Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Anti-Inflammatory Potential of Silver Nanoparticles Synthesized Using Eulophia Ochreata (Lindl.) Extract

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The concept of biological synthesis of nanoparticles, known as green synthesis, presents a challenging yet environmentally friendly approach. Among various biological agents, plants offer a safe and easily accessible route for the synthesis of nanoparticles, holding potential for large-scale production.

This research explores the green synthesis of AgNPs using extracts from Eulophia Ochreata Lindl, a ground orchid commonly known as 'Amarkand'. The plant's abundant metabolites serve as effective reducing and capping agents, facilitating the synthesis of stable metal nanoparticles. The study aims to synthesize, characterize, and evaluate the anti-inflammatory effects of AgNPs using Eulophia Ochreata extract.

The methodology involved the collection, authentication, and extraction of plant material, followed by the synthesis of AgNPs through a green synthesis process. Various characterization techniques, including UV-Visible spectroscopy, FTIR analysis, particle size analysis, SEM, TEM, and XRD, provided comprehensive insights into the synthesized AgNPs' morphology, structure, and properties.

The evaluation of anti-inflammatory activity revealed promising results, demonstrating dose-dependent inhibition of RBC hemolysis and protein denaturation by AgNPs derived from Eulophia Ochreata extract. These findings highlight the therapeutic potential of AgNPs as anti-inflammatory agents, offering a sustainable and effective alternative to conventional drugs.

In conclusion, this study exemplifies the convergence of nanotechnology and green chemistry, providing a sustainable solution for nanoparticle synthesis with biomedical applications. The anti-inflammatory efficacy of AgNPs synthesized using Eulophia Ochreata extract underscores their potential for future therapeutic interventions, covering further research into their mechanisms and clinical implications.

Keywords: nanoparticles, Eulophia Ochreata Lindl, green synthesis.

1. Introduction

Biological synthesis of nanoparticle is a challenging concept which is very well known as green synthesis. Among the different biological agents, plants provide safe and beneficial way to the synthesis of metallic nanoparticle as it is easily available, so there are possibilities for large scale production.[1] Environmentally friendly or "green" processes in chemistry and chemical technologies are gaining popularity and are crucially needed due to global environmental concerns.

Nanotechnology is the science and engineering of devices and materials on the scale of atoms or small groups of atoms having material size ranged from 0.1 to 100 nm. Metal nanoparticles like silver nanoparticles (AgNPs)have been extensively used due to its unique physical and chemical features, which consists of high electrical conductivity and thermal, optical, antimicrobial, and biological properties . Owing to its unique properties, AgNPs are widely used in biomedical applications such as anti-inflammatory, wound dressings , antiseptic fabrics , topical creams , and sprays. The present work is based on a green synthesis of AgNPs using extract of Eulophia Ochreata Lindl. Plant metabolites, abundant reducing and capping agents, are recognized for their effectiveness in synthesizing stable metal nanoparticles..[2]

E. ochreata, from the family Orchidaceae is a ground orchid, commonly known as 'Amarkand'. It is a perennial tuberous herb occurs in rainy seasons in the forest.[3]

In the Ethno botanical survey of the forests of Maharashtra it was found that the tubers are used as an ingredient in food, tonic and as rejuvenating herb. Astringent, antifatigue, aphrodisiac, anthelminthic, and as a blood purifier properties of this plant have been identified and exploited by the tribal people. It has been extensively used to relive cold, cough and cardiac problems. [4,5] Based upon these well-known remedial values, E.ochreata, tubers were selected for further work. The lack of well-documented scientific evidence will predominantly impede the progress of isolated molecule in the avenue. New research findings indicate that specific compounds present in orchids demonstrate anti-tumor effects, potentially inhibiting the proliferation of cancer cells[6]. The World Health Organization recommends traditional medicine due to its efficacy and safety; however, in recent years, there has been a growing concern about the environmental impact of conventional synthesis methods for nanoparticles.[7] Therefore, many researchers in this context utilise natural compounds for the biogenesis of silver nanoparticles.[8]

In consideration of this, there is a need for species possessing both therapeutic and aesthetic value. This research was undertaken to synthesize, characterize, and evaluate the anti-inflammatory effects of AgNPs using Eulophia Ochreata extract.

