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Formation of Silver Nanoparticles Using *Phyllantus buxifolius* Leaf Extract and Its Antioxidant Activity

Dini Hanifa[1*], Purnawan Potana Putra[1], Akmal Djamaan[1], Marta Aulia Zahra[1], and Maharani Auliasanrah Siregar[1]

[1.]Faculty of Pharmacy, Universitas Andalas, Limau Manis, Padang, Indonesia, 25163

Corresponding author: dinihanifa@phar.unand.ac.id

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Abstract: Green synthesis is an approach for producing silver nanoparticles (AgNPs) that more popular because the use of bioreductor, making it safer and more eco-friendly. The use of *Phyllanthus buxifolius* as a source of reducing and stabilizing agents in the formation of AgNPs and its contribution to antioxidant activity is still limited. Therefore, this study aims to synthesize AgNPs using *P. buxifolius* leaf extract and evaluate its antioxidant activity. The leaves of *P. buxifolius* were extracted using ethanol in a 1:10 ratio through maceration, then followed by solvent evaporation using a rotary evaporator. $AgNO_3$ was mixed with a 10% *P. buxifolius* leaf extract solution. The UV-Vis spectrum was analyzed at wavelengths ranging from 400 to 800 nm. Particle size, morphology, functional groups, and crystal structure of AgNPs were characterized. Antioxidant activity was assessed using the DPPH method to determine the IC_{50}, with ascorbic acid as the reference standard. To confirmed the formation of silver nanoparticles, a color change from colorless to brown were observed. These nanoparticles exhibited a Surface Plasmon Resonance (SPR) peak at 420 nm, were non-spherical with the particle size around 50 nm, and showed a crystalline structure. Additionally, the silver nanoparticles synthesized with *P. buxifolius* extract demonstrated comparable antioxidant activity to standard ascorbic acid, indicating their potential to be developed as antioxidant agents for health and therapeutic uses.

Keywords: antioxidant; green synthesis; nanoparticles; *Phyllanthus buxifolius*; seligi.

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1. INTRODUCTION

Nanotechnology is a field of great interest in modern recent studies that focuses on the design, synthesis, and manipulation of particles measuring around 1-100 nm. Nanoparticles are being employed in various fields particularly in medicine, cosmetics, food, the environment, optics, electronics, and drug delivery (Zouaghi et al., 2026). Many world scientists have been interested in nanotechnology which is one of the research areas that continues to grow. The main advantage of nanoparticles lies in their different surface properties compared to large-sized materials, allowing for the modification of material properties such as optical, magnetic, electronic, and mechanical, and their small size provides higher bioactivity (Joudeh and Linke, 2022). This encourages the development of research in the field of nanomaterials and nanotechnology, especially in nanoparticle synthesis.

Among the various types of metal nanoparticles, silver nanoparticles have attracted the most attention due to their broad applications in various fields, particularly in medicine, and their relatively simple synthesis process. Silver nanoparticles are widely used as antimicrobial, anticancer, anti-inflammatory, cosmetic, biosensor, and other materials (Kaushal et al., 2023). These advantages make silver nanoparticles a focus of research in developing more efficient and environmentally friendly synthesis methods.

The synthesis of metal nanoparticles, including silver, can be carried out using various methods, one of the most common methods is reduction using chemical agents. However, this method often uses synthetic chemical compounds that can have a negative impact on the environment and are less safe for medical applications. Therefore, an environmentally friendly approach, referred to "green synthesis" has emerged as an alternative. Stabilizers and natural reducing agents derived from plant extracts or microorganisms is used to reduce harmful impacts. The effectiveness of plant-mediated green synthesis is related to its rich content of secondary metabolites, such as alkaloids, flavonoids, phenolics, terpenoids, vitamins, and other bioactive compounds that act as reducing agents (Alharbi et al., 2022). This makes green synthesis not only safer for the environment but also more potential for medical applications.

