International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Characterization of Root-Mediated Zinc Oxide Nanoparticles of *Clitoria ternatea* and Evaluation of Antioxidant and Antidiabetic Potential

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Abstract:
The present study involves the evaluation of the antidiabetic potential, phytochemical properties, and green synthesis of ZnO NPs prepared using different root extracts of *Clitoria ternatea* Linn. Herbs having bioactive phytoconstituents are attracting much attention in nanobiotechnology because of their therapeutic potential and environmental sustainability. During this investigation, *Clitoria ternatea* root extracts (methanol extract, ethanol extract, and water extract) were prepared and quantitatively and qualitatively analyzed for some of the phytochemical constituents in them. The preliminary screening established the appearance of important secondary metabolites like flavonoids, phenolics, alkaloids, tannins, saponins, glycosides, and terpenoids, with a high medicinal potential in the extracts of the root of the plant. The quantitative estimation showed that the total phenolic, total flavonoid, and saponin contents were remarkable, especially in the methanolic root extract.
Root extracts were prepared and subsequently used as organic reducing and stabilizing agents for the green synthesis of zinc oxide nanoparticles. In addition to visual observation of the colour change, confirmation of the formation of ZnO NPs was done by UV-Visible spectroscopy, FTIR, XRD, SEM, and EDX investigations of sample solutions. The characteristic absorption peaks were observed between 325 and 340 nm, which was confirmed by the UV-Visible spectral studies during the synthesis of nanoparticles. FTIR studies showed that the hydroxyl, carbonyl, and phenolic groups were responsible for the stabilization in the case of nanoparticles, while XRD studies showed the formation of crystalline synthesized nanoparticles of ZnO.
Concentration-dependent inhibition against the alpha-amylase enzyme was shown with the methanolic extract of the root-mediated ZnO NPs, showing a higher concentration of inhibition potential. Further, the antioxidant activity of the root-mediated ZnO nanoparticles obtained by the DPPH radical scavenging assay was found to be high.
The results of the present investigation indicate that the root extract of *Clitoria ternatea* is an effective biological source to synthesize biologically safe ZnO nanoparticles with good antidiabetic and antioxidant activity. The study underlines the therapeutic relevance of root- mediated green nanotechnology and its prospects in the development of anti-diabetic drugs based on nanotechnology as a major field of nanotechnology.

Keywords: The Root Extract of *Clitoria ternatea*, ZnO Nanoparticles, Green Synthesis, Antidiabetic Activity, Alpha-Amylase Inhibition, Phytochemical Analysis, Antioxidant Activity.

1. Introduction

The therapeutic properties and the bioactive phytochemicals of medicinal plants have made them popular and extensively utilized in traditional medicinal systems such as Ayurveda, Siddha, and Unani. *Clitoria ternatea* Linn. is one of these. Butterfly pea (Fabaceae) is a medicinal plant with significant antioxidant, anti-inflammatory, antidiabetic, and neuroprotective effects that have been traditionally used. Flavonoids, phenolics, alkaloids, tannins, saponins, and glycosides are some of the phytoconstituents found in its roots that make it medically useful.[1,7]

Diabetes mellitus is a chronic metabolic disorder which is defined as high blood glucose, caused by low insulin production or by insulin not working properly. In oxidative stress, prolonged hyperglycemia can lead to complications that are primarily linked to high production of Reactive Oxygen Species (ROS), including cardiovascular diseases, nephropathy, neuropathy, and retinopathy. Hence, plant antioxidants and natural antidiabetics have become a popular source of alternative medicine for diabetes.[2,6]

