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RESEARCH PAPER

Characterization and optimization of biogenic Silver Nanoparticles derived from Ziziphus mauritiana for their Antimicrobial, Antioxidant and Anti-inflammatory assays

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ABSTRACT

Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension. Nanoparticles are used immensely in medical and industrial fields due to its small size, orientation, physical properties. The biological approach is the most emerging approach of preparation because this method is easier than the other methods, eco-friendly and less time consuming. In this study, green synthesis of nanoparticles was done using root extract *Ziziphus mauritiana* and its antimicrobial, antioxidant and anti-inflammatory assay was performed. Green synthesis was done by using of Ziziphus mauritiana root extract and $AgNO_3$ solution. The nanoparticles were characterized by UV-vis Spectroscopy, DLS, Zeta Potential, FTIR and SEM. Antimicrobial, antioxidant and anti-inflammatory assays were performed according standard procedures. The different types of compounds present in *Ziziphua mauritiana* root extract reduced the Ag+ to Ag^o. The synthesized silver nanoparticles showed satisfactory antimicrobial, antioxidant and anti-inflammatory properties. The novel approach of bio-nanotechnology can be applied in variety of fields like Nano medicine, Pharmaceuticals, bioremediation etc. These procedures are simple, cost effective and environment friendly hence can be used in a largely in bio medical applications.
Keywords: - *Nanotechnology, Green Synthesis, Nanoparticles, Root Extract.*

1. INTRODUCTION

Nanotechnology has the huge possibilities of changing the world. It is widely acknowledged as 'Key Technology' of 21st Century. According to the US Government's NNI "nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nano meters, where unique phenomena enable novel applications". Nobel metal Nanoparticles like gold, silver have the unique optical, electrical and catalytical properties and that's why they are given preference over other metals. Currently larger spectrum researches are going on for the optimization of Nano particles. Based on their shape and size and for this the chemical and

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physicals properties are being altered [1,2].

Biosynthesis of Nano particles. have gained quick attention because of the growing needs to develop eco-friendly and efficient technologies in material synthesis. Noble metals Nano particles. have widespread use in various fields like medicine, energy, electronics and catalysis [3]. Because of their nano size, they have the ability to communicate with the biomolecules present on the surface of cells which helps in understanding the biochemical and physiochemical properties of the cells. Also, they are used in targeted drug delivery systems and treatments of cancer due to their specificity. They are not only confined to Biomedical applications but they are widely used in environmental remedy, food industries, biomaterials, water treatment etc. [4] For the application purposes, it is important for a nanoparticle to stable, shape selective and compatible with the interacting environment. These parameters can be achieved by synthesizing nanoparticles under controlled conditions. There are number of ways to synthesize nanoparticles including Physical methods, chemical methods and biological methods. From the last five years, green synthesis has been given importance over the conventional methods. The abundance of raw materials makes green synthesis a cost-effective process and also no harmful chemicals are involved in the process [5]. Biogenic synthesis of nanoparticles is compatible with the principle of green chemistry. The resulting nanoparticles produced through biogenic synthesis are comparatively stable than that of physical and chemical methods. Further experiments and studies are under process to improvise the green synthesis of nanoparticles [6]. Hence, in this study an attempt was made to green synthesize silver nanoparticles using the root extract of *Ziziphus mauritiana* and its study its antimicrobial, antioxidant, anti-inflammatory properties.

MATERIALS AND METHODS

Sample Preparation

The roots of the plant *Ziziphus mauritiana* was collected from arid areas of Jaipur, washed, dried and powdered. The aqueous root extract was prepared by adding 5 gm of this powdered sample to 100 ml of Autoclaved double Distilled water. It was sonicated using Probe Sonicator for 50 minutes at 50% amplitude and 30 second pulse. After sonication the sample was filtered using Whatman Filter paper No. 1 and was stored at 4˚C for further use. The pH was set to 12.

Preparation of Silver Nanoparticle solution: 1 Millimolar $AgNO_3$ solution was prepared in a conical flask by adding 16.987 milligrams of $AgNO_3$ to 100 ml of Autoclaved Double Distilled water. The flask was covered with silver foil to avoid the penetration of light into the flask.