P. buxifolius (Seligi), is one of the *Phyllanthaceae* family, has traditionally been used to treat joint pain and skin infections. This plant contains secondary metabolite compounds, include flavonoids, phenolic, tannins, quinones, and triterpenoids (Hardiansyah and Lamid, 2022). Previous research has shown that *P. buxifolius* leaves contain a total phenolic content of 48.44 ± 0.45 GA/g and a total antioxidant content of 26.90 ± 0.19 AA/g (Hanifa and Djamaan, 2023). Furthermore, *P. buxifolius* leaf extract is known to possess various pharmacological activities, such as analgesic, antipyretic, antilipidemic, and anticholesterolemic effects, which have been shown to increase HDL levels and lower LDL levels in the blood serum of mice (Agustiningsih et al., 2014; Handayani and Hastuti, 2023; Hastuti et al., 2015). Other studies have also reported that *P. buxifolius* leaf extract contains bioactive compounds with significant antibacterial activity (Romulo et al., 2018). With its diverse pharmacological activities, *P. buxifolius* has great potential for further development as a beneficial natural-based therapeutic agent.

Plant-derived flavonoids, terpenoids, and phenolic compounds are responsible for reducing silver ions into silver nanoparticles. Meanwhile, other biomolecules such as proteins, amino acids, enzymes, alkaloids, tannins, saponins, terpenoids, and vitamins assist in nanoparticle capping and stabilization. However, the size, shape, and activity of nanoparticles produced through plant extracts are influenced by several factors, such as the type and concentration of compounds in the extract, temperature, and duration of the synthesis reaction (Fahim et al., 2024; Hanifa et al., 2024, 2020).

Phenolic and flavonoid compounds, which are major antioxidants in plants, contribute to the antioxidant activity of both plant extracts and the synthesized silver nanoparticles (Hanifa and Djamaan, 2023). The increasing antioxidant activity of AgNPs, due to the presence of phytochemical compounds encapsulated within the silver nanoparticles (Bedlovičová et al., 2020). Differences in antioxidant activity are likely associated with variations in the bioactive compounds present in the extract functioning as a bioreductor, as well as the concentration of the synthesized silver nanoparticles (Cai et al., 2022).

Biosynthesis of silver nanoparticles using extracts from plants belonging to Phyllanthaceae family, such as *Phyllanthus niruri*, *Phyllanthus acidus*, and *Phyllanthus emblica* have been reported previously. This synthesis produced AgNPs with particle sizes in range 5-250 nm (Basumatary et al., 2024). Compounds such as phyllanthin, hypophyllanthin, ellagitannin, and ellagic acid contain in the plant extract were known to take part in the silver ion reduction process (Iqbal et al., 2021; Qian et al., 2023). However, research on *Phyllanthus buxifolius* as a biomaterial for silver nanoparticles (AgNPs) is still very limited, even though this species has the potential to be a source of phytochemicals for green synthesis. Furthermore, evaluation of the antioxidant activity of AgNPs synthesized using *P. buxifolius* has also remained unreported.

In this study, *P. buxifolius* was utilized as a bioreductor to produce silver nanoparticles. The presence of secondary metabolites in the leaf extract is associated with their role as bioreductor, facilitating the reduction of silver ions (Ag^+) from $AgNO_3$ to form Ag^0 nanoparticles. The obtained nanoparticles were then characterized by appropriate methods including absorption spectrum, particle size, morphology, functional groups, crystallinity, and antioxidant activity.

2. MATERIAL AND METHODS

2.1 Material

P. buxifolius leaves were collected from Padang, West Sumatera, Indonesia during September 2024. Laboratory grade silver nitrate ($AgNO_3$), iron (III) chloride, hydrochloric acid, magnesium powder, Mayer's reagent, sulfuric acid, acetic anhydride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, ethanol, distilled water.

