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GREEN SYNTHESIS OF GOLD NANOPARTICLES USING *COLEUS AMBOINICUS* LEAVES EXTRACT, ITS CHARACTERIZATION AND ANTIBACTERIAL BIOASSAY

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Abstract: With the antibiotic resistance growth, there is rise of interest in discovering novel antimicrobial agents. The antibiotics restored with metal nanoparticles because of its enduring antibacterial properties. This investigation aimed for gold nanoparticles biosynthesis and determines the antibacterial efficacy against some human bacterial species.The gold nanoparticles was characterized by UV, FT-IR and DLS. The impacts of synthesized gold nanoparticles were tested against *E.coli* (Gram-negative), *S. aureus* (Gram positive), *S.pneumoniae* (Gram positive) and *K. pneumonia* (Gram-negative) by disc diffusion method (Kirby Bauer test).The gold nanoparticles were synthesized from the plant *Coleus amboinicus* leaves extract. The spectrum of UV-Vis revealed a higher peak at 546 nm; the FTIR showed the phenolic compounds in the plant extract that were responsible for the formation of gold nanoparticles and the DLS revealed the size to be 104.5 nm. The gold nanoparticles indicate the minimum and maximum activity for antibacterial pathogens towards *E.coli, K. pneumonia, S.pneumoniae and S. aureus.* Thus, the synthesized gold nanoparticles might be a better option to develop an antibacterial agent against selected bacterial human pathogens.

Index Terms - *Coleus amboinicus*, gold nanoparticles, Antibacterial activity, nanotechnology.

1. INTRODUCTION

Nanotechnology is an enlarging possibilities that empowering advanced solutions to be evolved using survive resources. Nanoparticles are synthesized by both physical and chemical methods that are slightly dangerous. In nanotechnology field, the most demanding barrier is synthesis of highly stable metal nanoparticles in various size, shapes and chemical composition based on eco-friendly development (Singh et al. 2016). The metal nanoparticles were initially synthesized by using chemicals as reducing agents. But due to its toxic and risk for environment and human health, the method of using chemicals were replaced by using plants and fungi etc., (biological system) called as biological methods.

In nanotechnology, the metals, metallic oxides and semiconductors were used for a wide range of implementation in environment, information, medicinal zones and energy (Heera and Shanmugam, 2015). The gold nanoparticles (AuNPs) have much interest among the nanomaterials due to gold is an oxidation-resistant and inert substance so it used in technologies and devices of nanoscale (Bindhu and Umadevi, 2014). For this reason, it has lot of applications like transport of drugs, chemicals and catalysis, diagnostics, therapeutics and biological imaging. The gold nanoparticles are the most investigated nanoparticles compared to all metals (Sathiyaraj et al. 2021). Additionally, the AuNPs functionalization and reduction with natural and synthetic compounds are well realized. So, in recent days the biosynthesis of gold nanoparticles got a much more attention due to the non-toxic property and its various biomedical uses (Priya Velammal et al. 2016).

From the past years, the nanoparticles used plants, plant components and their wastes, bacteria and fungi for their synthesis (Otunola et al. 2017). The synthesis of gold nanoparticles using plant extracts is an interesting area in the nanotechnology field, because it has eco friendly benefits and economic than the other methods of synthesis (Suzan et al. 2014). For the nanoparicles production the plants are considered as better for providing natural capping agents, non toxic and in expensive for isolation and culture media of microbes (Krithiga et al. 2015).

Now a days, the metal nanoparticles green synthesis is an major issue in nanoscience and also in attention in growing of metal nanoparticles biosynthesis using microbes. The biosynthesized nanoparticles applications are used in different spectrum of possible areas such as treatment of cancer, drug delivery, therapy of genes, analysis of DNA, biosensors, magnetic resonance imaging reaction rate enhancing and antimicrobial agents.

