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Vasyl Stefanyk Precarpathian National University

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Abhimanyu P. Pawar[1], Kishor S. Naktode[1], Arvind J. Mungole[2]

Green synthesis of silver nanoparticles from whole plant extract analyzed for characterization, antioxidant, and antibacterial properties

[1]*Department of Chemistry, Nevjabai Hitkarini College, Bramhapuri, Maharashtra, India,* abhaypawar1988@gmail.com
[2]*Department of Botany, Nevjabai Hitkarini College, Bramhapuri, Maharashtra, India*

In this analysis, a green synthesis method utilizing a plant extract derived from *Rumex nepalensis* (Spreng.) was employed to synthesize silver nanoparticles. The synthesized nanoparticles were thoroughly characterized for their structural, surface morphological, optical, antioxidant, and antibacterial properties. Structural analysis revealed a face-centered cubic structure, while FTIR analysis confirmed the presence of biosurfactant molecules in the leaf extract that acted as reducing agents. SEM and TEM analyses further confirmed the spherical shape of the nanoparticles, with a size range of 19-28 nm. The evaluation of the silver nanoparticles demonstrated their antioxidant and antibacterial properties. These nanoparticles exhibited activities in both antioxidant and antimicrobial realms, showcasing their potential as dual-functional agents. This study highlights the effectiveness of the green synthesis method using *Rumex nepalensis* (Spreng.) extract for the production of silver nanoparticles with desirable properties for various applications.

Keywords: Green synthesis, Plant extract, silver nanoparticles, Antibacterial activity, Antioxidant activity.

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Introduction

In the past century, Nanoscience and nanotechnology are recognized as major focal points in materials science research. These fields involve the production of nanoparticles, which are particles with a size of less than 100 nm [1]. Nanoparticles can act as building blocks for various physical and biological systems, opening up new opportunities in fields such as medicine, electronics, and energy. They exhibit novel properties that are maintained with particular features like morphology, shape, and size [2]. As a result, nanotechnology has contributed to the development of biosensors, space technology, polymers, ceramics, biomedicine, catalysis, cosmetics, [3-9] and many more, leading to advancements and improvements in various industries. Metal-based nanoparticles have become increasingly popular in various fields due to their unique physicochemical properties. Platinum, Titanium Oxide, Copper Oxide, Magnesium Oxide, Palladium, Gold, Iron Oxide, etc. are some of the widely used metal-based nanoparticles. These nanomaterials have shown promising results in applications such as drug delivery, imaging, tissue engineering, and plant growth promotion. Researchers from diverse fields have been also shown interest towardthe zero-valent metals nanoparticle like Ag, Fe, and Zn with potential applications [10-11]. Their antimicrobial properties make them a suitable candidate for disinfection and sterilization purposes [12]. Additionally, their unique optical and electrical properties make them ideal for use in electronic devices and sensors [13]. With their numerous applications, silver nanoparticles hold great potential for advancing technology and improving the quality of life. The formation of silver nanoparticles has been studied through diverse approaches, including chemical, physical, and biological methods [14]. However, silver nanoparticles synthesis using physical and chemical methods has some drawbacks, such as the use of expensive instruments, poor cost-effectiveness, high energy requirements, and the use of toxic chemicals such as organic solvents as stabilizing or reducing agents, which can be harmful to living organisms, including humans [15]. As a result, researchers have been searching for alternative methods for synthesizing silver nanoparticles that are more environmentally friendly and cost-effective.