2.2 Methods

2.2.1 Preparation of *P. buxifolius* Leaf Extract.

The leaves of *P. buxifolius* were washed with running water and dried. Following drying, the materials were ground into fine powder and sieved. Leaf extract was prepared by mixing *P. buxifolius* leaf powder and distilled water at a 1:10 w/v ratio and stirring for 15 minutes at 90ºC with a magnetic stirrer. The mixture was then cooled and filtered to separate the extract from the residue. The solvent was subsequently evaporated with a rotary evaporator, resulting in *P. buxifolius* leaf extract. The obtained extract was kept in a closed, airtight container at 4°C.

2.2.2 Phytochemical Screening of *P. buxifolius* Leaf Extract.

Phytochemical screening was employed to determine the presence of secondary metabolite chemicals in the extract. Phenolic compounds were identified with $FeCl_3$, which appeared in blue to purple-black coloring. Flavonoids were identified by reacting concentrated hydrochloric acid with magnesium powder, resulting in an orange to red color change. Alkaloids were examined with hydrochloric acid and Mayer's reagent, resulting in the formation of a cloudy solution or white precipitate. Saponins were detected by shaking the extract and then adding a concentrated hydrochloric acid, which led to the formation of a stable foam. Meanwhile, triterpenoids and steroids were assessed using concentrated sulfuric acid and acetic anhydride, where red or purple color change corresponds to triterpenoids, and a green or bluish-green ring indicates the presence of steroids. (Alamgir, 2018).

2.2.3 Formation of Silver Nanoparticles Using *P. buxifolius* Leaf Extract.

Silver nanoparticles were synthesized using a mixture of 0.5 mL of 0.1 M $AgNO_3$ solution with *P. buxifolius* leaf extract at concentrations of 0.01, 0.02, 0.03, 0.04, and 0.05% in a 50 mL Erlenmeyer flask. The volume was then adjusted to 50 mL with distilled water. The synthesis was carried out under continuous stirring at room temperature at 150 rpm (Hanifa et al., 2024). The color change of the solution from yellow to brown indicates the formation of silver nanoparticles.

2.2.4 Characterization of Silver Nanoparticles Mediated by *P. buxifolius* Leaf Extract

a. UV-Vis Spectra Analysis

A total of 2 mL colloidal solution was collected and examined using UV-Vis Spectrophotometer (Thermo Scientific Genesys 20) at scanning wavelength of 400-800 nm. To separate and eliminate any residue not associated to the silver nanoparticles, the colloidal solution was centrifuged at 10,000 rpm for 30 minutes. The precipitated nanoparticles were then collected from the bottom of the tube, rinsed three times with distilled water. After two hours of drying at 60°C in an oven for 2 hours, samples were stored for further characterization.

b. Size and Morphology Characterization

Particle size analysis was performed using Particle Size Analyzer (PSA) (Horiba SZ-100) operating at scattering angle of 90° and temperature of 25°C, with a measuring range of 0.35–10,000 nm..

Transmission Electron Microscope (Hitachi HT7700) was used to examine the morphology of the silver nanoparticles. A 5 µL aliquot of the colloidal nanoparticle sample was deposited onto a copper grid and allowed to dry prior to analysis. The samples were then observed with an accelerating voltage of 120 kV.

c. Crystallinity Characterization

The crystalline structure of silver nanoparticle was determined using X-Ray Diffractometer (XRD) (X'Pert Powder DY 3688). A sample holder was filled with approximately 10 mg of silver nanoparticles. The measurements were conducted at room temperature using a Cu target with Kα radiation, running at 40 kV and 30 mA. Analysis was performed over a 2θ range of 10°–100°. The resulting diffraction peaks, including their intensities and 2θ values, were analyzed to identify the crystalline structure of the synthesized silver nanoparticles (Hanifa et al., 2024).

d. Functional Group Analysis

FTIR spectroscopy (Shimadzu FTIR Tracer-100) was used to analyze the functional groups of *P. buxifolius* leaf extract and produced silver nanoparticles. About 2 mg of the sample was incorporated to 200 mg of KBr powder, pressed into pellets, and mounted in the sample holder. The spectra were recorded across a wavenumber range of 400-4000 cm^{-1}. The vibrational bands were then examined to characterize chemical functionalities present in both the extract and the silver nanoparticles (Hanifa et al., 2024).

