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Research Article

Production of Biologically Synthesized Silver Nanoparticles using *Glinus Oppositifolius* Aqueous Leaf Extract; Characterization and Evaluation of its Anti-Bacterial Efficacy

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ABSTRACT

The green synthesis of silver nanoparticles and their applications have attracted to applicable in many areas of research because of their therapeutic and clinical, and different applications, due to easily, safer, and lesser time and plant material. The synthesized nanoparticles achieved through greener way with the aqueous leaf source of Glinus oppositifolius. In the present work, bio-synthesized nanoparticles are characterized by advanced tools like UV-Vis Spectrophotometer, DLS-Zeta potential as well as the synthesized material was carried out the efficacy on two selected gram positive (Bacillus subtilis and Staphylococcus aureus) and two-gram negative (Klebsiella pneumonae and Escherichia coli) bacteria. The AgNPs formation was primarily confirmed through UV-Vis spectrophotometer scan ranges from 190 to 750 nm acquired intensive peak at 439 nm. The DLS particle size and zeta potential analysis revealed size and stability of the nanoparticle around 16.11 nm and – 31.4mV zeta potential values. The greener way synthesized silver nanoparticles of selected plant species AgNPs showed significant antibacterial activity on above mentioned bacteria. The DPPH activity expressed as significantly when compared with plant extract.

INTRODUCTION

The idea of nanotechnology was first proposed by Feynman in 1959. Nanotechnology has created new opportunities in several industries, such as food packaging, animal husbandry, electronics, agriculture, medicine, and health care. It is also one of the most recent industrial developments (Balachandar *et al.,* 2019). The nanomaterial opened very big window in material science research of physical, chemical and engineering. It concentrated on synthesis of nanoparticles, having at least one dimension range of below 100 nm. It possessed with different physiochemical components and most of them undefined materials (Ramesh *et al.,* 2018). Nanoparticles (NPs) have

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been integrated into nanotechnology and used as versatile tools in a large diversity of scientific and technic practices due to their remarkably advanced physicochemical properties (Ravindran *et al.,* 2013). Nanoscale particles have enhanced properties owing because of atomic interactions on their surface, resulting in less coordination than that in bulk materials. Nanoparticles can be made of metals or non-metals depending on their basic forms. Metallic nanoparticles mainly consist of gold, silver, copper, magnetic (cobalt, nickel), and semiconducting materials. In contrast, non-metallic nanoparticles are primarily composed of carbon-based materials. Intensive research has been conducted on metallic nanoparticles because they possess distinctive electrical, optical, and catalytic properties (Bharathi *et al.,* 2018)

Nanomaterials provide more advantages to environment and more compatibility with excellent biomedical applications including anti-microbial, anti-cancer, larvicidal, immunosuppressive, biosensor, catalysis and drug delivery (Vijayanand *et al.,* 2019). Nanomaterials are divided in different types such as nanoparticles, Nano rods, Nano films, fullerenes etc. Among these types, nanoparticles have the ability to exhibit the zero dimensions with significant biochemical properties. Last 10 years, the uniform sized nanoparticle synthesis is increased worldwide, and the size based morphology of the nanoparticles is frequently used to deal with advanced technology and environmental challenges (Ebrahimzadeh *et al.,* 2020). The smaller sized nanoparticles are synthesized in ordinary laboratory conditions and low cost, high surface area. Various routes are available in nanoparticles synthesis; they are physical, chemical, biological. Among these, biological route is considered more efficient than other routes due to the low toxicity, low cost, increased surface nature and volume zero (Al-Brahim and Mohammed, 2020). Sometimes, the chemical mediated nanoparticles seriously affected the environment and others directly or indirectly due to the toxic nature (Lakhan *et al.,* 2020). AgNPs are essential nanomaterials studied extensively because of their electrical, optical, and biological properties. Consequently, these nanoparticles have been used for numerous applications, including bio sensing, drug delivery, Nano device fabrication, and medicine (Jain *et al.,* 2008).