One such method is the biological method (biosynthesis/green synthesis), which uses natural resources such as plant extracts and microorganisms to reduce and stabilize the nanoparticles. This method has the advantage of being eco-friendly, very simple, and potentially more cost-effective than chemical methods [16]. In the biological method, plant extracts offer several advantages as a platform for nanoparticle synthesis, such as the ease of extraction, lower cost, and the absence of complex cell culture maintenance or downstream processing steps required with microorganisms [17]. Plant extracts contain various biomolecules such as flavonoids, terpenoids, and alkaloids that act as reducing and stabilizing agents for nanoparticle synthesis [18]. Therefore, green synthesis of silver nanoparticles by using plant-mediated has gained more attention. Reports have highlighted the use of different plant components, such as leaves, stems, fruits, and seeds, in the green synthesis of silver nanoparticles. This method has shown superior results than chemical and physical methods. Numerous studies have reported the use of plant extracts for green synthesis/biosynthesis of silver nanoparticles. These plant extracts have been obtained from various sources such as *Bergenia ciliate* [19], *Zingberofficinale* [20], *Calliandrahaemato cephala* [21], *Ricinus communis* [22], *Crocus sativus L.* [23], *Calotropis gigantean* [24], *Capparis zeylanica* [25], *Conocarpus Lancifolius* [26], *Myrtus communis* [27], and so on. Antifungal and antibacterial properties of silver nanoparticles have been demonstrated in well diffusion assays against pathogenic microbes, including Salmonella sp., Escherichia coli Staphylococcus aureus, Bacillus subtilis, Aspergillus niger and Aspergillus flavus [28 -29]. Green synthesized silver nanoparticles from *Sargassum muticum* [30], *Brassica oleracea* [31], *Rhodiolaimbricata* [32], *Mangiferaindica* [33], *Alpinia katsumadai* [34] *etc*. have been studied for their antioxidant activity by some researchers. The scarcity of research on the antioxidant and antibacterial properties of silver nanoparticles synthesized with plant extracts, as well as many more nanoparticles synthesized from different plant-based sources by several workers, motivated the authors to cost-effectively and eco-friendly green-synthesize silver nanoparticles using an extract from the promising plant *Rumex nepalensis* (Spreng.).To analyze the nanoparticles, UV-Vis spectroscopy, X-ray diffraction (XRD), Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM), and Scanning Electron Microscopy (SEM) were utilized and also studied their potential activities (antioxidant and antibacterial).

I. Experimental section

Materials and methods:

The preferred plant *Rumex nepalensis* (Spreng.) was collected from the Bramhapuri area of Chandrapur district in Maharashtra state, India, and used to prepare an extract. Silver nitrate ($AgNO_3$), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Butylated hydroxyl toluene (BHT), were procured from Sigma-Aldrich chemical.

Preparation of extract from whole plant material

To prepare the plant extract, the collected *Rumex nepalensis* (Spreng.) plant was carefully cleaned with de-ionized water and slice into small pieces before being dried under shade. The entire plant was then powdered with the help of a mortar and pestle, and 30 g of the powder was added to 300 mL of distilled water in a clean, dry round bottom flask. The mixture was then boiled at 60-70°C for at least 30 minutes before being cooled to room temperature. The solution was then filtered through a Whatman number 41 filter paper, and the obtained filtrate was stored in a refrigerator for further use in the preparation of silver nanoparticles.

Bio-synthesis of silver nanoparticles:

The addition of a 50 Ml *Rumex nepalensis* (Spreng.) plant extract to a 250 mL conical flask, along with a 50 mL solution of 1 mM silver nitrate solution added dropwise from a burette covered with black paper. The mixture is stirred with magnetic stirrer at room temperature until a color change (brown to dark brown) is observed which primary indicates the formation of silver nanoparticles after complete addition of the silver nitrate solution. That color changed solution was centrifuged for 20 minutes at 10,000 rpm and washed three times with double distilled water to remove plant metabolites. At the end, formed silver nanoparticles were collected and further kept for characterization studies.

Characterization of nanoparticles:

The nanoparticles were characterized using various methods: X-ray absorption spectrometry [35-36] determined elemental analysis, electronic structure, and composition; scanning electron microscopy [37] analyzed size and morphology; Fourier transmission infrared spectroscopy [38] identified functional groups; transmission electron microscopy [39] analyzed size; energy dispersive X-ray elemental analysis [40] assessed chemical composition and purity; and UV-visible spectroscopy [41] confirmed nanoparticle formation and synthesis.

The characterization of silver nanoparticles produced via reduction of silver nitrate with a plant extract was carried out using a spectrophotometer (EQUIP-TRONICS, Model EQ-826) by recording spectra in the range of 190 to 1100 nm. Thermo Nicolet iS50 FTIR Spectrometer analyzed samples by FT-IR in 4000-100 cm^{-1} range. The Brucker AXS D8 X-ray diffraction technique with copper as an X-ray source was used to perform crystallographic and structural analyses of prepared silver nanoparticles. TEM (Jeol/JEM, 2100, at an accelerating voltage of 200 kV), and SEM-EDX (Jeol 639Ola/OXFORD XMX N), used for silver nanoparticles size, morphology, and composition analysis. SAIF, Kochi (India) performed all of the analysis.

