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Research

Evaluation of antioxidant potential of silver nanoparticles derived from erythrina indica leaf extract

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Abstract

In the present work, an eco-friendly and cost-effective protocol for the green synthesis of silver nanoparticles (AgNPs) using an aqueous extract of *Erythrina indica* leaf was reported. Conventional heating method was adopted to prepare silver nanonanoparticles. The nanoparticles were characterized using FTIR, XRD, SEM and EDAX analysis. The appearance of peak at 3281 cm^{-1} generally proved the OH group, which is responsible for the reducing property of polyphenolic compounds, present in aq. extract of *Eithrina indica*. SEM analysis showed the shape of nanoparticles and the size ranges from 386-545nm. EDAX confirmed the purity of silver nanoparticles whereas XRD pattern confirmed the crystalline nature of NPs. The peak present at 3 keV in EDAX confirmed the presence of silver ion. The anti-oxidant activity was measured by DPPH method using ascorbic as standard. Furthermore, the AgNPs were found to exhibit significant antioxidant activity at 315 µg/ml with antioxidant potential at 82%.

Keywords: Erythrina indica, $AgNO_3$, DPPH, nanoparticles

INTRODUCTION

Recent advances in nanotechnology have shown promising result for the development of diverse Nano formulations due to their high biocompatibility, ease of surface functionalization, ability to target cancer, and ability to distribute drugs. Nanotechnology has garnered worldwide interest owing to its extensive applicability in various domains such as optoelectronics, biological sciences, mechanics, chemical industries, and drug delivery. [1–3] Green nanotechnology is an emerging field that focuses on creating eco-friendly nanoproducts and using them to promote sustainable development [4]. Metal-based nanoparticles are produced by either constructive or destructive means from metals and then synthesised into nanometric size. It is possible to create nanoparticles from almost all metals [5-9]. The commonly used metals for nanoparticle synthesis are aluminum, cadmium, cobalt, copper, gold, iron, lead, silver and zinc. The nanoparticles have distinctive properties such as size less than 100nm, surface characteristics like high surface area to volume ratio, pore size, surface charge and surface charge density, crystalline and amorphous structures, shapes like spherical and cylindrical and colour, reactivity and sensitivity to environmental factors such as air, moisture, heat and sunlight etc. Nanodrugs can release medication at a constant rate over a desired period of time.

Due to the presence of anti-oxidant compounds in herbal extracts, an attempt has been made to utilise them as good reducing agent and convert into nanoparticles. The increased pharmacological activity of the crystal powder that was produced from the acquired nanoparticles was then assessed. [10] Therefore, the current study's objective was to assess the pharmacological effects of AgNPs produced utilising *Erythrina india* leaf extract in a simple, efficient, and reliable manner.

MATERIALS AND METHODS

Plant collection

Newly-grown leaves of *Erythrina indica* were harvested from the vicinity of Chennai and Tiruvallur district in Tamil Nadu. The collected plant was authenticated by botanical survey of India Chennai.

Preparation of Plant Material

Using a mortar and pestle, 10 gm of the pre-cut leaves were coarsely ground in order to prepare the plant material for extraction. Through careful grinding, the plant material's surface area was further increased

and the cell walls were efficiently disrupted, allowing the release of bioactive chemicals. This stage increases the extraction process's efficiency and raises the yield of the target compounds by guaranteeing the consistency and homogeneity of the ground material.

Extraction Method

After adding 100 mL of Milli-Q water to the ground leaves, the mixture was heated to 80°C. Water was utilised as the extraction solvent because of its non-toxic nature and capacity to remove polar chemicals from plant material. Optimising the extraction efficiency involves carefully controlling the mixture's heating to help the target compounds dissolve and release from the plant matrix. The extraction efficiency required and the preservation of heat-sensitive chemicals were carefully considered when setting the temperature at 80°C. [11] After filtration, the *E. indica* leaf extract produced as a clear filtrate, which can be further analysed and used for a variety of purposes. This extract can be an invaluable tool for scientific research and therapeutic development because it is rich in bioactive chemicals that were present in the plant material. [12]

Synthesis of nanoparticles by Conventional Heating Method

In order to initiate the synthesis of silver nanoparticles (AgNPs), 90 mL of a 1 mM silver nitrate ($AgNO_3$) solution was carefully combined with 10 mL of the produced *Erythrina indica* leaf extract. Using a water bath, conventional thermal heating was used to reduce silver ions (Ag+) to metallic silver (Ag0). A reaction mixture produced in which the plant extract functions as a reducing agent and the $AgNO_3$ supplies the silver ions required for the creation of nanoparticles. [13] Seeing the reaction mixture's colour change from yellow to dark brown is a crucial component of the technique. This change in colour acts as a visual sign that AgNPs have formed successfully. This noticeable colour shift is caused by the special optical characteristics of silver nanoparticles, which offer a rapid and accurate way to evaluate nanoparticle.

