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Biological Synthesis of Gallium Nanoparticles using Extracts of *Andrographis paniculata*

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Abstract: *Nanoparticles have potential applications in medical field. Recently, plant extract mediated synthesis of nanoparticles has attracted wide attention due to its rich bioactive constituents. This green synthesis process is relatively inexpensive, simple and eco-friendly. Andrographis paniculata is a medicinal herb with anti-bacterial, anti-inflammatory, anti-cancer, anti-viral, anti-malarial, anti-fungal and anti-snake venom activities. In this perspective, the paper reports the synthesis of gallium nanoparticles using root and flower extracts of Andrographis paniculata as reducing agent for the first time. The biologically synthesized nanoparticles are characterized using UV spectroscopy, FT-IR spectroscopy, SEM with EDX, TEM and XRD analyses.*

Keywords: *Gallium nanoparticles, Andrographis paniculata, Green synthesis, Bacterial Infections*

1. Introduction

A number of nanoparticles based therapeutics has been approved clinically for infections, vaccines and renal diseases [1]. Metallic nanoparticles are most preferred due to their physical and chemical properties such as high surface-to-volume ratio and heat transfer. Among the various nanoparticles, metal nanoparticles assume special importance because they are easier and cheaper to synthesize [2]. Metal nanoparticles like silver and gold show different colours due to their Surface Plasmon Resonance phenomenon.

A lot of literature has been reported till to date on biological synthesis of nanoparticles using microorganisms including bacteria, fungi and plants, because of their antioxidant or reducing properties responsible for the reduction of metal compounds in their respective nanoparticles. Plants are majorly applied for green synthesis of metallic nanoparticles as they are potentially advantageous over microorganisms due to the ease of improvement and less biohazard [3]. The use of environmentally benign materials like plant root and flower extract for the synthesis of nanoparticles offers numerous benefits of eco-friendliness for pharmaceutical and biomedical applications as they do not produce or use any toxic chemicals [4]. They are also beneficial compared to microbes because of the presence of broad variability of bio-molecules, which can act as capping/stabilizing and reducing agents and so increases the rate of reduction and stabilization of synthesized nanoparticles.

Iron (Fe) is crucial for metabolism and growth of most microbes. Gallium (Ga) is a metal which is similar to iron. Unlike $Fe3^{+}$, $Ga3^{+}$ cannot be reduced, and therefore enzymes are

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rendered inactive when bound to Fe binding sites of the enzyme. Furthermore, many Fe binding proteins, such as bacterial siderophores, are unable to distinguish $Ga3^{+}$ from $Fe3^{+}$. Therefore growth of bacterial pathogens and virus can be inhibited by Ga through disruption of Fe dependent pathways. Ga is also not susceptible to classical drug efflux pumps and therefore, less vulnerable to antibiotic resistance mechanisms. Gallium nitrate is a FDA-approved drug for the treatment of hypercalcemia of malignancy and could serve as therapy against human infections [5].

As, Ga in the form of nanoparticles will possess long lasting presence inside the host cell, an attempt was made to biologically synthesize gallium nanoparticles using *Andrographis paniculata*.

2. Methodology

2.1 Plant

The plant chosen for the present work is *Andrographis paniculata*, commonly known as the "king of bitters", is an herbaceous plant. The common name of the plant is Green Chirayta and known as "Kalmegh" in the India [6]. *Andrographis paniculata* is a traditional medicinal plant used in India and Sri Lanka. The plant grows erect to the height of 30 to 110 cm in moistly and shady places. The slender stem is dark green, squared in cross section with longitudinal furrows and wings along the angles. The flowers are small white in color with purple shades. The lance shaped leaf has hairless blades measuring up to 8 cm long by 2.5 cm. The fruit is a capsule around 3 cm long and few mm wide which contains many yellow brown seeds. The roots and leaves are used for treating common cold, upper respiratory tract infections, digestive complaints such as diarrhea, constipation, colic pain also for infections such as leprosy, malaria, cholera. They exhibit anti-fungal, anti-inflammatory, anti-viral activities due to which a wide range of diseases can be cured [7].

Phytochemical constituents present in this plant are polyphenols, andrographolide, neoandrographolide, dehydroandrographolide, among which andrographolide is the active compound [6]. Among all other phytochemicals, andrographolide, neoandrographolide, dehydroandrographolide, are the most important bio protectants having wide range of therapeutic applications [8].

A recent study has demonstrated that the synthesis of nanoparticles using the leaf extract of *Andrographis paniculata* displayed good anti-plasmodial activity [9]. Sulchona et al., (2012) reported the synthesis of silver nanoparticles using leaves extract of *A. paniculata* [10]. However, there are no reports that demonstrate the anti-bacterial activity of nanoparticles derived from flower and root aqueous extract of *A. paniculata* against human pathogens.

