PREPARATION OF SILVER NANOPARTICLES FROM AQUEOUS EXTRACT OF *GMELINA PHILIPPENSIS* CHAM. FOR ANTIOXIDANT ACTIVITY: GREEN SYNTHESIS, CHARACTERIZATION AND ITS OPTICAL APPLICATION

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Abstract:

The silver nanoparticles synthesized by the green method have acquired a lot of attention due to one-step, eco-friendly and economical sustainability. This paper deals with the synthesis of silver nanoparticles using aqueous leaf extract of *Gmelina philippensis* Cham. The synthesized AgNPs were characterized by UV-visible spectroscopy to unfold metal stability and optical traits. Due to the Surface Plasmon Resonance, the prepared silver nanoparticles exhibit a distinct absorption peak in UV-Visible spectroscopy. The XRD diffraction, and TEM study reveals that the average diameter of synthesized silver nano-particles was found approximately 20 nm. The crystalline nature of synthesized silver nanoparticles was confirmed by XRD. While AFM and DLS studies confined the dimension of silver nanoparticle found in range of 20–75 nm. The chemical functional groups prior to aqueous leaves extract of *Gmelina philippensis* Cham. (ALEGP) were investigated by FTIR. The bioactive components such as, flavonoids, alkaloids, saponins, terpenoids, phenols, amino acids, tannins are responsible for the synthesis and stabilization of silver nanoparticles in aqueous medium confirmed from FTIR and Raman spectra. The photo-excitation peak was recorded at 328 nm NUV region, while the emission peaks was monitored at 362 nm exhibit violent color spectrum. Herein, CIE chromaticity coordinates: X = 0.175, Y= 0.0053) exposes the optical bands of synthesized silver nanoparticles. The antioxidant activity of silver nanoparticles was evaluated using DPPH methods. The optical and morphological property of synthesized silver nanoparticles using ALEGP is alternative for designing photonic devices as well as in medicinal field.

Keywords: Biosynthesis, Silver nanoparticles, Optical study, Antioxidant activity

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1. Introduction

The rapidly developing field of nanotechnology in material science is focused on the synthesis of nanoparticles with sizes between 1nm to 100 nm [1]. Metal-nanoparticles behaves differently than macro-sized materials due to their high surface and volume ratio [2]. Silver nanoparticles are widely used as metallic nanoparticles due to their wide applications in the field of photonics, and optoelectronics [3], pharmaceutical industry, and biomedical field, as they have unique optical, magnetic, electrical, mechanical, and chemical properties [4]. Top-down and bottom-up approaches are used to create the nanoparticles. The solid mass is mechanically broken down into smaller particles at the nanoscale level using a top-down approach, and these particles are then sized to the desired level. However, using this method makes it difficult to achieve the desired narrow size. The second method starts with an atomically small material that is produced to the necessary nanoscale. The bottom-up strategy is typically preferred for the synthesis of nanoparticles as it begins with simple particles that are easy to cluster and progresses to nanoparticles. It increases the flexibility of the synthesized nanoparticles over size and shape. Whereas, it is difficult to control the size, structure and surface chemistry of the nanoparticle using chemical methods such as the sol-gel method, the gas phase method, hydrothermal method, and the hydrolysis method, [5].

In a number of scientific fields, the development in the synthesis of silver nanoparticles appears to have a significant impact. The low yield and use of toxic materials in physical and chemical methods makes them unsuitable for the synthesis of silver nanoparticles. By using a variety of biological agents like plants, yeasts, enzymes, algae and fungi silver nanoparticles can produced. The biological approaches are able to overcome the majority of these drawbacks.

Plant-mediated nanoparticle synthesis is preferred among the many known synthesis techniques because it is simple to use, widely accessible, affordable, environmentally friendly, and suitable for human use [6][7]. Alkaloids, flavonoids, phenols, tannins, carbohydrates, saponins terpenoids, proteins, and amino acids are just a few of the phytochemicals and specific metabolites found in plants, that may speed up and improve the production of silver nanoparticles compared to microbes [8]. These plant-based phytochemicals are responsible for producing stable and well-dispersed silver nanoparticles as they act as a capping agent [9,10]**.** Numerous studies have been conducted on the biogenesis of silver nanoparticles using a variety of plant extracts, including Azadirachta indica [11], Cuscuta reflexa [12], Emblica officinalis, Garcinia mangostana, and Moringa oleifera [13]. These studies consistently make use of uniformly shaped nanoparticles and size in the range of 10-100 nm[2,14–16].

Free radicals, which include superoxide anion, hydroxyl radicals, and non-radical species like hydrogen peroxide and singlet oxygen, are the various types of activated oxygen that make up reactive oxygen and are responsible for the majority of oxidative diseases [17][18]. So, to balance this free radical, there is a need for active antioxidant defense. The phytochemicals present in plant extract act as antioxidants[19][20], which play a vital role in neutralizing free radicals by reducing singlet or triplet oxygen [21]. Because of their antioxidant properties, silver nanoparticles have the potential to treat degenerative Alzheimer's and cancer diseases[22]. It is assumed that the antioxidant materials that are present in plant extract are adsorbed on the surface of nanoparticles, which enhances its antioxidant activity.