Molarity Calculation:

Concentration (Molar) = Mass (Mg)

Volume (ml) x Molecular weight(g/mol)

Synthesis of Silver Nanoparticles

The Nanoparticles were synthesized according to the procedure done by Logeswai P et.al., (2015) along with slight modifications [7]. 100 ml of 1 Millimolar AgNO3 was heated at 55-60 ° C for 10 minutes at 250 rpm. After 10 minutes, about 10 ml of pH-maintained sample was added to AgNO3 solution. After 60 minutes of heating the color of reaction mixture changed from colorless to dark brown, which indicates the formation of nanoparticles and reduction of Ag^+ to Ag^0. The sample was then purified by centrifuging it at 13,000 rpm for 30 minutes.

Characterization of Nanoparticles

The formation of nanoparticles was monitored using UV – Vis Spectrophotometer by Surface plasmon Resonance (SPR) peaks. To determine hydrodynamic particle size of nanoparticles in silver colloidal solution a DLS was performed. Surface charge of silver nanoparticles was identified using Zeta Potential. FT-IR was done for the qualitative study of the nanoparticles i.e., to study the functional groups present in the nanoparticles. Also, SEM was done to analyse the exact shape and size of the synthesized nanoparticles.

Antimicrobial Assay

Antimicrobial assays were performed on pathogenic strains like *Listeria monocytogenes, S. aureus* and *E. coli* using standard Agar diffusion method on Nutrient Agar Plate. Different concentrations of AgNPs were loaded into the wells (25 µl, 50 µl, 75 µl and 100 µl.). Streptomycin was used as reference. The plates were incubated at 37 ℃ for 18-24 hours and zones were measured.

Antioxidant Assay (DPPH free radical scavenging assay)

The antioxidant activity of *Ziziphus* root extract was measured using free radical method by Williams-Brand W. *et.al.* (1995) using 2,2-diphenyl-1-picryl-hydrazyl [8]. 0.2 mM solution was prepared in methanol and it was kept in dark for 2 hours until the absorbance was stabilized. 100 µl of DPPH was added to different concentrations of AgNPs (5%,10%,15%,20% & 25%). It was incubated for 30 minutes and absorbance was measured at 517 nm against methanol as blank. Ascorbic Acid was used as reference.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Anti-Inflammatory Assay (*In-vitro)*

Also known as Human Red Blood Cell membrane stabilization method. 5 ml of blood was mixed with equal volume of Alsever's solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42

% NaCl) and centrifuged at 3000 rpm for 10 minutes. The obtained packed cells were washed with Normal saline for three times and 10% HRBC suspension was made. A mixture of 1 ml of phosphate buffer, 2 ml of hypo saline, 0.5 ml HRBC suspension was made and various concentrations of AgNPs (5%,10%,15%,20% & 25%) were added. These reactions were incubated at 37℃ for 30 minutes. After incubation the reactions were centrifuged at 3000 rpm for 20 minutes. The absorbance of supernatant solution was taken using UV – Vis Spectroscopy at 560 nm against Normal saline as blank. Disprin was used as reference.

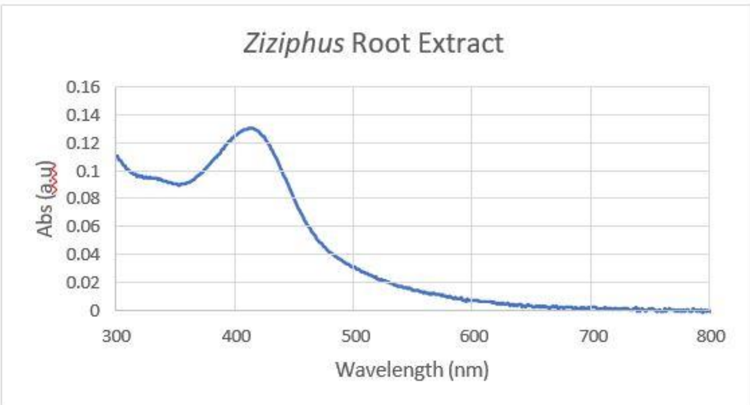
$$\text{Percentage Protection} = \frac{100 - \text{OD of test sample} \times 100}{\text{OD of Control}}$$

RESULTS AND DISCUSSION

Characterization of Nanoparticles

UV-Vis Analysis

UV–Vis spectral study is an authentic technique to monitor the progress of the reaction during the reduction of Ag+ ions. In this study, narrow cone-shaped spectrum curve was obtained from UV–Vis analysis and SPR of silver occurred at 416 nm. (Graph 1) The Ag+ ion reduction was confirmed by the apparent transformation in the colour of the reaction mixture from light brown to dark brown (Pic. 1). The samples were prepared using 1 mL, 5 mL, and 10 mL of *Ziziphus mauritiana* extract taken from the standard solution and amount of silver nitrate was kept fixed in all these tests.



