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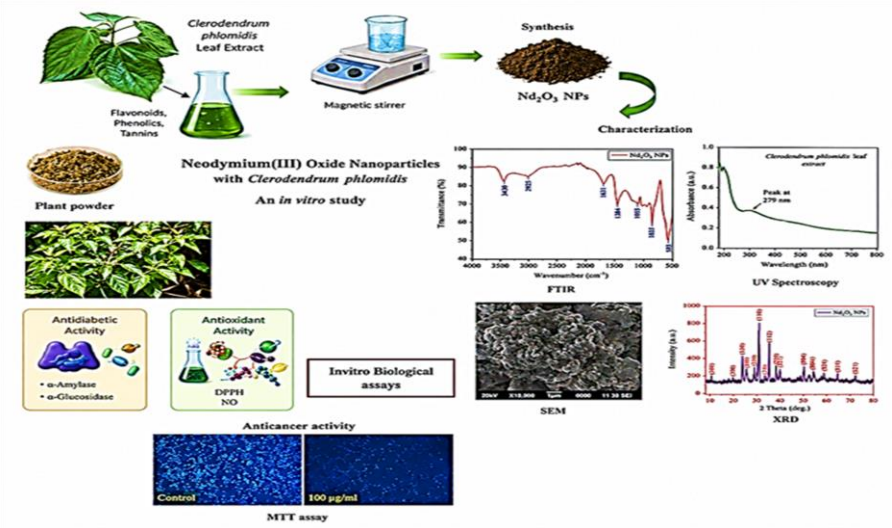
Biosynthesis of Nd_2O_3, or Neodymium(III) Oxide Nanoparticles with *Clerodendrum phlomidis* and their anti-diabetes and anti-cancer activity: An invitro study.

[1]Ayesha Nashita, [2*]Rekha Anantharaman

ABSTRACT:

Background: Green nanotechnology involves the utilization of phytochemicals as natural reducing and stabilizing agents that offer an alternative to the traditional synthesis of nanoparticles, which can be considered sustainable. *Clerodendrum phlomidis* leaf extract is abundant in flavonoids, phenolics, and tannins was used to biosynthesis neodymium oxide (Nd_2O_3) nanoparticles in this work. **Materials and methods:** The synthesized Nd_2O_3 nanoparticles were characterized by UV -Vis spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, X-ray Diffraction (XRD) and Field emission scanning electron microscopy (FE-SEM) confirmed the functional groups, crystal structure, and aggregated nano-granular morphology. The antioxidant activity properties were evaluated by 2,2-diphenyl-1-picrylhydrazl (DPPH) and nitric oxide (NO) assays. The anti-proliferative effect of Nd_2O_3 nanoparticles synthesized using *C. phlomidis* on A549 cells was quantified by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. **Results**: FT-IR showed significant O-H, C=O, C-N, and N-O bands, which are the indicators of phytochemical capping, the examination of the UV-Vis showed that there was a large absorption peak at 425 nm. XRD patterns confirmed the formation of cubic-phase Nd_2O_3 that was crystalline. SEM images showed agglomerated porous structures consisting of small nanoparticles. Nd_2O_3 nanoparticles exhibited significant activity in DPPH assay and NO assay exhibited radical scavenging at 86.71% and 89.56% at higher concentration (100 µg/mL) respectively. The α-amylase and α glucosidase demonstrated 80.54% and 80.52% at higher concentration of 320 µg/mL. At 200 µg/mL, MTT assay revealed a 33% reduction in A549 cell viability. **Conclusion:** Nd_2O_3 nanoparticles synthesized using *C. phlomidis* demonstrated significant in-vitro antidiabetic, antioxidant properties and cytotoxic activities. It creates environmentally friendly multifunctional nanoparticle-based therapies for the treatment of metabolic and inflammatory diseases.

KEYWORDS: Anti-cancer activity, anti-diabetes activity, *Clerodendrum phlomidis*, invitro study, Nd_2O_3, or Neodymium Oxide



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1. INTRODUCTION

Biosynthesize using plant extracts is an attractive and environmentally friendly procedure for the production of neodymium oxide (Nd_2O_3) nanoparticles with improved biological functions. Nd_2O_3 is a suitable candidate owing to its unusual physicochemical, redox, catalytic and biological properties such as surface reactivity, antioxidant potential high stability and an emerging therapeutic relevance. Additionally, to date, only limited studies have been performed on Nd_2O_3 nanoparticles synthesize using green approaches and the biomedical applications thereof of such materials confirm the novelty and relevance of the present work. In contrast to the conventional chemical synthesize of nanoparticles, which usually involves hazardous reagents and requires a great amount of energy input, green synthesize uses phytochemicals from plants as nature-inspired reducing and stabilizing agents that cap the nanoparticles. Examples include flavonoids, terpenoids, alkaloids, saponins, and phenolics, which afford the formation of nanoparticles under mild conditions with additional medicinal values. [1]