Gmelina Philippensis Cham. is a small, 2 to 3.5 m-tall ornamental plant from the Verbenaceae family. This plant's stem has a rounded shape and is monopodially branched. The leaves are oval-shaped. Yellow flowers with a light scent are present in the terminal inflorescence. The flowers blooms in between the month of April and July. According to the literature survey this is the first report to use *Gmelina philippensis* Cham. for the creation of silver nanoparticles [23]. The aim of the current study is to create novel, simple, one-step AgNPs using a green method at room temperature, by reducing silver nitrate to silver nanoparticles by using aqueous leaves extract of *Gmelina philippensis* Cham. (ALEGP). The synthesized silver nanoparticles, various characterized by employing, UV-Visible, XRD, TEM, DLS, AFM, FT-IR, Raman and PL. Raman scattering and photoluminescence experiments were used to investigate the optical emission of silver nanoparticles caused by Surface Plasmon Resonance (SPR), which is based on the collective oscillation of conduction electrons caused by an electromagnetic field. We expect that these little findings will be useful in trials to use metal-enhanced fluorescence in numerous applications such as in biosensing materials and photonic devices. Additionally, efforts were made to assess its antioxidant activity.

2. Materials and Methods

2.1. Collection of Plant Material

The plant leaves of *Gmelina philippensis* Cham. **fig.1**. were collected from Botanical Garden of Government Vidarbha Institute of Science and Humanities, Amravati, (M.S.), India. The collected leaves were washed with distilled water. The leaves were mechanically ground, then stored in an airtight container after being shade dried at room temperature for 15 days.

Figure 1: Plant of *Gmelina philippensis* Cham.

2.2. Preparation of Plant Extract

A Soxhlet extractor was used for the extraction. 10 gm of powdered leaves were mixed with 250 ml of deionized water in a thimble, placed in a thimble mixed with 250 ml of deionized water. The extraction was done at 60 ˚C for 48 hours. Brown color of leaves extract was obtained as shown in **fig.2**.

Figure 2: Preparation of Plant extract in Soxhlet apparatus

2.3. Synthesis of silver nanoparticles by using aqueous Leaf extract of Gmelina philippensis Cham.

In presence of sunlight, 45 ml of a 1 mM silver nitrate solution was continuously stirred with 5 ml of ALEGP. Just after the addition, the change in color from colorless to a light brown color indicated that the silver nanoparticles were formed, that became dark brown with time. For further confirmation the synthesized silver nanoparticles were examined by various spectroscopic methods. The complete procedure for producing silver nanoparticles by mixing plant extract to silver nitrate solution as shown in **fig. 3 (a-h)** that shows the change in color after 15 min., after 3 hours, after 24 hours, after 72 hours, after 5 days and after 7 days.

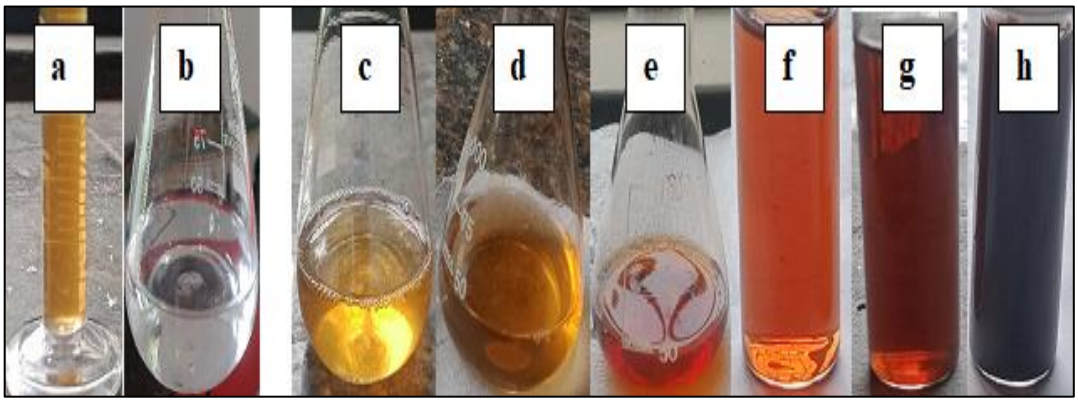

Figure 3: Visual change of color after addition of leaf extract to silver nitrate (a), Leaf extract (b), Silver nitrate (c), after 15 mints. (d), after 3 hours (e), after 24 hrs. (f) 72 hrs. (g) 5 days (h) after 7days

3. Characterization

3.1 UV-Visible Spectrophometer.

The UV-Visible spectroscopy technique is a simple, remarkably sensitive method for the analysis of metal-nanoparticles. Using a Shimadzu double-beam spectrophotometer, the surface plasmon resonance (SPR) of silver nanoparticles was examined at 200–800 nm. Deionized water is used as a blank. Silver nanoparticle formation was verified by a change in color, from colorless to reddish brown, which over time turned dark brown. Six absorbance measurements (after 15 min., 3

hours, 24 hours, 72 hours, 5 days and 7 days) were recorded after the addition of leaf extract to silver nitrate solution. After complete reduction of $AgNO_3$, AgNPs were fully formed at different wavelength intervals (200-800 nm).

3.2. X-ray diffraction

Using Rigaku Japan's X-ray diffraction technology, the synthesized silver nanoparticles exhibit crystalline nature. The X-ray diffractometer was used to measure the diffraction patterns of synthesized AgNPs coated on glass slides. It operated at a voltage of 40 kV and a current of 50 mA with Cu-K radiation over a 2θ range of 20˚–90˚

3.3. TEM analysis

The shape of the synthesized AgNPs was observed by TEM analysis at 20-200 KV (Philips, CM-200). The sample were prepared by placing a drop of fresh suspension onto the carbon and copper TEM grids, and the solvent was allowed to evaporate for 24 hours at room temperature.

3.4. AFM

The surface topological studies of synthesized silver nanoparticles were carried out using an Atomic Force Microscope AGILENT-N9410A-5500. For AFM characterization thin film of synthesized silver nanoparticles was placed on glass slides by dropping 10 mL of the sample and was allowed to dry for 30 minutes.