Biological processes, known as green synthesis, primarily conducted via medicinal plants, offer advantages over chemical and physical methods because they are cost-effective, eco-friendly, and readily available. Green synthesis methods using microorganisms and plants have attracted great attention because of their eco-friendly and biocompatible procedures (Le Ouay and Stellacci F, 2015; Kharissova *et al.,* 2013). Among them plant extract based biosynthesis of metal nanoparticles particularly silver and gold have been reported by many researchers [Badri Narayanan and Sakthivel, 2008; Prathap Chandran *et al.,* 2006; Dubey *et al.,* 2010]. Plants are regarded as a highly desirable system for nanoparticle synthesis due to their great ability to produce a wide range of bioactive secondary metabolites with high reducing potential.



Fig 1. Selected Plant *Glinus oppositifolius*

Table:1. Preliminary Phytochemical screening from leaves of Glinus oppositifolius

Phytochemical constituents	Leaf						
	Aqueous	Methanol	Ethanol	Acetone	Ethyl acetate	Petroleum ether	Chloroform
Proteins	+	+	+	-	-	-	-
Amino acids	+	+	-	-	-	-	-
Reducing sugars	+	+	+	+	-	-	-
Alkaloids	+	+	+	-	-	+	+
Anthraquinones	-	+	-	+	+	-	+
Anthocyanins	-	-	-	-	-	-	-
Coumarins	-	+	+	-	-	-	+
Flavonoids	+	+	+	+	+	+	-
Glycosides	+	+	+	-	-	+	+
Lignins	-	-	-	-	-	-	-
Leucoanthocyanins	-	+	+	-	-	-	-
Phenols	+	+	+	+	+	+	-
Saponins	+	+	+	-	-	-	+
Steroids	+	+	+	-	-	-	+
Tannins	+	+	+	-	-	+	-
Terpenoids	+	+	+	-	-	-	+
Triterpenoids	+	+	-	+	-	-	-

Table 2: Quantitative estimation of secondary metabolites from Glinus oppositifolius

S. No	Phytoconstituent	Leaf (mg/gdw).
1	Starch	0.753±0.001
2	Sugar	0.462±0.004
3	Lipids	0.324±0.003
4	Proteins	0.575±0.004
5	Flavonoids	1.584±1.570
6	Phenols	1.357±0.001
7	Tannins	1.251±0.002

'±' indicates Standard error
Units-mg/gdw.

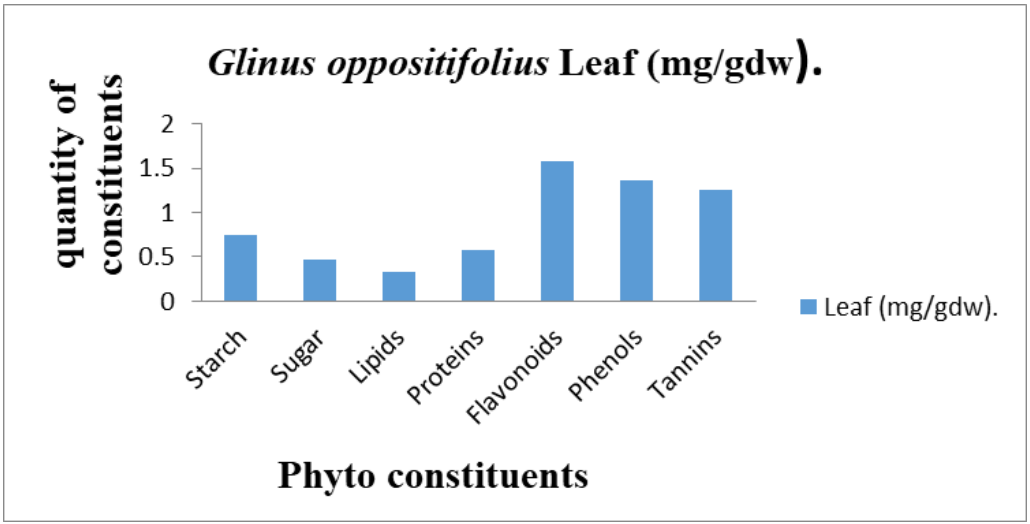


Fig.2 Graphical representation of Quantitative analysis of phyto-active compounds

MATERIAL AND METHODS

Collection of Plant material and preparation of aqueous extract

Glinus oppositifolius fresh leaves were collected from natural habitats, plant was identified 3 km from Tirupati. The leaf material was brought into the lab then the material was washed with running tap water and followed by distilled water, later moisture content and dust particles removed using sterile cotton cloth and the material was cut into small pieces; this taken and was shade dried for two weeks. Afterwards made this ground fine powder; 50 grams of powder taken and extracted by 200 ml Milli pore water boiling on water both for 30 min with helping 500 mL sterile Erlenmeyer flask. The extract was filtered using by What man no. 1 filter paper and it was stored at refrigerator until the synthesis of silver nanoparticles (AgNPs).