Figure 1: *Andrographis paniculata* and its flower

2.2. Preparation of root and flower extracts

Fresh roots and flowers of *Andrographis paniculata* were collected from Kalasalingam University, Tamil Nadu, India and were cleaned thoroughly in running tap water. The roots and flowers were shade dried and were cut into small pieces. 10g of roots and flowers were boiled at 60^{o}C separately in 100 ml of distilled water for 30 minutes. The crude aqueous extracts were cool down to room temperature. The root and flower extracts were then filtered out using Whatman No.1 filter paper [11].

2.3. Optimization of Incubation time

For effective synthesis of nanoparticles, incubation time was optimized. To 120 ml of 1mM gallium nitrate solution, 18 ml of root and flower extract was added separately in each flask. The samples were withdrawn after 10, 20, 30, 40, 50 and 60 minutes and centrifuged at 10,000 rpm for 10 minutes. Each pellet of nanoparticle was dispersed in distilled water and analysed using UV spectroscopy. The time at which the sample has maximum absorbance is taken as optimal synthesis time.

2.4 Synthesis of gallium nanoparticles

To 20ml of gallium nitrate, 3 ml of root and flower extract were added separately in different flasks. The reaction mixture was incubated at room temperature for 50 minutes and 40 minutes respectively. Purification of nanoparticles was done by repeated centrifugation at 10,000 rpm for 10 minutes. The nanoparticle pellet was stored at 4^{o}C for further analysis. The nanoparticles were subjected to sonication before characterization

2.5 Characterization of biologically synthesized gallium nanoparticles

The characterization of nanoparticles was done by the examining size, shape and quantity of particles. A number of techniques are used for this purpose, including UV-visible spectroscopy, Scanning Electron Microscopy (SEM), Fourier Transmission Infrared Spectroscopy (FT-IR), X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS).

2.5.1 UV-Visible spectra analysis

The gallium nanoparticles were characterized using UV-Visible spectrophotometer (Shimadzu) in the wavelength range of 200 nm to 700 nm.

2.5.2 FT-IR analysis

The functional groups of plant extracts present on the surface of gallium nanoparticles were investigated using FT-IR spectra (Shimadzu) scanned in the range of 4000 – 400 cm^{-1}.

2.5.3 SEM and EDX analysis

The morphology of the nanoparticles was studied using SEM analysis (Carl Zeiss, Germany) and EDX (Bruker) was used for elemental analysis.

2.5.4 XRD analysis

XRD is used to study the crystalline nature and size of the nanoparticles. XRD analysis was done using BRUKER, ECO D8 ADVANCE X-Ray Diffractometer, operated at a voltage of 40 kV and a current of 20 mA using Cu Kα radiation. The crystallite domain size can be calculated from the width of the XRD peaks, using the Scherrer formula [12]:

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

Where,

D is the average crystallite domain size

λ is the X-ray wavelength (1.54060)

β is the full width at half maximum (FWHM)

θ is the diffraction angle.

2.5.5 TEM analysis

To identify the size, shape and morphology of nanoparticles, TEM analysis was done using JEOL/JEM 2100 at voltage 200 kV. A drop of nanoparticles was placed on carbon coated copper grids and the solvent was allowed to evaporate prior to analysis.

2.5.6 Particle Size Analyzer

Particle Size Analyzer (HORIBA) was used to determine the particle size distribution, average size and polydispersity Index (PI) of nanoparticles.

3. Results and Discussion

With the addition of root and flower extract to colourless gallium nitrate solution, the colour changed into dark brown and light brown respectively (Figure 2 and 3). Gallium nanoparticles synthesized from flower and root extracts exhibited a peak at 308 nm and 262 nm respectively in UV spectra (data not shown). The maximum absorbance for gallium nanoparticles synthesized using flower and root extracts was obtained at 40 and 50 minutes respectively (Table 1 and 2).