3.5. DLS/ Zeta potential

Using Horiba SZ-100 instruments, including a zeta potential analyzer and a DLS particle size analyzer, the size distribution profile and charge quantification of synthesized AgNPs were determined by measuring the dynamic fluctuations of intensity of light scattering caused by Brownian motion. The measurement provided the average hydrodynamic diameter as well as the peak values of the distribution.

3.6. FTIR study

Fourier-transform infrared spectroscopy (Shimadzu Japan, IR affinity-1) was used to identify the functional groups present in silver nanoparticles. The sample was made by combining it with KBr and hand-pelletizing it. A spectrum between 4000 cm^{-1} and 400 cm^{-1} was recorded.

3.7. Raman spectra

At room temperature, Raman spectra of synthesised silver nanoparticles were captured using X-plora Plus, Horiba, France. The CCD-based monochromator was used for collection and detection of scattered light in the spectral range 0 to 2500 cm^{-1}.

3.8. Photoluminescence Investigation.

The optical property of newly synthesized silver nanoparticles was studied by using Photoluminescence spectroscopy. At room temperature, photoluminescence was measured using a Hitachi with a pulsed ultra violet He-Cd laser source at a 325 nm excitation wavelength.

3.9. Antioxidant Activity

The scavenging ability of synthesized silver nanoparticles using ALEGP was calculated by DPPH method. In test tubes, stock solution of DPPH was mixed with 100 ug/ml of ascorbic acid, leaf extract, and synthesized silver nanoparticles. A calorimeter was used to measure the absorbance of the blank solution (Ethanol + DPPH solution) at 420 nm as a positive control. Similarly, the absorbance of ascorbic acid, leaf extract, and synthesized silver nanoparticles was recorded at 420 nm. The absorbance was recorded before and after the addition of DPPH and to remove the error, subtracting the absorbance value of ascorbic acid, leaf extract, and synthesized silver nanoparticles before the addition to those of the final absorbance. The radical scavenging activity was calculated as follows,

Radical scavenging effect (%) = Absorbance of control - Absorbance of sample/ Absorbance of control × 100

4. Result and Discussions

4.1. UV-Visible Spectroscopy

UV–Visible spectroscopy is an important technique that gives idea about the formation and stability of metal nanoparticles in aqueous solution. **Fig.4.** shows the absorbance spectra of aqueous leaf extract, 1mM aqueous solution of silver nitrate and silver nanoparticles synthesized using ALEGP at different time interval (i.e., 15 min., 3 hrs., 24 hrs., 72 hrs., 5 days and after 7 days). The surface plasmon resonance peak appeared at 435, 433, 420, 445, 433, and 448 nm disappeared after 20 days of reaction time and AgNPs are completely vanished. By the above observation, it was concluded that the intensity of peak of surface plasmon resonance increases with increase in reaction time. Due to continuous increase in number of silver nanoparticles formed, as a result of reduction of silver ions present in the aqueous solution of silver nitrate by leaf extract. Mie theory states that spherical nanoparticles have only one SPR band [24–28]**.**

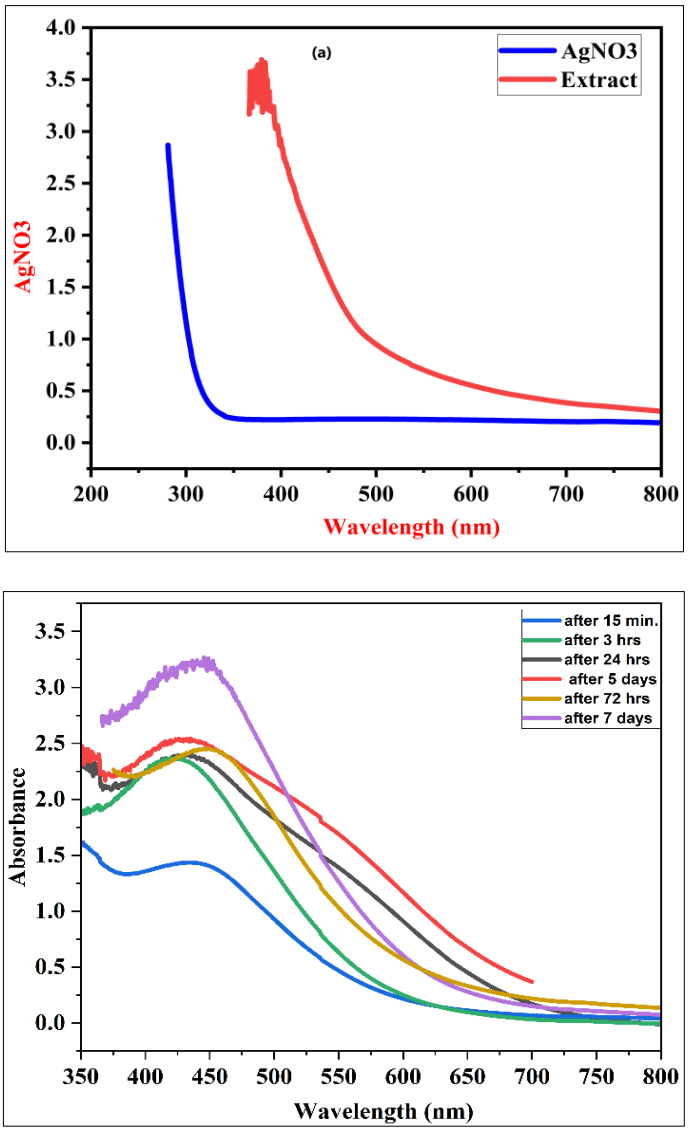

Figure 4: UV-Visible absorption spectra of aqueous plant extract and 1mM $AgNO_3$ solution (a), Synthesized silver nanoparticles using ALEGP at different time interval (b)