Chemicals and preparation of Ag (NO3)2 solution

10 grams of Ag $(NO3)_2$ procured from Sigma Aldrich, then 1 mM Silver nitrate solution was prepared with the sterile distilled water, it was stored in the amber colour bottle until the synthesis process.

Synthesis of Silver Nanoparticles (AgNPs)

AgNPs were synthesized according to modified literature protocols (Siddhant e*t al.,* 2017 and Hebeish *et al.,* 2017). In briefly 25 ml of leaf aqueous extract was poured into 500 ml sterile conical flask; then it was titrated by 250 ml of 1 mM prepared Ag $(NO3)_2$ with temperature at 60-80^{0}C for 60 minutes. The reaction mixture was centrifuged at 15,000 for 15 min to remove the presence of admixture, and this was used for further characterization and anti-bacterial activities.

Characterization of synthesized Silver nanoparticles

The UV-Visible absorption spectrum of AgNPs was performed by using Nano drop 800 nm spectrophotometer. AgNPs analysed by the Fourier Transform Infra-Red (FT-IR) spectra range of 4000 to 500 cm1 with an ALPHA interferometer (ECO-ART) to find study of the morphology in the reaction mixture which are actively involved in the bio-reduction of AgNPs. To determine the size of the nanoparticle and size distribution in aqueous AgNPs solution performed by the recent tool Dynamic Light Scattering (DLS) Malvern-Zeta analyzer. To find the size, shape, and agglomeration pattern of the synthesized AgNPs was analysed by the Hr-TEM H-3300 advanced 300 kV from Hitachi.

Antibacterial Activity

Antibacterial study evaluated by using standard protocol followed by the disc diffusion assay [Anonymous, 1996]. The bacterial cultures were procured from the department of Microbiology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The synthesized Go-AgNPs were examined for antibacterial activity against selected two gram negative (*Klebsiella pneumonae* and *Escherichia coli)* and two gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains. For this 20 µl of plant extract (40 mg/mL), 20 µl Ag $(NO3)_2$, 20 µl (40 mg/mL), and Streptomycin were applied on separate sterile Whatman No.1 filter paper discs (7 mm diameter) and allowed to dry before being place on nutrient agar medium. The entire process was performed in triplicates and incubated at 37^{0}C for 24 hours. Eventually the results were obtained by the measuring the zone diameter in centimetre and this results are tabulated.

DPPH Activity

DPPH stock solution was prepared by dissolving 10mg of DPPH in 100mL Methanol, which resulted a solution mixture with an absorbance of around 1.305 at 517 nm. In the test tubes 3 mL DPPH workable solutions (1mL of DPPH stock solutions+ 2mL of Methanol) were combined with 100µl of leaf extract respectively, as a standard, 3mL of DPPH workable solutions often mixed with 100µl of methanol. After 30min of incubation in darkness, the absorbance was used to compute the percentage of antioxidants (Baliyan *et al.,* 2022; Ramakrishna and Savithramma, 2019). Similar results were seen in earlier work AgNPs synthesized from *Plantago lanceolata* leaf extract (Karakas *et al.,* 2023) and AgNPs synthesized from *Plantago lanceolata* leaf extract (Karakas *et al.,* 2023).

Percentage of antioxidants activity= [(Ac-As)/Ac] X 100

Ac- Control reaction absorbance; As- Testing specimen absorbance

RESULTS AND DISCUSSION

UV-Visible spectroscopy

The aqueous leaf extract of *Glinus oppositifolius* is mixed with the 1mM of Silver nitrate solution ($Ag(NO3)_2$, the colour intense was observed as deep brown from light brown, which is primary method to confirm the synthesized nanoparticles are Ag. The broad peak was acquired at 439nm (Fig.3), this is due to the reaction mixture is contain SPR (Surface Plasmon Resonance) of AgNPs. The Surface Plasmon Resonance revealed about dispersion, shape, size, and stability types of morphological features of nanoparticles. These types of results were recorded in synthesized AgNPs with leaf source aqueous extract of *Walsura trifoliata* (Venkata Subbaiah and Savithramma, 2022).

