Green Synthesis of Silver Nanoparticles Using *Phyllanthus acidus* and Their Catalytic and Biomedical Applications

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ABSTRACT

Phyllanthus acidus, a pantropical plant native to Brazil and widely cultivated in Asia and the Caribbean, is valued for its acidic fruits rich in vitamins and minerals. Traditionally used to treat rheumatism, respiratory issues, and liver disorders, *P. acidus* has recently been employed for eco-friendly silver nanoparticle (AgNP) synthesis. This study investigated AgNP production using *P. acidus* fruit extract, rich in flavonoids and polyphenols. Formation of AgNPs was indicated by a color change from colorless to dark brown and confirmed by a UV-visible absorption peak at 450 nm due to surface plasmon resonance. The synthesized AgNPs were spherical with an average size of 17 nm and exhibited uniform morphology. Biomolecules in the extract acted as reducing and stabilizing agents. Energy-dispersive X-ray analysis detected 55.8% metallic silver, with minor plant residue elements. FTIR analysis identified functional groups related to flavonoids and proteins involved in nanoparticle stabilization. X-ray diffraction confirmed the crystalline face-centered cubic structure of AgNPs. Zeta potential of -31.0 mV indicated strong stability. The AgNPs demonstrated concentration-dependent antioxidant activity, surpassing the crude extract. They also catalyzed degradation of dyes—methylene blue (91%), malachite green (79%), and methyl orange (58%)—within 24 hours, showcasing environmental remediation potential. Additionally, the AgNPs exhibited anticoagulant activity comparable to EDTA, suggesting biomedical applications. Overall, *Phyllanthus acidus* provides a sustainable, effective source for stable AgNP synthesis with promising antioxidant, catalytic, and anticoagulant properties.

Keywords: Green synthesis; Silver nanoparticles (AgNPs); Phyllanthus acidus; Antioxidant activity; Catalytic dye degradation

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INTRODUCTION

Nanoparticles are the fundamental components of nanotechnology. Particles between one and one hundred nanometers in size are known as nanoparticles. The three main types of nanoparticles—organic, inorganic, and carbon-based—have more advanced characteristics than their larger counterparts. Due to their small size, nanoparticles have improved qualities including high reactivity, strength, surface area, sensitivity, stability, etc. Different nanoparticles have various forms, sizes, and architectures [1]. Nanoparticles are utilized for a wide range of purposes, including energy storage, catalysis, sterilization, electronics, sensor technology, biomarkers, and the treatment of some malignancies. They are also used in a number of commercial products, including solar energy and oxide fuel cells. Due to their unique qualities, including antimicrobial activity, strong oxidation resistance, and high thermal conductivity, nanoparticles have gained interest recently [2]. Chemical or biological methods can be used to create nanoparticles. The secret to a viable and environmentally friendly future is nanotechnology.

Nanoparticles are perfect for biological and environmental applications because of their antibacterial, antifungal, disinfectant, and toxicological characteristics [3]. Plant extracts are rich in phytochemicals like flavonoids and phenolics, which act as natural reducing, capping, and stabilizing agents in nanoparticle synthesis. This eco-friendly method is cost-effective, faster than microbial processes, and avoids harmful chemicals.Various plant parts—leaves, stems, bark, and flowers—are used to produce these extracts. They enable the green synthesis of metal nanoparticles such as silver, gold, copper, zinc, and iron.

Phyllanthus acidus (L.), Skullcap is a member of the kingdom Plantae, the subphylum Angiosperms, the class Dicotyledons, the order Euphorbia, the family Euphorbiaceae, and the phyllophyllum. Their regional names include cirmai, grosella (Puerto Rico), jimbilin (Jamaica), karamay (Philippines), Malay gooseberry, Tahitian gooseberry, ground gooseberry, star gooseberry, and Indian gooseberry (Indonesia and Malaysia) [5]. Their regional names include cirmai, grosella (Puerto Rico), jimbilin (Jamaica), karamay (Philippines), Malay gooseberry, Tahitian gooseberry, ground gooseberry, star gooseberry, and Indian gooseberry (Indonesia and Malaysia).. *P. acidus* is commonly used in the treatment of several disorders connected to pain, inflammation, and oxidative stress such as rheumatism, bronchitis, asthma, respiratory disease, liver disease, diabetes, and gonorrhoea [6] The plant is beneficial for curing coughs, dermatitis, skin disorders, and sweating and boosting eyesight, memory, and other bodily functions [7]