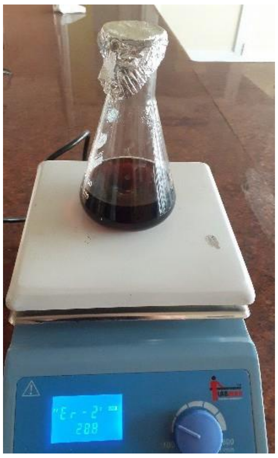


Figure1: (a) UV–Vis absorption spectra of synthesized silver nanoparticles (b) Silver Nanoparticle synthesis

DLS and Zeta Potential

The particle size distribution and zeta potential of the prepared AgNPs colloid were determined by the DLS technique using Zetasizer Nano ZS 90 (Malvern Instruments Ltd., UK) within the range of 0.1–

10 000 nm at a scattering angle of 90° and 25 °C. The results of DLS analysis showed the average size of nanoparticles to be 28.78 nm (Graph No. 2).

The stability of the nanoparticles was seen at −9.22 mV (Graph No. 3). The negative values of nanoparticles specified the good stability, well dispersed and possibility of presence of secondary metabolites as a capping or reducing agent.

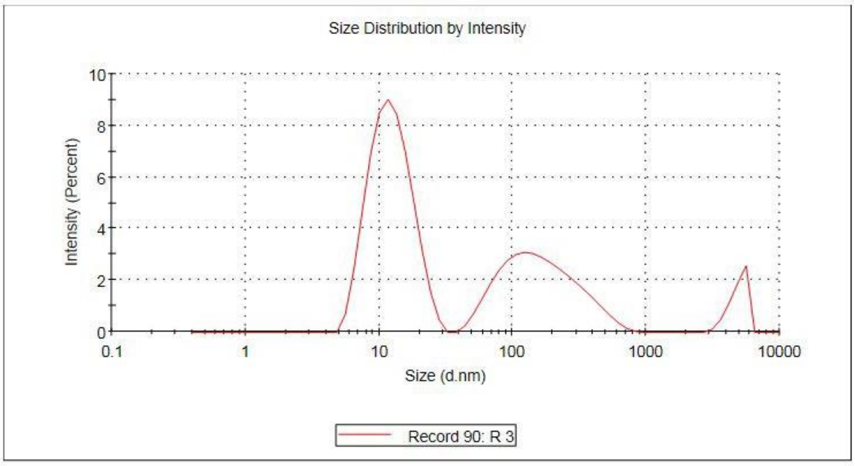


Figure2: Particles size distribution for synthesized AgNPs

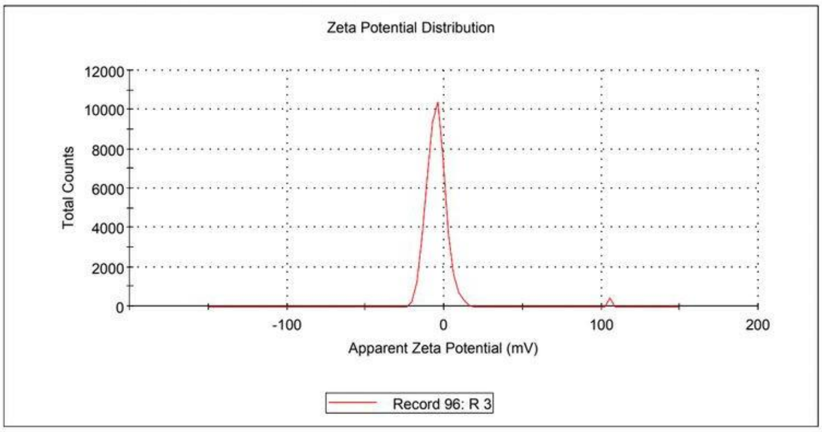


Figure3: Zeta potential for synthesized AgNPs

FT-IR Analysis

FT-IR spectroscopy was used to identify the surface and functional groups and its interaction with the AgNPs. The spectrum showed major absorption peaks at 629.16, 1222.2, 1371.1, 1636.85, 2144.66 and 3329.50 cm^{-1}, which signify that the plant molecules act as capping agents that were bound on AgNPs. The absorption peak at region 3329.50 cm^{-1} was the reason for -OH stretching vibration. The absorption peak at 2144.66 cm^{-1} represented the C–H stretching. Hence, this spectral data confirmed the presence of proteins by the amine or amide I band at the region of 1636.85 cm^{-1}. Also, the absorption band 1371.1 cm^{-1} appended by AgNPs with the C= O functional groups. The peaks at