2. MATERIALS AND METHODS

Chemicals:

Eulophia Ochreata Lindl was collected from Bhima Shankar regioan near Pune , Maharastra, India. Acetone (99%),Ethanol (98%), sodium hydroxide (NaOH, 99%) purchased from Loba Chemicals India. Distilled water was used throughout the study where necessary. SHIMADZU UV-1900 instrument is used for UV analysis. Particle size determination, SEM analysis was

performed at Diya Labs Mumbai. All additional chemicals and reagents were of very high analytical purity.

1. Plant material collection and extract preparation:

Collection of the plant material.

The tubers of Eulophia ochreata Lindl and rhizomes of Zingiber cassumunar Roxb were collected from the Bhimashankar region Taluks maval, Maharashtra.

Authentication of plant.

The plant life changed into recognized. It was authenticated via Dr.G.G.Podar, HOD, Botany Department, Y.C.College of Science, Karad and Voucher specimen turned into deposited at the same college as wide variety AAK1

Preparation of Extract:

About 20 gm of finely cut tubers were kept in a beaker containing 200 mL double distilled water and boiled for 30 min. The extract was cooled down and filtered with Whatman filter paper no.1 and extract was stored at 4 ^{0}C for further use.

2. Synthesis of Silver Nanoparticles

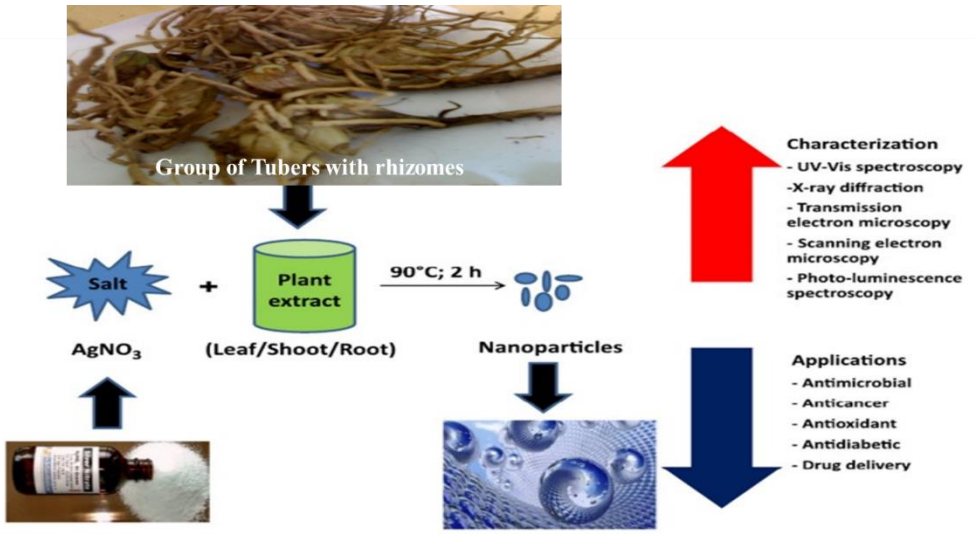

Fig. 1.Synthesis of Silver Nanoparticles.

1)The powders of Eulophia ochreata Lindl Tubers with rhizomes were taken (60gm).

2) Then this EU powder was mixed in a beaker with 600 ml of distilled water and boiled for 30 minutes. After boiling, the solution was cooled down for 10 min and double filtered by Whatman paper.

3)The solution of silver nitrate was prepared by dissolving o.16 g of silver nitrate in 100ml of

distilled water in a beaker and kept in the airtight bottle until further use.

4)Then 6ml of EU extract were mixed using a mechanical stirrer for 10 to 15 minutes with a 10 ml solution of silver nitrate in the beaker and kept for 24 hours at room temperature.

5)After the 24h ,it was observed that the colour of the solution changed due to the formation of AgNPs.

6)Then the sample was centrifuged for 10 min at 12000 rpm.

Fig .2. Centrifugation of AgNPs

7)The sample was washed thoroughly with ethanol, acetone and distilled water. The sample was transferred to petri dish and dried at 90^0C for 2 h on a water bath[9].