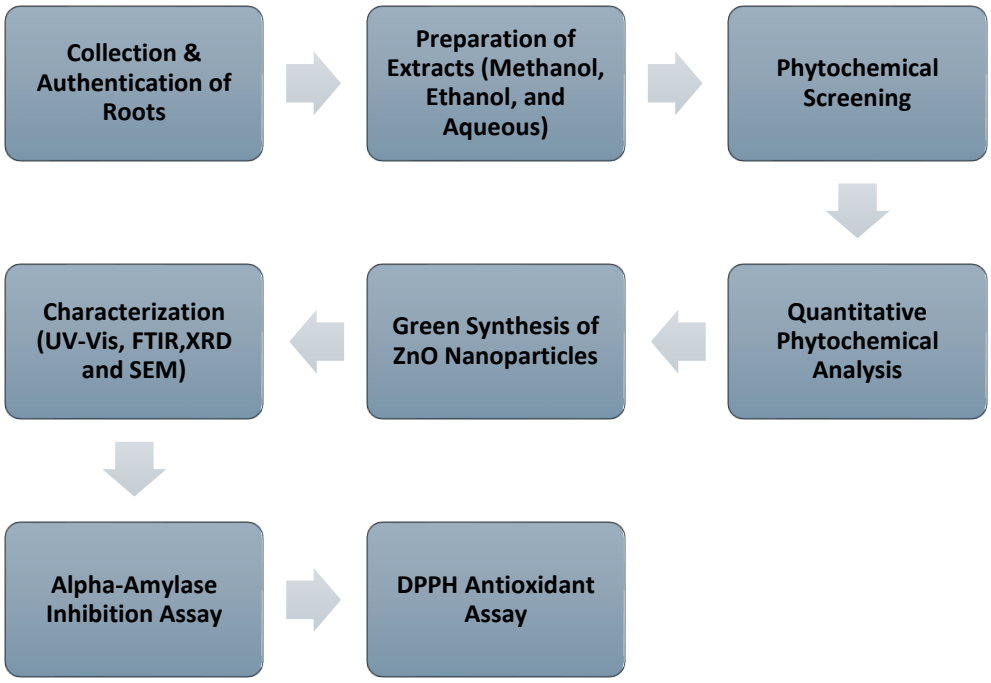
Nanotechnology has become a significant part of life in biomedical research, especially in drug delivery and development of new therapies. The zinc oxide nanoparticles (ZnO NPs) have been of interest due to their physicochemical stability, biocompatibility, and antioxidant and antimicrobial properties. The Green synthesis method with plant extracts offers an eco-friendly and cost-effective alternative to conventional methods for the production of nanoparticles. The root extracts of *Clitoria ternatea* can be used as a reducing, stabilizing, and capping agent in the synthesis of nanoparticles.[3]

The current study is designed to investigate the phytochemical composition and antidiabetic potential of green-synthesized zinc oxide nanoparticles (ZnO) with the extract of the root of *Clitoria ternatea*. The research includes qualitative and quantitative phytochemical screening, synthesis of nanoparticles, and physicochemical characterization. In addition, the antidiabetic activity will be assessed by alpha-amylase inhibition and glucose diffusion assays, and antioxidant activity will also be assessed. The results could offer scientific understanding about the therapeutic potential of *Clitoria ternatea*-mediated ZnO Nanoparticles and their possible application in plant-based nanotherapeutic diabetes management.[4.5]

Table 1: Major Phytochemicals Reported in *Clitoria ternatea* Root Extracts and Their Biological Significance

Phytochemical	Biological Role	Therapeutic Importance
Flavonoids	Free radical scavenging	Antioxidant, antidiabetic
Phenolics	Electron donation and ROS reduction	Antioxidant activity
Alkaloids	Metabolic regulation	Antidiabetic, antimicrobial
Tannins	Enzyme inhibition	Glucose regulation
Saponins	Membrane interaction	Hypoglycemic activity
Terpenoids	Anti-inflammatory effects	Protective medicinal role
Glycosides	Biological signalling	Cardioprotective and therapeutic

Figure 1. Experimental Workflow of the Study



Schematic representation of the experimental procedure used for extraction, phytochemical analysis, green synthesis, characterization, and biological evaluation of ZnO nanoparticles.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

Clitoria ternatea Linn. roots were collected from naturally grown plants, which were healthy and fresh in the Udaipur region, Rajasthan, during the post-rainy months. Ethnomedicinal importance and traditional uses were considered in the selection of the plant. The collected root materials were extensively cleaned by flushing them through with running tap water and then distilled water, which should wash away any soil particles and contaminants as well. A qualified taxonomist from the Department of Botany authenticated the plant specimen, and herbarium specimens were prepared and preserved to be used for future reference. Shade drying at room temperature was done for about 14 days so that the heat-sensitive phytoconstituents are not degraded. The roots were dried completely, then ground with a mechanical grinder to make coarse powder and stored in airtight containers for further analysis.[8]