2.2.5 Antioxidant Activity of Silver Nanoparticles from *P. buxifolius* Leaf Extract

A total of 0.5 mL of ascorbic acid standard solution and sample solutions at various concentrations (5, 10, 15, 20, 25 mg/L) were pipetted. Each of the solutions was then vortexed with 1.5 mL of DPPH 0.1 mM solution before being incubated at 37°C in dark conditions. The absorbance was measured at 517 nm after the incubation. The blank and control solutions followed the same process. From the absorbance measurement results, the inhibition percentage is calculated to find the IC_{50} value. The formula for calculating the inhibition percentage is as follows (Ramadhani et al., 2022):

$$\% \, Inhibition = \frac{Blank\ absorbance - Sample\ absorbance}{Sample\ absorbance}\ x\ 100\%$$

A linear regression equation was formulated between the sample concentration and the percent inhibition. IC_{50} was determined by entering the percent inhibition value of 50.

3. RESULTS AND DISCUSSION

3.1 Extraction of *P. buxifolius* leaves

The dark brown extract that was obtained from the ethanol extraction of powdered *P. buxifolius* leaves at a ratio of 1:10 w/v is shown in Figure 1. The extract was utilized as a bioreductor to form silver nanoparticles (AgNPs).

Figure 1. *P. buxifolius* leaves extract

3.2 Phytochemical properties of *P. buxifolius* Leaf Extract.

The phytochemical content of *P. buxifolius* leaf extract is presented in Table 1. Various secondary metabolites were detected in the ethanol extract used in this study. including phenolics, alkaloids, saponins, and triterpenoids. Prastika et al. reported that the ethanol extract of *P. buxifolius* leaves contains phenolic, flavonoids, and terpenoids (Prastika and Muflihah, 2024). Phenolic compounds are known as major antioxidant constituents and are essential in facilitating the reduction of silver ions in the extract.

Table 1. *P. buxifolius* leaf extract phytochemical screening test

No	Phytochemical Test	*P. buxifolius* leaf extract
1	Phenollic	+
2	Flavonoid	-
3	Alkaloid	+
4	Saponin	+
5	Terpenoid	+
6	Steroid	+

3.3 Formation of Silver Nanoparticles

Silver nanoparticle biosynthesis was carried out through the bioreduction of silver ions using *P. buxifolius* leaf extract. The silver nanoparticles colloid was confirmed by the appearance of a brown coloration (Figure 2).

Figure 2. Colloidal silver nanoparticles mediated by *P. buxifolius* leaf extract (extract concentration variations from left to right 0.01, 0.02, 0.03, 0.04, and 0.05%)

The color change reflects the reduction process and is caused by the excitation of surface plasmon resonance (SPR) in the metallic nanoparticles. It occurs during the first 10 minutes, indicating a nucleation phase. Further darkening occurs, reflecting continued particle growth without visible precipitation. The resulting color intensity can vary depending on many factors, such as concentration, reaction time, pH, temperature, and reducing agents (Fahim et al., 2024; Nabila et al., 2026).

3.4 Characterization of Silver Nanoparticles Mediated by *P. buxifolius* Leaf Extract

a. UV-Vis Spectrum Analysis

The absorption peaks of silver nanoparticles were obtained at a wavelength of 420 nm (Figure 3), confirming the formation of silver nanoparticles. The appearance of a peak at a wavelength of around 400-450 nm, is likely due to the excitation of surface plasmon resonance. The Localized Surface Plasmon Resonance (LSPR) property of metal nanoparticles is one of the characteristics that depends on the size, shape, and distribution of the synthesized metal. Silver nanoparticles contain free electrons that are stimulated through the absorption of visible light, and transmitted to a higher energy level, returning to the ground energy level after emitting a photon (Hasan et al., 2022).