3. Characterization of Silver nanoparticles[10] -

3.1UV –Visible Spectroscopy –

The progress of AgNP synthesis was observed using UV-Visible spectrophotometer (SHIMADZU UV-1800). The AgNPs formation was observed through the UV-visible spectrometer. The UV-visible spectrophotometer has a quartz cuvette with path length of 1 cm. The absorption spectrum was recorded in the range of 350-500 nm^2 .Then the samples were scanned at wavelength of 350nm – 500nm.

3.2FTIR Analysis.-

The secondary metabolites present in the plant extract and the functional groups on the AgNPs were identified by using FTIR characterization technique. The different functional groups present in the sample are responsible for the stabilization and the reduction process for the AgNPs synthesis.

3.3.Particle Size Analyzer -

The Litesizer 500 instrument is used for the particle size analysis.Particle size can affect final formulation, performace,appperance and stability. Particle size analysis was carried out for the sample which is lyophilized and dispersed by ultrasonicator for the determination of size.

3.4SEM –

Scanning electron microscopy (SEM) analysis is employed to characterization of size, shape & morphologies of formed nanoparticle SEM gives high-resolution images of the surface of a sample is desired. The scanning electron microscope works as same principle as an optical microscope, but it measures the electrons scattered from the sample rather than photon.SEM

Analysis of AgNPs.

3.5 TEM- The approximate sizes and shapes of AgNPs were examined using TEM by dropping an aqueous solution containing the silver nanoparticles onto the carbon-coated grids and drying under an infrared lamp. Micrographs were obtained using a Philips Morgagni (M-268) operating at 80 kV. TEM provides the direct visualizes of the image which is obtained from the transmitted electron. It gives the structural and chemical behaviour of the nanoparticles at a high electron beam with high resolution.

3.6 XRD-Crystalline nature of metallic silver nanoparticles was examined using an X-ray diffractometer (XRD) from Bracer, D8 advance, Germany. XRD-6000 equipped with Cu Ka radiation source using Ni as filter at a setting of 40 kV/30 mA.

4. Invitro anti-inflammatory Activity[11] -

4.1 Invitro Evaluation by Membrane Stabilizing Property:

The author of research work (AAK 37 years), Blood was collected from her median cubital vein. The solution was prepared by dissolving 2% dextrose, 0.8% Sodium citrate, 0.05% citric acid ,0.42% sodium-chloride , distilled water which was then sterilized. The 1ml of collected blood and 1ml of sterilized solution was mixed together, further it was centrifuged at 1000-2000 rpm. cells and 10% (V/V)Isosaline solution, suspension was prepared. Various concentrations of the SNPs of Eulophia ochreata Lindl extract, 1mlPhosphate buffer, 2ml Hyposaline, 0.5ml HRBC suspension was prepared. The reference drug used for study was Diclofenac sodium. Distilled water was used as control. The incubation of above assay mixtures were carried out at 37ºC for30 minutes and centrifuged. From supernatant solution, haemoglobin content was calculated at 560nm using UV. The percentage inhibition of haemolysis was calculated. The equation shown as below:

Percentage inhibition of Haemolysis= Abs of Control- Abs of test/ Abs of control) * 100

Where, Absorbance of control= Absorbance of hypotonic buffered saline solution alone and Abs of test = Absorbance of test sample (extracts and diclofenac) in hypotonic medium.

4.2 Evaluation of in vitro anti-inflammatory activity by Protein denaturation method:

The 10 ml of solution was prepared. It consists of : 0.4 mL of egg albumin (fresh hen's egg), 5.6 mL of phosphate buffered saline pH 6.4 ,4 mL of different concentrations of SNPs of Eulophia ochreata Lindl . Now concentrations was 25, 50, 100, 200, 400 and 800µg/mL. Double-distilled water used as control. Incubation of the mixtures was done at (37^0C ±2) in a incubator for 15 min. It was heated at 70^0 C form five min. Cool the solution and absorbance was noted at 660 nm by using UV spectrophotometer Shimadzu, using vehicle as blank . The standard drug Diclofenac sodium its absorbance was calculated at the final concentration of (50, 100 &200 µg/mL) .The equation used for calculation is as follows

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control-Absorbance of Extract}}{\text{Absorbance of Control}} \times 100$$

3. RESULT AND DISCUSSION

UV-Visible spectroscopy

The formation of silver nanoparticles was tracked through UV-VIS absorption spectra, revealing a noticeable color transformation from grey to dark brown within 60 minutes due to reduction in Ag+ to Ag0 indicating the formation of nanosilver. A distinctive absorption peak emerged at 420 nm, attributable to the surface plasmon resonance of silver nanoparticles in the reaction mixture.