Antioxidant activity of silver nanoparticles:

Antioxidant activity states to the ability of a substance to prevent or slow down the oxidative damage caused by free radicals. To determine the scavenging capacity percentage, the DPPH assay was used with Brand Williams's method to evaluate the antioxidant activity of silver nanoparticles [42]. To evaluate the antioxidant activity of different Ag NPs, solutions of varying concentrations (20, 40, 60, 80, and 100 µg/mL) were prepared in methanol along with a standard reference, butylated hydroxytoluene (BHT). To each solution, 3 mL of 4% DPPH solution was added, followed by thorough mixing, and incubation in a dark place at room temperature for 30 minutes. The absorbance of the reaction mixture at 517 nm was then measured using a UV-visible spectrophotometer. BHT was used as a comparison standard for the experiment, and the entire procedure was performed in triplicate to ensure the accuracy and reproducibility of the results [43].

The percentage of scavenging capacity was determined with the following formula:

% RSA = [(abs$_{517}$ nm of control – abs$_{517}$ nm of sample) / abs$_{517}$ nm of control] × 100

(% RSA- percentage radical scavenging activity, Abs -absorbance)

Antimicrobial activity of silver nanoparticles:

Antimicrobial activity refers to the ability of a substance to inhibit or kill microorganisms. It is measured by evaluating the substance's ability to create a zone of inhibition when tested against specific microorganisms. The larger the zone of inhibition, the stronger the antimicrobial activity of the substance. The antimicrobial activity of silver nanoparticles (Ag NPs) at different concentrations (25 µl, 50 µl, and 100 µl) was evaluated against four bacterial organisms: *E. coli*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*. The well diffusion method was used to assess the antimicrobial activity, and the results were compared with the standard antibiotic, amikacin 30 mcg.

II. Results and Discussion section

UV-visible spectroscopy:

UV-visible spectroscopy is a useful technique for characterizing the optical absorption properties or energy structure of nanoscale materials. One of the simplest methods to verify nanoparticle synthesis is to observe the color change that occurs due to surface plasmon resonance, where the excitation of outer surface electrons decreases with decreasing particle size. This technique relies on the relationshipbetween particle size and optical properties [44]. The spectroscopic analysis of silver nanoparticles synthesized using *Rumex nepalensis* (Spreng.), Fig.1 showed a broad absorption spectrum between 350 and 800 nm with peak value at 395 nm, indicating the presence of silver nanoparticles [45-46].

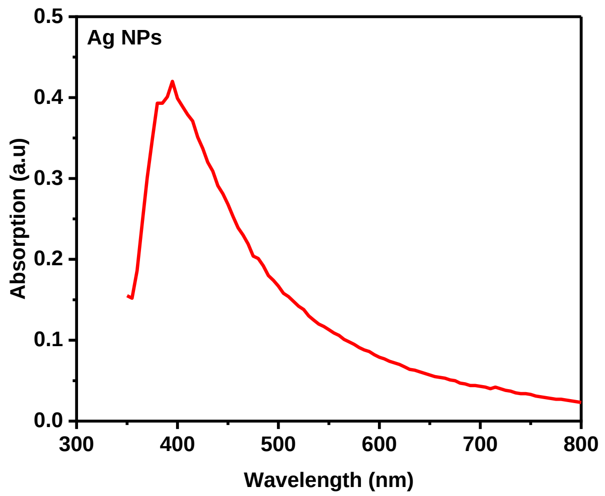

Fig. 1. UV–vis spectrum of prepared silver nanoparticles.

FT-IR analysis

FTIR measurements were conducted to identify main functional groups and their possible roles in the synthesis and stabilization of silver nanoparticles. The spectrum of synthesized silver nanoparticles can be found in the provided Fig.2. The N-H stretching band at 3725.3 cm^{-1} and 3423.32 cm^{-1} indicated the availability of primary and secondary amides, while the OH stretching band at 3423.32 cm^{-1}suggested the presence of alcohol functional groups [47].The peaks at 2922.01 cm^{-1}and 2851.46 cm^{-1}corresponded to the asymmetric stretching of -CH groups, indicating the presence of alkyl groups. The spectral bands at 1624.95 cm^{-1}, 1382.70 cm^{-1}, and 1021.74 cm^{-1} suggested the presence of alkenes, carbonyl compounds, and C-O stretching, respectively [48-49]. The observed spectral peaks at 826.07 cm^{-1}, 778.73 cm^{-1}, and 609.03 cm^{-1} could be attributed to the presence of $C=CH_2$ stretching, acetylenic C-H bend with alkynes, and alkyl halide group [50-51].