Silver nanoparticles synthesized from *Dimorphocalyx glabellus* leaf extract also showed the provision of broadened peaks at 430-450 nm confirming the successful formation of nanoparticles. The presence of a smaller additional peak or shoulder in the spectrum indicates aggregation, while a sharp decrease in absorbance above 500 nm indicates minimal aggregation, and the stability of the nanoparticles (Pandian et al., 2025). Similar findings have been reported that spherical silver nanoparticles typically exhibit absorption in the 400–450 nm range (Soshnikova et al., 2018).

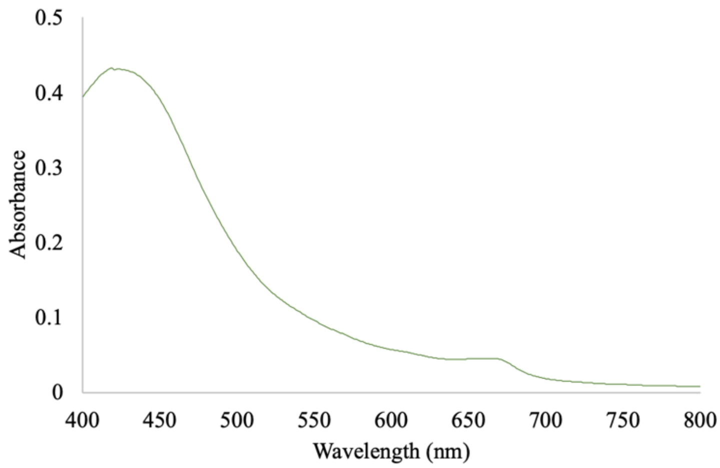

Figure 3. UV-Vis spectrum of silver nanoparticles mediated by *P. buxifolius* leaf extract 0.01%

b. Size and Morphology Characterization

TEM analysis showed an irregular shape of silver nanoparticles and absence of aggregation between particles (Figure 4). Similar shape has been reported in other plant-mediated synthesis such as persimmon peel and *Ficus carica* leaf extract (Al-Malki and Alharbi, 2025; Nabila et al., 2026). Two distinct peaks were observed in PSA result, indicate the particle size within the nanoscale range (<100nm). Overall, the silver nanoparticle formed showed an average diameter size of 50 nm (Figure 5).

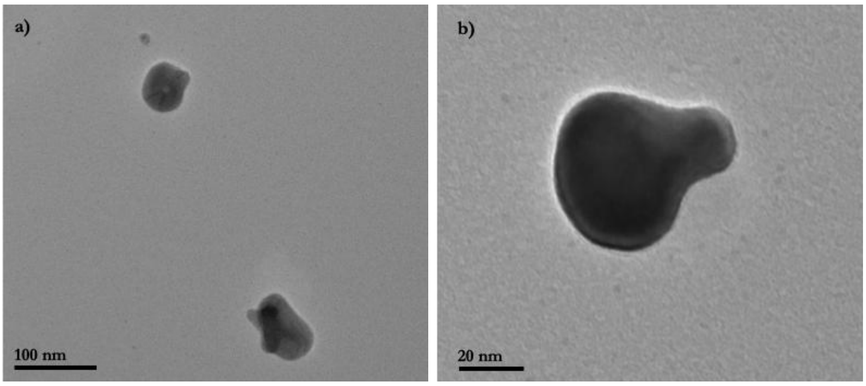

Figure 4. TEM micrographs of silver nanoparticles mediated by *P. buxifolius* leaf extract

The National Nanotechnology Initiative, states that nanoparticles range in size from 1-100 nm (Zouaghi et al., 2026). Various works have reported several plant extracts utilized in biosynthesized silver nanoparticles, including *Acanthus ilicifolius*, *Aerva lanata* and *Bupleurum rotundifolium*, which have particle sizes of 80 nm, 5-15 nm, and 10-16 nm, respectively, and were spherical (Islam et al., 2026; Palithya et al., 2022; Shahabadi et al., 2025).