2.2 Preparation of Root Extracts

Solvent extraction process using three different solvents (methanol, ethanol, and distilled water) on the powdered root material was carried out in order to get extracts that contain different phytochemical constituents. The extraction of root powder was carried out for 6–8 hours in the Soxhlet system using 250 mL of the solvent, and around 25 g of root powder was obtained. The extracted materials were filtered with Whatman No.1 filter paper and evaporated in a hot air oven at 50–55°C to remove surplus solvent. The concentrated extracts were stored in sterile containers and kept at 4°C for further phytochemical analysis and synthesis of nanoparticles.[9]

Table 2: Preparation of Different Root Extracts of *Clitoria ternatea*

Extract Type	Solvent Used	Extraction Method	Storage Condition
Methanolic Root Extract	Methanol	Soxhlet Extraction	4°C
Ethanolic Root Extract	Ethanol	Soxhlet Extraction	4°C
Aqueous Root Extract	Distilled Water	Soxhlet Extraction	4°C

2.3 Preliminary Phytochemical Screening

Qualitative phytochemical analysis of methanolic, ethanolic, and aqueous root extracts was conducted for the presence of important secondary metabolites by standard biochemical methods. A conventional phytochemical screening test was applied to analyze for alkaloids, flavonoids, phenolic acids, tannins, saponins, terpenoids, glycosides, protein, carbohydrates, and steroids in the extracts. Characteristic colour changes or precipitates were formed in the reactions suggested, confirming the presence of such phytochemicals.[10]

Table 3: Phytochemical Tests Performed for Root Extract Analysis

Phytochemical	Test Used
Alkaloids	Mayer's Test
Flavonoids	Alkaline Reagent Test
Phenolics	Ferric Chloride Test
Tannins	Lead Acetate Test
Saponins	Foam Test
Terpenoids	Salkowski Test
Glycosides	Keller-Killiani Test

2.4 Quantitative Phytochemical Estimation

2.4.1 Total Phenolic Content (TPC)

The sum of the phenolic substances in the root extract was measured by the Folin–Ciocalteu phenol red method. Briefly, 1 mL of extract was mixed with Folin–Ciocalteu reagent, followed by sodium carbonate solution. The reaction mixture was kept in the dark for 30 minutes, and absorbance was measured at 765 nm on the UV-Visible spectrophotometer. Gallic acid was used as the standard, and the results were presented as mg gallic acid equivalent (GAE)/g extract.[11]

2.4.2 Total Flavonoid Content (TFC)

The aluminium chloride colorimetric assay was used to ascertain the total flavonoid content. The root extract was treated with different concentrations of sodium nitrite, aluminium chloride, and sodium hydroxide solutions, followed by measuring the absorbance at 510 nm. The standard compound used was rutin, and the content of flavonoids was calculated as mg rutin equivalent (RE)/g extract.

2.4.3 Total Saponin Content (TSC)

The root extracts were analysed for their total saponin content spectrophotometrically using diosgenin as a standard. The absorbances were taken at 544 nm and expressed as mg Diosgenin equivalent (DE)/g extract.

2.5 Green Synthesis of Zinc Oxide Nanoparticles

The root extracts of methanolic, ethanolic, and aqueous extracts of *Clitoria ternatea* acted as reducing and stabilizing agents in the green synthesis of zinc oxide nanoparticles. Zinc nitrate solution (0.01 M) was prepared and mixed with root extracts in a continuous magnetic stirring system. This reaction mixture was heated to 60–70°C and kept boiling until the formation of a pale white precipitate was observed, which confirmed the formation of nanoparticles in the reaction mixture. The collected nanoparticles were then washed with distilled water and ethanol several times by centrifugation at 10,000 rpm for 15 minutes. The purified precipitate was dried in a hot air oven at 80°C, and then it was calcined at a high temperature to get the pure powdered nanoparticles of ZnO.[12]

2.6 Characterization of ZnO Nanoparticles

2.6.1 UV–Visible Spectroscopy

It was confirmed by UV–Visible spectrophotometric analysis that ZnO nanoparticles were formed. A double-beam UV–Visible spectrophotometer was used for the wavelength scan of the nanoparticle suspension between 200 and 800 nm. The characteristic peaks of the ZnO nanoparticles were recorded.