DLS and Zeta potential

Dynamic Light Scattering (DLS) serves as an advanced analytical technique for examining the surface charge, size, and size distribution of nanoparticles. This method relies on the interaction of Brownian motion of spherical particles with light that traverses a colloidal solution (Saxena *et al.,* 2010). The measurement of Zeta potential is crucial in estimating the stability, distribution, and aggregation of biologically synthesized nanoparticles by evaluating the repulsive forces arising from fluctuations in charge densities. In this investigation, the *Glinus oppositifolius* leaf (Go)-AgNPs demonstrated an average size of 145 nm, a negatively charged zeta potential of -31.4 mV (Fig. 4). The results of the AgNPs are depicted in the Figures 2 a&b. Similar kind of results was reported by Vijayalakshmi Sakaray et al. (2024) from *Acalypha indica* Plant Extract AgNPs.

Antibacterial study

The antibacterial study revealed that the synthesized nano particles significant activity against four clinically isolated bacteria, but the efficient activity was seen against gram negative bacteria when compared to gram positive bacteria. The zone of inhibition is mentioned in the table (Table 3; Fig. 5&6; Table 3). The highest zone of inhibition of Go-AgNPs was recorded against *K. pnemonea*e. This kind of study accordance with the previous results of Green synthesis of silver nanoparticles using *Trema orientalis* L. extracts (Richa Das *et al.,* 2025). Among the bacterial activity, the highest zone of inhibition was observed in *K. pneumoniae* (-ve) and *B. subtilis* (+ve); AgNPs are more susceptible for gram negative bacteria than gram positive. Antimicrobial activities of AgNPs are relying on the size and shape of the nanoparticles. The size of the nanoparticles also gives more space to interact

with microorganisms. According to the earlier studies smaller-sized nanoparticles have showed maximum antimicrobial activity than larger-sized particles due to they have a large surface to interact with bacteria efficacy which leads to cell death.

2, 2- diphenyl-1- picrylhydrazyl anti-oxidant (DPPH) (DPPH) Activity

Synthesized PmL-AgNPs was determined by DPPH method. The activity depends on the reduction of DPPH radical from DPPH-H, a hydrogen donating anti-oxidant. The IC 50 values of in-vitro *Glinus oppositifolius* leaf aqueous extract and biologically synthesized Go-AgNPs anti-oxidant activity mentioned in the table (Table 4). The results revealed that the DPPH anti-oxidants activity was done by the increased concentrations of the test samples. Plants contain wealth of bio-active compounds like flavonoids, tannins related to the phenolic compounds along with other polyphenols, which are a significant group of phytochemicals that acts as primary anti-oxidants of free radical scavengers (Alvur *et al.,* 2022; Ko *et al.,* 20220. Based on acquired results, revealed that the synthesized AgNPs are exhibited excellent antioxidants activity when compared to selected plant extract and it was showed very near to Ag $(NO_3)_2$ solution (Fig. 7&8; Table.4). The highest DPPH activity was observed at 25µl as 1.305±0.012. Similar results were observed in earlier work eco-friendly AgNPs synthesized from *Plantago lanceolata* leaf extract (Karakas *et al.,* 2023).