The evolving interest in green nanotechnology has expanded the therapeutic scope of medicinal plants even further. The plant-based synthesize of metal-oxide nanoparticles is finding significant momentum as it is eco-friendly, cost-effective, and nontoxic. Herein, phytochemicals act as reducing and capping agents in nanoparticle formation and help in improving their stability and bioactivity. [2] These biogenic nanoparticles generally exhibit higher functional properties compared with conventionally synthesized nanoparticles due to the presence of biomolecules derived from plants attached to the surface of the nanoparticles, which enhance biocompatibility and add more bioactivity. [3]

Clerodendrum phlomidis, a member of the Lamiaceae, is used as a Ayurvedic medicine in against different diseases, including inflammatory disorders, Diabetes Mellitus, fever, and microbial infections. [4] The leaves of this plant contains secondary metabolites such as flavonoids, phenolic acids, tannins and glycosides which exhibit potent antioxidant, antidiabetic, anti-inflammatory and anti-inflammatory actions. These phytochemicals make *C. phlomidis* an ideal candidate green synthesize since they have the ability to reduce neodymium hydroxide precursors into Nd_2O_3 nanoparticles and surface adsorption is used to stabilize the nanoparticles. [5] The synergistic interactions result in the by-products. Adding *C. phlomidis* extracts to the production of Nd_2O_3 nanoparticles is one that introduces the problem of probability of augmenting the treatment impacts of the plant as well as the nanoparticles. [6]

The antioxidant property study of *C. Phlomidis* leaf extract and its oxide nanoparticles is relevant because oxidative stress is very closely related to inflammation, aging, and chronic diseases. Other antioxidant assays that can be conducted are 2,2-diphenyl-1-picrylhydrazl (DPPH) and nitric-oxide (NO) scavenging, which quantitatively give evidence of neutralizing the free radicals by the extract. Previous studies on *C. Phlomidis* have already revealed strong antioxidant activity due to its high phenolic content. [7] The formation of nanoparticles can enhance this activity further due to increased surface reactivity and facilitation of sustained active compound release. Similarly, the assessment of antibacterial and antibiofilm activities is crucial in view of growing antibiotic resistance. Though the extracts of *C. Phlomidis* have exhibited broad-spectrum antibacterial activity, nano-formulations might enhance this effect. [8] Green-synthesized oxide nanoparticles have been reported to impede microbial adhesion, interfere with biofilm architecture, and impede quorum-sensing pathways involved in bacterial communication. The green synthetic approaches using the bioresources (plants and microbes) for the synthesize of the nanoparticles seem to be a cost-effective, convenient, reasonable and ecofriendly methods. [9] In a sustainable, cost-effective and eco-friendly manner, this research work demonstrates the greenness of the

synthesized nanoparticles (NPs) as an alternative method that limits harmful chemical compounds used in NP synthesize. Moreover, phytochemical constituents in the plant extracts also act as capping & reducing agents for NP synthesize. Most importantly, biosynthesized NPs exhibit better therapeutic properties than NPs prepared through conventional techniques. [10] Biosynthesized NPs also show significant antimicrobial, antioxidant, anticancer and antidiabetic potential. [11]

Given the expanding frequency of diabetes and inflammation-related illnesses, there is a growing demand for innovative treatment agents that are efficient, safe, and economically practical utilizing *C. phlomidis* leaf extract, this work focuses on the production of Nd_2O_3 nanoparticles and assesses their antidiabetic and antioxidant capabilities utilizing in vitro tests. [12] The study intends to aid in the creation of novel, plant-based nanotherapeutics for the treatment of inflammatory and metabolic disorders by investigating the structural traits and biological activities of these biosynthesized nanoparticles. [13]

The antidiabetic potential of green-synthesized Nd_2O_3 NPs is attributed mainly to the inhibition of key carbohydrate-digesting enzymes, such as α-amylase and α-glucosidase, which generally reduce postprandial hyperglycemia. [14] Phytochemical-capped nanoparticles are highly capable of rendering strong antioxidant defense against oxidative stress and free-radical damage to pancreatic β-cells in diabetes. [15] Indeed, *C. phlomidis* has been known for its antidiabetic activity; therefore, attaching its phytochemicals onto the surfaces of Nd_2O_3-NPs may enhance the inhibition of enzymes and antioxidant defense. [16] Therefore, the biosynthesize of Nd_2O_3 nanoparticles using *C. phlomidis* offers a green, cost-effective, biologically enhanced method for producing multifunctional nanomaterials. [17] Their in vitro antidiabetic and anti-cancer activity brings scientific validation for their therapeutic potential and lays the foundation for future biomedical applications.