In recent years, *Phyllanthus acidus* is used as starting material for the synthesis of nontoxic, eco friendly and cost effective silver nanoparticles [8-9]. However from the literature search, it is comprehended that the synthesis of AgNPs from *P.acidus* fruit extract has not been applied for uses like anticoagulant and dye reduction. Hence, the present study is carried out with the aim to offer a novel approach to the green synthesis of AgNPs by using the fresh whole fruits of *Phyllanthus acidus* as metal reducing and capping agent as well as to obtain evidence for the antioxidant , anticoagulant and dye reduction properties of the synthesised AgNPs.

MATERIALS AND METHODS

Collection and Identification of Plant

The *Phyllanthus acidus* fruit was collected in the local area of Poonamalle in the Thrivallur district. The fruit was taxonomically identified and authenticated by Dr.Akansha Pandey, Research Assistant, Department of Botany, Central Ayurveda Research Institute, Central Council for Research in Ayurvedic Sciences Ministry of Ayush, Government of India, A Government Hospital Campus Arumbakkam, Chennai, India.

PREPARATION 0F PLANT EXTRACT

1 kg of entire, fresh *Phyllanthus acidus* fruits was dried in a hot air oven for 8 days at 50 degrees. The fruit was ready to be ground after drying. Fruit powder weighing 40g was ready for further use. 200 ml of sterile, distilled water was added to 20g of fruit powder, which was then placed in a rotary evaporator for 8 hours. The extract was ultimately gathered on a petri plate. A hot plate was used to dry the extract.

GREEN SYNTHESIS OF SILVER NANOPARTICLES

In 1L beaker, 100g of whole, fresh *Phyllanthus acidus* fruit was combined with 1000 ml of double-distilled water. The suspension was boiled for 20 minutes at 60 ^{0}C while being stirred. In order to prepare the extract for use later, it was filtered through Whatman No.1 filter paper and kept at 4^0 C. About 800ml of 1 mM silver nitrate ($AgNO_3$) was taken in a 1L clean glass beaker and kept on a magnetic stirrer for the production of Ag-NPs. About 400ml of *Phyllanthus acidus* fruit extracts were added to AgNO3 solution drop by drop while being constantly stirred. After two and half hours, AgNO3 reduction took place, which was visible in the reaction mixture's color shift, the mixture was held on the magnetic stirrer. The resulting Ag-NPs were

centrifuged in cooling media for 20 minutes at a speed of 10,000 rpm before being rinsed with double-distilled water. Ag-NPs were meticulously collected and dried. Then, the characterization and application experiments were conducted using Ag-NPs.

CHARACTERIZATION OF SYNTHESISED AgNPs

UV-Vis Spectroscopy

A useful technique for examining the optical characteristics of metal nanoparticles, which vary in direct proportion to their size and shape, is UV-Vis spectroscopy. In this study, a UV-Vis spectrophotometer (model: Shimadzu double beam spectrophotometer) was used to monitor the synthesized Ag-NPs between the wavelengths of 200nm and 600nm.

SEM and EDX

The morphology of the produced silver nanoparticles was examined using Scanning Electron Microscope (SEM). Each sample of manufactured silver nanoparticles was made into a thin film by dropping a tiny amount onto the grid. With the same tool, energy-dispersive X-ray spectroscopy (EDX) analysis was performed. To give both qualitative and quantitative status in elements.

Fourier Transform Infrared Spectroscopy

Using FTIR, it was feasible to identify and foresee potential physicochemical interactions between the constituents of a formulation. Ag-NPs with a wavelength range of 1000 to 3500 nm were the subject of the measurements, and KBr pellets were added to the samples to get spectra.