4.2. X-ray diffraction Analysis.

The crystalline nature of synthesized silver nanoparticles obtained from ALEGP was confirmed by XRD diffractogram. The XRD diffraction peaks of synthesised silver nanoparticles were obtained at 38.01^0, 64.48^0 and 77.34^0 with miller indices value (111), (220), and (311) as shown in **fig.5**. The highest diffraction peak at 38.01^0 (111) can be indexed to silver nanoparticles. The silver nanoparticles that were synthesized were confirmed to be crystalline by the peaks obtained from XRD, as reported in [28-32][34]**.** The average crystallite size of silver nanoparticles was calculated, from the full width at half maximum (FWHM) diffraction peaks by the Scherrer formula.

$$D = \frac{0.9.\ \lambda}{\beta Cos\theta} \quad (1)$$

Where β-Width of diffraction peak in radian
λ- wavelength of X- rays (1.54 ˚A)
Θ-diffraction angle
D-average diameter of crystallite.
The average diameter of synthesized silver nano-particles was found 20.5 nm from the Scherrer formula.

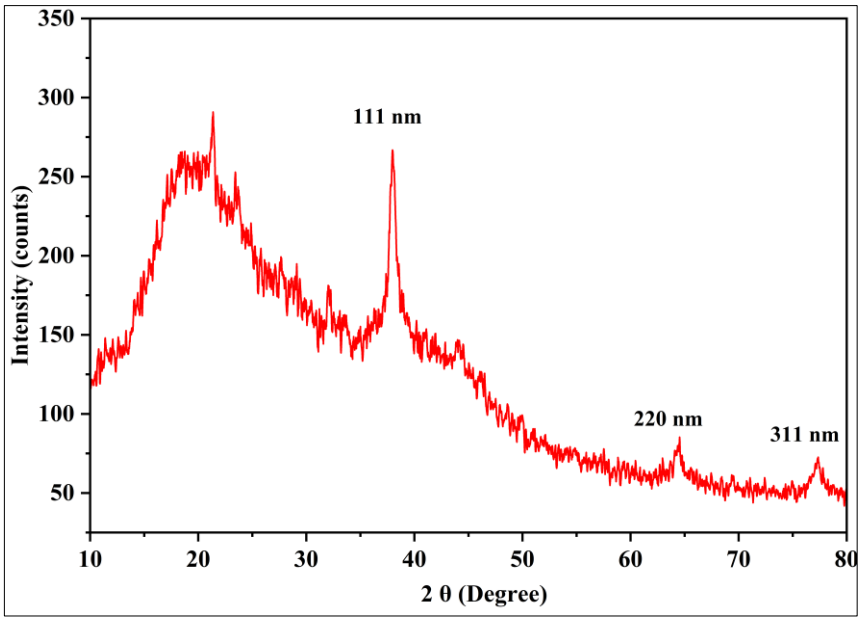

Figure 5*:* XRD spectrum of synthesized silver nanoparticles.

4.3. TEM Analysis.

The particle morphology and crystalline nature of synthesized silver nanoparticles were done by TEM analysis. TEM images **(fig. 6)** revealed that the silver nanoparticles synthesized from ALEGP is spherical in shape having particle dimension in the range of 20 to 100 nm that are uniformly distributed and are crystalline in nature [35].

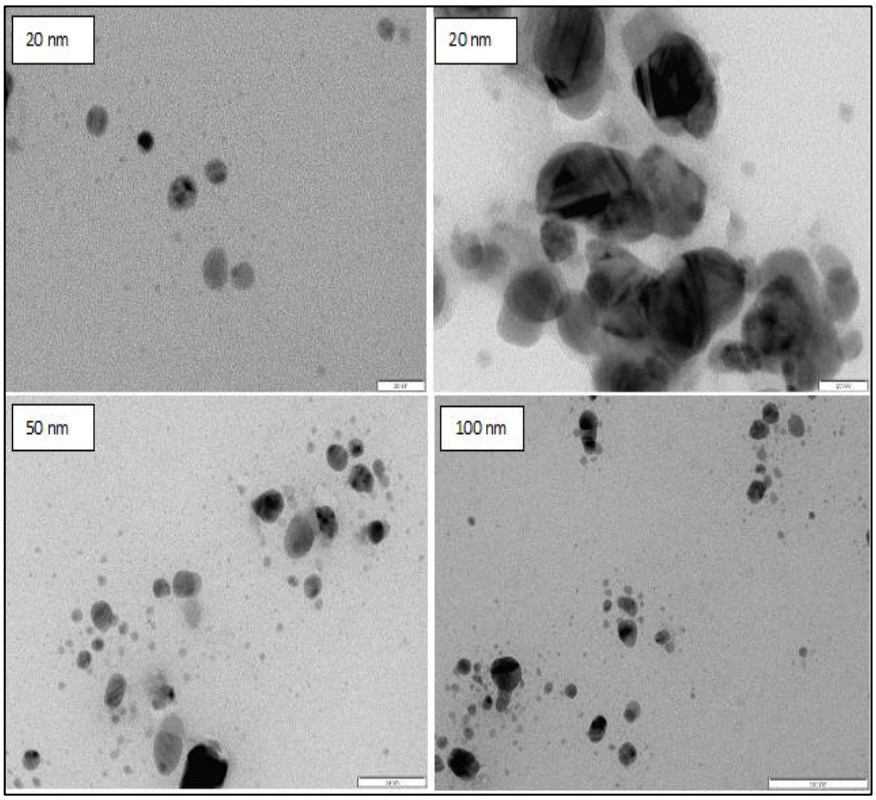

Figure 6*:* TEM Images of synthesized silver nanoparticles.