2.6.2 Fourier Transform Infrared Spectroscopy (FTIR)

To explore functional groups that are responsible for the synthesis and stabilization of nanoparticles, a Fourier transform infrared (FTIR) analysis was conducted. FTIR spectrophotometer was used to characterize the dried ZnO nanoparticle samples within the spectral range of 400-4000 cm^{-1}.

2.6.3 X-Ray Diffraction (XRD) Analysis

Structural analysis and crystalline nature of synthesized ZnO nanoparticles were determined using X-ray diffraction analysis. Cu-Kα radiation was used for recording the diffraction pattern in an appropriate range of scan.

2.6.4 Scanning Electron Microscopy (SEM)

The produced nanoparticles of ZnO were studied by Scanning Electron Microscopy in terms of their morphology and surface texture. Coated samples of dried nanoparticles were again examined under the SEM at appropriate magnification.

2.6.5 Energy Dispersive X-Ray (EDX) Analysis

The synthesized ZnO nanoparticles were subjected to EDX analysis to reveal the elemental composition and purity of the nanoparticles. Two peaks for the elements Zn and O indicated successful synthesis of the nanoparticles.

2.7 Evaluation of Antidiabetic Activity

2.7.1 Alpha-Amylase Inhibitory Assay

The root extracts and synthesized ZnO nanoparticles show their alpha-amylase inhibitory activity by the DNSA method. Aliquots of these samples were treated with alpha-amylase enzyme solution, and then starch was added to the samples to give different concentrations of the sample, and then alpha-amylase enzyme was added. Dinitrosalicylic acid reagent was then added, followed by a boiling water bath. The absorbance was determined at 540 nm using a spectrophotometer. The standard antidiabetic drug was acarbose.[22]

This was done using the following formula:

$$\% \, Inhibition = \frac{Control - Sample}{Control} \times 100$$

2.7.2 Glucose Diffusion Inhibition Assay

The glucose diffusion inhibitory activity was evaluated using the dialysis membrane method. Test samples and glucose solution were placed in the dialysis tubing and then placed in distilled water. The amount of glucose diffused into the external solution was estimated at regular time intervals using a glucose estimation method. A comparative study was carried out to find out the inhibitory effect of the glucose movement with the help of root-mediated ZnO NPs.[23]

2.8 Antioxidant Activity

This method is also known as the DPPH Radical Scavenging Assay.

Root extract and synthesized ZnO NPs were investigated for their antioxidant activity using the DPPH radical scavenging activity. Samples were prepared with different concentrations, and DPPH solution was added to each sample, and it was kept in a dark condition for a 30-minute period. Absorbance was measured at 517nm using a UV–Visible spectrophotometer against a blank solution. The standard antioxidant was ascorbic acid.

The percentage radical scavenging activity was calculated as:

$$\% \, Scavenging = \frac{A_0 - A_1}{A_0} \times 100$$

2.9 Statistical Analysis

Each experiment was repeated three times, and the results are presented as mean ± standard deviation (SD). The data were analyzed statistically with the software SPSS and Microsoft Excel. The significance of the differences among different experimental groups was determined using one-way analysis of variance (ANOVA), and p-values less than 0.05 were taken as significant.

3. Results

3.1 Collection and Authentication of Plant Material

Fresh roots of *Clitoria ternatea* Linn. were collected from healthy and disease-free plants growing in their natural habitat during the appropriate growing season. The collected specimens were carefully cleaned to remove adhering soil and other foreign materials before further processing. Botanical authentication was carried out by a qualified taxonomist to confirm the identity of the plant species. A voucher specimen was deposited in the departmental herbarium for future reference. The authenticated roots were washed thoroughly with distilled water, shade-dried at room temperature until a constant weight was obtained, and subsequently pulverized into a coarse powder using a mechanical grinder. The powdered material was stored in airtight containers under dry conditions until further extraction and phytochemical analysis.[6]

The successful authentication ensured the botanical identity and purity of the plant material used throughout the investigation. The roots exhibited characteristic morphological features including a light brown external surface, fibrous texture, and distinct odour, which were consistent with the standard taxonomic description of *Clitoria ternatea* Linn.