2. MATERIALS AND METHODS

2.1. Plant Extract Preparation

Fresh *Clerodendrum phlomidis* leaves collected from Coimbatore at the month of June 2025 and it was authenticated by the botanist Dr. P. Palani, Director, Centre for Advanced Studies in Botany at the University of Madras, Chennai, Indian. To get rid of debris, the leaves were completely sanitized by washing it for 2 to 3 times with distilled water. To retain phytoconstituents, the leaves were shade dried. The dried leaves were powdered using a mechanical grinder. For the subsequent extraction process, the powder was maintained in a tightly sealed container and extracted using distilled water (aqueous extraction). The resulting extract was stored and maintained at 0-40 °C in an amber-colored glass container. [18]

2.2. Biosynthesize of Neodymium oxide Nanoparticles

C. phlomidis leaf extract was used in a green method to create Nd_2O_3 nanoparticles. Briefly, 40 mL of the prepared leaf extract was added to a 0.2 M $NdCl_3$ solution under continuous stirring. Subsequently, 1.0 mL of the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM BF_4) was introduced to the reaction mixture and it was agitated at room temperature for 4 hours. Following the reaction, the product was recovered by centrifugation, and filtration was used to gather the solid fraction. The recovered precipitate was thoroughly washed with distilled water and then the material was dried at 80 °C in an air oven. Finally, the dried material was calcinated at 550 °C for 5 hours in a muffle furnace to obtain Nd_2O_3 nanoparticles. [19]

2.3. Characterization of Synthesized Nanoparticles

The purified Nd_2O_3 nanoparticles (NPs) were characterized between 200 and 800 nm wavelengths using a UV spectrophotometer (Shimadzu Corporation, Kyoto, Japan). FT-IR spectroscopy was used to identify the functional groups that involved in the reduction and stabilization of Nd_2O_3 NPs using FT-IR spectrometer from range 4000–400 cm^{-1}. To obtain the XRD pattern of Nd_2O_3 NPs, a Bruker D8 Advance X-ray diffractometer was used within a 2θ range

(Bruker AXS, Germany)with Cu Ka radiation (k=1,54). FE-SEM with EDAX was used to analyze the surface morphology and elemental composition of Nd_2O_3 NPs by FE-SEM,JEOL LtdJSM-IT1800). Dried Nd_2O_3 NPs samples were deposited on aluminum stubs and sputter coated with gold prior to SEM imaging. [20]

2.4 Antioxidant Activity

2.4.1. DPPH assay

Scavenging of radicals was evaluated by DPPH assay using 0.1 mM standard stock solution of DPPH, prepared in methanol and regular ascorbic acid. Working standard solution is prepared fresh for each experiment, in which the stock mixture is diluted to an appropriate concentration of 20 µM by methanol. In five different concentrations (50, 40, 30, 20 µg/mL and 10 µg/mL), the test sample *C. phlomidis derived* Nd_2O_3 nanoparticles were prepared with 200 µL DPPH working solution and the solution was mixed thoroughly. Following, the solution was maintained for 10 minutes in dark at room temperature. A UV-Vis spectrophotometer was used to evaluate the absorbance (OD) at 517 nm. As a control, DPPH was used and methanol as a blank solution. [21]

$$Activity\ of\ Scavenging(\%) = \frac{Absorbance\ of\ the\ standard - Absorbance\ of\ test}{Absorbance\ of\ standard} x100\%$$

2.4.2. Nitric Oxide Assay

The nitric oxide (NO) scavenging activity of *Clerodendrum phlomidis* derived Nd_2O_3 nanoparticles was evaluated using the Griess reaction. Briefly, 1 mL of sodium nitroprusside (10 mM) prepared in phosphate-buffered saline (pH 7.4) was mixed with 1 mL of nanoparticle samples at different concentrations (typically 5–100 µg/mL). The reaction mixtures were incubated at 25-27 °C for 150 minutes under light to allow NO generation. After incubation, 1 mL of the reaction mixture was combined with 1 mL of freshly prepared Griess reagent (equal volumes of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride), and the pink chromophore formed was measured at 546 nm. [10] A control (without nanoparticles) and a standard antioxidant such as ascorbic acid was included for comparison. The percentage NO scavenging was calculated using the following equation:

$$\%\ Inhibition = \frac{Absorbance\ of\ the\ standard - Absorbance\ of\ test}{Absorbance\ of\ standard}\ x\ 100\%$$

2.5 Cytotoxicity Assay

2.5.1. Anticancer Activity Assessment Using the MTT Assay

The anticancer activity of biosynthesized Nd_2O_3 nanoparticles and plant extract was evaluated against A549 lung cancer cell line by MTT assay. The A549 cells were purchased from National Centre for Cell Sciences (NCCS), Pune. A volume of 100 µL of cell suspension was added into every well of a 96-well plate and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 hours. Trypan blue exclusion assay were used to determine the cytotoxicity of aqueous leaf extracts against A549 cell line. The lyophilized extracts were then re-suspended in DMSO and filtered through a sterile 0.2 µm cellulose acetate filter. The cells were treated with 1, 10 and 100 µg/mL of each respective extract for an incubation period of 24 hours. The optical density (OD) value was measured at 550 nm using microplate reader. [20] The anticancer effect of the samples on A549 cells was expressed as the percentage of cell viability, calculated using the formula:

$$\%\ Cell\ viability\ = \frac{Absorbance\ of\ stimulated\ cells}{Absorbance\ of\ control\ cells}\ x\ 100$$