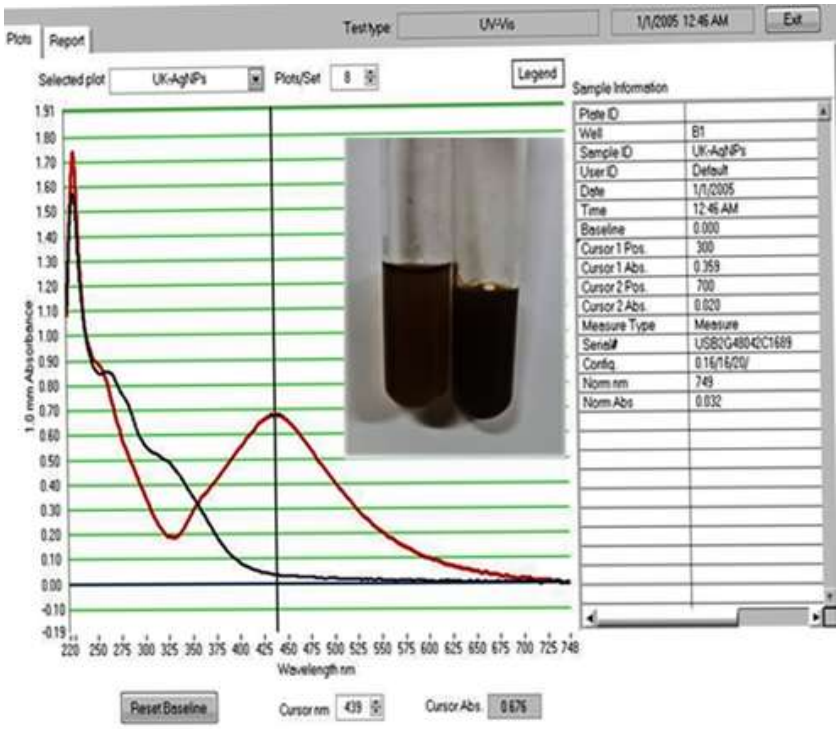


Figure 3: Uv-graph Synthesis of AgNPs from *Glinus oppositifolius* leaves acquired by UV-Vis Spectroscopic analysis

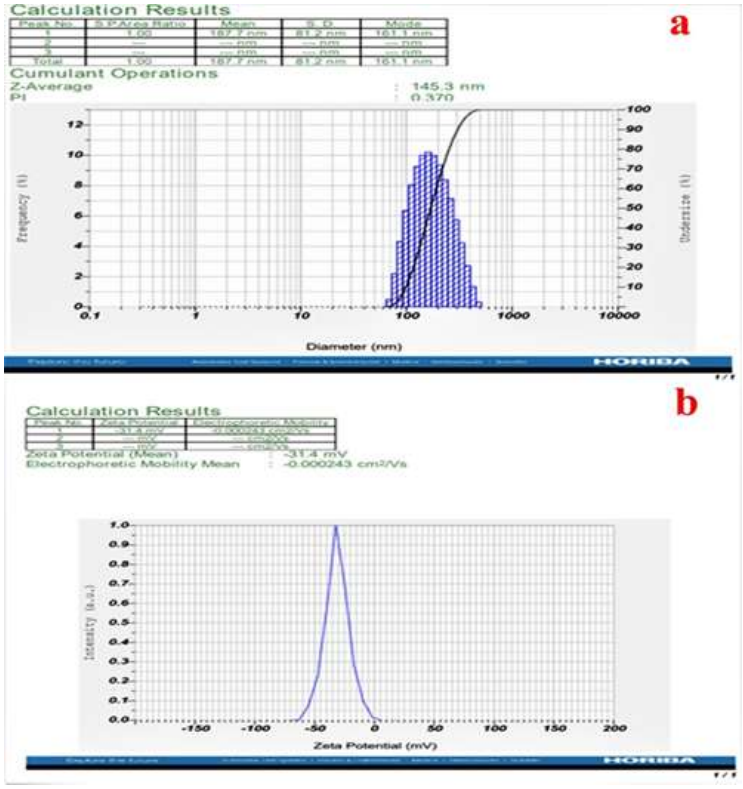


Figure 4: Synthesis of AgNPs from *Glinus oppositifolius* leaves acquired a) Particle size and b) Zeta potential of the synthesized AgNPs