This approach resulted in a slower rate of colour change from yellow to dark brown. [14] However, further analytical verification was carried out to confirm the presence and characteristics of the synthesized nanoparticles.

Characterization of AgNPs

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed within the wavenumber range of 400-4000 cm^{-1} to identify specific functional groups present on the surface of the AgNPs. These functional groups, likely originating from the plant extract used during synthesis, play a crucial role in both reducing silver ions and stabilizing the formed nanoparticles. FTIR spectroscopy provides valuable insights into the chemical composition and surface chemistry of the AgNPs.

X-Ray Diffraction (XRD)

XRD analysis was conducted using an XDL 3000 powder X-ray diffractometer, operating at settings of 30 mA, kV, and Cu Kα of 1.5405˚A. This technique was employed to determine the crystallinity of the AgNPs, revealing whether they possess a crystalline or amorphous structure. Additionally, XRD allows for the calculation of average nanoparticle size based on the diffraction pattern obtained.

Scanning Electron Microscopy (SEM)

SEM imaging offers high-resolution visualization of the morphology and size distribution of the AgNPs. This technique allows for detailed analysis of nanoparticle shape (e.g., spherical, rod-like) and size range within the sample. Sample was applied on carbon tapes, which were then mounted under a microscope to capture pictures and measurements after being subjected to a spray of gold and palladium ions for six minutes in a vacuumed sputter coater.

Energy-Dispersive X-ray Spectroscopy (EDAX)

EDAX analysis was employed to confirm the elemental composition of the AgNPs. By detecting characteristic X-rays emitted from the sample, EDAX verifies the presence of elemental silver, providing definitive proof of AgNP composition. This analysis further enhances our understanding of the chemical composition and purity of the synthesized nanoparticles particularly silver ions. It also followed the sample preparation similar to SEM [15]

In Vitro Antioxidant Activity (DPPH Radical Scavenging Assay)

The DPPH assay serves as a fundamental method to assess the antioxidant potential of various compounds, including plant extracts and synthesized substances. Its primary objective is to measure the ability of a substance to scavenge free radicals, specifically the DPPH radical, providing valuable insights into their antioxidant activity. The DPPH radical scavenging assay was conducted following Bruchez M method[16]. A DPPH solution was prepared in methanol at a concentration of 0.0135 mM. Different concentrations of the aqueous Nano extract (5-320 µg/ml), standard (ascorbic acid), and control were mixed with 2.5 mL of the prepared DPPH solution. After vortexing, the mixtures were incubated at room temperature for 30 minutes. The absorbance of the mixtures was measured at 517 nm using a UV-visible spectrophotometer.

The percentage of DPPH inhibition (scavenging activity) was calculated using the formula:

% DPPH Inhibition = [(absorbance of Control – absorbance of Test)/absorbance of control] × 100

RESULTS AND DISCUSSION

IR

The FTIR spectrum of Ag NP s exhibited the peaks at 3281cm $^{-1}$, 2919 cm $^{-1}$, 1624 cm $^{-1}$ and 1317cm^{-1}.The peak 3281 cm^{-1} indicated the presence of OH group in polyphenolic compounds in *Erithrina indica*. The peaks at 2919 cm $^{-1}$ indicated the aldehydic (C-H) group. The peaks at 1624cm $^{-1}$ confirmed the presence of C=O group. However, the presence of peak at 3281 cm $^{-1}$ generally proved the OH group that is responsible for the reducing property polyphenolic compounds present in aq. extract of *Eithrina indica*. (Fig1)