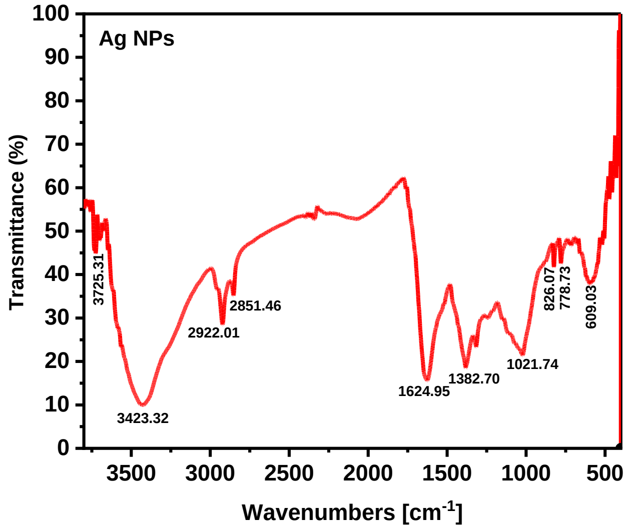

Fig. 2. FTIR spectra of synthesized silver nanoparticles.

Overall, the FT-IR spectrum study concluded that the plant extract contains polyphenolic, amine, and carbonyl functional group-containing components that can act as capping agents and reducing agents. These functional groups participated in the reduction of silver ions.

Table 1.
Calculated parameters for silver nanoparticles include crystallite size, interplanar spacing, and dislocation density

Plane	2-Theta value (Degree)	FWHM	Crystallite size (D) nm	Theta value (Degree)	Interplanar Spacing(d) (Å)	Dislocation Density $\delta=1/D^2$	Crystallinity of material
(210)	27.916	0.26297	31.1290208	13.958	3.19347535	0.00103197	70.2571
(122)	32.333	0.26057	31.7433573	16.1665	2.76658815	0.00099242	
(111)	38.496	0.4203	20.0206146	19.248	2.33666625	0.00249485	
(200)	46.321	0.32451	26.6268189	23.1605	1.95851518	0.00141046	
(242)	54.905	0.36275	24.6792213	27.4525	1.67088529	0.00164186	
(141)	57.548	0.36488	24.8393695	28.774	1.600271	0.00162076	
(220)	64.499	0.30319	30.9818362	32.2495	1.4435714	0.0010418	
(311)	76.804	0.46289	21.8999454	38.402	1.24006895	0.00208504	

XRD analysis

XRD (X-ray diffraction) is a powerful analytical technique that provides information about the crystal structure and composition of materials based on X-ray scattering patterns. The crystalline nature of silver nanoparticles was confirmed by Fig.3. displaying characteristic diffraction peaks, with distinct peaks at 2θ values of 27.917°, 32.333°, 38.496°, 46.321°, 54.905°, 57.548°, 64.499°, and 76.804° indexed to miller indices (210), (122), (111), (200), (242), (141), (220), and (311) for silver nanoparticles from *Rumex nepalensis* (Spreng.). These results were matched with JCPDS database file no. 04-0783and therefore, confirming FCC (face centered cubic) structure for crystalline silver [52-54]. Unassigned spectra seen possibly from bioorganic remnants on synthesized nanoparticle surfaces during crystallization [55].

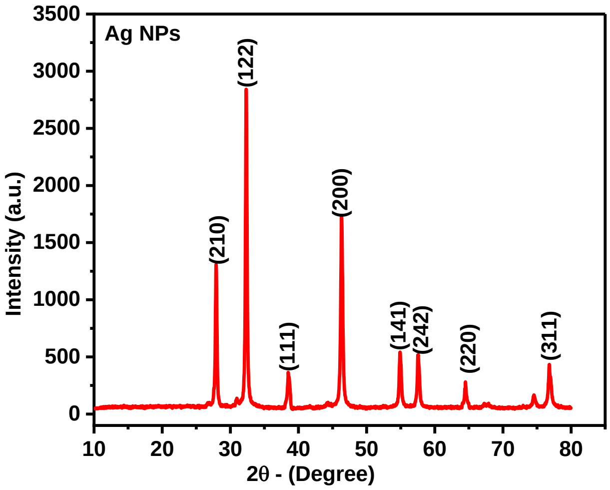

Fig. 3. XRD spectrum of synthesized silver nanoparticles.