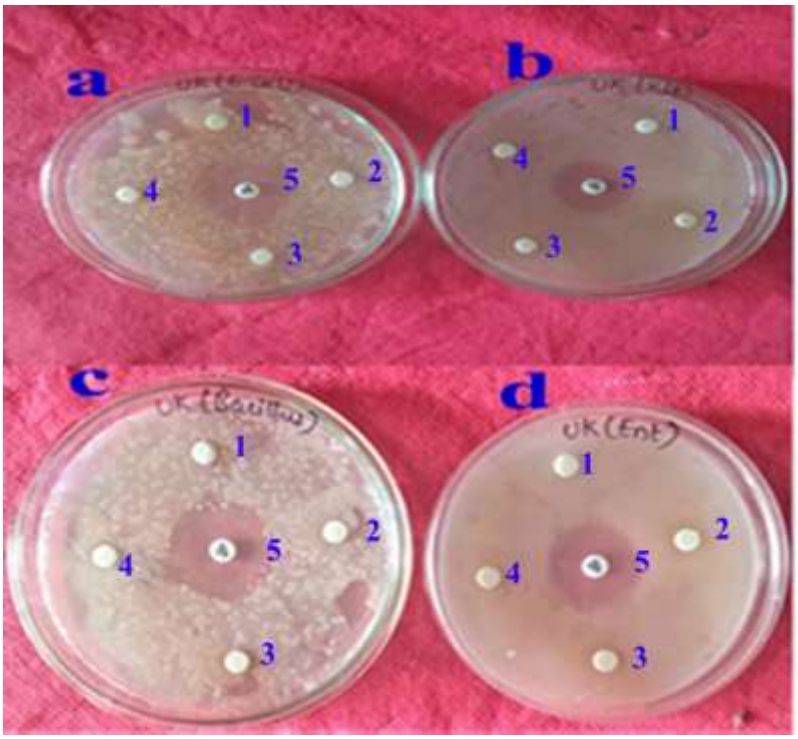


Fig 5. Antimicrobial activity of *Glinus oppositifolius*
a). *E. coli* b). *K. pneumonia* c). *B. sabtilis* d). *Enterococcus* aqueous leaf extract biologically synthesized GoL- 1). Ag $(No_3)_2$ solution 2.) acqueous leaf extract, 3) and 4). AgNPs two concentrations (25µl and 75µl), 5). Antibiotic Streptomycin.

Table 3: Antibacterial activity of *Glinus oppositifolius*

Name of the Organism	Zone of Inhibition				
	Plant extract	Ag(NO3)2	AgNPs (25µl)	AgNPs (75µl)	Streptomycin
B. subtilis	11.9±0.088	8.1±0.057	10.5±0.094	21.1±0.033	21.1±0.066
Enterococcus	8.9±0.066	10.3±0.11	9.2 ±0.124	17.2±0.088	22.1±0.057
K. pnemonea	12.8±0.057	9.8±0.066	9.7±0.235	17.8±0.033	29.1±0.115
E. coli	10.9±0.033	12.1±0.088	9.5±0.169	14.9±1.25	28.4±0.066