X-ray Diffraction Spectroscopy

The crystallinity and phase composition of the produced Ag-NPs were determined using an X-ray diffractometer. The main method for figuring out how elemental silver develops is to look at the production of single-phase crystalline silver. From 100 to 800 diffraction intensities were measured. The Debye-Scherrer formula is used to compute the average crystal size.

$$\mathbf{D = 0.94\ \lambda / \beta\ Cos\ \theta}$$

Where D is the average crystalline size of nanoparticles,

λ is the wavelength of the X-ray radiation source

β is the angular full-width at half maximum of the XRD peak at the diffraction angle θ.

Zeta Potential Measurement

The electrostatic potential at a particle's shear plane, known as the Zeta potential, depends on the particle's surface charge and immediate surroundings. Zeta potential measurements offer a crucial test for a colloidal system's stability.

Antioxidant activity by DPPH Method

The scavenging activity of DPPH free radical by Phyllanthus acidus fruit extracts was done according to the method reported [10]. Various Ag-NP concentrations of 10μg-50μg/ml was separately combined with 3 ml of 0.1mM DPPH and incubated for 30 minutes in the dark. An aqueous extract of Phyllanthus acidus was used to scavenge DPPH free radicals. Several Ag-NP concentrations of 10–50 μg /ml were separately mixed with 3 ml of 0.1 mM DPPH and incubated for 30 minutes in the dark. Using a UV-Vis spectrophotometer at 517 nm and methanol as a blank, the samples' absorbance was measured after incubation. The Standard was ascorbic acid, and the control was DPPH methanol reagent without sample. The following formula was used to get the percent of inhibition.

$$\%\ of\ Inhibition = \frac{Absorbance\ control - Absorbance\ test}{Absorbance\ of\ control} \times 100$$

Catalytic degradation of dye

The decolorization of malachite green, methylene blue, and methyl Orange was tested by combining 1 ml of the biosynthesized Ag-NPs at a concentration of 100 g/ml with 9 ml of the dye alone in 10 ppm malachite green, methylene blue, and methyl orange. All of the response test tubes were shaken on a rotary shaker for up

to 24 hours at 100 rpm with an ambient temperature of 30-20 C. Using a UV-Vis spectrophotometer with a 619nm wavelength, the absorbance values of the reaction mixtures were calculated.

$$\%\ of\ Dye\ reduction = \frac{Absorbance\ control - Absorbance\ test}{Absorbance\ of\ control} \times 100$$

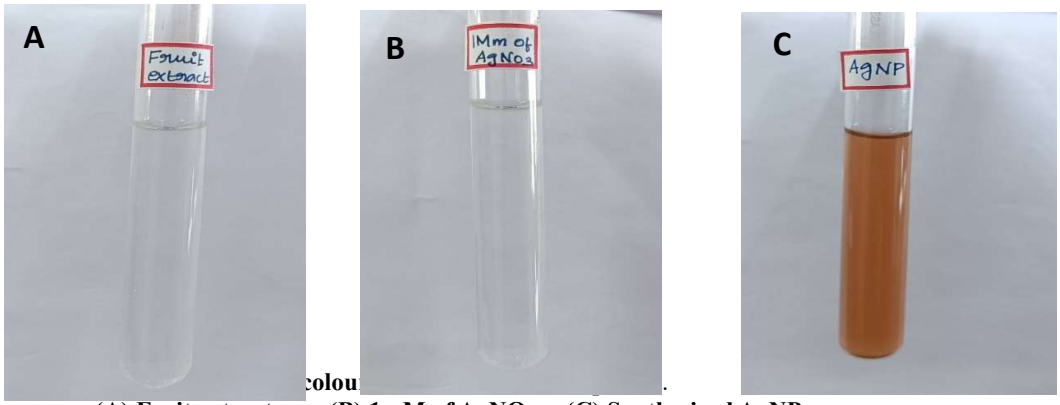
Anticoagulant Assay

Using recently collected human blood, the synthesized nanoparticles were tested for their ability to prevent clotting. By combining 1 ml of 500 g/ml silver nanoparticles with 2 ml of fresh blood obtained from a healthy human volunteer, and then holding the mixture at room temperature to watch for blood coagulation visually, the anticoagulant activity of silver nanoparticles was examined. As positive and negative controls, blood samples were taken in EDTA bottles and regular clean bottles. After being observed visually, the anticoagulant properties of the nanoparticles were examined via an optical microscope by taking pictures of blood samples spread across plates.

