an absorption peak around 440 nm, a face-centred cubic crystal structure with an average crystal size of 9.13 nm, and a crystallinity of 70.09%. Surface morphology observations showed that the silver

nanoparticles were spherical, with particle sizes ranging from 15-80 nm, predominantly distributed between 30-40 nm. After removing the residual guava leaf extract, the silver nanoparticle sample demonstrated good dispersion in the solution, indicating effective capping by polymers during the reduction process, preventing aggregation due to electrostatic interactions between the nanoparticles. Analysing the conditions affecting the synthesis and stabilisation of silver nanoparticles opens a path for developing green silver nanomaterials in Vietnam, with prospects for large-scale production and applications in disinfection, healthcare [33], pest control, and mould prevention [34]. The assessment of the application potential and commercialisation of this material will be addressed in subsequent studies.

CRediT author statement

Dong Duc Tran: Methodology, Original draft preparation, Data collection, Investigation, Funding acquisition, Formal analysis; Van Hoang Bui: Writing, Reviewing, Methodology, Investigation; Quang Nguyen Dinh: Writing, Reviewing, Methodology, Investigation; Duc Cuong Nguyen: Visualisation, Investigation, Formal analysis, Editing; Cong Trinh Bui: Writing, Reviewing, Investigation, Formal analysis; Thao Vu Thi: Experimental Design, Visualisation, Investigation, Funding acquisition, Writing, Reviewing and Editing, Final approval of manuscript.

ACKNOWLEDGEMENTS

This research was funded by the research project QG.23.38 of Vietnam National University - Hanoi. Tran Duc Dong was funded by the Master, PhD Scholarship Programme of Vingroup Innovation Foundation (VINIF), code VINIF.2023.ThS.035.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] S. Hasan (2015), "A review on nanoparticles: Their synthesis and types", *Res. J. Recent. Sci.*, **4**, pp.1-3.

[2] T.B. Taha, A.A. Barzinjy, F.H.S. Hussain, et al. (2022), "Nanotechnology and computer science: Trends and advances", *Memories - Materials, Devices, Circuits and Systems*, **2**, DOI: 10.1016/j.memori.2022.100011.

[3] U. Choudhari, S. Jagtap, S. Rane (2023), "Emerging applications of nanotechnology for e-nose", *Nanotechnology-Based E-Noses*, *Fundamentals and Emerging Applications*, pp.57-100, DOI: 10.1016/B978-0-323-91157-3.00008-8.

[4] I. Khurana, P. Allawadhi, D. Neeradi, et al. (2023), "Introduction to nanoengineering and nanotechnology for biomedical applications", *Emerging Nanotechnologies for Medical Applications*, *Micro and Nano Technologies*, pp.1-34, DOI: 10.1016/B978-0-323-91182-5.00002-4.

[5] N. Joudeh, D. Linke (2022), "Nanoparticle classification, physicochemical properties, characterization, and applications: A comprehensive review for biologists", *Journal of Nanobiotechnology*, **20(1)**, DOI: 10.1186/s12951-022-01477-8.

[6] Y. Ma, L. Shi, F. Liu, et al. (2019), "Self-assembled thixotropic silver cluster hydrogel for anticancer drug release", *Chemical Engineering Journal*, **362**, pp.650-657, DOI: 10.1016/j.cej.2019.01.096.

[7] D. Yan, Q. Yao, F. Yu, et al. (2020), "Surface modified electrospun poly(lactic acid) fibrous scaffold with cellulose nanofibrils and Ag nanoparticles for ocular cell proliferation and antimicrobial application", *Materials Science and Engineering: C*, **111**, DOI: 10.1016/j.msec.2020.110767.

[8] R.A. Praphakar, M. Jeyaraj, M. Ahmed, et al. (2018), "Silver nanoparticle functionalized CS-g-(CA-MA-PZA) carrier for sustainable anti-tuberculosis drug delivery", *International Journal of Biological Macromolecules*, **118**, pp.1627-1638, DOI: 10.1016/j.ijbiomac.2018.07.008.