1222.2 corresponding to the C–H stretching of the aromatic group and 629.16 cm^{-1} represents the corresponding OH group of a phenolic group. (Graph No. 4)

A similar study was done by Velmurgan P *et.al.,* (2013) in which the root extract of *Zingiber officinale* was used to synthesize silver nanoparticles. In FTIR analysis, an absorption peak was observed at 2950 cm^{-1} which indicates the characteristic of -OH stretching and presence of Phenolic group. Hence, our study result showed that phytochemical constituents like alkaloids, phenolic compounds, amino acids carbohydrates and particularly tannins are responsible for reduction of silver ions and also protect the AgNPs from aggregation and therefore increases its stability by acting as caping agents [9].

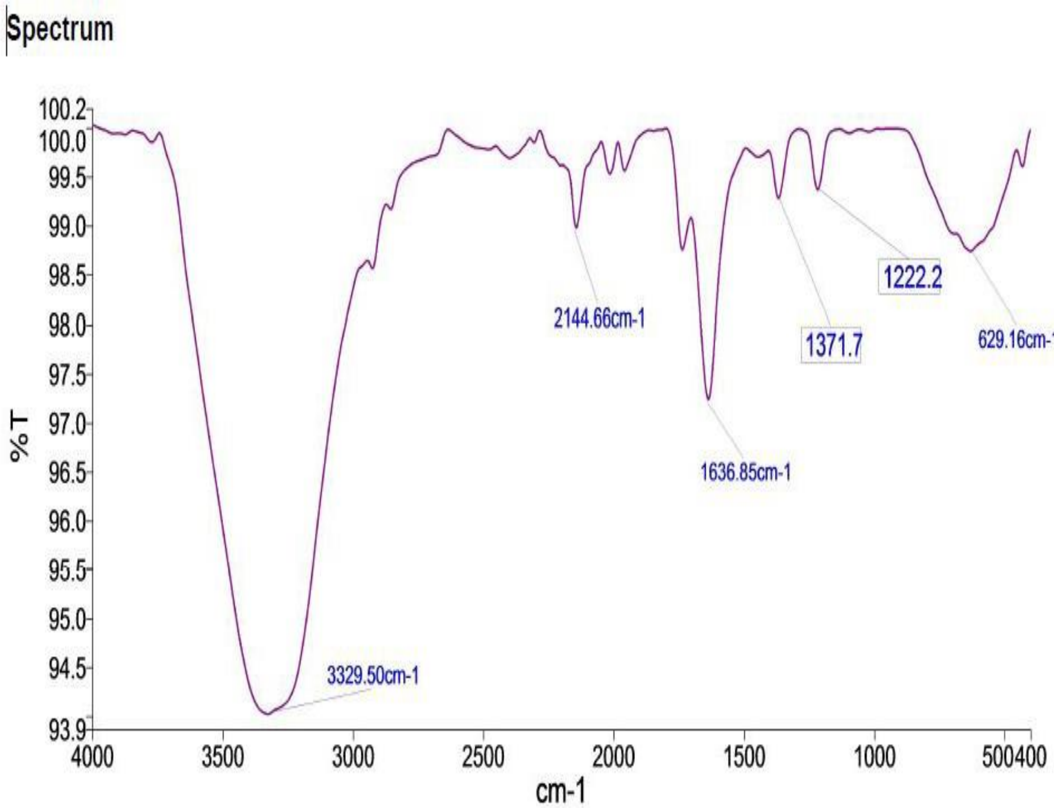
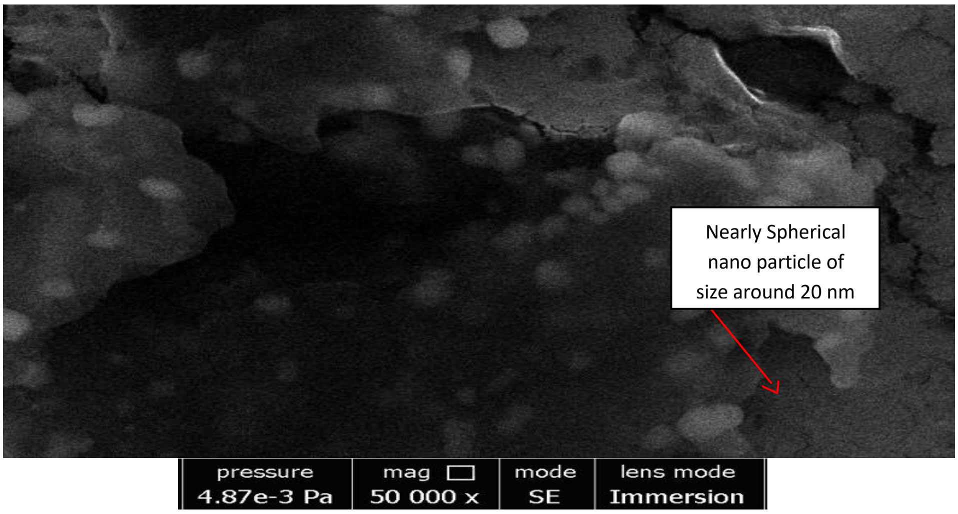