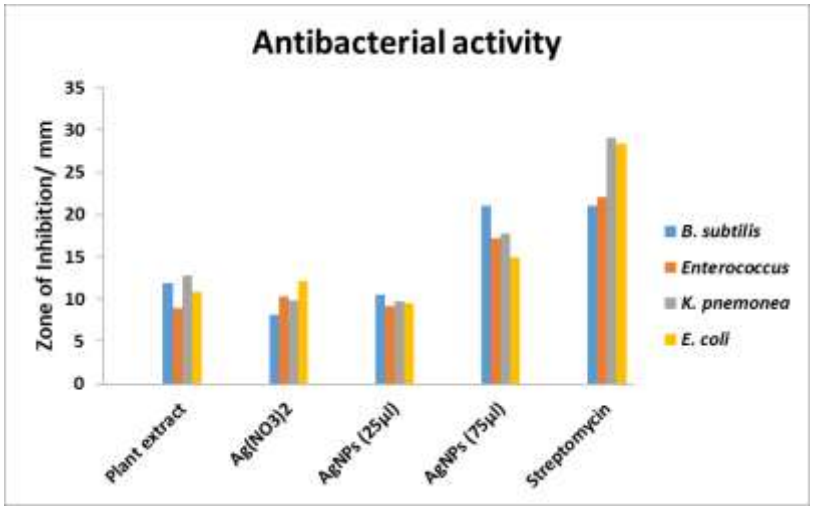


Figure 6: Antimicrobial activity of AgNPs from *Glinus oppositifolius*

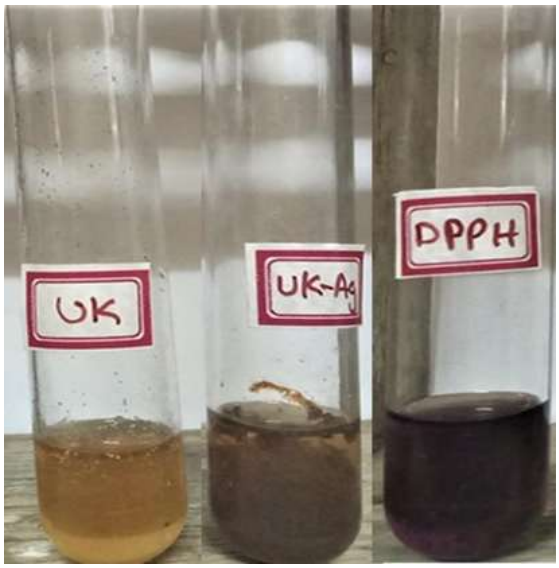


Figure: 7. DPPH activity of AgNPs from *Glinus oppositifolius*

Table.4 DPPH anti-oxidants activity of AgNPs from *Glinus oppositifolius*

Samples	25µl	50 µl	100 µl
Blannk	1.278±0.005	2.556±0.004	4.962±0.014
Extract	0.378±0.003	0.756±0.003	1.507±0.002
AgNPs	1.305±0.012	2.61±0.004	5.22± 0.020

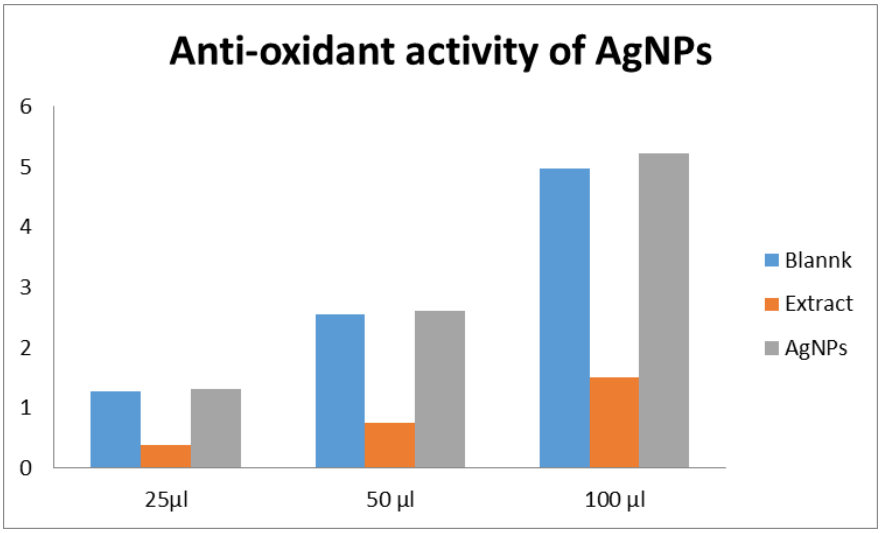


Fig 8. DPPH anti-oxidants activity of AgNPs from *Glinus oppositifolius*

CONCLUSION

In this investigation, we adopted a green technique to synthesise Silver-NPs, by means of *Glinus oppositifolius* leaves extract. In UV-Vis absorption curve, the SPR Peak was observed around 439 nm, revealing the amalgamation of AgNPs. The DLS zeta potential study revealed about stability, dissemination and aggregation levels of bio-synthesized nanoparticles and the size of the nanoparticles around 16.11 nm distributed evenly and -31.4 mV zeta potential values. Additionally, the nanoparticles were studied to show their antibacterial activity against Gram-positive and Gram-negative bacteria. The anti-bacterial studies of the synthesized AgNPs on selected two gram positive *Bacillus subtilis* and *Enterococcus* ; two gram negative bacteria *Klebsiella pneumonae* and *Escherichia coli* showed significant activity and AgNPs play role in inhibition of bacterial colonies, and also expressed effective DPPH activity with dose dependant manner. Above mentioned biological properties were evaluated, and AgNPs were found to have potent anti-bacterial and anti-oxidants. The phytochemicals present in plant extract have significantly stimulated the rate of synthesis, with stabilized and enhanced bioactivity. This method of synthesis can be a promising way and used for large-scale production. Consequently, these biologically synthesized nanoparticles are environmentally benign. Antimicrobial agent, DPPH activities are best quality production of nanoparticles with lesser volume of plant extract. The plant assisted nanoparticles are useful for coming days to prepare novel drug to treat different diseases and moreover these are non-hazardous to the environment, most stable products. Go-AgNPs are that will be help to develop into efficacious drug delivery systems as they are smaller in size.

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