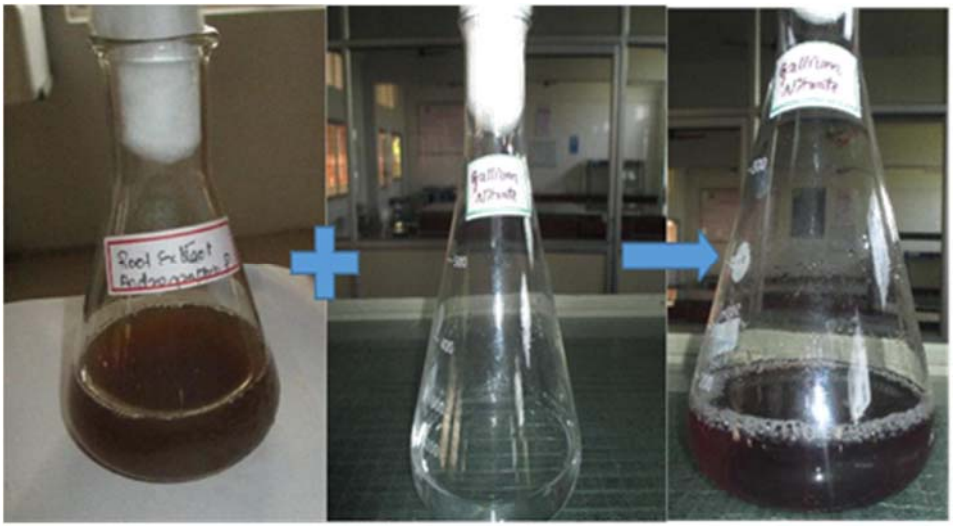

Figure 2. Synthesis of Gallium nanoparticles using root extract of *A. paniculata*

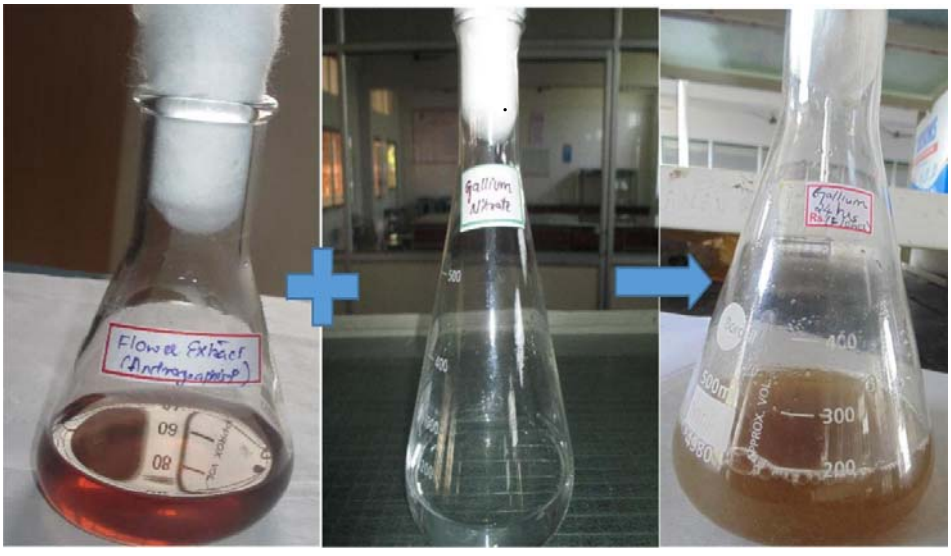

Figure 3. Synthesis of Gallium nanoparticles using flower extract of *A. paniculata*

Table 1: Optimized time for gallium nanoparticles synthesized using flower extract

Time (minutes)	Maximum Wavelength	Absorbance
10	309	1.569
20	307	1.55
30	343	2.370
40	307	0.725
50	348	2.436
60	308	1.560

Table 2: Optimized time for gallium nanoparticles synthesized using root extract

Time (Minutes)	Maximum Wavelength	Absorbance
10	239	2.237
20	239	1.529
30	238	3.675
40	253	4
50	238	3.014
60	238	2.561

FT-IR analysis was done to identify the possible reducing biomolecules in the root and flower extracts. Figures 4 and 5 shows the FT-IR spectra of gallium nanoparticles from the root and flowers extracts of *Andrographis paniculata* respectively. The signals and its corresponding functional group assignments are given in Table 3 and Table 4.

The reducing agents promote formation of metallic nanoparticle from the corresponding ionic compounds. The reduction reaction involves plant biomolecules (secondary metabolites) such as sugars, proteins, organic compounds etc. These secondary metabolites are known as key sources for controlling the various acute diseases [13]. The plant extracts contain numerous functional group such as C=C (Alkenyl), C=N (amide), O=H (phenolic and alcohol), N-H (amine), C-H and COO- (carboxylic group). These chemical groups are responsible for nanoparticle production [14].