A yield of 51% was calculated after centrifuging at 6000 rpm. silver nanoparticles produced from Eulophia ochreata lindl had a wavelength of 420 nm. single peak was observed in the UV–vis spectrum of AgNPs synthesized from EU extract. Phenols, steroids, favonoids, tannins, steroids, and saponins are among the metabolites found in EU extract that can reduce Ag+ ions. The presence of secondary metabolites in EU extract can be linked to the plant extracts' ability to serve as a stabilizing and capping agent.

FTIR Analysis

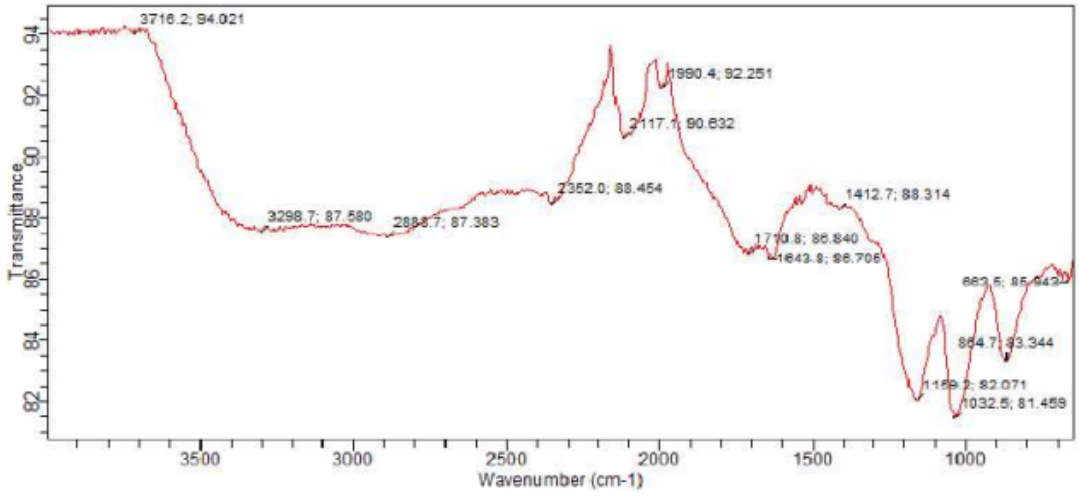

Fig 3: FTIR spectra of green synthesized SNPs from tuber extract of Eulophia ochreata Lindl

FTIR spectrum of synthesized SNPs was carried out to know the possible biomolecules responsible for capping and stabilization of nanoparticles. For this the FTIR spectrum was analysed between the scan ranges from 4000 to 500 cm–1 . Here the broad peaks obtained at 3298.7 cm–1 assigned for O—H (Stretch) bond of phenols,2888.7 cm–1 assigned for C=O stretching,2352 cm–1 assigned for C-N Stretching, 1642.9 cm–1 assigned for N—H (Bend) bond of primary amines (fig.2). These FTIR studies suggested that the hydroxyl groups of phenols and amide groups forming a layer to the nanoparticles and acting as capping agents to prevent agglomeration and providing stability to the medium. The C–O band at 1032 cm−1 may be assigned to the polyols (i.e., flavones, terpenoids, and carbohydrates) present in the plant extract. C-O was observed in spectra at 1032 cm−1 implying that the system's stabilization was caused by the carbonyl group of the reducing sugars adhering to the silver (Ag)[12].