4.4. DLS/ Zeta potential

DLS analysis revealed that synthesized silver nanoparticles had an average size of about 20 nm that supports the TEM observation and the X-ray diffraction patterns. The synthesized silver nanoparticles showed only one peak depicted in **fig.7**. The size obtained was confirmed from the previously synthesized silver nanoparticles [36][25][37].

To investigate their improved storage stability and surface charge, the zeta potential of synthesized silver nanoparticles was measured. The surface zeta potential of synthesized silver nanoparticles stands at -20 mV, indicating their stability [38]. The negative value indicated that the synthesized silver nanoparticles were highly stable and also avoids agglomeration [30].

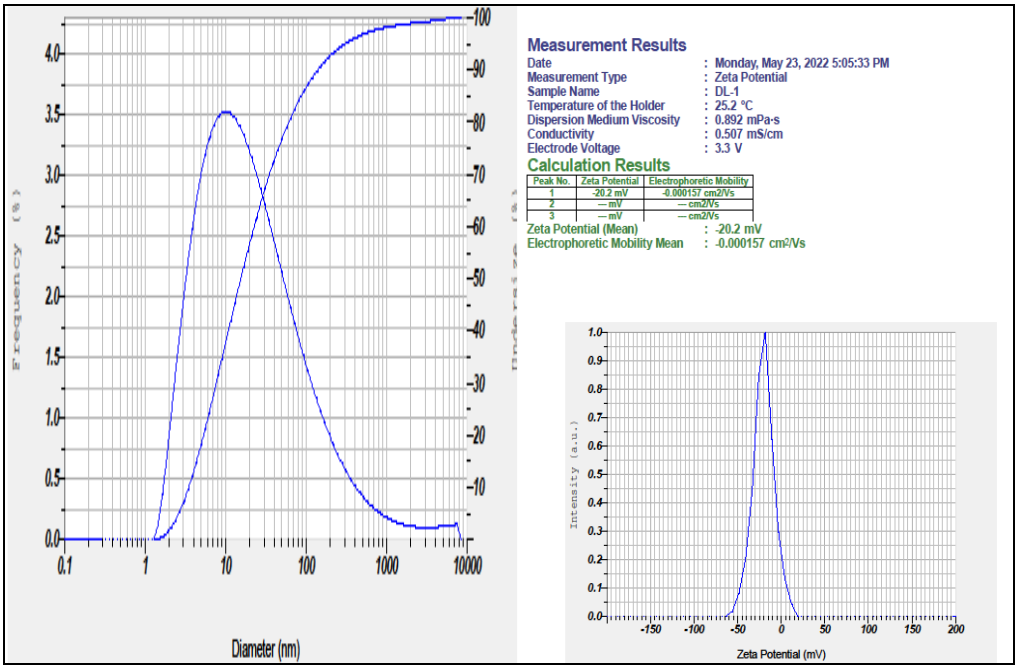

Figure 7: DLS size distribution and Zeta potential of silver nanoparticles

4.5. AFM Technique

Atomic Force Microscopy determine the surface morphology and particle dimension of synthesized silver nanoparticles by using the ALEGP. **Fig.8** shows AFM images of the synthesized silver nanoparticles, which were found 70 nm in size. This result is relatively consistent with TEM analysis [39].

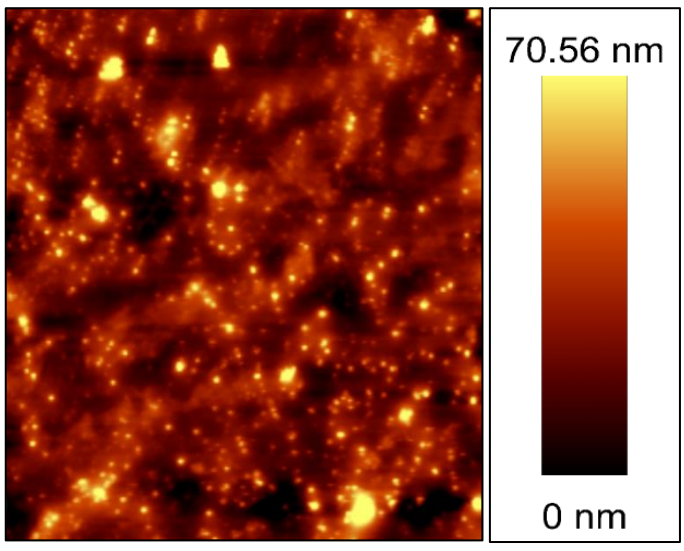

Figure 8: AFM images of silver nanoparticles

4.6. FT-IR Investigation.

FT-IR measurements determines chemical behavior and Functional groups in the aqueous leaf extract **(fig. 9a),** and also in the biosynthesis and stabilization of silver nanoparticles **(fig.9b**). The broad absorption peak recorded at 3387 cm^{-1} is due to the H-O stretching of phenolic compounds, which shifted to 3464 and 3286 cm^{-1} in silver nanoparticles. while, the broad peak at 1602 cm^{-1} in leaf extract was due to the C-N stretching of amide I, this band was shifted in AgNPs at 1612cm^{-1} due to proteins that may bind to AgNPs via the amine

groups [40]. The peaks at 1452 and 1415.70 cm^{-1} attributed due to asymmetric bending of methyl groups of flavonoids and lipids in leaf extract, but in silver nanoparticles it was shifted to 1465 cm^{-1} [41]. The leaf extract of *Gmelina philippensis* Cham. showed a moderate absorption band of aldehydic C-H stretching of CH_2 at 2924.09 cm^{-1}. The C-H stretching of the methyl group corresponds to the peak at 2924 cm^{-1}. The absorption peak at 1114.86 cm^{-1} corresponds to C-O-C vibrations, whereas the peak at 910.40 cm^{-1} attributes due to C-O-H vibrations [40-41]. In synthesized silver nanoparticles the absorption peak of symmetrical C-H stretching of hydrocarbons appeared at 2854 cm^{-1} [29].