Statistical analysis

All the experiments were conducted at least three times (n=3) and the values were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION



colou . (A) Fruit extract, (B) 1mM of $AgNO_3$, (C) Synthesized AgNPs

Figure 1 represents the colour of fruit extract. *Phyllanthus acidu*s contain phytochemical such as flavonoids, terpenoids, and phenolic compounds. In the present study, an attempt has been made toward the green synthesis of silver nanoparticles using *Phyllanthus acidus* fruit extract. Recent studies have shown that AgNPs contribute to the absorption band at approximately 400–500 nm in the UV-visible spectra. However, the preliminary evidence of Ag-Nps formation was the color changes of the reaction mixture in the reaction process. These results can be seen from the colorless to dark brownish color after reaction with Phyllanthus acidus fruit, indicating the reduction of Ag+ to Ago in Ag-NO3 Solution

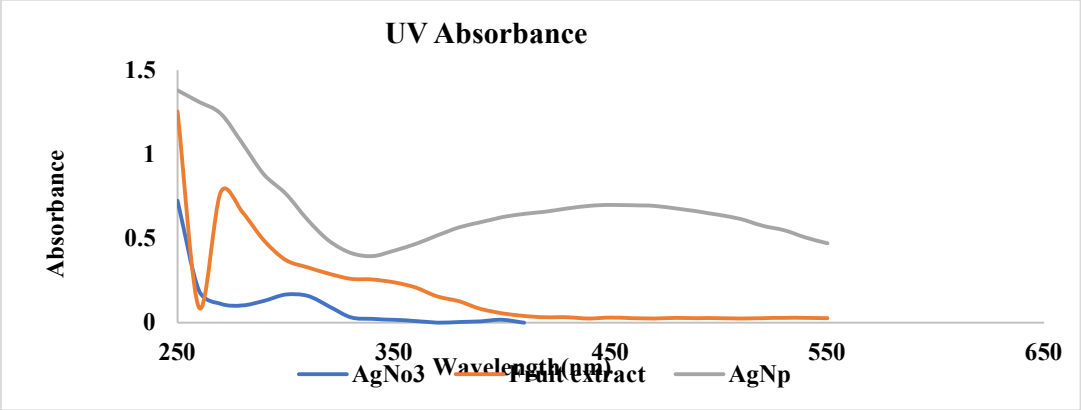


Figure: 2: UV absorbance of silver nanoparticles synthesized using *Phyllanthus acidus* fruit

Figure2 shows the absorbance spectrum of the AgNPs solution after the 2 and 1/2 hour incubation period. A characteristics absorption maximum was found at 450 nm due to the surface plasma resonance of AgNPs. The characteristic absorption spectrum affirms the stability and creation of AgNPs .

Figure: 3 Scanning Electron Micrograph of Silver nanoparticles synthesized using *Phyllanthus acidus* fruit

Phyllanthus acidus-mediated silver nanoparticles were examined using an SEM linked with EDX and the results are shown in (Fig.3) The silver nanoparticles are spherical in shape with an average diameter of 17 nm. The silver nanoparticle's well-defined outer surface indicates the stability of the nanoparticles. A uniform morphology with almost similar size was observed. Phyllanthus acidus possess polyphenols and flavonoids, which acted as reducing and stabilization agent in the formation of silver nanoparticles [11]. The silver nanoparticles' surface area to volume ratio is inversely correlated with how quickly they dissolve, meaning smaller nanoparticles (5 nm) dissolve more quickly than larger ones (50 nm). As a result, the retention of nanoparticles in the site is quite high.

The formation of silver nanoparticles (AgNPs) by plant extracts occurs via two main mechanisms. First, silver ions bind to protein surfaces through electrostatic interactions and are reduced by plant biomolecules, leading to the formation and growth of silver nuclei. Second, polyphenols and organic acids in the extract directly reduce silver ions and act as capping agents, eliminating the need for separate reducing or stabilizing agents[12].