Further, the average crystallite size of nanoparticles in a material based on the width of their X-ray diffraction peaks can be calculated by Scherrer equation. The equation relates the peak broadening (in radians) to the crystallite size (in nanometers), the X-ray wavelength (in angstroms), and the peak angle (in degrees) according to the following formula:

$$D = (K * \lambda / \beta \cdot \cos\theta)$$

Where D is the average crystallite size, λ is the wavelength of the X-rays used, θ is the Bragg angle (the angle at which the peak occurs), β is the full width at half maximum (FWHM) of the peak, and K is a dimensionless shape factor (usually assumed to be 0.9). The average crystallite size of synthesized silver nanoparticles was found to be 26.4900 nm. Other properties, such as crystallinity (70.2571%), dislocation density (0.0015), and individual interplanar spacing (d) were also measured and reported in the table 1.

TEM image of silver nanoparticles

Transmission electron microscopy can be used to image and characterize nanoparticles at the nanoscale, providing insights into their size, shape, and distribution. It allows for the measurement of particle size, size distribution, and morphology, making it a valuable technique for nanomaterials research and development.as depicted in Fig. 4a and 4b, The TEM analysis revealed that the silver nanoparticles exhibited a high degree of dispersion and were predominantly spherical in shape and had an average size range of 19-28 nm.The use of selected area electron diffraction (SAED) in Fig. 5a suggests that the prepared silver nanoparticles from *Rumex nepalensis* (Spreng.) are highly crystalline in nature, as evidenced by the presence of a spherical pattern. The SAED pattern is a result of the interference of electron waves that have interacted with the crystal structure of the nanoparticles.

EDAX Analysis

Energy dispersive X-ray analysis (EDAX) is a technique for elemental analysis of a sample by measuring energy and intensity of X-rays emitted from the sample. It provides information on elemental composition, chemical, and crystallographic structure. The EDX spectrum of synthesized silver nanoparticle (Fig. 5b.) reveals a significant silver signal at around 3 keV. The presence of chlorine and other organic components in the spectrum could suggest that it originated from the photochemical components of the plant extract [56]. This highlights the potential of EDX in identifying the elemental composition and source of elements in a sample.

SEM image of silver nanoparticles

Scanning electron microscopy is important analytical technique used to identify the surface morphology and topography of nanomaterials, as seen in the images of formed silver nanoparticles through Rumex nepalensis (Spreng.) in Fig.6a and 6b. The SEM images show that the silver nanoparticles have an almost spherical shape and form sponge-like bunches due to surface agglomeration. The size of the nanoparticles is influenced by several factors, including the procedure, the condition, the concentration of reducing agents, and the various phytoconstituents exist in the plant extracts [57-58].

Antioxidant activity:

The DPPH radical scavenging activity of Ag nanoparticles synthesized through green methods is depicted in Fig. 7. The DPPH free radical accepts an electron or hydrogen from a donor atom, resulting in a reduction [59]. Antioxidants donate hydrogen to the odd electron in DPPH, converting it to hydrazine [60]. The use of UV-Visible spectrophotometer makes DPPH a simple and quick way to assess antioxidant activities. The dose-dependent radical scavenging behavior of Ag nanoparticles was observed [50 µg/ml – 18.224%; 100 µg/ml – 28.349%; 150 µg/ml – 41.589%; 200 µg/ml – 50.156 %], with a significant result at 250 µg/mL [68.069 %]. Similar studies have also demonstrated that

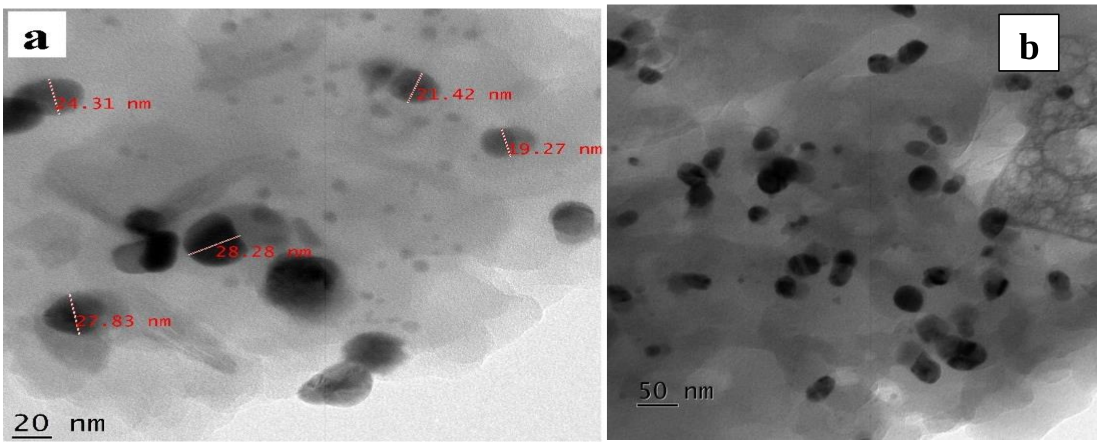

Fig. 4. TEM Images of synthesized silver nanoparticles at 20 and 50 nm scale.