The bioactive compounds of plants act as a reducing agents for metal ion reduction to metal nanoparticles with well defined shape, size and remarkable anti-microbial efficacy (Ahmed et al. 2016). The plant *Coleus amboinicus* L. is commonly called as Indian Borage, it is a medicinal plant with tender fleshly perennial it carries various phytochemicals like patchoulane, flavanoids, monoterpenoid (carvacrol), bicyclic sesquiterpene (caryophyllene) had many medicinal applications. It is used in the treatments such as malaria fever, renal, hepatopathy, cough, asthma, bronchitis, hiccoughs, colic, convulsions and vesical calculi (Bhatt et al. 2013). The gold nanoparticles are widely used for invivo studies and have no side effects (Chen et al. 2014), the synthesis of nanoparticles deserves merit for green chemistry. There was no previous report on the *Coleus amboinicus* used for the synthesis of AuNPs. Our present study determined the *Coleus amboinicus* leaves extracts moderate gold nanoparticles synthesis against gram positive and gram negative bacteria. The synthesis AuNPs was confirmed by UV–Visible spectroscopy (UV–Vis), Fourier Transform Infra-Red Spectroscopy (FTIR) and Dynamic Light Scattering Technology (DLS).

II. TECHNIQUES AND METHODS

Plant collection and authentication

The leaves were collected from the Enathi Rajappa College campus, Pattukkottai, Tamilnadu, India and it was verified, authenticated as *Coleus amboinicus* with the help of the standard manuals such as "The flora of the Presidency of Madras"(Gamble, 1967) and "Indian Medicinal Plants" (Kirtikar and Basu,1994).

Preparation of leaves sample

40g of *Coleus amboinicus* fresh leaves were taken and added into the round bottom flask. The water bath was set up using 100ml of double-distilled water at 70-80°C. Insert the round bottom flask into the water bath and heat for two hours. After two hours, the flask was taken from the waterbath and the filter the leaves extract through the whatmann filter paper. All the extracts were concentrated using rotary flash evaporator and stored at 5°C in air tight bottle until further use.

Synthesis of gold nanoparticles (AuNPs)

For the AuNPs synthesis, the aqueous extract 10ml was mixed with the 90ml solution of $AuCl_3$ (1 mM) and the colour changed was noted. The solution of AuNPs was swirled for purification into 10,000 rpm at 4°C for 15 minutes. The collected pellet was then air dried and kept at room temperature for future process use after being dangled with deionized water.

Analysis of stability for synthesized AuNPs

The stability analysis of biosynthesized AuNPs was carried out against pH and temperature.

Effect of pH

The synthesized AuNPs were employed against pH stress such as acidic and basic. 1m of HCl and 1M of NaOH solutions were prepared using de-ionized water and added the few drops of these solutions for various pH range adjustment from acidic to basic (3-8). It was kept overnight and the results were observed in the form of scanning graph by running the samples using UV-Vis spectrophotometer.

Effect of Temperature

The plant leaves extracts and AuNPs were carried out to heat stress by keeping the flask on the hot plate. For observing the rise in temperature, the thermometer was placed in the nanoparticles solutions. The samples were identified at various temperature ranged from 25 to 100°C. The data was noted in the scanning graphs and check out by using UV-visible spectrophotometry.

Characterization of AuNPs

The Characterization of gold nanoparticles was performed in sequence using UV-Visible Spectrophotometer, DLS (Dynamic Light Scattering) and FT-IR measurement.

UV–Vis analysis

The UV-Visible spectrophotometer employed for the complete bio reduction property confirmation of gold ions into gold nanoparticles in plant aqueous leaves extract. For the analysiso of spectrum, the 3ml of the reaction mixture was poured in a quartz cuvette and the spectra analysis of UV-Visible was recorded on the wavelength of 300-800nm (Mani et al. 2021).

Fourier Transform Infrared spectroscopy (FTIR)

The FTIR was used to identify the functional groups of AuNPs synthesis. The mixture of plant leaves extracts with AuNP were placed in the sample container. Then the mixture was analyzed by using the FTIR (IR, SHIMADZU) at the resolution mode of 4cm (Gandhi et al. 2021). The results were recorded at the range of 400-4000 cm-1.