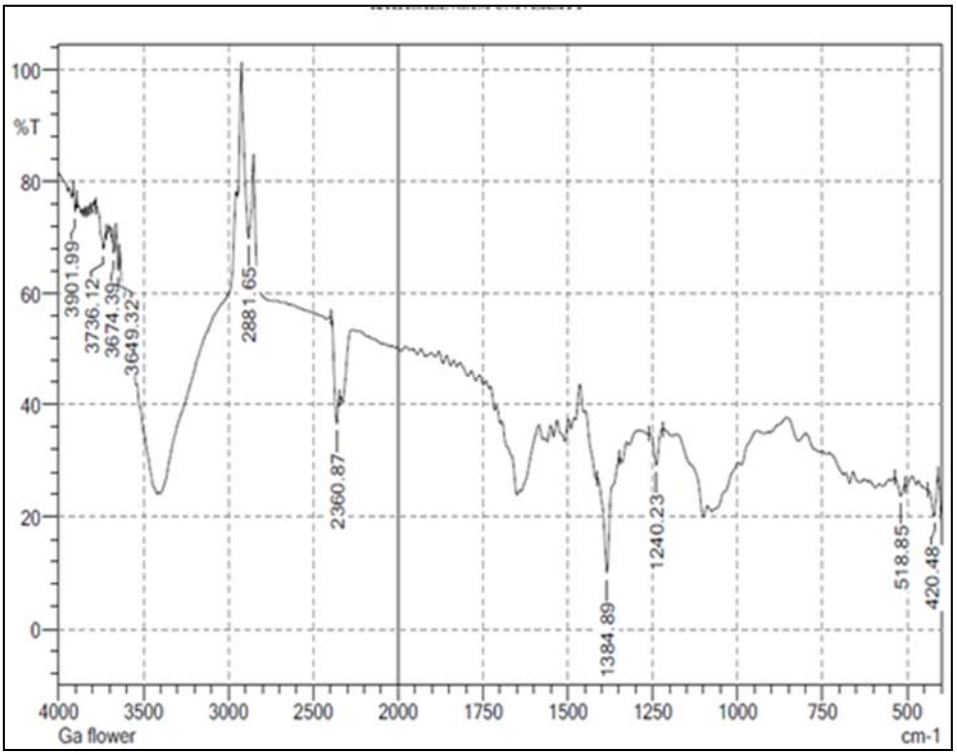

Figure 4. FT-IR spectrum of gallium nanoparticles synthesized using flower extract of *A. paniculata*

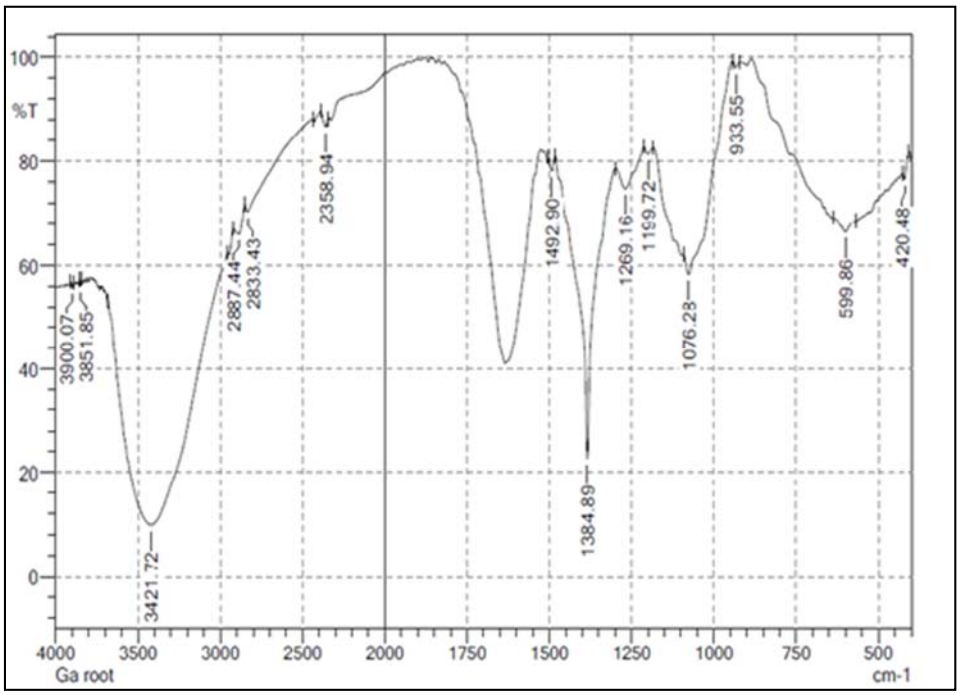

Figure 5. FT-IR spectrum of gallium nanoparticles synthesized using root extract of *A. paniculata*.

TABLE 3: FT-IR bands corresponding to their functional group of gallium nanoparticles synthesized from root extract of *A. paniculata*

Nanoparticles	Wave number	Functional group
Gallium nanoparticles (root extract)	1076.28 cm^{-1}	Bending vibration of C-OH groups [15].
	1384.89 cm^{-1}	Asymmetrical stretching for nitro compounds [9]
	2887.44 cm^{-1}	CH group of alkanes [16]
	3421.72 cm^{-1}	N-H stretch vibration of peptide linkages [15]

TABLE 4: FT-IR bands corresponding to their functional group of gallium nanoparticles synthesized from flower extract of *A. paniculata*.