Particle Size Analyzer:

Table No.1 .Particle Size Analysis

Hydrodynamic diameter	51.0 nm	Mean intensity	289.4 kcounts/s
Polydispersity index	31.2 %	Absolute intensity	974790.4 kcounts/s
Diffusion coefficient	1.0 µm²/s	Intercept g1²	0.6182
Transmittance	0.0 %	Baseline	0.996

1)The development of AgNPs was confirmed. Interestingly, major effffects of plant extract on the synthesis of AgNPs were observed related to the obtained size and structure, which meant that extract had a tendency to synthesize AgNPs with different natures.

2)The results are summarized in Table no.1. and indicate that using NHO, particles of 51 nm were produced by performing analysis .

SEM:

FESEM determined the morphology of the green synthesized SNPs from tuber extract of Eulophia ochreata Lindl, shown in (Figure 4). The FESEM image shows the spherical shape, and these particles are not agglomerated. The SEM study revealed that the average particle size of the synthesized AgNPs was 19.80 ± 2.43 nm. For drug delivery, antibacterial and anti-inflammatory studies, the interaction potential of nanoparticles with cells is based on gravitation, diffusion and convection forces. The evident homogeneity of the particles indicates their presence in a uniform form, highlighting the significance of nanoparticle homogeneity in the various activities they exhibit.

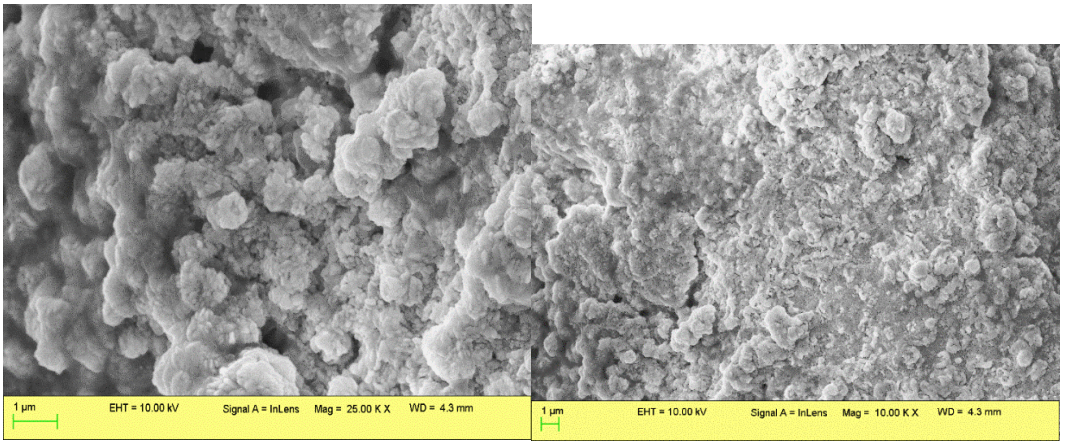

Fig 4: SEM of synthesized AgNPs

TEM

TEM is a powerful tool in determining the morphologies, shapes, and sizes of NPs. By using TEM, various studies characterized different shape and size of nanoparticles synthesized by biological resources [13,14]. In the present study, TEM results revealed spherical shape for silver nanoparticles. NPs (Fig. 5).