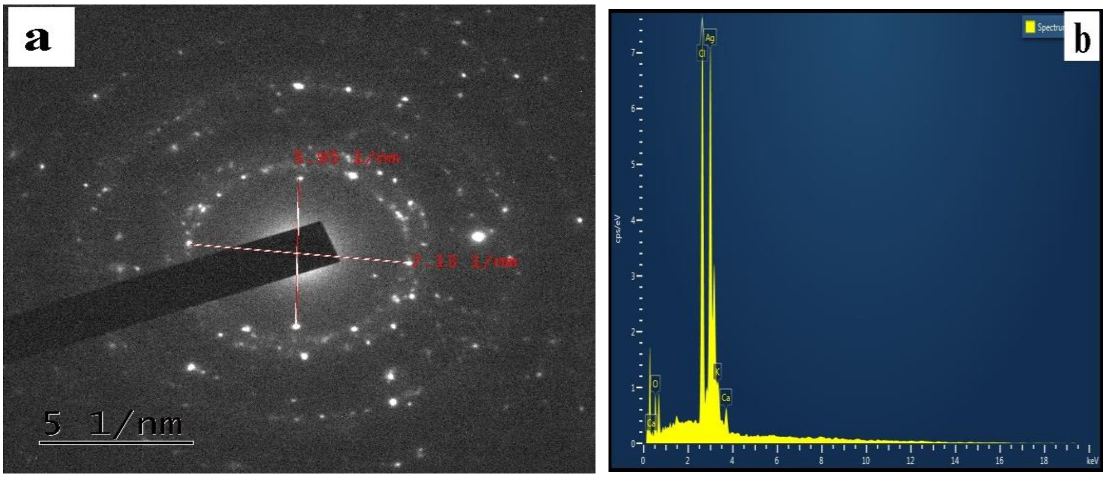

Fig. 5. SAED pattern and EDAX images of synthesized nanoparticles.

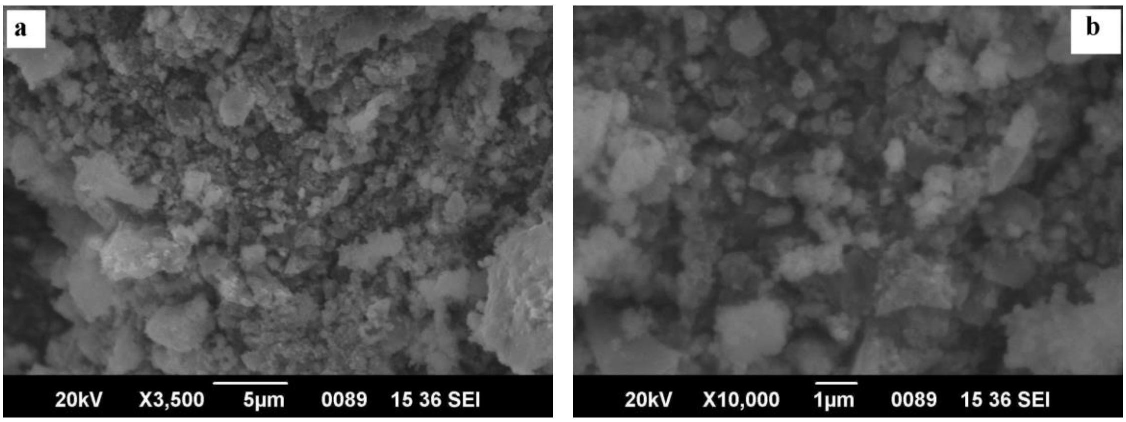

Fig. 6. Scanning electron microscopy images of synthesized silver nanoparticles.

nanoparticles can enhance the antioxidant properties of materials [61]. The antioxidant activity of silver nanoparticles may be enhanced by the presence of alkaloids, phenolic compounds, flavonoids, and other compounds in *Rumex nepalensis* (Spreng.), which are well-coupled to nanomaterials. As a result, silver nanoparticles may have potential in treating many currently deadly diseases.