Figure4: FTIR spectral analysis of synthesized Nanoparticles

SEM Analysis

SEM provides further insight into the morphology and size details of the silver nanoparticles. The nanoparticles obtained in this study were analysed with SEM to reveal their shape and size which revealed nearly spherical shaped nanoparticles which were slightly larger in size as compared to that of DLS. The size of nanoparticles ranged from 20-80 nm in size. The increase in size might be due to the presence of layer of stabilizing agents naturally present in the root extract as well as due to aggregation of smaller nanoparticles together [10].



Clusters of Nano particles with size ranging from 20-40 nm

Pic No. 2: SEM images of synthesized Nanoparticles

Antimicrobial Assay

Three pathogenic strains i.e., *L. monocytogenes*, *S. aureus* and *E. coli* were tested against different concentrations of synthesized Nanoparticles, Silver Salts, Root extract and Streptomycin which was used as Reference. Amongst all the pathogens, the zone of inhibition of Nanoparticles were observed only against *L. monocytogenes* while other two strains were resistant to the synthesized nanoparticles. The other studies also suggest that the silver nanoparticles lead to bacterial cell death by penetrating the cell wall and degrading the plasma membrane. This creates pores in the cell wall and eventually leading to death of the organism. [11]

According to Kim S. *et.al.*, that oxidative stress induces cytotoxicity rather than the independent silver ions and hence no zones were seen against silver salts [12]. This may also be due to larger surface of area of nanoparticles as to that of silver ions which therefore provides better attachment towards microorganisms. [Table No. 1, Pic. No. 3 & 4]

Table No. 1: Zone of Inhibition shown by Microorganisms

AgNPs	25 µl	50 µl	75 µl	100 µl	Streptomycin	Silver Salts	Root extract
L. monocytogenes	No Zone	5 ± 0.1 mm	7± 0.1 mm	8 ± 0.1 mm	1.4 ± 0.1 mm	No Zone	No Zone
S. aureus	-	No Zone	No Zone	No Zone	1.7 ± 0.1 mm	No Zone	No Zone
E. coli	-	No Zone	No Zone	No Zone	0.7 ± 1 mm	No Zone	No Zone