2.6. Anti-diabetic Assay

The antidiabetic activity was performed using the α-amylase inhibition assay. Briefly, 100 µl of α-amylase enzyme solution was incubated with 100 µl of plant extract at different concentrations for 10 minutes at 25 °C. [19] Following the addition of starch solution, 20 mM sodium phosphate buffer, 3,5-dinitrosalicylic acid (DNSA), and 6 mM sodium chloride, the reaction mixture was incubated for 30 minutes at 25 °C. Then, the mixture was heated to 70 °C for 5 minutes in order to stop the reaction. The percentage inhibition (%) of α-amylase activity measured the absorbance at 530 nm. [22]

$$\%\ Inhibition\ of\ \alpha - amylase = \frac{Absorbance\ of\ the\ control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control}\ x\ 100\%$$

3. RESULTS

3.1 Characterization of Nd_2O_3 nanoparticles using *C. phlomidis* leaf aqueous extract

3.1.1 UV-visible spectral analysis:

A color shift occurred when *C. phlomidis* leaf aqueous extract was introduced to a 1 mM $NdCl_3$ solution. Initial solution was pale violet is due to the f–f electronic transitions of neodymium. [20] The phytochemical biomolecules present in the leaf extract complex with Nd^{3+} ions or reduce and stabilize nanoparticles changed the solution to dark brown color. UV-VIS spectrum characterization of the colloidal solution was measured at 200-800 nm and revealed the presence of neodymium (III) oxide nanoparticles and a highest absorption peak range at 425 nm. (Fig 1).

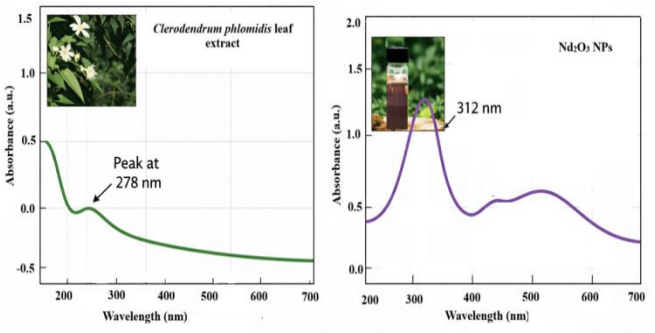

Fig 1: UV-Vis spectra for the synthesize of Nd_2O_3 nanoparticles

3.1.2 Fourier transform infrared (FT-IR) analysis of *C. phlomidis*-mediated Nd_2O_3 nanoparticles

FT-IR spectra of the prepared *C. phlomidis plant* leaf extract and *C. phlomidis*-mediated Nd_2O_3 nanoparticles were recorded in the range of 4000–400 cm^{-1} to identify the functional groups responsible for nanoparticle synthesize and stabilization (Fig 2). Also, the range of 1200-1000 cm^{-1} indicates C-O stretching of alcohols, ethers and esters whereas the range below 800-600 cm^{-1} indicates C-H bending and the rest of the fingerprint vibrations. A distinct peak at approximately 3460 cm^{-1} confirms hydrogen-bonded O–H groups. The band at 1638 cm^{-1} matches the interaction of C=O groups or amide groups to suggest the connection of biomolecules to the nanoparticle surface. The highest peak at approximately 1449 cm^{-1} is due to C-H bending vibrations whereas the peak at approximately 1065 cm^{-1} shows C-O stretching polysaccharide or phenolic compounds. Notably, the appearance of peaks at approximately 585 cm^{-1} validates the typical metal-oxygen (Nd-O) stretching vibration, which confirms the development of neodymium oxide nanoparticles.

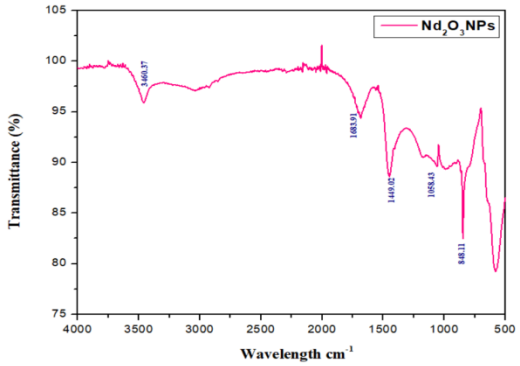

Fig 2: FT-IR spectral analysis of Nd_2O_3 synthesized nanoparticles using *C. phlomidis* leaf extract

3.1.3 X-ray diffraction (XRD) pattern analysis

The XRD pattern of the green synthesized Nd_2O_3nanoparticles by *Clerodendrum phlomidis* leaf extract shows a series of well-crystallized diffraction peaks at the position of 2 theta value of 21.8°, 29.3°, 32.6°, 33.6°, 36.5–37.0°, 38.3°, 40.2°, 41.6°, 44.2–45.5°, 54.7–56.1°, 59.9–60.9°, 65.3°, and 72.1°. These sharp and deep peaks show that the Nd_2O_3 NPs made are very crystalline. The diffraction angles seen can be indexed to the characteristic planes of cubic Nd_2O_3, which proves the successful synthesize of the neodymium oxide with no observable impurity peaks (Fig 3). There are no additional diffraction lines implying that there are no such phases or unreacted precursors left in the product. The widening of the peaks particularly the stronger ones, also point to the nanometer dimension of the crystallites. Based on the Debye Scherrer equation, the mean size of crystallites of the Nd_2O_3 NPs can be estimated to be within the nanometer range (usually a few tens of nanometers) hence their classification as nanoparticles.