Nanoparticles	Wave number	Functional group
Gallium nanoparticles (flower extract)	518.85 cm^{-1}	Occurrence of vibration of alkyl halides [9].
	1384.89 cm^{-1}	Asymmetrical stretching for nitro compounds [9].
	2881.65 cm^{-1}	CH group of alkanes [16].

The SEM images of gallium nanoparticles synthesized using root and flower extracts are shown in Figure 6 and 7 respectively. The corresponding EDX graphs are shown in Figure 8 and 9 respectively.

Figure 6 shows that the gallium nanoparticles synthesized from flower extract are of cuboidal shape and the respective EDX confirms the presence of gallium nanoparticles. From Figure 6 it was also observed that gallium nanoparticles synthesized from root extract are of spherical in nature and its corresponding EDX confirms the presence of gallium nanoparticles.

Figure 10 and 11 shows the XRD pattern of gallium nanoparticles synthesized using root and flower extract. The broad peak position at 13º confirms the presence of gallium [17].

The crystallite size of gallium nanoparticles synthesized using root and flower extracts of *Andrographis paniculata* is given in Table 5. The crystallite size of nanoparticles ranges between 22 nm and 42 nm.

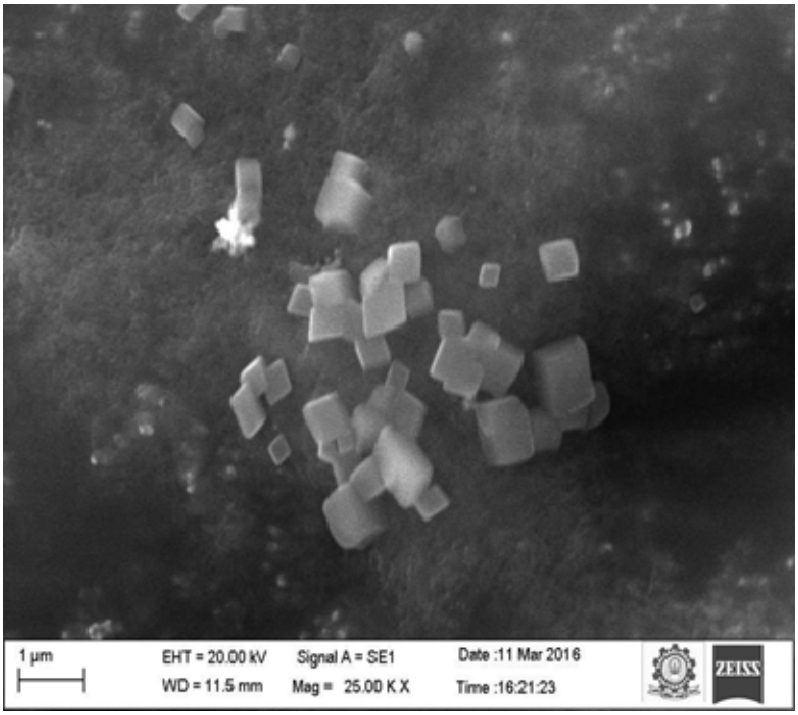

Figure 6. SEM image of gallium nanoparticle synthesized using flower extract of *A. paniculata*

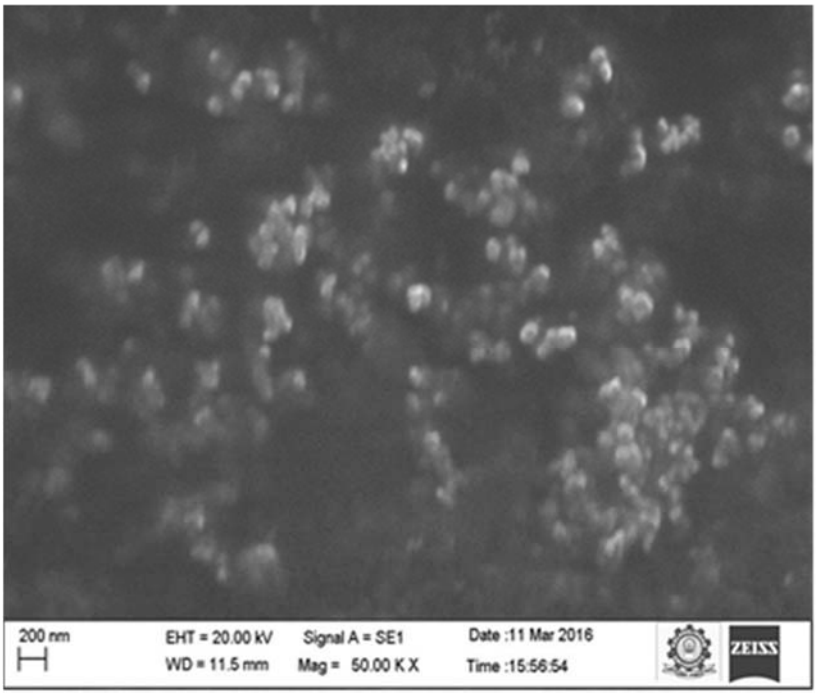

Figure 7. SEM image of gallium nanoparticles synthesized using root extract of *A. paniculata*