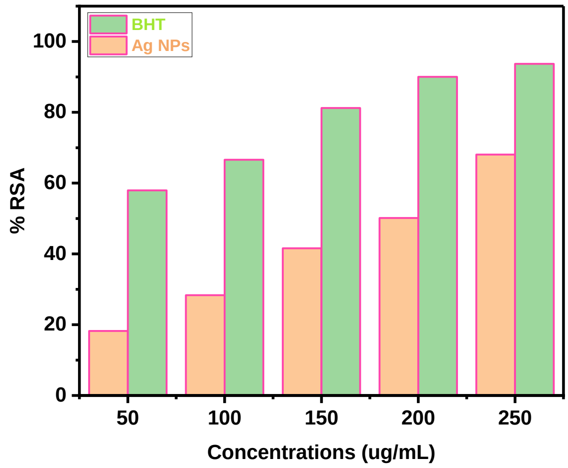

Fig. 7. DPPH scavenging activity of silver nanoparticles.

Antimicrobial activity

The study evaluated the antimicrobial activity of silver nanoparticles (Ag NPs) at different concentrations (25 µl, 50 µl, and 100 µl) against four bacterial organisms: *E. coli, S. aureus, P. aeruginosa*, and *K. pneumoniae* (Fig.8). The well diffusion method was used to assess the inhibitory effect, and the results were compared with the standard antibiotic, amikacin 30 mcg in table 2.

Amikacin effectively inhibited the growth of *E. coli*, as indicated by a 20 mm zone of inhibition. However, the silver NPs at a concentration of 25 µl did not exhibit any inhibitory effect against *E. coli*. At higher concentrations of 50 µl and 100 µl, the silver NPs demonstrated zones of inhibition measuring 10 mm and 17 mm, respectively. For *S. aureus*, amikacin displayed a zone of inhibition measuring 25 mm, indicating its sensitivity. The silver NPs at concentrations of 25 µl, 50 µl, and 100 µl showed zones of inhibition measuring 17 mm, 20 mm, and 22 mm, respectively. This suggests that the Ag NPs possessed moderate antimicrobial activity against S. aureus, slightly lower than the effectiveness of amikacin. In the case of *P. aeruginosa*, amikacin exhibited a zone of inhibition measuring 18 mm, indicating sensitivity. However, the silver NPs at concentrations of 25 µl, 50 µl, and 100 µl displayed zones of inhibition measuring 10 mm, 10 mm, and 14 mm, respectively, indicating relatively lower antimicrobial activity compared to amikacin. Regarding *K. pneumoniae*, amikacin demonstrated a zone of inhibition measuring 22 mm, indicating strong sensitivity. However, the silver NPs at a concentration of 25 µl did not show any inhibitory effect. At concentrations of 50 µl and 100 µl, the silver NPs exhibited zones of inhibition measuring 11 mm and 13 mm, respectively. At a high

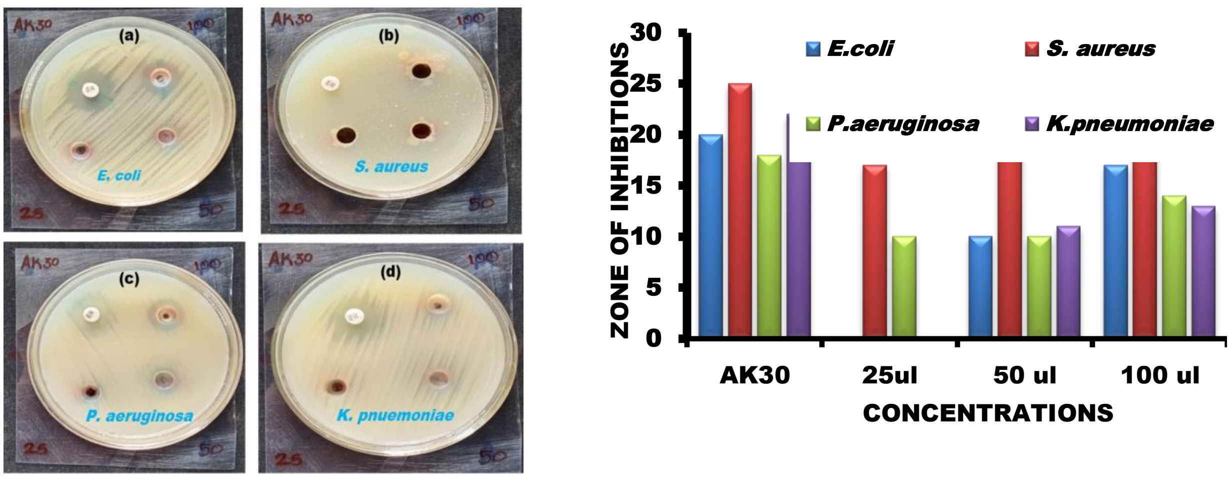

Fig. 8. Provides visual representation of the antibacterial activity of synthesized silver nanoparticles was assessed against four bacterial organisms: a) *E. coli, b) S. aureus, c) P. aeruginosa, d) K. pneumoniae* and e) relative antibacterial activity of silver NPs and amikacin (AK30 µg).