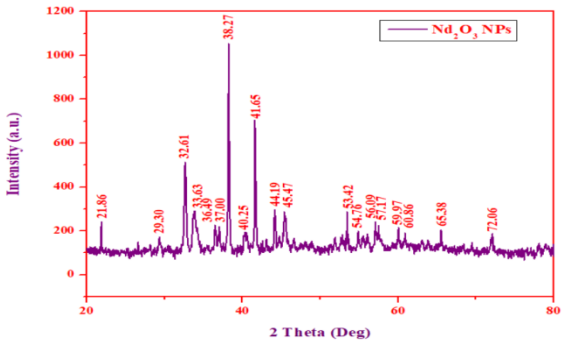

Fig 3: XRD analysis of Nd_2O_3 nanoparticles

3.1.4 Surface morphology analysis by scanning electron microscopy (SEM)

The SEM micrographs of the green synthesized Nd_2O_3 nanoparticles with *Clerodendrum phlomidis* extract indicate that the particles are stacked tightly and agglomerated into irregular clusters which have a rough and flaky surface morphology. On increased magnification, it is revealed that these clusters consist of much smaller nano-sized grains, which means that the primary nanoparticles are apt to form aggregations, because of their high surface energy (Fig 4).

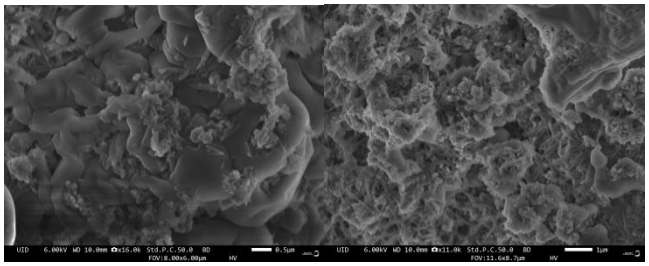

Fig 4: SEM analysis of Nd_2O_3 nanoparticles

3.2 Antioxidant activity

3.2.1 DPPH assay

Free radical scavenging capacity of DPPH at the concentration of 10-100 µg/mL was used as the antioxidant activity of the Nd_2O_3 nanoparticles prepared with *Clerodendrum phlomidis* (Fig 5). The Nd_2O_3 NPs (10 µg/mL) exhibited a scavenging activity of 34.51% which was slightly less than that of ascorbic acid (37.81%) but greater than that of the plant extract (28.36%). As the concentration rose to 20 and 30 µg/mL, the activity of the nanoparticles slowly increased to 41.01% and 46.81% respectively, even though it was still below the standard and the crude extract.

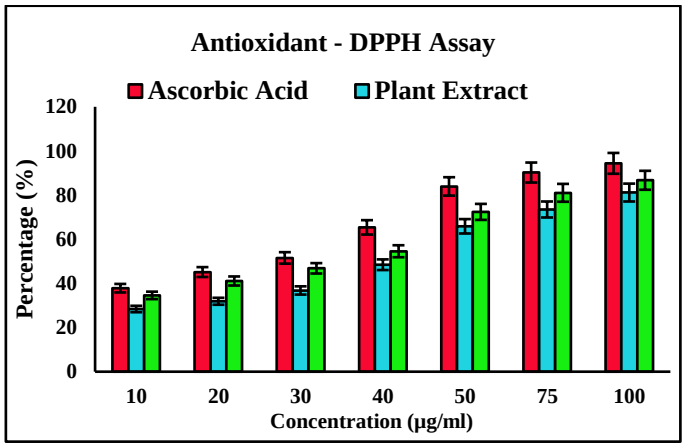

Fig 5: Antioxidant activity of Nd_2O_3 nanoparticles

Nd_2O_3 NPs showed 54.51% inhibition at 40 µg/mL, whereas ascorbic acid and plant extract showed 65.41% and 48.46% scavenging, respectively, a high degree of dose dependent increase in the activity of the nanoparticles. In addition, the nanoparticles exhibited 72.41% and 81.01% scavenging activity at 50 and 75 µg/mL, respectively. These values were compared to ascorbic acid (83.91% and 90.21%) and were significantly higher than those of the plant extract (65.86% and 73.46%). At the maximum concentration of 100 µg/mL, Nd_2O_3 nanoparticles exhibited 86.71% DPPH scavenging activity, compared to 94.41% for ascorbic acid and 81.16% for the plant extract (Fig.5). In general, these findings indicate that *Clerodendrum phlomidis* mediated Nd_2O_3 nanoparticles by possess strong, concentration-dependent antioxidant activity,with performance better than the crude extract and comparable to the standard reference antioxidant

3.2.2 NO assay

The nitric oxide (NO) scavenging activity was checked over a concentration level of 10-100 ug/ml (Fig. 6). The Nd_2O_3 nanoparticles were found to have inhibition (35%) at the lowest concentration (10 µg/mL), a little lower than the ascorbic acid (38.62%) but higher than the plant extract (31.1%) indicating a moderate level of baselines scavenging. With the increase in the concentration, the scavenging activity of Nd_2O_3 nanoparticles was gradually increasing up to 44.72% - 49.32% in the concentration of 20 to 30 µg/mL respectively, and this was always between the standard

antioxidant and the crude extract. The nanoparticles had a dose dependent increase in nitric oxide scavenging activity with 59.32% inhibition at 40 µg/mL, as compared to 68.92% with ascorbic acid, and 50.72% with the plant extract, demonstrating a definite increase of this activity with an increase in concentration.