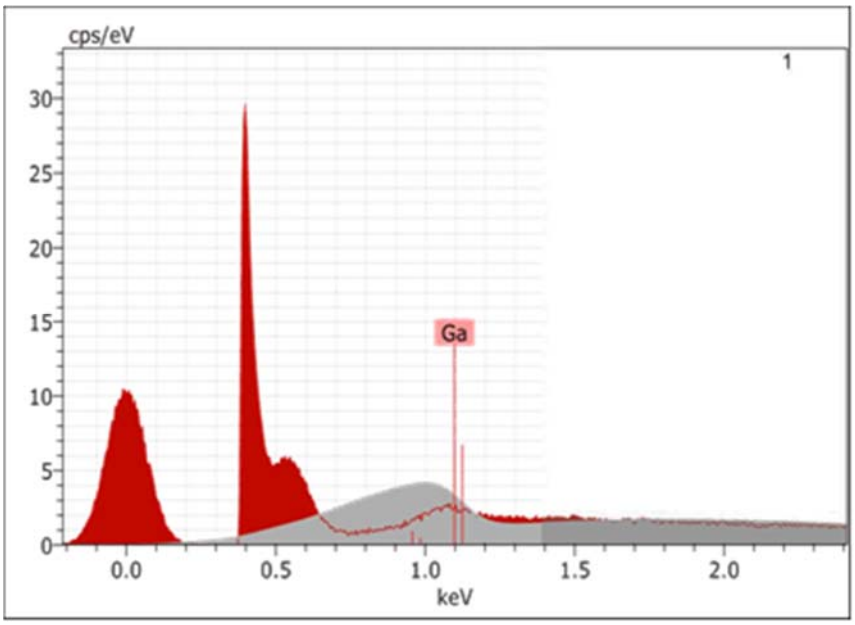

Figure 8. EDX image of gallium nanoparticles synthesized using flower extract of *A. paniculata*

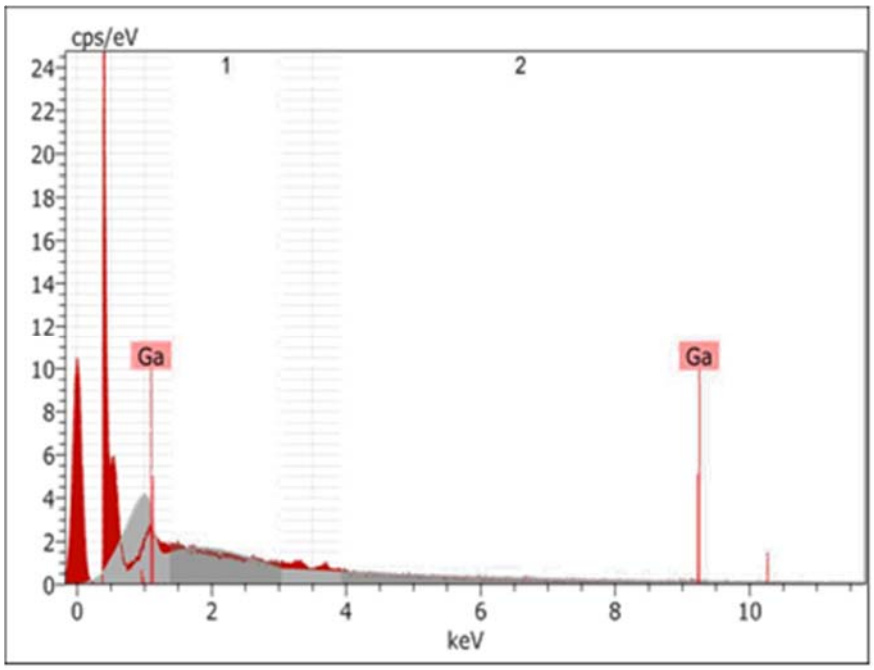

Figure 9. EDX graph of gallium nanoparticles synthesized using root extract of *A. paniculata*