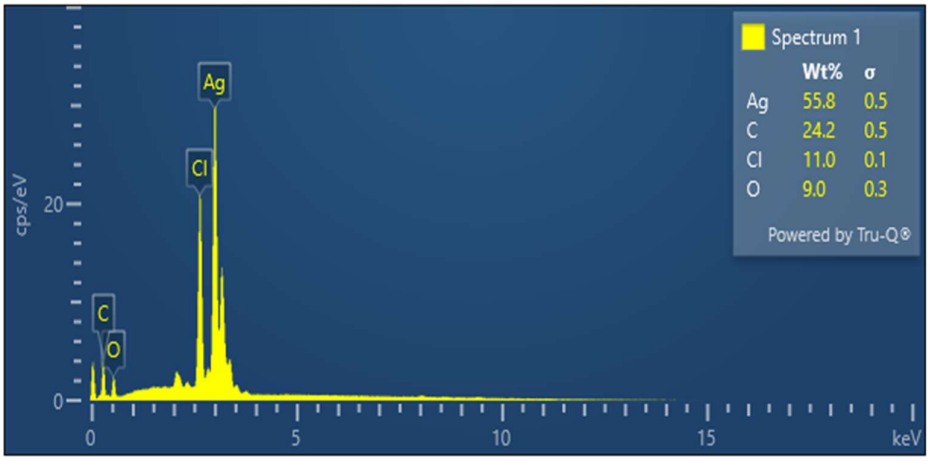


Figure: 4 Energy Dispersive X-ray Spectroscopy (EDX) of silver nanoparticles synthesized using *Phyllanthus acidus* fruit

EDX is a reliable technique for confirming the formation of elemental silver and analyzing sample composition. In Fig. 4, the EDX spectrum shows a strong silver peak at 3 keV, characteristic of metallic Ag, confirming the synthesis of silver nanoparticles using Phyllanthus acidus fruit extract [13]. Minor peaks for C, Cl, and O were also observed, indicating the presence of plant-derived residues. Elemental analysis revealed 55.8% Ag, along with small amounts of Cl (11%), C (24%), and O (9%).

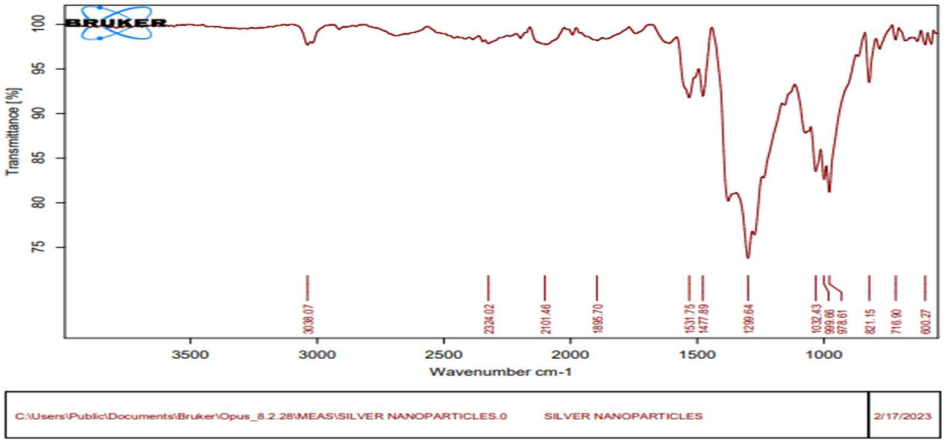


Figure 5: Fourier Transform Infrared Spectroscopy (FTIR) spectrum of silver nanoparticles synthesized using *Phyllanthus acidus* fruit

Figure 5 shows the FTIR spectrum of silver nanoparticles synthesized using Phyllanthus acidus fruit extract. The observed peaks at 3038, 1531, 1477, 1299, 1020, and 978 cm^{-1} indicate the involvement of various functional groups in nanoparticle synthesis and stabilization. Specifically, the peak at 3038 cm^{-1} corresponds to unsaturated system stretching, 1020 cm^{-1} to C–O stretching of ethers, 1531 cm^{-1} to N–O group stretching, and 978 cm^{-1} to C=C stretching of monosubstituted alkenes. These functional groups suggest that flavonoids, polyphenols, and proteins in the extract act as both reducing and capping agents during the green synthesis of AgNPs.