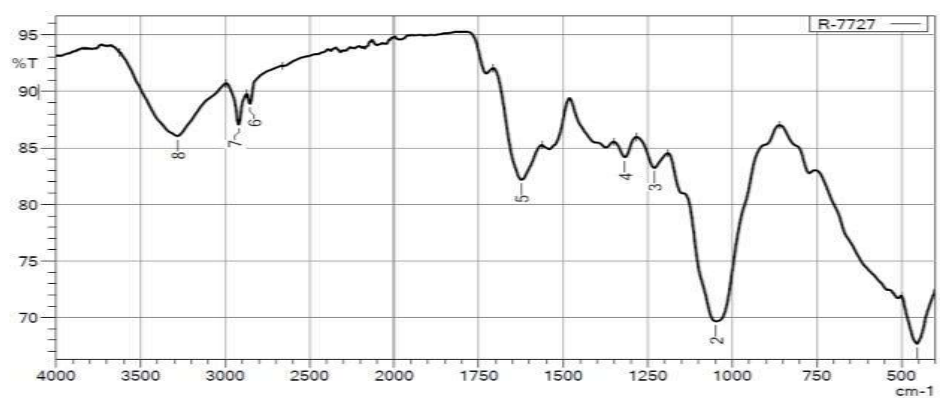
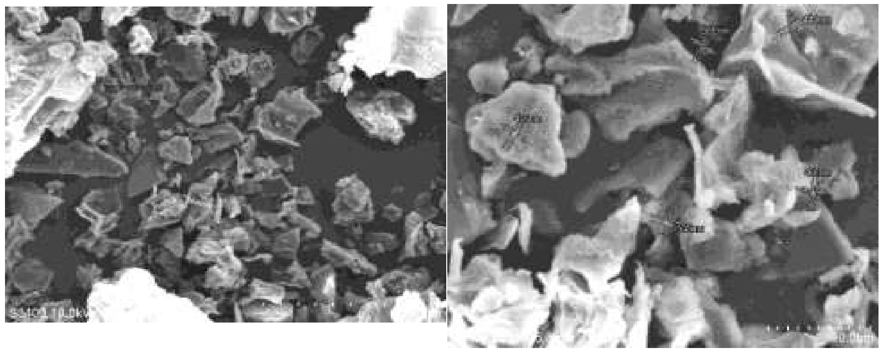


Fig1: FT IR spectrum of Ag NP

SEM

The utilization of scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX or EDAX) represents a synergistic approach in the comprehensive characterization of nanoparticles derived from *Erythrina indica* leaf extraction. Through SEM analysis, researchers gain unprecedented insights into the morphology, size, and surface features of the nanoparticle ensemble, while EDX analysis delves deeper into the elemental composition, thereby unraveling the chemical fingerprint of the Nano formulation. SEM analysis serves as a linchpin in unraveling the morphology of nanoparticles derived from *Erythrina indica* leaf extraction. Through meticulous examination of SEM micrographs, researchers discern a diverse array of nanoparticle morphologies, ranging from spherical and dendritic to rod-like and irregular shapes. Each morphological archetype bears testament to the complex interplay of synthetic parameters and environmental factors that govern the nucleation, growth, and aggregation kinetics of nanoparticles during synthesis. From the figure 2 it can be



interpreted that AgNPs were not agglomerated and dispersed but in intact and the size ranges from 386-545nm. (Fig 2)

Fig 2: SEM image of silver nanoparticles

XRD

The XRD pattern is generally used for the confirmation of the crystalline nature of AgNPs. In the current study, an XRD investigation was performed to determine the crystal size of the samples (Fig. 3). From the XRD results, different peaks were recorded for the samples, the size of the crystals was determined to be 8.5 Å for the synthesized AgNPs. The intensities of diffraction were recorded 33.5°, 66.5° were consigned to reflections from the 2θ region.They are previously reported in which the XRD pattern incorporated in the complete spectrum of 2θ ranging from 10° to 90°. These peaks were due to the biomolecules present in the plant extract and responsible for the fabrication of AgNPs [17]. In this regard, the XRD data reveal a crystalline fraction constituting 33.5% of the nanoparticle ensemble, underscoring the presence of ordered atomic arrangements represented by well-defined lattice planes and sharp diffraction peaks. This crystalline component, arising from the inherent clustering of bioactive constituents within the leaf extraction matrix, serves as a hallmark of structural coherence and stability within the nanoparticle formulation. Concomitantly, the XRD results unveil the prevalence of an amorphous phase, comprising the majority share at 66.5% of the nanoparticle ensemble. Unlike their crystalline counterparts, amorphous nanoparticles lack long-range order and exhibit a disordered atomic arrangement, reflected in broad diffraction peaks devoid of distinct intensity maxima. Despite their apparent structural disorder, amorphous nanoparticles offer unique advantages, including enhanced solubility, bioavailability, and facile tunability of physicochemical properties, rendering them indispensable in pharmaceutical and biomedical applications.