[9] M.A. Odeniyi, V.C. Okumah, B.C.A. Tayo, et al. (2020), "Green synthesis and cream formulations of silver nanoparticles of *Nauclea latifolia* (African peach) fruit extracts and evaluation of antimicrobial and antioxidant activities", *Sustainable Chemistry and Pharmacy*, **15**, DOI: 10.1016/j.scp.2019.100197.

[10] M.E.K. Kraeling, V.D. Topping, Z.M. Keltner, et al. (2018), "*In vitro* percutaneous penetration of silver nanoparticles in pig and human skin", *Regulatory Toxicology and Pharmacology*, **95**, pp.314-322, DOI: 10.1016/j.yrtph.2018.04.006.

[11] O. Gherasim, A.M. Grumezescu, V. Grumezescu, et al. (2020), "Bioactive surfaces of polylactide and silver nanoparticles for the prevention of microbial contamination", *Materials*, **13(3)**, DOI: 10.3390/ma13030768.

[12] K.D. Mandakhalikar, R. Wang, J.N. Rahmat, et al. (2018), "Restriction of *in vivo* infection by antifouling coating on urinary catheter with controllable and sustained silver release: A proof of concept study", *BMC Infectious Diseases*, **18(1)**, pp.1-9, DOI: 10.1186/s12879-018-3296-1.

[13] M.P. Mishra, R.N. Padhy (2018), "Antibacterial activity of green silver nanoparticles synthesized from *Anogeissus acuminata* against multidrug resistant urinary tract infecting bacteria *in vitro* and host-toxicity testing", *Journal of Applied Biomedicine*, **16(2)**, pp.120-125, DOI: 10.1016/j.jab.2017.11.003.

[14] S. Liao, Y. Zhang, X. Pan, et al. (2019), "Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant *Pseudomonas aeruginosa*", *International Journal of Nanomedicine*, **14**, pp.1469-1487, DOI: 10.2147/IJN.S191340.

[15] N.A. Samoilova, M.A. Krayukhina, D.A. Popov, et al. (2018), "3'-sialyllactose-decorated silver nanoparticles: Lectin binding and bactericidal properties", *Biointerface Research in Applied Chemistry*, **8(1)**, pp.3095-3099.

[16] J.R. Morones, J.L. Elechiguerra, A. Camacho, et al. (2005), "The bactericidal effect of silver nanoparticles", *Nanotechnology*, **16(10)**, DOI: 10.1088/0957-4484/16/10/059.

[17] E.A. Skomorokhova, T.P. Sankova, A.N. Savelev, et al. (2020), "Size-dependent bioactivity of silver nanoparticles: Antibacterial properties, influence on copper status in mice, and whole-body turnover", *Nanotechnology, Science and Applications*, **13**, pp.137-157, DOI: 10.2147/nsa.s287658.

[18] S. Iravani, H. Korbekandi, S.V. Mirmohammadi, et al. (2014), "Synthesis of silver nanoparticles chemical, physical and biological methods", *Research in Pharmaceutical Sciences*, **9(6)**, pp.385-406.

[19] V.K. Sharma, R.A. Yngard, Y. Lin (2009), "Silver nanoparticles: Green synthesis and their antimicrobial activities", *Advances in Colloid and Interface Science*, **145(1-2)**, pp.83-96, DOI: 10.1016/j.cis.2008.09.002.

[20] H. Barabadi, O. Hosseini, K. Jounaki, et al. (2023), "Bioinspired green-synthesized silver nanoparticles: *In vitro* physicochemical, antibacterial, biofilm inhibitory, genotoxicity, antidiabetic, antioxidant, and anticoagulant performance", *Materials Advances*, **4(14)**, pp.3037-3054, DOI: 10.1016/j.scp.2023.3037.