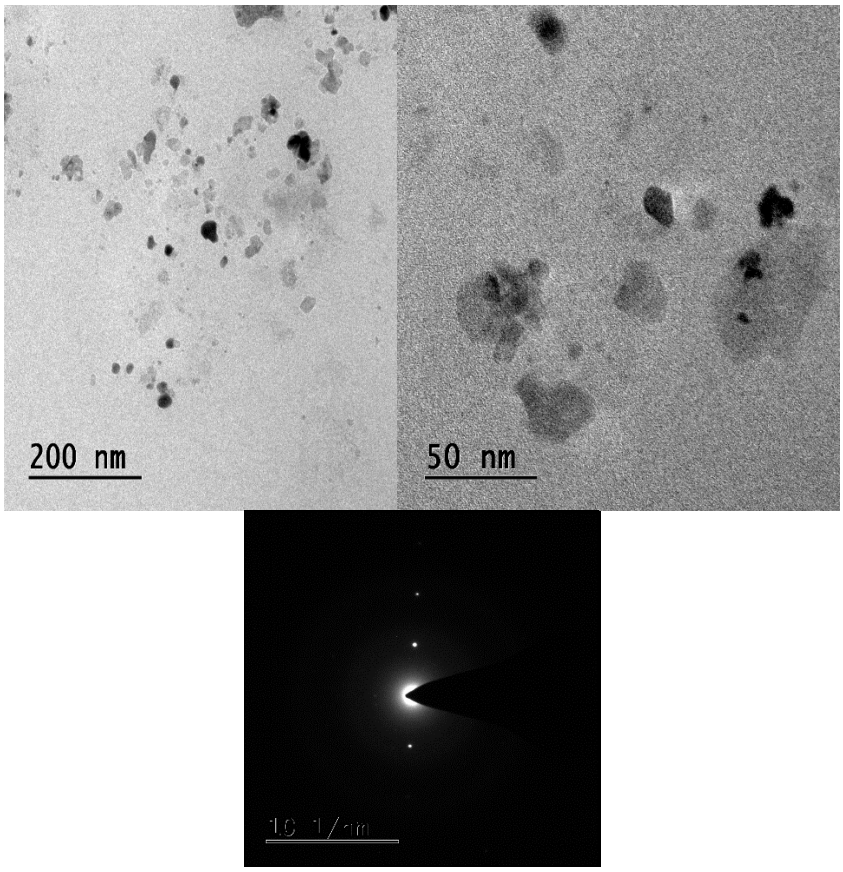

Fig 5: TEM of synthesized AgNPs

XRD:

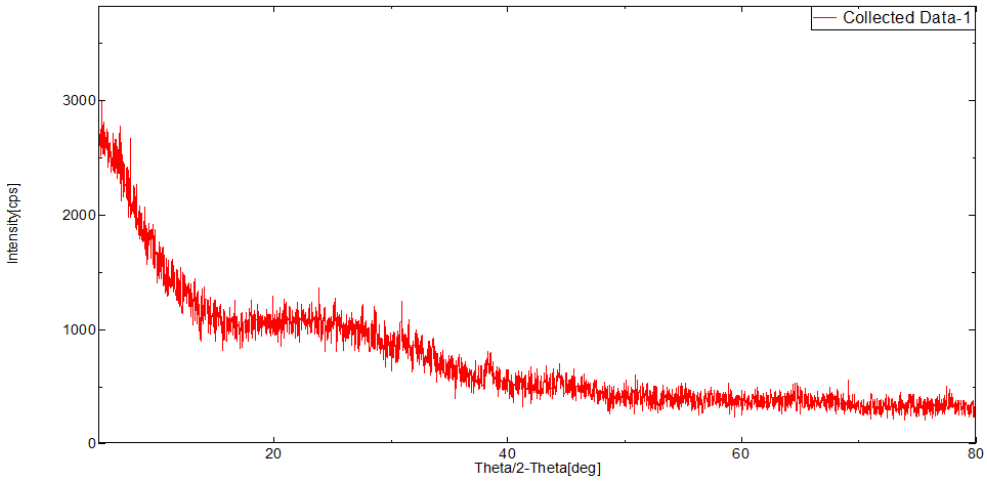

Fig 6: XRD of synthesized AgNPs

The XRD pattern of AgNPs is shown in Fig. 6. XRD pattern reveals four intense peaks at 31°, 39°, 52°, and 69°, which correspond to the face cubic centre (fcc) of AgNPs.

Invitro Evaluation by Membrane Stabilizing Property

Table 2: Effect of SNPs Eulophia ochreata Lindl and Std Diclofenac on membrane stabilizing property.

Treatment	Concentration (µg/ml)	% Inhibition of haemolysis	SEM
SNPs Eulophia ochreata Lindl	25	25.45	3.10
	50	38.95	0.96
	100	48.60	1.10
	200	81.41*	0.57
	400	86.96*	2.10
	800	87.31*	0.82
Standard Diclofenac	50	45.99	0.085
	100	71.87*	0.171
	200	75.47*	0.086

Values are expressed in mean ± SEM of 3 replicates ,where *P< 0.05 consider significant