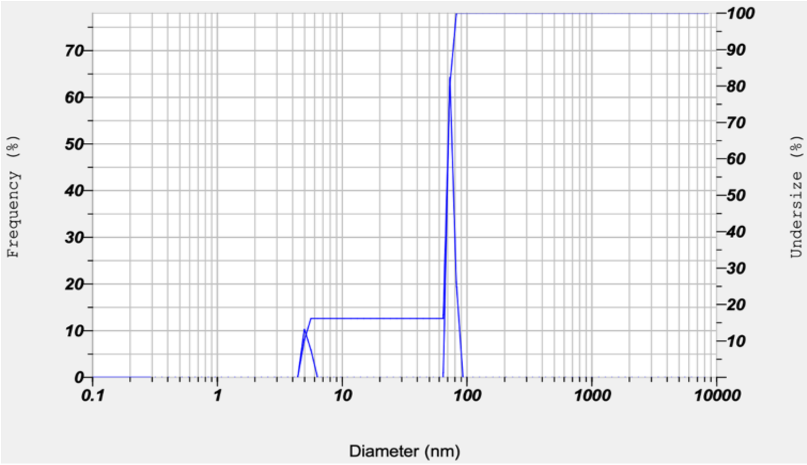

Figure 5. Distribution of silver nanoparticle size mediated by *P. buxifolius* leaf extract

The zeta potential value of silver nanoparticles mediated by *P. buxifolius* leaf extract has a value of -30.7 mV, indicating that the formed nanoparticles were stable. Negative zeta potential indicates the presence of negatively charged functional groups in the extract likely participated in the AgNPs synthesis. A zeta potential value greater than ±30 mV is generally taken to indicate good colloidal dispersion stability due to the repulsive forces between particles (Satria et al., 2026).

c. Crystal Structure Characterization

The XRD analysis of silver nanoparticles shows diffraction peaks at 2θ region = 38.16°, 44.27°, 64.52°, 77.46°, and 81.50°. These peaks correspond to Miller indices (111), (200), (220), (311), and (222) respeectively. These crystallographic planes possess a face-centered cubic (fcc) structure (Figure 6). The diffraction pattern is similar to the standard X-ray diffraction (ICSD #604631). The strong diffraction peaks reflect the high degree of crystallinity and thermodynamic stability of AgNPs with face-centered cubic structure (Satria et al., 2026).

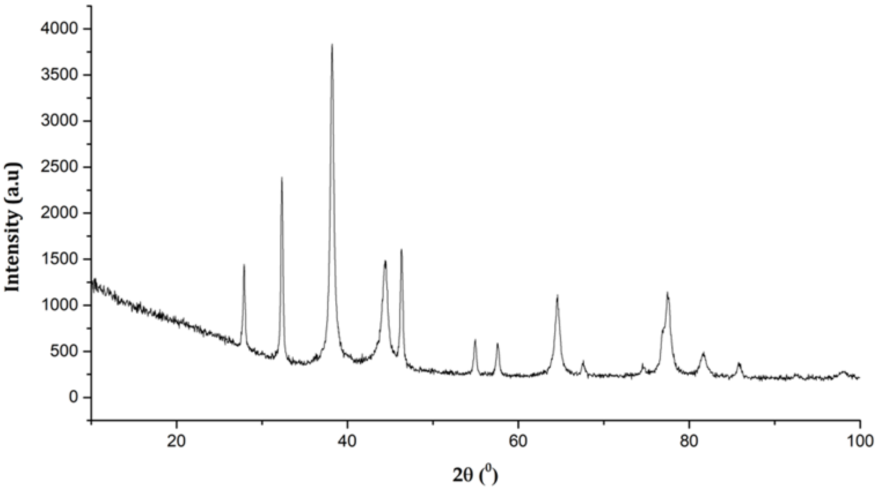

Figure 6. Diffractogram of silver nanoparticles by *P. buxifolius* leaf extract as bioreductor