Dynamic Light Scattering Technology (DLS)

Dynamic Light Scattering Technology Zetasizer (Malvern Instruments, UK) was used to investigate the particle size, particle size distribution, polydispersibility index (PDI) and zeta potential of GNPs. Particle size can be determined by measuring the random changes in the intensity of light scattered from a suspension or solution. Light from the laser light source illuminates the sample in the cell. The scattered light signal is collected with one of two detectors, either at a 90 degree (right angle) or 173 degree (back angle) scattering angle. The provision of both detectors allows more flexibility in choosing measurement conditions (Koperuncholan, 2015).

Determination of Antibacterial activity

The antibacterial activity of plant leaves extract and AuNPs were determined using *E.coli* (Gram-negative), *S. aureus* (Gram positive), *S.pneumoniae* (Gram positive) and *K. pneumonia* (Gram-negative) by disc diffusion method (Kirby Bauer,1957). The selected organisms were sub-cultured on petri plates containing a sterilized Mueller- Hinton (MH) agar medium and the inoculums were spread on the petri plates. Then, the plates were incubated in aerobic chamber for 24 hours. After the formation of visually identified inhibition zone was measured in diameter. The results were compared with 10 mg/mL of standard control (Amoxicillin).

III. RESULTS AND DISCUSSION

Synthesis of AuNPs

The gold nanoparticles synthesis was first confirmed and monitored visually. By adding the different concentration of 1 mM solution of gold chloride ($AuCl_3$) with plant leaves extract, the black colour solution immediately changed into ash colour that indicates the process of synthesis and reduction (Fig.1). The reaction mixture colour gets changes after 24 hours propounded the absolute bioreduction and higher amount of formation of gold nanoparticles.

Coleus amboinicus was used as a stabilizing and reducing agent in this work and 1mM of $AuCl_3$ was utilized as a gold precursor. For the AuNPs formation, the $AuCl_3$ was segregated into ions of Au+ and Cl_3 – in the solution of reducing agent. As a result, the Au+ ions are capped and bounded by phytochemicals to produce the gold nanoparticles in the stable form. The AuNPs formation has been noticed by determining the changes of colour in the reaction mixture, as reported in earlier by Mittal et al. 2013.

Stability studies

The synthesized gold nanoparticle stability was tested by determining the effect of pH and temperature.

Effect of pH

The important parameter that affects the synthesis of nanoparticles is pH due to the effect of AuNPs size and shape. The AuNPs absorption spectra at different pH of 3-4, 4-5, 5-6, 6-7 and 7-8 were determined under the UV-VIS spectrophotometer. The synthesis of AuNPs enhanced at pH of 6-7 (Fig.2).

By rising of pH ranges from 3-4 (acidic) to 6-7 (basic), the intensity of absorption also increased, it suggests that the process of bio-reduction for the AuNPs synthesis was achieved at alkaline pH. Thus the pH of neutral to basic in reaction mixture was observed to be most strong and optimal for the synthesis of AuNPs. Sarwar et al (2017) and Singh et al (2015) reported increased synthesis of AuNPs at increased pH of 7-8.

Effect of temperature

The another important parameter for the AuNPs synthesis reveals that they stable at the temperature ranges at 35-50 ºC (Fig.3). This result is coherence with the investigation reported by Golmoraj et al., 2011. Our findings suggested that the increased temperature leads to the stability reduction of AuNPs because of the bioactive compounds deprivation in AuNPs synthesis (Paulkumar et al., 2013 and Golmoraj et al. 2011). 70 to 100°C, most of the synthesized nanoparticles were degraded that showed the absence of absorbance in the specific region.

Characterization studies

Plant leaves extract solution and 1mM gold chloride solutions were analyzed by UV- VIS spectrophotometry, FTIR and DLS. The absorption spectrum of synthesized AuNPs (Fig.4) was found to be maximum absorption band in the range of 546 nm. It is the remarkable phenomenon that formed in metal nanoparticles that occurs in higher peak on the surface of the particles. The metal nanoparticle's optical features were extensively known to be significantly determined by their form and size. Due to the tremendous nature and reduced size of the gold nanoparticles, the earlier investigations disclosed that the spherical gold nanoparticle gives absorption bands in the UV–visible spectra at the nm of 525-55 (Saqr et al. 2021). The gold nanoparticles absorption was established approximately 547 nm in most of the findings (Balasubramanian et al., 2020; Nadaroglu et al., 2017). The gap in energy band was extinct by the Tauc plot method (Ramesh et al. 2019) and it was observed to be 1.9 eV. The lower gap energy band was found at *Coleus amboinicus* plant extracts –AuNps successfully absorbed the UV light (Raja et al. 2019).