[21] H. Barabadi, H. Vahidi, M. Arjmand, et al. (2024), "Exploring the biological properties of *Saccharomyces cerevisiae*-derived silver nanoparticles: *In vitro* structural characteristics, antibacterial, biofilm inhibition and biofilm degradation, antioxidant, anticoagulant, thrombolytic, and antidiabetic performance", *Inorganic Chemistry Communications*, **162**, DOI: 10.1016/j.inoche.2024.112291.

[22] S.A. Akintelu, Y. Bo, A.S. Folorunso (2020), "A review on synthesis, optimization, mechanism, characterization, and antibacterial application of silver nanoparticles synthesized from plants", *Journal of Chemistry*, **2020(1)**, pp.1-12, DOI: 10.1155/2020/3189043.

[23] A.A. Ntoumba, F.E. Meva, W.E. Ekoko, et al. (2020), "Biogenic synthesis of silver nanoparticles using guava (*Psidium guajava*) leaf extract and its larvicidal action against *Anopheles gambiae*", *Journal of Biomaterials and Nanobiotechnology*, **11(1)**, pp.49-66, DOI: 10.4236/jbnb.2020.111004.

[24] J.M. Ashraf, M.A. Ansari, H.M. Khan, et al. (2016), "Green synthesis of silver nanoparticles and characterization of their inhibitory effects on AGEs formation using biophysical techniques", *Scientific Reports*, **6(1)**, pp.1-10, DOI: 10.1038/srep20414.

[25] D. Bose, S. Chatter (2015), "Biogenic synthesis of silver nanoparticles using guava (*Psidium guajava*) leaf extract and its antibacterial activity against *Pseudomonas aeruginosa*", *Applied Nanoscience*, **6(6)**, pp.895-901, DOI: 10.1007/s13204-015-0496-5.

[26] K.R. Markham, T.J. Mabry (1975), "Ultraviolet-visible and proton magnetic resonance spectroscopy of flavonoids", *The Flavonoids*, pp.45-77, DOI: 10.1007/978-1-4899-2909-9_2.

[27] A. Singh, S. Jha, G. Srivastava, et al. (2013), "Silver nanoparticles as fluorescent probes: New approach for bioimaging", *Int. J. Sci. Technol. Res.*, **2(11)**, pp.153-157.

[28] Y.Z.N. Htwe, W.S. Chow, Y. Suda, et al. (2019), "Effect of silver nitrate concentration on the production of silver nanoparticles by green method", *Materials Today: Proceedings*, **17**, pp.568-573, DOI: 10.1016/j.matpr.2019.06.336.

[29] K. Anandalakshm, J. Venugobal, V. Ramasamy (2016), "Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity", *Applied Nanoscience*, **6**, pp.399-408, DOI: 10.1007/s13204-015-0449-z.

[30] W. Ruksiriwanich, C. Khantham, A. Muangsanguan, et al. (2022), "Guava (*Psidium guajava L.*) leaf extract as bioactive substances for anti-androgen and antioxidant activities", *Plants*, **11(24)**, DOI: 10.3390/plants11243514.

[31] C.S.M. Martins, H.B.A. Sousa, J.A.V. Prior (2021), "From impure to purified silver nanoparticles: Advances and timeline in separation methods", *Nanomaterials*, **11(12)**, DOI: 10.3390/nano11123407.

[32] Y. Meng (2015), "A sustainable approach to fabricating Ag nanoparticles/PVA hybrid nanofiber and its catalytic activity", *Nanomaterials*, **5(2)**, pp.1124-1135, DOI: 10.3390/nano5021124.

[33] L. Ge, Q. Li, M. Wang, et al. (2014), "Nanosilver particles in medical applications: Synthesis, performance, and toxicity", *International Journal of Nanomedicine*, **9**, pp.2399-2407, DOI: 10.2147/IJN.S55015.

[34] S. Allahvaisi (2016), "Effects of silver nanoparticles (AgNPs) and polymers on stored pests for improving the industry of packaging foodstuffs", *Journal of Entomology and Zoology Studies*, **4(4)**, pp.633-640.