Hanifa et al. also found a similar crystal structure of synthesized Ag nanoparticle from the bioreduction using surian (*Toona sinensis*) leaf extract. It exhibited distinct diffraction peaks which corresponds to the planes of face centered cubic silver. The crystal size can vary depending phytochemical compounds presence in extract and reactant concentration during silver nanoparticle synthesis (Esmaile et al., 2020; Hanifa et al., 2024).

d. Functional Groups Characterization

Figure 7 shows the FT-IR spectrum of synthesized silver nanoparticles and *P. buxifolius* extract. Broad absorption bands at 3336 cm^{-1} and 3321 cm^{-1}, representing phenolic –OH stretching, 1391 cm^{-1} and 1437 cm^{-1} representing C-C aromatic and C–N stretching vibrations, and absorption bands at 1014 cm^{-1} and 1022 cm^{-1} representing C–O vibrations.

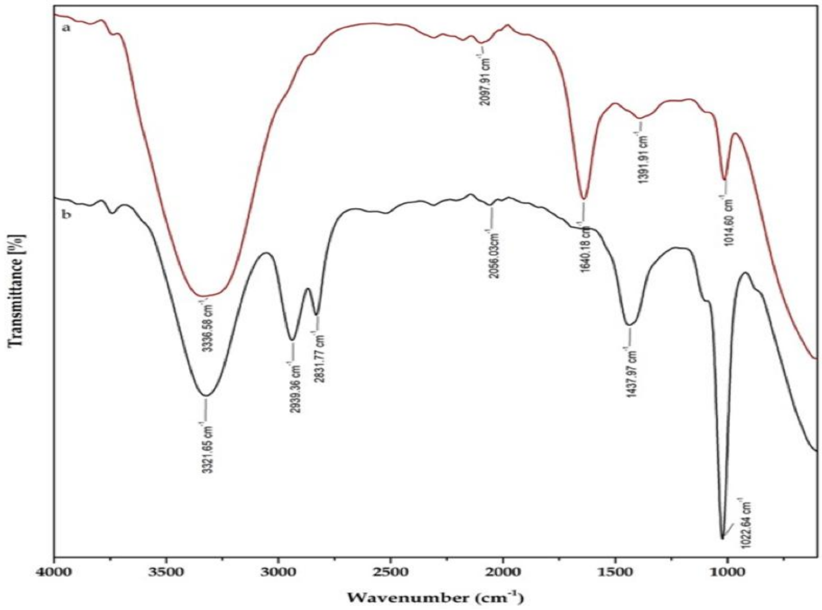

Figure 7. FTIR spectrum of a) silver nanoparticles mediated by *P. buxifolius* leaf extract and b) *P. buxifolius* leaf extract

FT-IR analysis was conducted to identify the functional groups involved in the reduction of Ag^+ ions and stabilization of silver nanoparticles (Ag^0). *P. buxifolius* leaf extract exhibited strong absorption band at 3321 cm^{-1}, corresponding to hydroxyl (O-H) groups stretching vibrations of phenolic compounds or amine (N-H) groups stretching from amino acids or proteins. The peak at 1437 cm^{-1} corresponds to C-C aromatic and C–N stretching vibrations of flavonoids, phenolic, and nitrogen-containing biomolecules. The absorption band in the 1022 cm^{-1} region is associated with C–O stretching vibrations, which may originate from phenolic compounds, flavonoids, and polysaccharides present in the *P.buxifolius* ethanolic extract.