The FTIR spectra in this investigation ranged from 438 to 3449 cm_1. Fig. 5 showed that the bands noticed on the spectrum of FTIR at 2093.14 cm-1, 1641.83cm-1, 1460.44 cm-1, 1418.87 cm-1, 1384.31 cm-1, 1339.91 cm-1 and 1271.82 cm-1, 1039.86cm-1, 438.02cm-1,993.23cm-1 and 779.67cm-1. The spectrum of FTIR exhibit many peaks and the broad peaks were found at 3449 cm-1 corresponds to the –OH phenolic group in the plant flower extracts (Sk et al. 2020). The 2093 cm-1 absorption band belongs to aliphatic group of C–H stretching (Nithiyavathi et al. 2021). The carboxylate ion or free amine groups of the residues of amino acids were found at 1641 cm-1. A peak at 1460.44 cm-1, 1271.82 cm-1represent presence of –C=O group of ketone respectively. The peaks at 115.44, 993.23cm-1 and 779.67 cm-1 shows amide band and the peak at 1384 and 1039.86 cm-1 confirms the presence of amide group. The phenolic groups that aid in the synthesis of plant leaves extracts-AuNPs are represented by the majority of the peaks.

The DLS results of particle size analysis are showed in fig.6 indicates that all the phytosynthesised AuNPs are in the range of 104.5 nm. The zeta potential and particle size of at *Coleus amboinicus* plant extracts-AuNPs were determined by analysis of DLS (Mani et al. 2021a). It showed the negative zeta potential indicated

the presence of stability because of the negatively charged functional groups from the plant extracts-AuNPs (Hemlata et al. 2020; Mariadoss et al. 2019).

Antibacterial study

Antibacterial activities increased with higher volume of 50> 40>20µl with 1mg/ml,1.5mg/ml and 2mg/ml. Au nanoparticles showed great antimicrobial activities with the best zone of inhibition against *S.aureus* (29mm), *E.coli* (28.5mm), *K. pneumonia* (28 mm) and *S.pneumoniae* (23 mm) as shown in Fig.7. The antibiotic disc zone (Amoxicillin) also observed higher activity in *E.coli* (33.5mm).

Our study showed that the nanoparticles had a virtual antibacterial activity against both gram positive than the negative bacteria. This is due to the gram positive bacteria had an hard cell wall producing nanoparticles was difficult to enter while the gram negative bacterial cell wall are very easily destroyed (Renuka et al.2020; Sathiyaraj et al. 2020). The mechanism of gold nanoparticles engaged with the membrane of bacteria is not well accepted but many investigations showed that the morphology of membrane changes at the reaction of nanoparticles with bacteria results the plasma membrane transport and permeability increase, leads to the cell death Ezhilarasi et al. 2020). Damage of cell wall, mitochondria and DNA by gold nanoparticles has been suggested as mechanism of antibacterial (Perveen et al. 2018).

There are so many earlier reports of nanoparticles generated similar harm to cells of bacteria Bhushan et al. 2015; Veena et al. 2019). The nanoparticles antibacterial effectiveness was mainly influenced by morphology of samples, size of the particles and higher surface area to volume ratio. Nanoparticles are entirely smaller than the bacterial species so it allowed them to frequently entering the cell (Amanulla et al. 2019; Mobeen et al. 2019). So this findings report that the plant leaves extract- AuNPs showed the potent antibacterial properties.