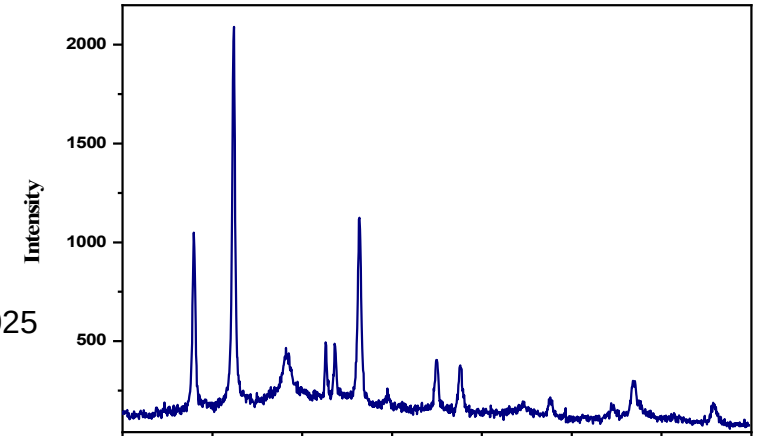


Figure:6 X-ray diffraction (XRD) pattern of silver nanoparticles synthesized using *Phyllanthus acidus* fruit

Figure 6 shows the x-ray diffraction pattern of the synthesized nanoparticles by Phyllanthus acidus fruit extract. The diffractogram revealed the crystalline nature of the synthesized AgNPs. The XRD pattern can be characterized by intense peaks at 27.94; 32.36; 46.37; 39.55; 43.66; 54.96; 57.61; and 76.96. The distinct peaks are indexed by (1049), (2018), (1125), (243), (474), (405), (361), and (233) crystallographic planes, respectively. These peaks match the standard Bragg reflections of elemental silver, indexed to the ICDD file no. 04-0783, and indicate a face-centered cubic (FCC) crystal structure with no additional phases detected [14]. The average crystalline size was found to be 17nm.

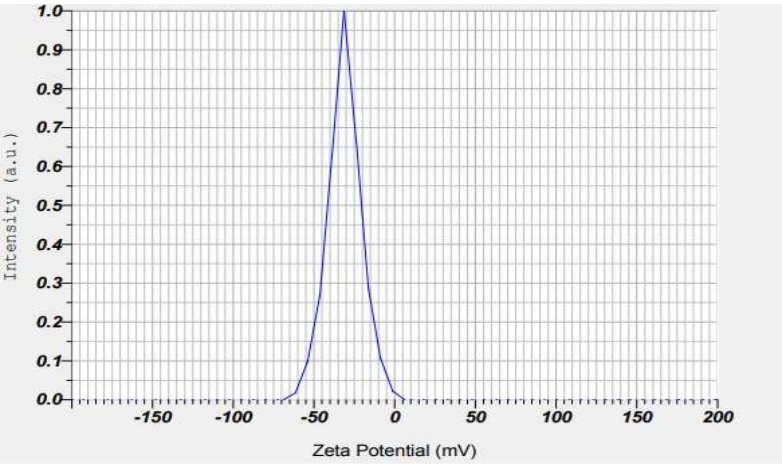


Figure: 7 Zeta Potential Measurement of Silver nanoparticles synthesized using *Phyllanthus acidus* fruit

Figure 7 depicts zeta potential of silver nanoparticles synthesized using *Phyllanthus acidus* fruit. The biosynthesized silver nanoparticles' zeta potential tests revealed a high peak at -31.0 mV , indicating that the nanoparticles' negatively charged surfaces scatter in the medium. The electrostatic repulsive forces between the nanoparticles due to negative charge may prevent their aggregation which may be attributed for the stable nature of silver nanoparticles. In fact that the zeta potential values were negative implying that the capping mechanism of phytochemical components was present in the leaf extract [15] .