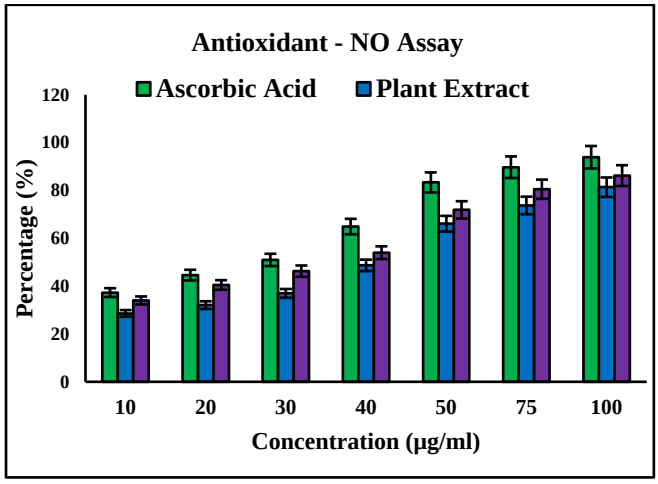

Fig 6: Antioxidant activity-NO assay of Nd_2O_3 nanoparticles using *Clerodendrum phlomidis*

Further, Nd_2O_3 nanoparticles reached 79.92% inhibition at 50 µg/mL and 84.12% at 75 µg/mL, while the plant extract showed 69.76% and 78.12%, respectively, and ascorbic acid maintained the highest activity. At the maximum tested concentration of 100 µg/mL, Nd_2O_3 NPs achieved 89.56% NO scavenging, which is significantly higher than the plant extract (85.42%) and close to the standard ascorbic acid (96.42%). The capacity of a chemical to undergo reduction is frequently correlated with its antioxidant activity. The results of the DPPH and nitric oxide tests showed that the biosynthesized Nd_2O_3 NPs were good at stopping free radicals. This could be due to various causes, including the capping agents (phytochemicals) on the surface of biosynthesized neodymium oxide nanoparticles, their dispersion, and their diminutive size (about 20 nm). Overall, these results demonstrate that *C. phlomidis*-mediated Nd_2O_3NPs possess strong, concentration-dependent NO scavenging activity, outperforming the crude extract, and approaching the efficiency of the reference antioxidant.

3.3 MTT assay

The anti-proliferative effect of Nd_2O_3 nanoparticles synthesized using *Clerodendrum phlomidis* on A549 cells was quantified by MTT assay, and the results clearly show a concentration-dependent decrease in cell viability (Fig 7a and 7b).

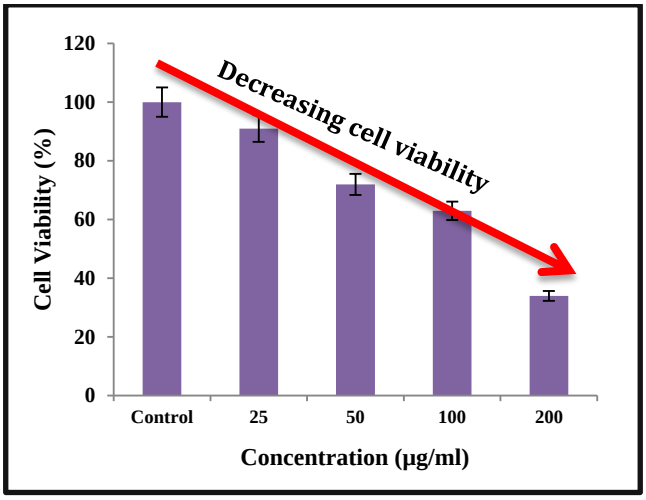

Fig 7a: MTT assay confirming the anti-proliferative activity of Nd_2O_3 NPs against A549 cell lines.

In the untreated control group, cell viability was considered 100%, indicating normal proliferation of A549 cells. On exposure to 25 µg/mL of Nd_2O_3 NPs, the viability dropped slightly to around the high 80–90% range, suggesting the onset of cytotoxic effects. At 50 µg/mL, a more noticeable reduction was observed, with viability falling to roughly 70–75%, indicating that a significant fraction of cells lost metabolic activity. Further increasing the concentration to 100 µg/mL reduced the viability to about 60–65%, confirming strong growth inhibition (26). The maximum cytotoxicity was recorded at 200 µg/mL, where the cell viability decreased sharply to 33% nearly one-third of the control, demonstrating pronounced anti-proliferative action of the nanoparticles. MTT assay results indicate that *C. Phlomidis*-mediated Nd_2O_3 NPs effectively suppress A549 cell proliferation in a dose-dependent manner and exhibit promising anticancer potential.