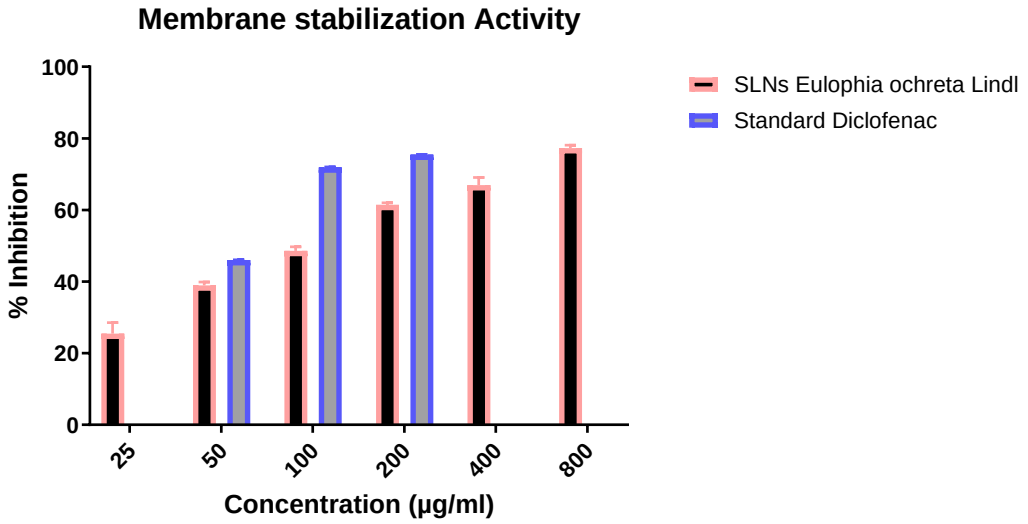

Fig 7: Graph of membrane stabilization activity

The research was carried out considering the points as need of society for newer anti-inflammatory agents obtained from environmental herbal source with significant readings and fewer side effects. When there is less stabilization of membrane, lyses of RBC membrane take

place due to the release of hemoglobin, so cell membrane stabilization is very importantly noted in developing new anti-inflammatory agents. The Positive result is noted when the lyses of membrane HRBC was prevented[15] The results obtained demonstrate that SNPs of Eulophia ochreata Lindl can significantly and dose-dependently inhibits RBC haemolysis (Table 2 and Fig 7).

In the study both the extracts at concentration range of 25-800 µg/ml protects significantly the erythrocyte membrane against lysis. Also Diclofenac sodium offered a significant protection of the RBC's ultimately stabilization of membrane. The extracts at concentration range of 25-800 µg/ml protect the human erythrocyte membrane against lysis. At concentration of 200 µg/ml, SNPS Eulophia ochreata Lindl produced 81.41% significant inhibition of RBC haemolysis as compared with 75.47 % produced by diclofenac sodium($P<0.05$). The maximum percentage of stabilization was observed in SNPs of Eulophia ochreata Lindl was found to be 87.31% at 800µg/ml. SLNs of Eulophia ochreta Lindl have significant anti-inflammatory activity due to presence of secondary plant metabolites.

Evaluation of in vitro anti-inflammatory activity by Protein denaturation method

Table 3: Effect of SNPs Eulophia ochreata Lindl and Std Diclofenac on mean of inhibition of protein denaturation

Treatment	Concentration (µg/ml)	Mean of % inhibition of protein denaturation	SEM
SNPs Eulophia ochreata Lindl	25	29.81	3.28
	50	38.88	1.91
	100	47.9	1.80
	200	56.83*	3.68
	400	62.75*	4.58
	800	67.95**	3.00
Standard Diclofenac	50	48.03	2.98
	100	56.26*	1.98
	200	69.84**	0.88

Values are expressed in mean ± SEM of 3 replicates, where **$P<0.01$,*$P< 0.05$ consider significant