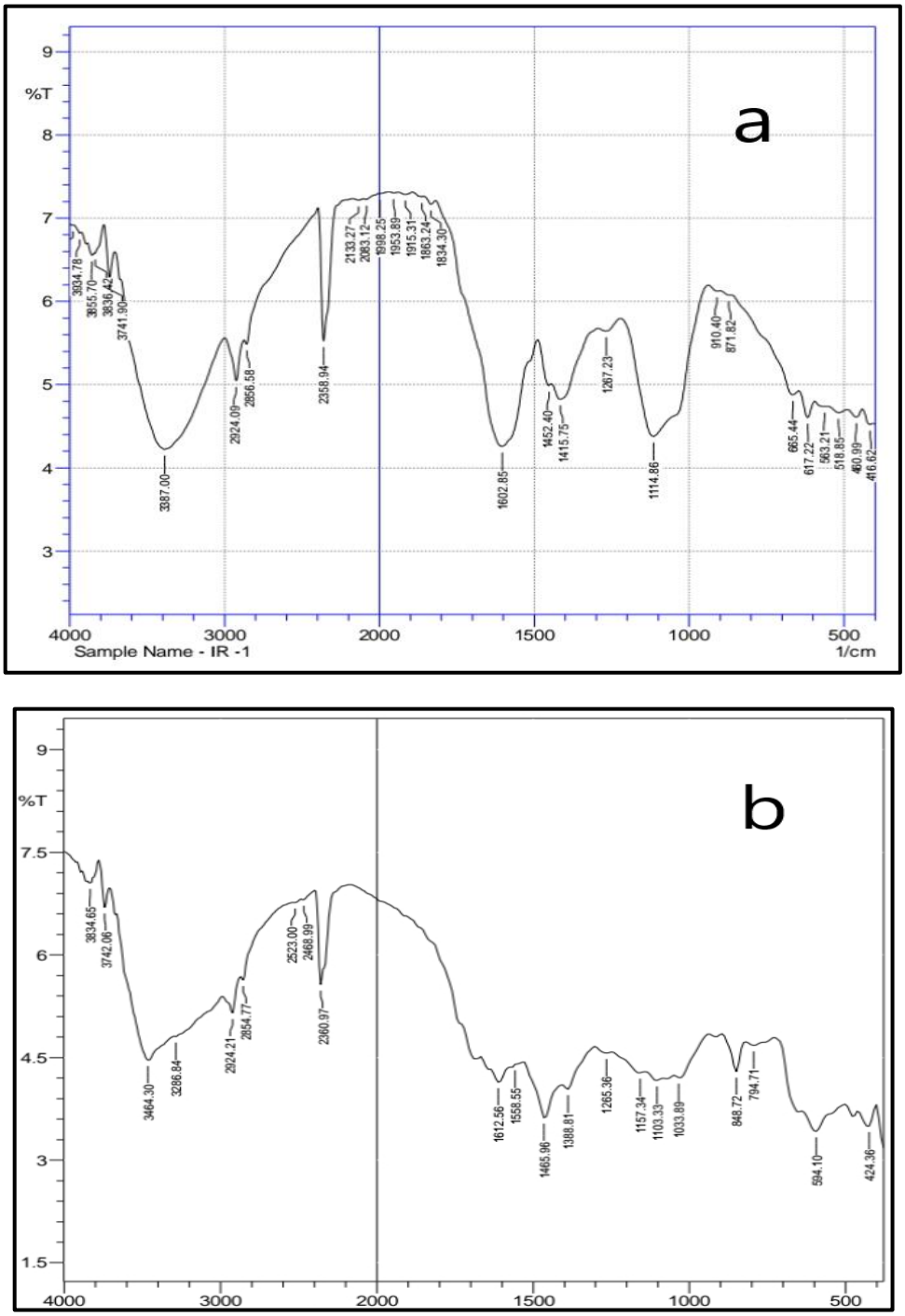

Figure 9: FT-IR Spectra of ALEGP (a) Synthesized silver nanoparticles (b)

The band at 1,558 cm^{-1} could be due to C-C stretching vibration. The peak at 1388 indicates the presence N=O symmetry stretching of nitro compound. Another medium peak at 1033 cm^{-1} indicates the presence of aliphatic amines due to C-N stretching, while as, 2395.59cm^{-1} represents N-H

stretching of secondary amine. The peak at 1558 cm^{-1} occurred due to stretching vibration of the C=O of carbonyl of tannins and nitro compounds. At 1104 cm^{-1}, a stretching peak of the C-N bond is observed, which can be attributed to the presence of plant-based amines. These investigated FTIR peaks match the reported work [4-9-23-24]. The shifted peaks in the synthesized silver nanoparticles indicate that functional groups from the leaf extract are involved in the formation of AgNPs. Therefore, from FT-IR results it can be concluded that the bioactive components such as flavonoids, alkaloids, saponins, terpenoids, phenols, amino acids, tannins are responsible for the synthesis and stabilization of silver nanoparticles in aqueous medium.

4.7. Raman Spectroscopy

The colloidal dispersion of synthesized silver nanoparticles was analyzed by surface enhanced Raman spectroscopy as depicted in **fig. 10**. The peak at 1169 cm^{-1} arises due to CO stretching, while the peaks at 1236 cm^{-1} and 1565 cm^{-1} were caused by stretching vibration of C-N, which can be attributed to some components of the amide III, and amide II groups, which indicated the interaction of nanoparticles with the backbone of peptide linkage. The other peak at 1420 cm^{-1} due to O-H bending, The peak at 2129 cm^{-1} was due to stretching of C≡C, whereas another peak at 2275 cm^{-1} was due to C≡N stretching respectively [42][43].