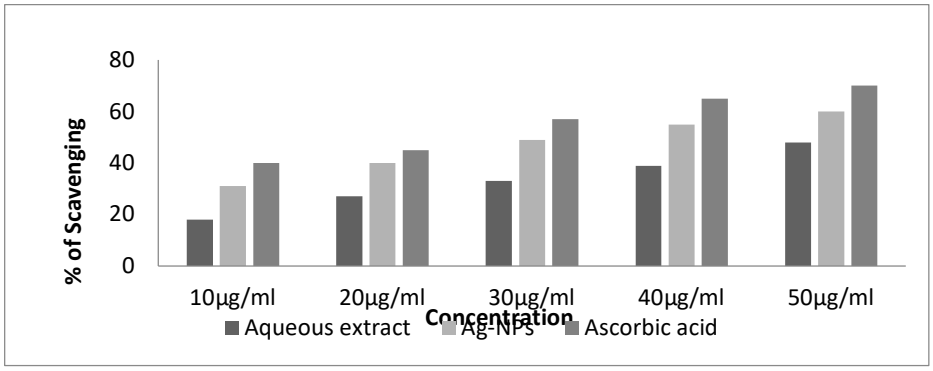


Figure: 8 Percentage inhibition of DPPH Radical Scavenging

Figure 8 presents the percentage inhibition of DPPH radical scaveging activity by synthesised AgNPs and aqueous extract. When the aqueus extract compared to synthesized AgNPs is more reducing power activity. The free radical scavenging activity of Ag-NPs was evaluated to scavenge the free radical DPPH. When DPPH accepts an electron donated by an antioxidant the DPPH is decolorized which can be quantitatively measured

from changes in absorbance. The biosynthesized Ag-NPs of *Phyllanthus acidus* showed concentration-dependent scavenging activity i.e., with an increase in the concentration of NPs, the scavenging activity also increased when compared to the aqueous extract of *Phyllanthus acidus* is relatively higher than the biosynthesized Ag-NPS.

Table: 1 Percentage of catalytic dye reduction by silver nanoparticles synthesized using *Phyllanthus acidus* fruit

S.NO	DYE	24 HOURS (%)
1	Methylene blue	91
2	Malachite green	79
3	Methyl orange	58

Methylene Blue (MB), a commonly used basic dye in medicine and staining, turns from blue to colorless upon reduction to leucomethylene blue [16]. Malachite Green (MG), a toxic cationic dye widely used as a coloring agent, and Methyl Orange (MO), an azo dye used as an indicator with known health hazards, were also effectively degraded. The results demonstrate the catalytic potential of *Phyllanthus acidus*-mediated Ag-NPs for environmentally friendly dye removal applications [17]. The catalytic dye reduction ability of green-synthesized Ag-NPs was evaluated over 24 hours by monitoring absorbance changes at 619 nm. Visual decolorization was initially observed, followed by colorimetric measurements to quantify dye degradation. As shown in Table 1**,** the Ag-NPs effectively reduced MB by 91%**,** malachite green (MG) by 79%**,** and methyl orange (MO) by 58%**.**

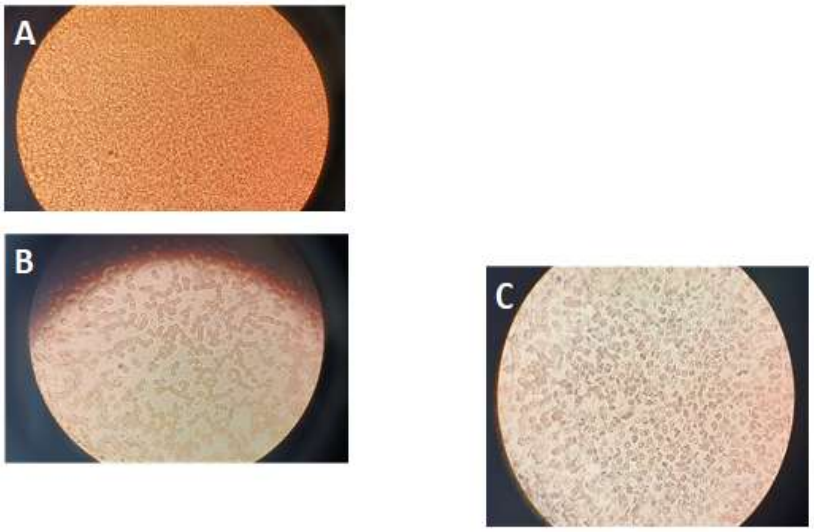


Plate 1 Anticoagulant assay (a) Clotted blood, (b) Blood with EDTA, (c) Blood with synthesized Ag-NPs