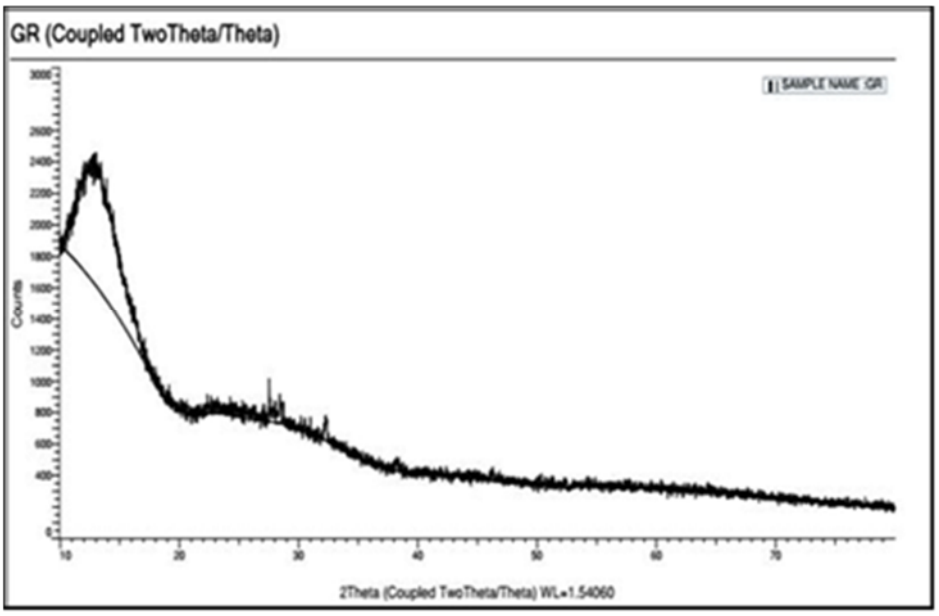

Figure 10 XRD pattern of the synthesized gallium nanoparticles using root extract of *A. paniculata*

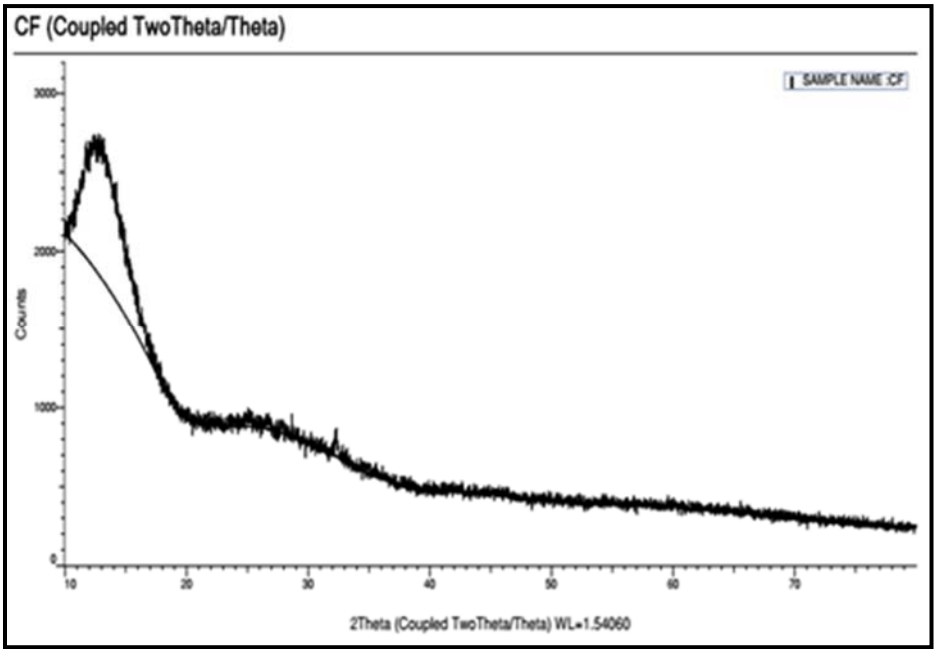

Figure 11. XRD pattern of the synthesized gallium nanoparticles using flower extract of *A. paniculata*

TABLE 5: Crystallite Size of the nanoparticles

Nanoparticles	Crystallite size, nm
Gallium nanoparticles from root extract	41.6
Gallium nanoparticles from flower extract	36.93

The TEM images of nanoparticles synthesized from the root extract is depicted in Figure 12. The discrete rings with bright spots in Figure 12 (d) indicate that the particles are crystalline in nature.

The particle size distribution of gallium nanoparticles synthesized using root extract is shown in Figure 13. Gallium nanoparticles have an average size of 243.8 nm, the polydispersity index is 1.505 and the particle size distribution ranges from 151.57 nm to 454.69 nm.