Silver nanoparticles infrared spectrum mediated by *P. buxifolius* leaf extract shows an absorption shift at 3336 cm^{-1}. This confirms the interaction of hydroxyl groups in phenolic compounds and N-H groups in proteins contained in the extract. Hydroxyl and amine groups known to play an important role in the reduction and stabilization of silver nanoparticles (Sadeghi and Gholamhoseinpoor, 2015).

The absorption shift observed from 1437 to 1391 cm^{-1} indicates a decrease in vibrational energy as a result of the chemicals in the extract interacting with the surface of silver nanoparticles through aromatic and amine functional groups. A shift band was also observed at 1022 cm^{-1} to around 1014 cm^{-1} after silver nanoparticles formation, suggesting changes of C-O stretching vibrations. This shift also indicates the involvement of oxygen-containing groups, such as phenolic and flavonoid compounds, as capping and reducing agents during the formation of nanoparticles.

Similar findings were by using *Azadirachta indica L.* extract as bioreductor. The significance shifts were corresponded to hydroxyl, carbonyl, and amino groups from the extract (Velusamy et al., 2015). The presence of phenolic, flavonoids, terpenoids, amino acids, vitamins, and tannins facilitates reduction of Ag ions and stabilized the surface of the produced Ag nanoparticles (Huq et al., 2022).

3.5 Antioxidant Activity Determination using DPPH Method

Antioxidant activity of the DPPH method using ascorbic acid standard and silver nanoparticles synthesized using *P. buxifolius* extract is expressed in IC_{50} values, as seen in Table. 2.

Table 2. IC_{50} of ascorbic acid as reference standard and silver nanoparticles bioreduced by *P. buxifolius* leaf extract

Ascorbic acid or AgNP	Concentration (mg/L)	Inhibition (%)	IC_{50} (mg/L)
Ascorbic acid	0	0.00	13.09
	5	21.34	
	10	36.56	
	15	56.72	
	20	77.12	
	25	94.81	
AgNP	5	17.92	17.88
	10	29.36	
	15	43.99	
	20	56.72	
	25	66.27	

The IC_{50} value of ascorbic acid was 13.09 mg/L, as well as the IC_{50} value of silver nanoparticles was 17.88 mg/L. These results indicate that silver nanoparticles synthesized with *P. buxifolius* extract have relatively strong antioxidant activity. Similar finding also reported that AgNPs biosynthesized by *Phyllanthus emblica* exhibited inhibition rates 85.43% that comparable to standard ascorbic acid (Sharma et al., 2024). Previous study reported silver nanoparticles mediated by *Linum usitatissimum* extract exhibited significant antioxidant activity (IC_{50} of 44.56 µg/mL) than extract only. The presence of phytocompounds, such as phenolics and flavonoids as capping on silver nanoparticles enhance their ability to stabilizing free radicals (Fahim et al., 2024).

Silver nanoparticles have significant antioxidant activity due to their large surface area, and the presence of phytochemical compounds of the extract bound to the surface. The presence of bioactive compound as capping agents enhances antioxidant capabilities. The addition of bioactive substances enhances its antioxidant activity through a synergistic effect, resulting in potent radical-scavenging activity (Fahim et al., 2024).

4. CONCLUSION

P. buxifolius leaf extract can be utilized as a silver ion reducing and capping agent in the formation of silver nanoparticles. The synthesized nanoparticles were 50 nm in size with an irregular shape and face-centered cubic crystal structure. Silver nanoparticles exhibited comparable antioxidant activity to standard ascorbic acid with an IC_{50} value of 17.88 (mg/L). These findings indicate that silver nanoparticles synthesized by *P. buxifolius* leaf extract could be potential to developed as antioxidant agents for health and therapeutic uses. Further research needs to investigate the compounds that are involved in the formation of nanoparticles.

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