The anticoagulant potential of silver nanoparticles (Ag-NPs) synthesized using Phyllanthus acidus fruit extract was evaluated in this study. Given the eco-friendly and biocompatible nature of the synthesis process, these Ag-NPs are suitable for biomedical applications, including anticoagulation. As shown in plate (1) , three blood samples were compared: (a) normal clotted blood, (b) blood treated with EDTA (a standard anticoagulant), and (c) blood treated with synthesized Ag-NPs. Microscopic examination revealed that the Ag-NPs effectively inhibited blood clotting, similar to EDTA. These findings suggest that Phyllanthus acidus-derived Ag-NPs exhibit promising anticoagulant activity and may offer potential therapeutic applications in managing coagulation disorders.

CONCLUSION

The present study introduces a novel, green approach for synthesizing silver nanoparticles (Ag-NPs) using fresh whole fruits of *Phyllanthus acidus*. The fruit extract effectively reduced silver nitrate to form stable, spherical Ag-NPs with an average size of 17 nm and crystalline structure. The biosynthesized nanoparticles demonstrated notable radical scavenging**,** dye degradation, and anticoagulant activities. Overall, this method is simple, rapid, cost-effective, and environmentally friendly, highlighting the potential of *P. acidus*-derived Ag-NPs for biomedical and catalytic applications.

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REFERENCES

1. E.J. Cho, H. Holback, K. Liu, S. Abouelmagd, J. Park and Y. Yoon, Mol. *Pharmaceutics*, **10**, 2093 (2013).
2. S. Machado, J. Pacheco, H. Nouws, J. Albergaria and C. Delerue-Matos, *Sci. Total Environ.*, **533**, 76 (2015).
3. A.M. Ealias and M. Saravanakumar, *Mater. Sci. Eng. Conf. Ser*., **263**, 032019 (2017).
4. M. Hassaan and S. Hosny, *Int. J. Atmos. Ocean. Sci.,* 2, 10 (2018).
5. S.-P. Tan, E.N.-Y. Tan, Q.-Y. Lim and M.A. Nafiah, J. Ethnopharmacol., **253**, 112610 (2020).
6. R. Chakraborty, B. De, N. Devanna and S. Sen, *Asian Pac. J. Trop. Biomed.,* **2**, 953 (2012).
7. R. Brooks, A. Goldson-Barnaby and D. Bailey, *Int. J. Fruit Sci.,* **20**, 1706 (2020).
8. C. Sowmya, V. Lavakumar, N. Venkateshan, V. Ravichandiran and D.V.R. Saigopal, *Chem. Cent. J.,* **12**, 42 (2018).
9. A. Bose Mazumdar Ghosh, A. Banerjee and S. Chattopadhyay, Nucleus (Calcutta), **65**, 437 (2022).
10. S.-T. Chang, J.-H. Wu, S.-Y. Wang, P.-L. Kang, N.-S. Yang and L.-F. Shyur, *J. Agric. Food Chem*., **49**, 3420 (2001).
11. K. Buddhachat, S. Chomdej, W. Pradit, K. Nganvongpanit and S. Ongchai, *Planta Med*., **83**, 87 (2017).
12. S. Rajeshkumar and L. Bharath, *Chem. Biol. Interact.,* 273, 219 (2017).
13. R.S.A.P.C. Sorna Kumar, P.C. Kunthavai and S. Gnaniah, *Int. Res. J. Pharm.,* **5**, 207 (2014).
14. S.A. Dhar, R.A. Chowdhury, S. Das, M.K. Nahian, D. Islam and M.A. Gafur, Mater. Today: Proc., **42**, 1867 (2021).
15. T. Varadavenkatesan, R. Selvaraj and R. Vinayagam, *J. Mol. Liq.,* 221, 1063 (2016).
16. A. Ilunga and R. Meijboom, *J. Mol. Catal. A: Chem.,* **411**, 48 (2016).
17. V. Thivaharan, S. Raja and V. Ramesh, *J. Mol. Liq*., **221**, 30 (2016).