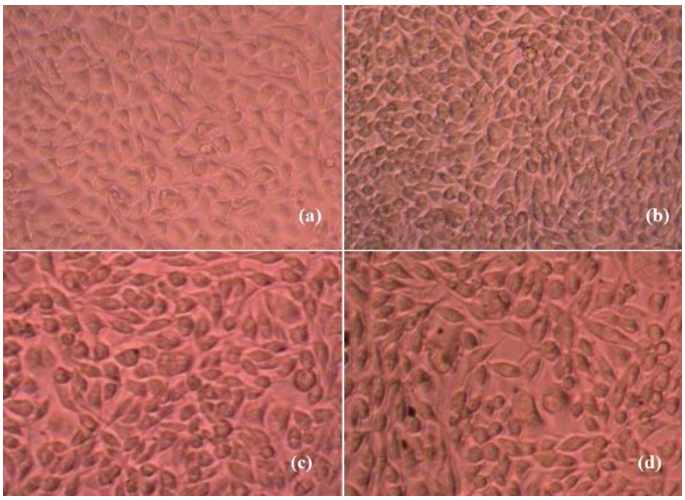

Fig 7b : Anticancer activity of A549 lung cancer cell line after 24 hrs of treatment (a) Control (untreated cells) (b) 25 µg/mL (c) 50 µg/mL (d) 100 µg/mL

3.4 Antidiabetic Activity

3.4.1 Alpha amylase inhibition assay

The antidiabetic effect of Nd_2O_3 nanoparticles prepared by *C.phlomidis* was screened by the virtue of alpha amylase inhibition assay, which demonstrated a concentration-dependent increase in activity compared with the plant extract and the control standard. At a concentration of 10 µg/mL Nd_2O_3 nanoparticles, the inhibition rate was 34.04%, which was slightly lower than the control (37.34%) but higher than the plant extract (28.64%). When the dosage was increased to 20 and 40 µg/mL, the Nd_2O_3 nanoparticles showed α-amylase inhibition of the nanoparticles resembling the control standard and plant extract were 40.54% and 46.34, respectively, which were intermediate values (Fig 8a). At 80 µg/mL, the inhibition increased to 54.04%, indicating a concentration-dependent activity. Further increases in concentration to 160 and 320 µg/mL, the inhibitory effect was 71.94% and 80.54%, which were comparable to the values of the control drug and consistently higher than the crude plant extract. Comprehensively, these results imply that *C. phlomidis*-mediated Nd_2O_3 nanoparticle exhibit strong α-amylase inhibitory activity and may serve as potential antidiabetic agent in a dose dependent manner.

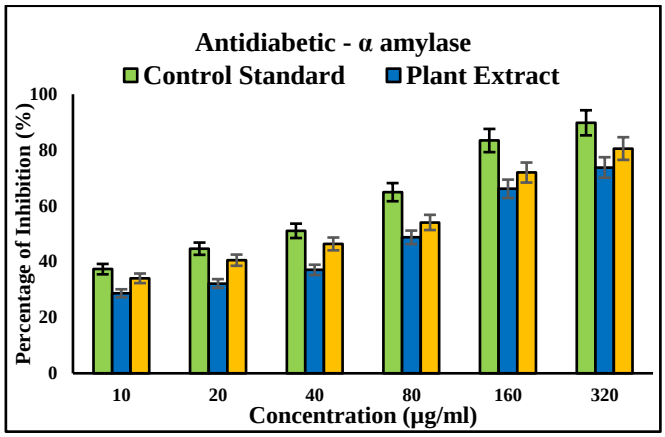

Fig 8a: Antidiabetic Activity: α amylase of Nd_2O_3 nanoparticles

3.4.2 Antidiabetic Activity – α glucosidase

The antidiabetic activity of Nd_2O_3 nanoparticles prepared with *Clerodendrum phlomidis* was tested by using the α-glucosidase as a biomarker, and the activity was shown to increase with the dosage. The nanoparticles were found to be 34.02% inhibitory at 10 ug/ml, which is slightly less than the standard (38.16%) although it is much higher than that of the plant extract (29.46%). The inhibitory activity increased under the concentrations of 20 and 40 µg/mL to 40.52% and 46.32%, respectively, and remained stable and constant when compared to the standard drug and the crude extract (Fig. 8b).