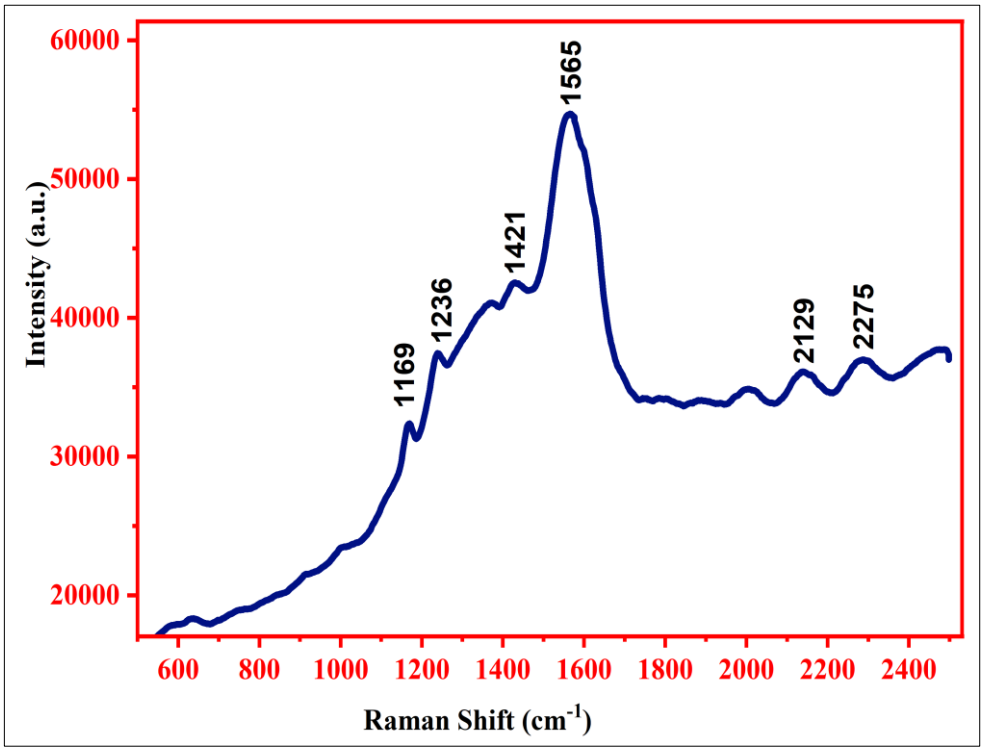

Figure 10: Raman spectra of synthesized silver nanoparticles using ALEGP

4.8. Photoluminescence studies

Photoluminescence is a common spectroscopic technique for studying emissions in metal particle systems. The synthesized silver nanoparticles using ALEGP were found to be photoluminescent. The photo-excitation (PLE) spectra of synthesized silver nanoparticles show one strong emission band at 328 nm in the NUV region, as shown in **fig. 11 (a).** The excitation of electrons from occupied "d" bands into the state above the Fermi level are responsible for the luminescence property of silver nanoparticles [46]. The Photo-emission were monitored around 362 nm in Violet region.

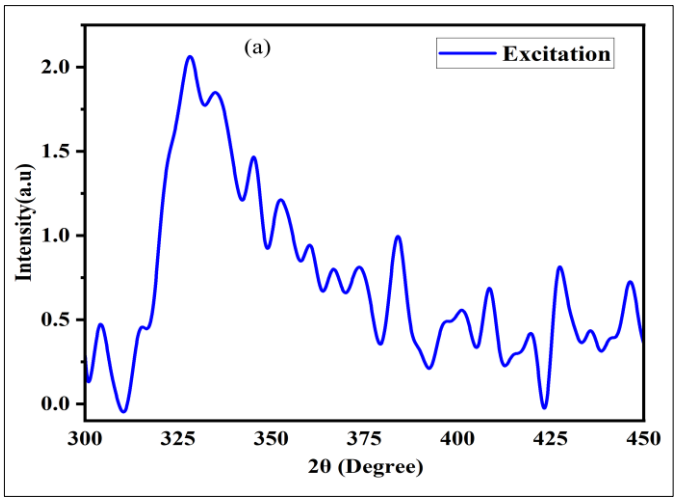

Figure 11: Excitation spectra recorded at 328 nm of the synthesized silver nanoparticles using ALEGP (a)

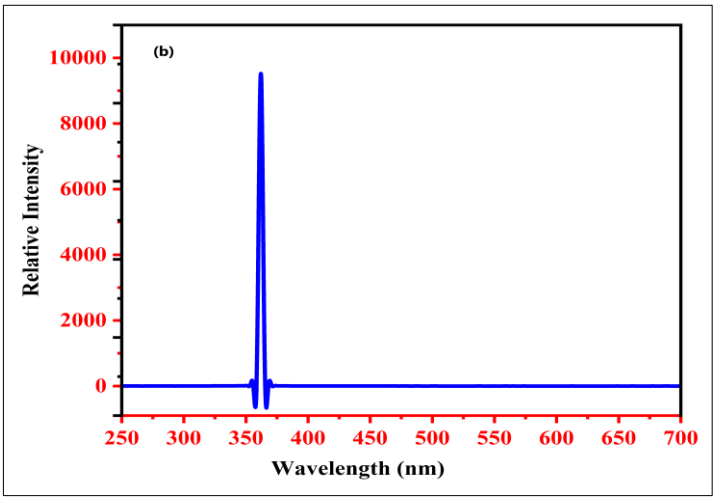

Figure 12: Photoluminescence emission spectra of synthesized silver nanoparticles using ALEGP were monitored at 362 nm (b)

The CIE coordinates of synthesized silver nanoparticles were found at x = 0.175, y = 0.0053, which indicates violet color emission **(fig.12)** [46]. The presence of biochemicals or antioxidants in plant extract may responsible for the luminescence at excitation 328 nm. The nanoparticles synthesized from olive leaf extract are also found to be luminescent, with an emission band at 425 nm [47].