Table 2.

Antimicrobial activity of silver NPs against microorganisms

ZONE OF INHIBITION (mm)				
Organisms	Concentrations			
	AK30	25 µl	50 µl	100 µl
E. coli	20	NI	10	17
S. aureus	25	17	20	22
P. aeruginosa	18	10	10	14
K. pneumoniae	22	NI	11	13

Note: E. coli =Escherichia coli, S. aureus= Staphylococcus aureus, P. aeruginosa= Pseudomonas aeruginosa, K. pnuemoniae = Klebsiella pnuemoniae, NI= No Inhibition. AK30= Amikacin 30 mcg.

concentration of 100 µl, the silver NPs showed moderate to strong antimicrobial activity against all tested organisms, including *E. coli, S. aureus, P. aeruginosa,* and *K. pneumoniae*. In summary, the study found that silver nanoparticles exhibited varying degrees of antimicrobial activity against the tested bacteria, with higher concentrations generally resulting in larger zones of inhibition.

Conclusion

Silver nanoparticles were successfully synthesized using whole plant extracts of *Rumex nepalensis* (Spreng.). This process was not efficient but also eco-friendly. Various characterization techniques such as UV-vis spectrophotometry, XRD, FTIR, and SEM confirmed the reduction of silver nitrate salt to silver nanoparticles.TEM images revealed a size range of 19 - 28 nm. The antimicrobial screening test demonstrated the formation of zones of inhibition, indicating the strong antimicrobial activity of the synthesized Ag NPs against human pathogenic bacteria and nanoparticles also exhibited strong antioxidant properties. The biologically synthesized silver nanoparticles hold great potential in the medical field due to their effective antimicrobial and antioxidant properties, making them valuable for various medical applications.

Utilizing plant sources for nanoparticle synthesis offers a promising alternative to the time-consuming process of cultivating and maintaining microbial cultures. This approach is poised to have a significant impact in the coming years, circumventing potential drawbacks associated with microbes. Building upon prior research, this study presents a novel route for nanoparticle synthesis, holding potential benefits for pharmaceutical formulations within the field of medical science, following rigorous experimentation.

Abhimanyu P. Pawar – M.Sc. in Chemistry, Assistant Professor;
***Kishor S. Naktode* – Ph.D,** M.Sc. in Chemistry, Assistant Professor;
***Arvind J. Mungole* –Ph.D,** M.Sc. in Botany, Assistant Professor.

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А.П. Павар[1], К.С. Нактоде[1], А.Дж. Мунголе[2]

"Зелений" синтез наночастинок срібла з цілого рослинного екстракту, їх фізичні характеристики, антиоксидантні та антибактеріальні властивості

[1]*Хімічний факультет, коледж Невджабай Хіткаріні, Брамхапурі, Махараштра, Індія, abhaypawar1988@gmail.com;*
[2]*Кафедра ботаніки, коледж Невджабай Хіткаріні, Брамхапурі, Махараштра, Індія*

Розглянуто метод зеленого синтезу наночастинок срібла з використанням рослинного екстракту, отриманого з Rumex nepalensis (Spreng). Синтезовані наночастинки ретельно вивчали щодо їх структурних, морфологічних, оптичних, антиоксидантних та антибактеріальних властивостей. Структурний аналіз виявив гранецентровану кубічну структуру. Аналіз FTIR підтвердив наявність молекул біосурфактанту в екстракті листя, які діяли як відновники. SEM і TEM дослідження додатково підтвердили сферичну форму наночастинок із діапазоном розмірів 19-28 нм. Оцінка наночастинок срібла продемонструвала їх антиоксидантні та антибактеріальні властивості. Ці наночастинки виявляли активність як в антиоксидантній, так і в антимікробній сферах, демонструючи свій потенціал як подвійних функціональних агентів. Проведене дослідження підкреслює ефективність методу зеленого синтезу з використанням екстракту Rumex nepalensis (Spreng) для виробництва наночастинок срібла з бажаними властивостями для різних практичних застосувань.

Ключові слова: «зелений» синтез, рослинний екстракт, наночастинки срібла, антибактеріальна активність, антиоксидантна активність.