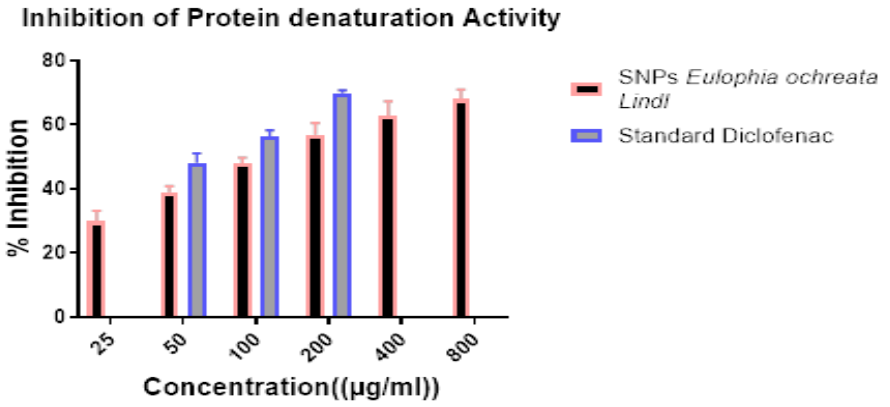

Fig 8 Graph of % inhibition of protein denaturation

The results for inhibitory effects of different concentrations of SNPs Eulophia ochreata Lindl are summarized in Table 3. The results obtained demonstrate that SNPs Eulophia ochreata Lindl exhibited a inhibition of protein (albumin) denaturation depends on concentration. The SNPs Eulophia ochreata Lindl throughout the concentration range of 25 to 800 µg/mL showed concentration dependent inhibition of protein denaturation(Table 3 and Fig 8). As well as Diclofenac sodium (at the concentration range of 50 to 200 µg/mL) was used as reference drug which also showed significant inhibition of protein denaturation. The extracts at higher concentration 200 µg/mL and above ($P<0.05$) are significant but failed to show significance at lower concentration below 200 µg/mL ($P>0.05$) as considerable activity in comparison with standard ($P<0.01$).

4. Discussion and Conclusion:

The research on the anti-inflammatory potential of silver nanoparticles (AgNPs) synthesized using Eulophia ochreata extract presents significant insights into the realm of nanomedicine and green synthesis methodologies. The synthesis and characterization of AgNPs demonstrated promising results, paving the way for further exploration of their therapeutic applications.

The study's findings underscored the superiority of AgNPs over solid particles due to their enhanced physical and chemical properties, attributed to their large surface area. The utilization of plant extracts, particularly from Eulophia ochreata, for synthesizing AgNPs proved to be a cost-effective, safe, and eco-friendly approach. This method not only addresses the increasing demand for nanoparticle synthesis but also aligns with the growing preference for sustainable and environmentally conscious practices in scientific research.

The synthesis process was meticulously outlined, involving the collection and authentication of plant material, extraction of active components, and subsequent reduction of silver ions to nanoparticles. Characterization techniques such as UV-Visible spectroscopy, FTIR analysis, particle size analysis, SEM, TEM, and XRD provided comprehensive insights into the morphology, size distribution, and crystalline structure of the synthesized AgNPs.

The UV-Visible spectroscopy confirmed the successful formation of AgNPs, as evidenced by the characteristic absorption peak at 420 nm. FTIR analysis elucidated the role of various biomolecules in capping and stabilizing the nanoparticles, highlighting the intricate interplay between plant extracts and nanoparticle synthesis. Particle size analysis revealed the homogeneous distribution of AgNPs, crucial for their functional properties and applications.

SEM and TEM imaging further corroborated the spherical morphology of the AgNPs, emphasizing their uniformity and potential for cellular interactions. XRD analysis confirmed the crystalline nature of the nanoparticles, laying the foundation for understanding their structural properties.

The evaluation of anti-inflammatory activity through membrane stabilization and protein denaturation assays yielded promising results. The AgNPs derived from Eulophia ochreata extract demonstrated dose-dependent inhibition of RBC hemolysis and protein denaturation, comparable to the standard drug Diclofenac sodium. These findings underscore the therapeutic potential of AgNPs as anti-inflammatory agents, offering a novel approach to combat inflammation with reduced side effects[15].

In conclusion, the study exemplifies the synergistic convergence of nanotechnology and green chemistry, offering a sustainable solution for nanoparticle synthesis with therapeutic implications. The anti-inflammatory efficacy of AgNPs synthesized using Eulophia ochreata extract holds promise for future biomedical applications, warranting further investigation into their mechanism of action and clinical relevance.

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