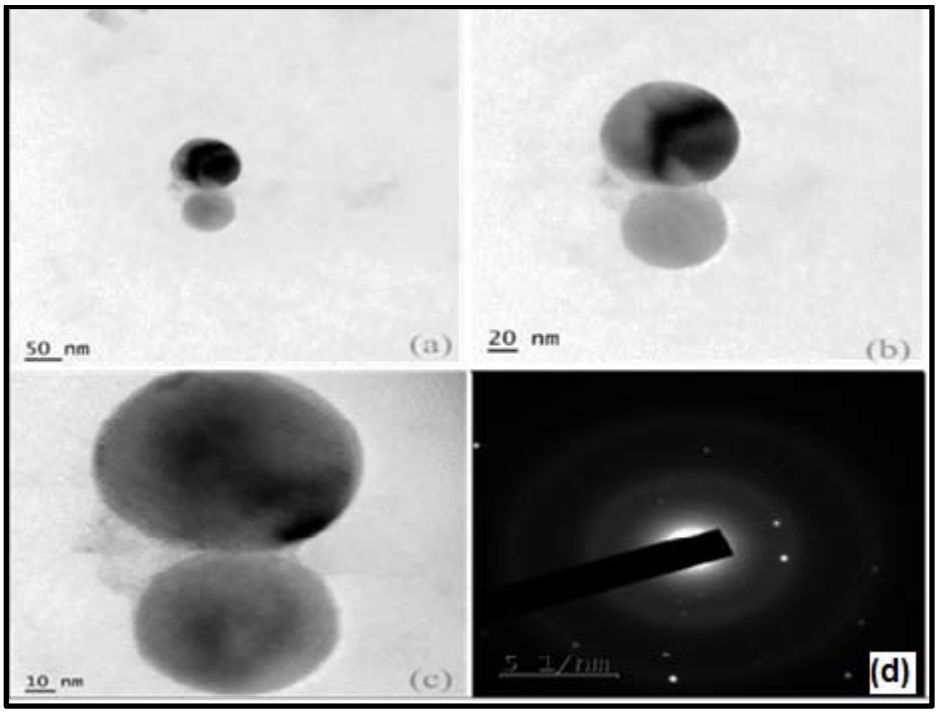

Figure 12. TEM images (a, b, c) of gallium nanoparticles in low and high magnification, (d) SAED pattern of gallium nanoparticles synthesized using root extracts of *A. paniculata*

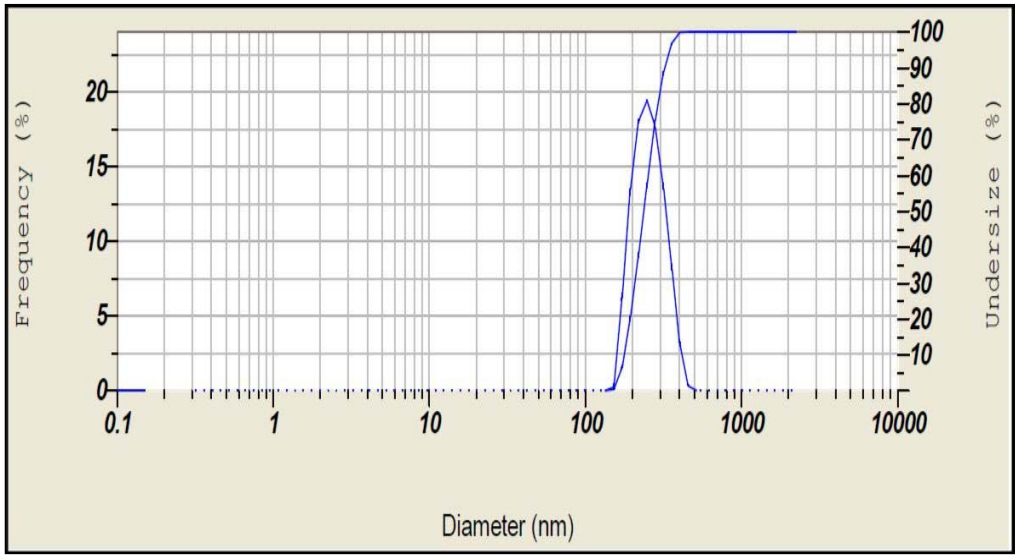

Figure 13. Particle size distribution of gallium nanoparticles prepared using root extract of *A. paniculata*.

4. Conclusion

Green synthesis of gallium nanoparticles was carried out using the root and flower extracts of *Andrographis paniculata*. The reaction time for nanoparticle synthesis was optimized. Biologically synthesized nanoparticles were characterized using UV spectral analysis, FT-IR spectra, SEM with EDX, XRD, TEM, and Particle size analyzer. The crystallite size of these nanoparticles was calculated theoretically using Scherrer formula and the size ranges from 22-40 nm. To our knowledge, this is the first report describing the biological synthesis of gallium nanoparticles. These gallium nanoparticles can be used in the treatment against bacterial infections.

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