IV. CONCLUSION

This study concludes for the nanoparticles synthesis, the leaves extract of *Coleus amboinicus* L. was employed as a stabilizing and reducing agents. The plant extract had an potency to producestable gold nanoparticles at optimum temperature of 35-50°C and with a size range of 546 nm identified and confirmed by various characterization techniques such as FTIR and DLS. The synthesized nanoparticles also showed potent antimicrobial activity against different gram positive and gram negative bacterial pathogenic species. As a result, biologically synthesized nanoparticles have the potential to become future anti infective and can be developed into medicines or topical agents.

Declaration of Competing Interest

The author declared that they have no known personal relationship or competing financial interest that could have to impact the work reported.

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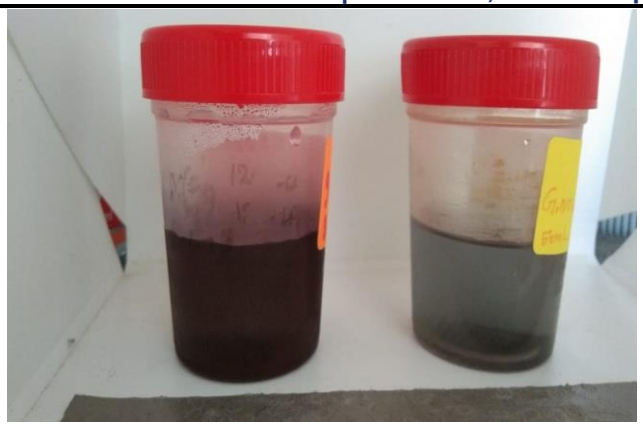


Fig.1 Synthesis of gold nanoparticles (GNPs)