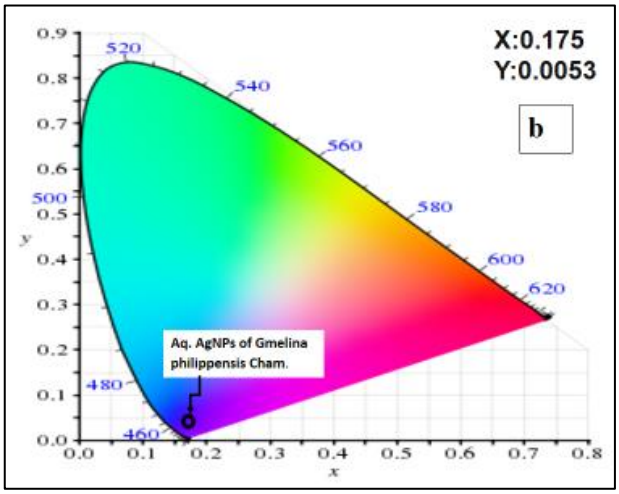

Figure 12: CIE diagram of Aqueous AgNPs extract from Plant leaf.

4.9. Antioxidant activity

DPPH method

DPPH is a stable radical that is frequently used to assess the antioxidant activity. The DPPH free radical which is purple in color shows a characteristic absorption at 420 nm, but the absorbance decreases when it is exposed to radical scavengers. Lower the absorbance value indicates the higher percentage of radical scavenging activity. The synthesized silver nanoparticles using ALEGP exhibited strong antioxidant activity than that of extract and ascorbic acid. The percentage radical scavenging activity of leaf extract and synthesized silver nanoparticles were found to be 76.77 %, 77.77% displayed in **Table No.1** below**.**

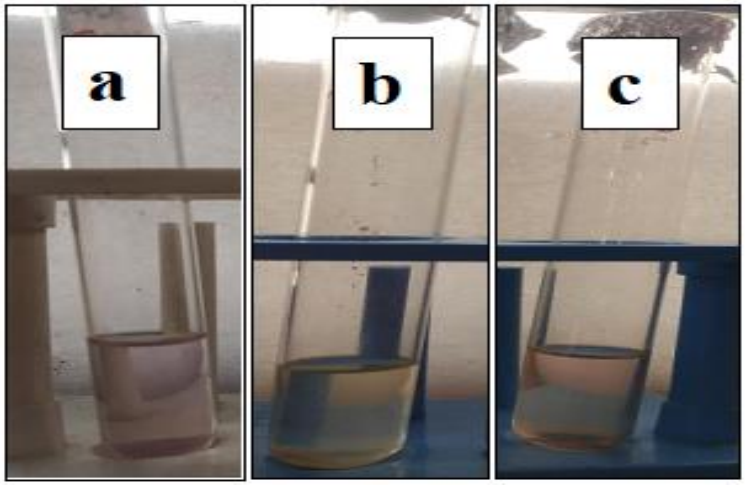

Figure 12: Color solution of Ascorbic acid (a), Aq. leaf extract (b), Synthesized silver nanoparticles. After addition of DPPH(c)

Table No. 1: Radical scavenging activity of AgNPs using ALEGP by DPPH method

Sample	Concentration	Absorbance	% RSA	Ascorbic acid
Aqueous Extract	0.2ml	0.127	76.48	0.54
AgNps	0.2ml	0.120	77.77	0.54

4.10. Conclusion

The biosynthesis of silver nanoparticles by using ALEGP is simple, safe, and one step process. The synthesis of silver nanoparticles is free from any toxic chemicals as leaf extract itself acts as a stabilizing or reducing agent. UV-Vis. analysis revealed that the synthesized silver nanoparticles was spherical in shape. XRD and TEM analysis showed the crystalline nature of synthesized silver nanoparticles was approximately 20 nm. AFM and DLS study confirmed that the size of silver nanoparticles synthesized by ALEGP was in the range of 20 nm-75 nm confirmed from TEM study. Herein, Zeta potential measures the stability of synthesized silver nanoparticles. The chemical and functional group present in ALEGP that are responsible for the bio -reduction and stabilization of silver nanoparticles were prior examined by FT-IR study. The Photo-excitation peak of the synthesized silver nano-particles were recorded at 328 nm and emission peak were monitored 362 nm indicating violet color. The silver nanoparticles have strong antioxidant activity. The DPPH free radical which is purple in color shows a characteristic absorption at 420 nm absorbance decreases when exposed to radical scavengers. The optical and morphological property of synthesized silver nanoparticles using ALEGP shows that it is a good alternative for designing photonic devices as well as in medicinal field.

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Conflict of interest: The authors in this paper declares no conflict of interest

Abbreviations

AgNPs : Silver nanoparticles
$AgNO_3$: Silver nitrate
XRD : X-ray diffraction
UV-Vis.: Ultra Violet-Visible spectroscopy
TEM : Transmission electron microscopy
DLS : Dynamic light scattering
FT-IR : Fourier Transform Infrared
PL : Photoluminescence
AFM : Atomic force microscopy

ALEGP : Aqueous leaf extract of Gmelina philippensis Cham.
DPPH : 2,2-diphenyl-1-picryl-hydrazyl-hydrate
SPR : Surface plasmon resonance

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