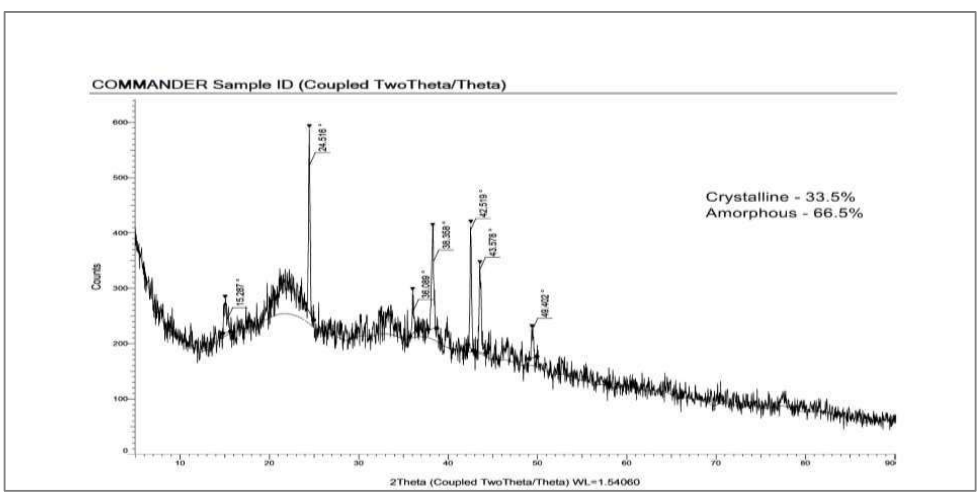


Fig 3: XRD pattern of Silver nanoparticles

EDX

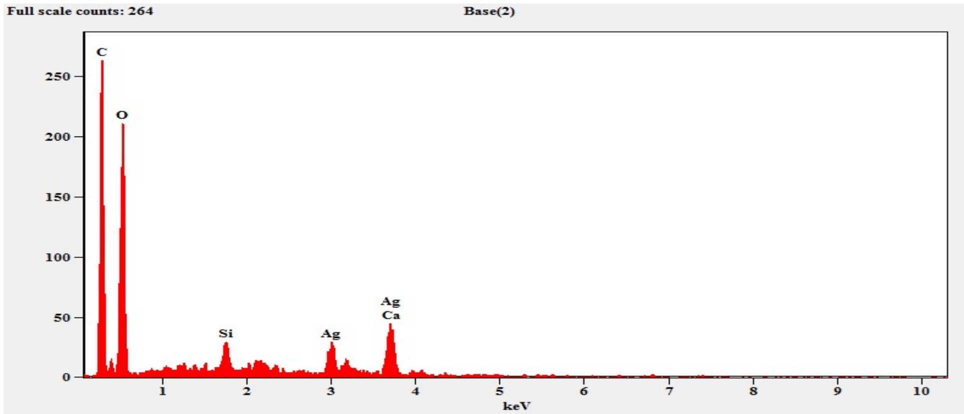


Fig 4: EDX pattern of nanoparticle

Element	Wt. %	AT%
CK	30.31	40.29
OK	55.80	55.68
SiK	1.74	0.99
Ca K	4.95	1.97
Ag L	7.21	1.07

The EDX pattern of synthesized AgNPs revealed strong signs for the silver element (Fig. 4). It was clearly confirmed from the EDX profile that the bio-inspired AgNPs have crystalline morphology and peaks of silver atoms are located in the range of 2–4 keV along with other elements, i.e. carbon, oxygen, silicon, potassium, and calcium [18].the presence of silver ion was confirmed by wt.% at 7.2 and At% at 1.07 and The peak present at 3 keV confirmed the presence of silver ion.(Fig:4)

Anti-oxidant activity

In this study, we aimed to evaluate the antioxidant activity of silver nanoparticle of *E. indica* using the DPPH radical scavenging assay. Figure 5a illustrated the standard solutions, while Figure 5b depicted the sample and control solutions. The antioxidant activity of the aqueous nano extract was compared with that of ascorbic acid as a standard.