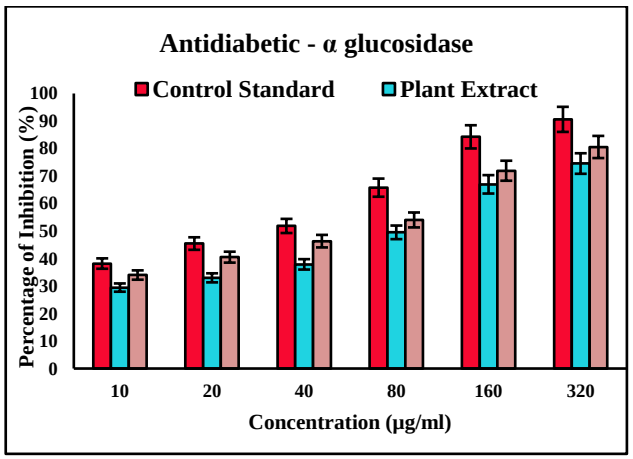

Fig 8b: Antidiabetic Activity: α glucosidase of Nd_2O_3 nanoparticles

The Nd_2O_3 nanoparticles inhibited 54.02% at 80 µg/mL which is higher than the extract (49.56%) and close to the standard (65.76%). Additional concentrations of 160 and 320 µg/mL improved the inhibition to 71.92% and 80.52% and the standard inhibition was 84.26 and 90.56% (30). In

general, these observations suggest that Nd_2O_3 nanoparticles obtained via the mediation of *Clerodendrum phlomidis* have an excellent α-glucosidase inhibitory activities, which are directly proportionate to the standard antidiabetic drug, and much better than the plant extract regarding concentration dependence.

4. DISCUSSION:

This study successfully develops Nd_2O_3 nanoparticles by a green synthetic route utilizing *Clerodendrum phlomidis* leaf extract and identifies their multi-functional biological potential. Nanoparticle synthesize and the role of phytochemicals (such as phenolics and proteins in reduction/stabilization mechanisms) were confirmed using UV–Vis spectroscopy, FT-IR analysis. A noticeable color change from pale violet to dark brown is a key visual evidence of the formation of neodymium oxide nanoparticles. [23, 24] This indicates the respective participation of the extract's phytochemical constituents including polyphenols, flavonoids, and other reducing agents in causing a variation in color upon addition of C. phlomidis leaf aqueous extract. [25] These biomolecules presumably exert a dual action of reducing Nd^{3+} ions in addition to acting as capping and stabilizing agents, enabling the formation of stable Nd_2O_3 nanoparticles. [26] In general, the FT-IR were shown to show that bioactive phytoconstituents that include phenolics, flavonoids, and proteins in C. *phlomidis* extract are important in the bioreduction of Nd_2O_3 nanoparticles, stabilization and capping. [27, 28] Shifts and changes in the peak intensity of the FT-IR spectrum of Nd_2O_3 nanoparticles mediated by *C. phlomidis* were found to be significant. [29]

The XRD patterns reflected good crystallinity of the samples. The Nd_2O_3 was successfully synthesized by *C. phlomidis* is the uniformity and regularity of the diffraction peaks indicating good crystallinity of the samples. [30] The SEM analysis confirmed that aggregates of nano-size, porous particles were obtained, which are favorable for catalytic and biological interactions. [31] These surfaces are found to be porous with many voids and pores, which could be due to the loss of volatile phytochemicals during the calcination process as well as be able to increase the surface area of the substance. [32] The general morphology implies that the biomolecules of plant origin are reducing and stabilizing factors, which control the formation of NPs of Nd_2O_3 and the development of interconnected networks. [33] The aggregated yet nano-grained structures are mostly preferable in relation to catalytic and adsorption-based applications due to the high number of active sites. [34] With regard to biological activity, the effect of the nanoparticles showed a strong dose-dependent antioxidant activity using DPPH and nitric oxide assays which were better than that of crude extract but comparable if not closer to standard ascorbic acid playing into account the orientation for surface-bound bioactive compounds and high surface area. [35] The MTT assay confirmed substantial cytotoxicity against A549 cancer cells purchased from National Centre for Cell Sciences (NCCS), Pune, showed a notable rise in the destruction of lung cancer cells at its highest concentration compared to the untreated group of cells, which indicating the anticancer potential. [36] Moreover, significant inhibition of α-amylase and α-glucosidase indicates a potent antidiabetic activity. [37] These findings collectively advocate the therapeutic significance of phenolic compounds synthesized Nd_2O_3 nanoparticles as a novel class of antioxidants, anticancer and antidiabetic agents.

5. CONCLUSION

To conclude, the present study demonstrates a novel and eco-friendly approach for the biosynthesize of Nd_2O_3 nanoparticles using *Clerodendrum phlomidis* leaf extract, effectively integrating green chemistry principles with nanomedicine. The phytochemical-mediated synthesize was successful, yielding well-formed nanoparticles with confirmed structural and morphological integrity. In vitro studies evaluations revealed notable anti-oxidant, anti-diabetic, and anti-cancer activities. The enhanced

bioactivity relatively compared to the crude plant extract highlights the synergistic effect of bioactive phytochemicals coupled with nanoscale properties of the synthesized particles. This research work presents a sustainable approach for developing multifunctional nanotherapeutic agents with promising applications in the management of diabetes and cancer management.

Limitations:

This study has one major limitation, the biological activities based on synthesized nanoparticles (Nd_2O_3) were only tested in vitro with no validation experiments took place in vivo. Furthermore, the current investigation did not include detailed mechanistic studies including toxicity assessment and long-term stability analysis as well as comparative evaluation with commercially available antidiabetic and anticancer drugs.

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