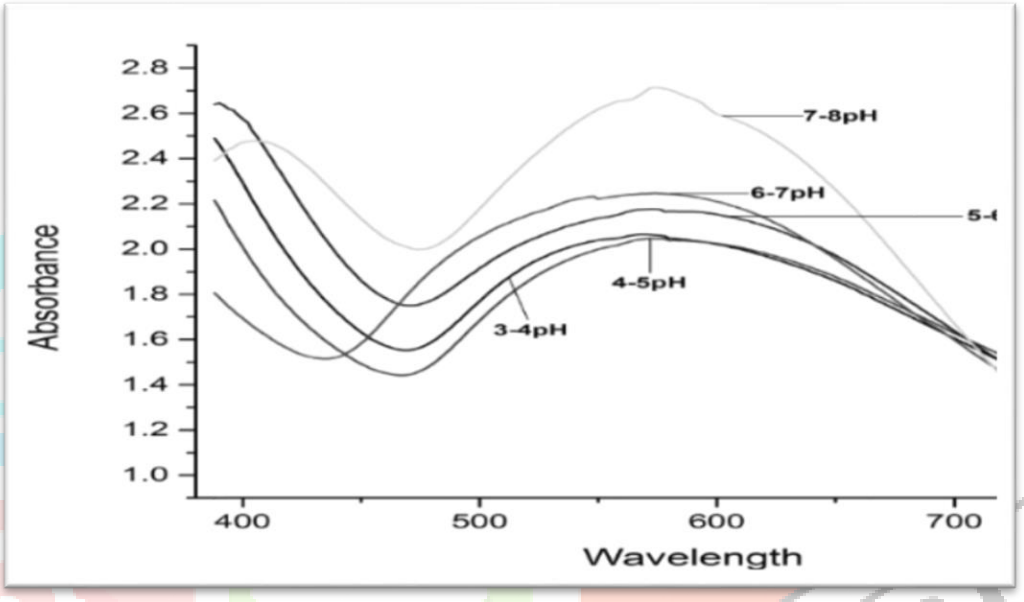


Fig.2 V-VIS Spectrum of AuNPs at different pH level

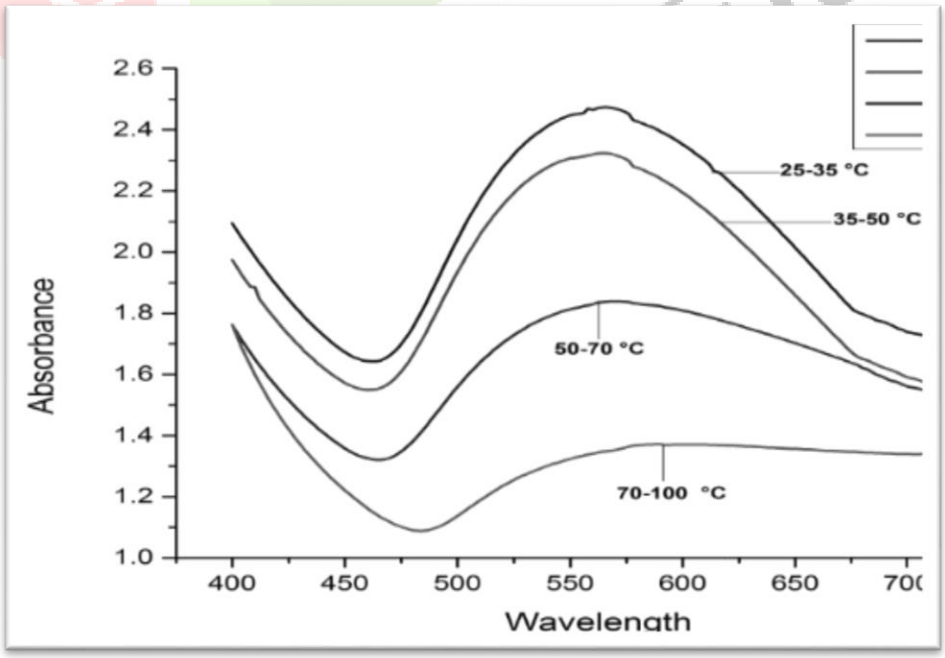


Fig.3 UV-VIS Spectrum of AuNPs isolated at different temperature ranges

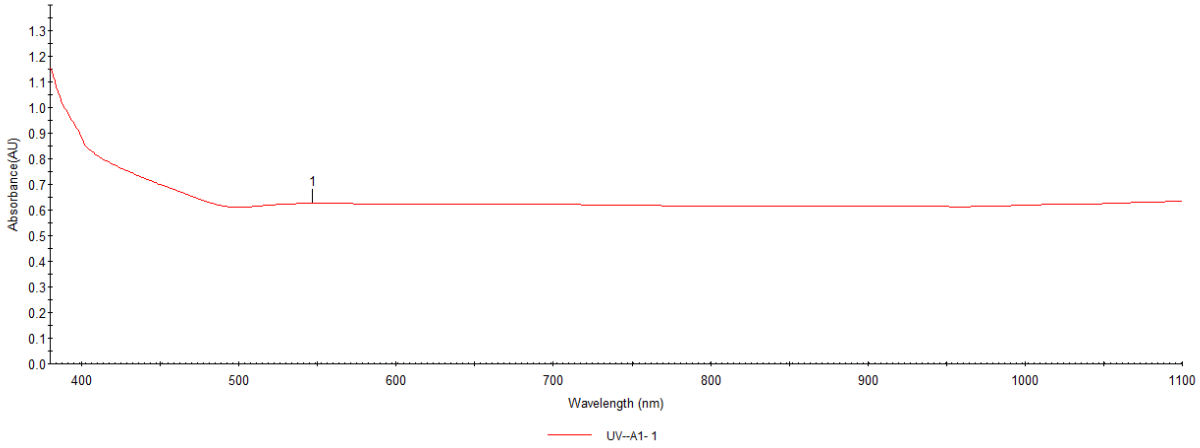


Fig.4 UV-VIS Spectrum of AuNPs isolated at different temperature ranges

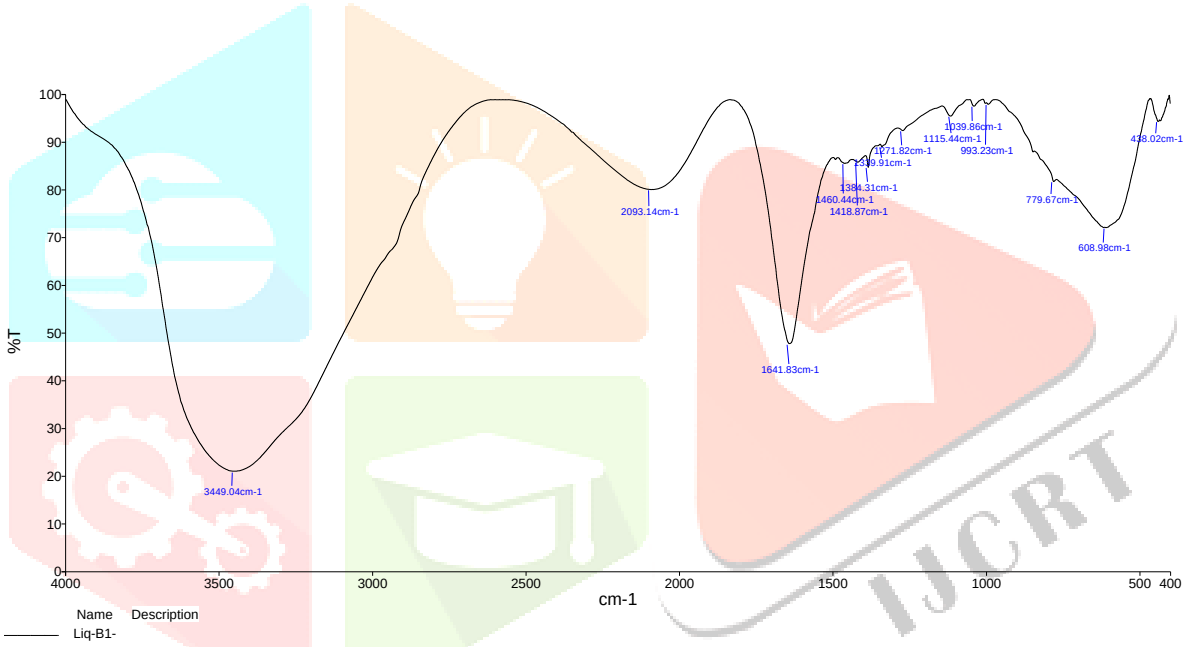


Fig.5 FTIR spectrum of AuNPs

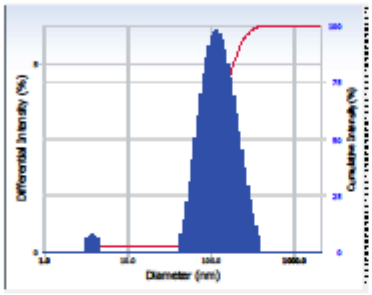
NanoPlus
Common

Intensity Distribution S/N : 411615

User	: Common	Group	:	Repetition	: 1/1
Date	: 05-May-22	File Name	: DLS-A2		
Time	: 15:03:15	Sample Information	:		
SOP Name	: SJC Size Method			Security	: No Security

Version 5.22 / 3.00

Intensity Distribution



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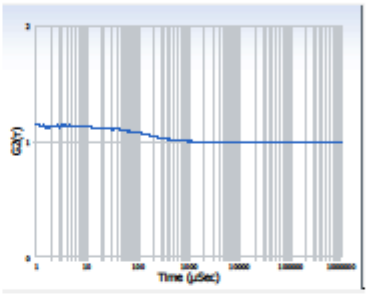

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	3.7	0.4
2	134.1	64.6
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	131.5	66.5

Residual : 3.004e-003 (O.K)

Cumulants Results

Diameter	(d)	: 104.5	(nm)
Polydispersity Index	(P.I.)	: 0.276	
Diffusion Const.	(D)	: 4.720e-008	(cm²/sec)