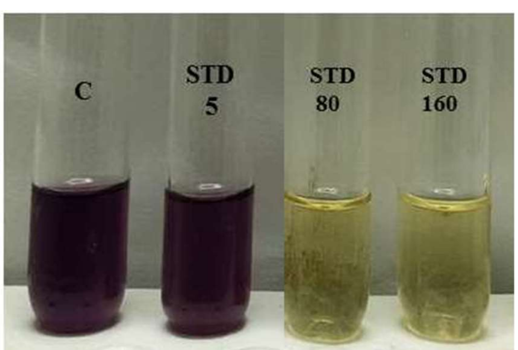


Fig 5a: Std solution

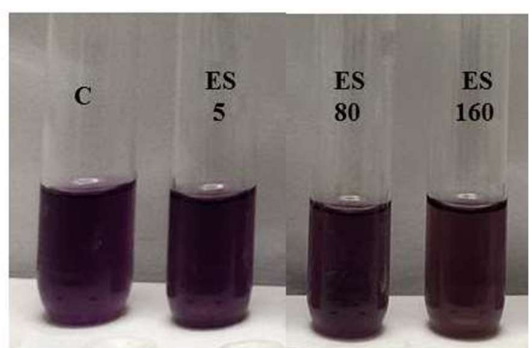


Fig 5b: Sample solution

The results revealed a concentration-dependent increase in DPPH inhibition for both the extract and the standard. A dose-response curve was plotted (Fig: 6 & Tab:1), and the IC_{50} value, representing the concentration at which 50% of the DPPH radicals were scavenged, was calculated. The IC_{50} value provides a quantitative measure of the antioxidant potency of the extract. The aqueous nano extract of *E. indica* exhibited promising antioxidant activity, as demonstrated by its ability to scavenge DPPH radicals *in vitro*. The study provides valuable insights into the antioxidant potential of the aqueous nano extract of *E. indica*. The findings highlight its efficacy in scavenging DPPH radicals, suggesting its possible utility in mitigating oxidative stress-related conditions. Future research should focus on elucidating the bioactive compounds responsible for the observed antioxidant activity and exploring their pharmacological properties. Additionally, *in vivo* studies are essential to validate the therapeutic efficacy and safety of the extract.

Table1 : DPPH inhibition value of AgNPs Vs std

Conc.(µg/ml)	AgNPs	Ascorbic acid
5	2.48	3.1
10	3.26	30.33
20	4.8	58.63
40	6.0	93.01
80	7.01	94.99
160	11.99	95.96
320	19.66	98.11

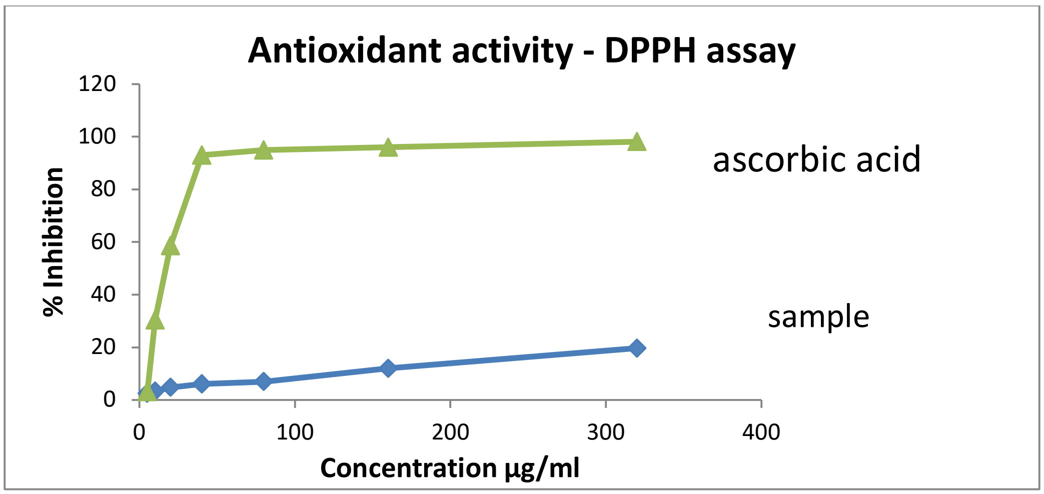


Fig. 6: Std calibration graph

Samples	IC_{50}µgm/ml
Ascorbic acid	17.05
AgNPs	315

CONCLUSION

Green biosynthesis of NPs has several advantages and it will facilitate the process easily scalable and economically emergent. A fast, environment-friendly and useful process for the production of AgNPs using *Erythrina indica* leaf extract has been developed. In the present study, the colour of the solution changes during the process by the phytochemicals present in the plant resulting in the formation of AgNPs that was confirmed by FT IR, SEM, XRD and EDAX . Furthermore, from SEM analysis, it is confirmed that the synthesized NPs are irregular crystalline in nature shape having a size ranging from 386 to 545 nm. The XRD study showed that the particles are crystalline in nature. The invitro activity data proved that compound has antioxidant potential. Further in vivo study is needed to arrive a concrete conclusion.

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