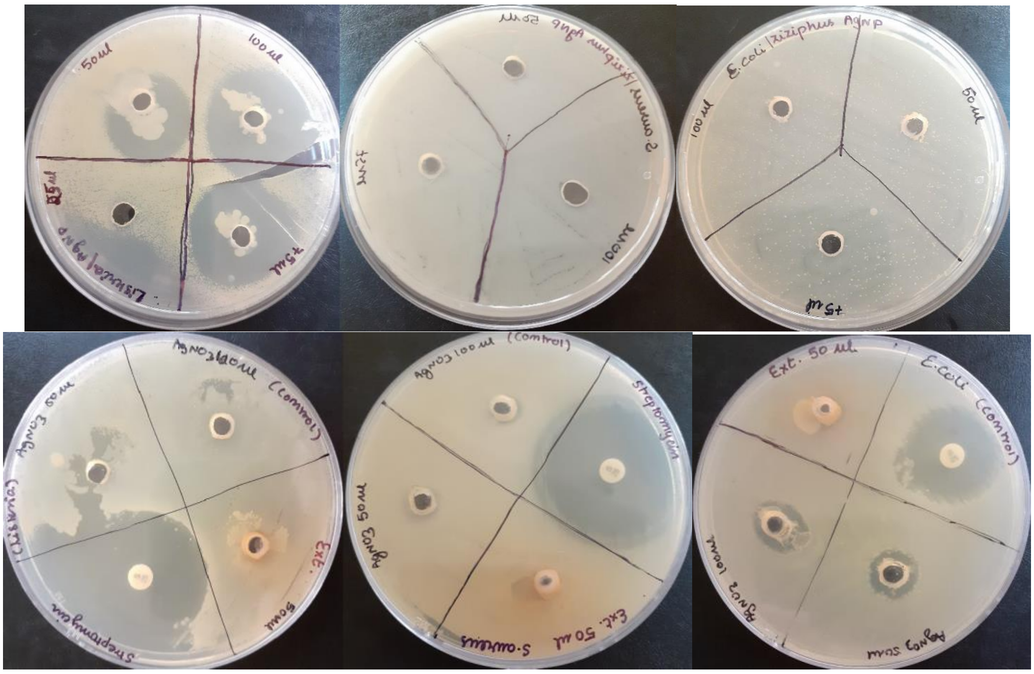


Figure4: Zone of Inhibition against Streptomycin, Silver salts and Root Extract

Antioxidant Assay

The antioxidant properties of synthesized nanoparticles were measured using DPPH scavenging assay. The results showed that synthesized nanoparticles exhibited better antioxidant properties compared to that of the reference material i.e., Ascorbic acid. The value for lowest activity was recorded at 10% of silver nanoparticles as well as of the reference material. The highest value was recorded at 20% of silver nanoparticles and at 25% of reference material. This indicates that the activity increased up to certain concentration and then decreased.

The antioxidant activity might be exhibited due to presence of several compounds like phenols, alkaloids, tannins, amino acids which are present in our root extract sample and has the capability to donate hydrogen to the free radicles to produce stable phenoxyl radicles [13].

Also, the studies done by Keshari A.K. *et.al*, (2020), Abdel-Aziz M. *et.al.*, (2014) suggest that green synthesized silver nanoparticles exhibit moderate to high level of antioxidant activity and hence can be used for therapeutic purposes [14,15]. [Table No. 2].

Table No. 2: Antioxidant Activity of synthesized silver nanoparticles

Concentration	AgNPs	Ascorbic Acid
5%	10.62 ± 0.10	11.12 ±0.12
10%	9.125 ±0.25	2.00 ±0.42
15%	13.37 ±0.76	3.87 ±0.56
20%	32.00 ±0.82	4.75 ±0.64
25%	17.00 ±0.41	11.50 ±0.82

Anti-Inflammatory Assay

Silver nanoparticles have the ability to inhibit the haemolysis by decreasing the efflux of intracellular components hence this test was done to measure the HRBC membrane stabilizing activity using different concentrations of synthesized silver nanoparticles. Previous studies suggest that the secondary metabolites of the plant extracts have the ability to inhibit the lyposomal activity of neutrophils [16]. In this study, percentage protection was observed to increase with increase in concentration of silver nanoparticles and after thereafter become stable. The highest activity was observed at 20% which was nearly similar to the reference material. [Table No. 3].

Table No. 3: Anti-inflammatory activity of nanoparticles at different concentrations

Concentration	AgNPs	Diclofenac
5%	2.64 ± 0.02	1.76 ± 0.72
10%	40.56 ± 0.76	38.27 ± 0.34
15%	55.81 ± 0.73	55.76 ± 0.93
20%	55.81 ±1.25	55.79 ±0.56
25%	55.80 ± 0.12	55.71 ± 0.33

Conclusion

Green synthesis is highly considered as an important tool to diminish the hostile effects of traditional methods for the synthesis of nanoparticles which are highly used in laboratories and industries. The use of bio-nanotechnology to synthesize the compounds with antimicrobial, antioxidant and anti-inflammatory properties is an area of current research by many scientists.

In this study, non-toxic, practical and environmentally benevolent green synthesis approach was used for green synthesis of silver nanoparticles using *Ziziphus mauritina* roots. From the analysis of UV-Vis spectroscopy, give sharper peak showing that the nanoparticles formed are varying in sizes and also stable. The SEM analysis shows the comparatively spherical shape of silver nanoparticles. The size as compared to DLS increased due to the presence of a layer of protein on the surface of nanoparticles. From the FTIR it was concluded that phenolic compounds, amino acids carbohydrates and particularly tannins may act as the stabilizing agent and protect the nanoparticles.

Also, the synthesized nanoparticles showed potent antimicrobial, antioxidant and anti-inflammatory properties. It is believed that the silver nanoparticles penetrate the cell wall of the microorganism and causes the cellular damage by interacting with Sulphur and phosphorous containing components like DNA, protein.

Hence, it can be concluded that silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications well as large scale commercial production.

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