Measurement Condition

Temperature	: 25.1	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8858	(cP)
Scattering Intensity	: 29922	(cps)
Attenuator 1	: 0.9	(%)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
1.0	0.0	0.0	6.8	0.0	2.0	46.5	0.8	3.4	316.8	1.0	98.9
1.1	0.0	0.0	7.4	0.0	2.0	50.2	1.3	4.7	342.1	0.7	99.6
1.2	0.0	0.0	7.9	0.0	2.0	54.2	1.8	6.5	369.4	0.4	100.0
1.3	0.0	0.0	8.6	0.0	2.0	58.5	2.4	9.0	398.9	0.0	100.0
1.4	0.0	0.0	9.3	0.0	2.0	63.2	3.0	12.0	430.7	0.0	100.0
1.5	0.0	0.0	10.0	0.0	2.0	68.2	3.6	15.6	465.0	0.0	100.0
1.6	0.0	0.0	10.8	0.0	2.0	73.7	4.2	19.8	502.2	0.0	100.0
1.7	0.0	0.0	11.7	0.0	2.0	79.5	4.7	24.5	542.2	0.0	100.0
1.8	0.0	0.0	12.6	0.0	2.0	85.9	5.1	29.6	585.5	0.0	100.0
2.0	0.0	0.0	13.6	0.0	2.0	92.7	5.5	35.1	632.2	0.0	100.0
2.2	0.0	0.0	14.7	0.0	2.0	100.1	5.7	40.8	682.7	0.0	100.0
2.3	0.0	0.0	15.9	0.0	2.0	108.1	5.9	46.7	737.2	0.0	100.0
2.5	0.0	0.0	17.1	0.0	2.0	116.8	5.9	52.5	796.0	0.0	100.0
2.7	0.0	0.0	18.5	0.0	2.0	126.1	5.8	58.3	859.5	0.0	100.0
2.9	0.0	0.0	20.0	0.0	2.0	136.1	5.6	64.0	928.1	0.0	100.0
3.2	0.4	0.4	21.6	0.0	2.0	147.0	5.4	69.3	1002.2	0.0	100.0
3.4	0.4	0.8	23.3	0.0	2.0	158.7	5.0	74.3	1082.1	0.0	100.0
3.7	0.4	1.2	25.1	0.0	2.0	171.4	4.6	78.9	1168.5	0.0	100.0
4.0	0.4	1.7	27.2	0.0	2.0	185.1	4.2	83.1	1261.7	0.0	100.0
4.3	0.4	2.0	29.3	0.0	2.0	199.9	3.7	86.7	1362.4	0.0	100.0
4.6	0.0	2.0	31.7	0.0	2.0	215.8	3.2	89.9	1471.1	0.0	100.0
5.0	0.0	2.0	34.2	0.0	2.0	233.0	2.7	92.6	1588.5	0.0	100.0
5.4	0.0	2.0	36.9	0.0	2.0	251.6	2.2	94.8	1715.3	0.0	100.0
5.8	0.0	2.0	39.9	0.0	2.0	271.7	1.8	96.6	1852.2	0.0	100.0

Fig.6 Dynamic light scattering analysis (DLS)

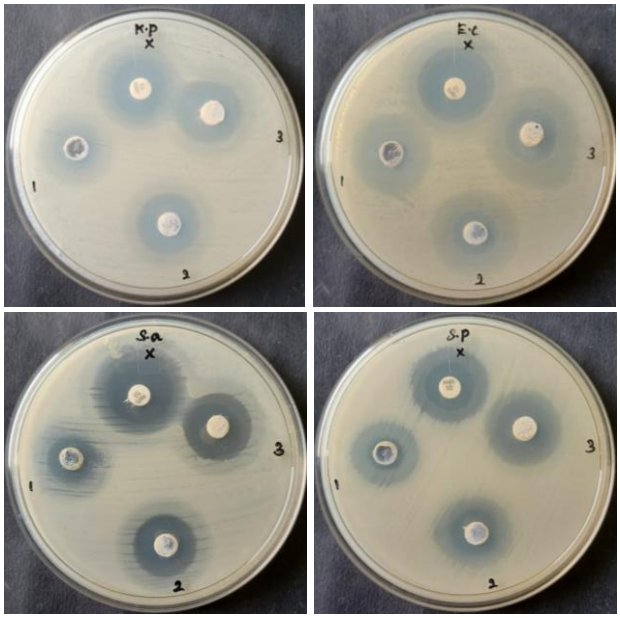

Fig.7 Antibacterial activity ±SD of green synthesized AuNPs against different microbes