3.2 Preparation of Root Extracts

The powdered root material was subjected to solvent extraction using methanol, ethanol, and distilled water to obtain extracts containing phytoconstituents with varying polarities. Extraction was carried out

using the Soxhlet extraction method for the organic solvents, whereas the aqueous extract was prepared by maceration under controlled laboratory conditions. Following extraction, the solvents were removed under reduced pressure using a rotary evaporator, and the concentrated extracts were dried to obtain crude residues.[9]

The methanolic extract yielded a dark brown semi-solid residue, while the ethanolic extract appeared as a brown viscous mass. The aqueous extract was comparatively lighter in colour and exhibited a gummy consistency. Variations in colour, texture, and extractive yield reflected differences in solvent polarity and extraction efficiency for the bioactive constituents present in the roots.

The dried extracts were preserved in airtight containers at 4°C until further phytochemical screening, nanoparticle synthesis, and biological evaluation.

Table 4: Physical characteristics of *Clitoria ternatea* root extracts

Extract Type	Colour	Texture	Odour	Solubility
Methanol Root	Brownish	Thick paste	Earthy	Miscible
Ethanol Root	Light brown	Semi-solid	Slight odour	Limited miscibility
Aqueous Root	Dark brown	Viscous	Strong earthy	Fully miscible

Table 5: Percentage extractive yield of different solvent extracts

Plant Part	Solvent	Quantity (g)	Yield (%)
Root	Methanol	20	3.05
Root	Ethanol	20	0.9
Root	Aqueous	20	2.49

3.3 Preliminary Phytochemical Screening

Qualitative phytochemical screening was performed to identify the major classes of secondary metabolites present in the methanolic, ethanolic, and aqueous root extracts of *Clitoria ternatea*. Standard phytochemical tests demonstrated the presence of several biologically active constituents, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, carbohydrates, proteins, amino acids, steroids, and terpenoids. The intensity of phytochemical reactions varied among the different solvent extracts, indicating differences in extraction efficiency associated with solvent polarity.[19]

The methanolic extract exhibited the richest phytochemical profile, showing strong positive reactions for phenolics, flavonoids, tannins, and saponins. The ethanolic extract also demonstrated the presence of most of the tested phytochemicals, although the reaction intensity was comparatively lower than that of the methanolic extract. The aqueous extract contained several polar phytoconstituents but showed relatively weaker responses for certain secondary metabolites.[20]

The presence of these bioactive compounds indicates that *Clitoria ternatea* roots possess considerable phytochemical diversity, which may contribute to their reducing, stabilizing, antioxidant, and antidiabetic properties during the green synthesis of zinc oxide nanoparticles.

Table 6: Qualitative phytochemical screening of *Clitoria ternatea* root extracts

Phytochemical Group	Test Applied	Observation	Result
Alkaloids	Wagner's test	No precipitate	–
Flavonoids	Alkaline reagent test	Intense yellow colouration	+

Glycosides	Keller–Killiani test	Reddish-brown ring	+
Tannins	Ferric chloride test	No blue-black coloration	–
Saponins	Froth test	No persistent foam	–
Phenols	Lead acetate test	Slight turbidity	±
Carbohydrates	Fehling's test	Brick-red precipitate	+
Proteins	Biuret test	Light violet coloration	±

3.3.1 Comparative Phytochemical Profile

Comparison of the three solvent extracts revealed that methanol extracted a broader spectrum of phytoconstituents than ethanol and distilled water. This observation suggests that methanol was the most efficient solvent for extracting polyphenolic compounds and other secondary metabolites responsible for reducing zinc ions during nanoparticle synthesis. The phytochemical profile obtained from the qualitative analysis further supports the selection of the methanolic extract for subsequent quantitative estimation and biological investigations.

3.4 Quantitative Phytochemical Estimation

Quantitative phytochemical analysis was performed to determine the concentration of major bioactive constituents present in the methanolic, ethanolic, and aqueous root extracts of *Clitoria ternatea*. The estimation of Total Phenolic Content (TPC), total flavonoid content (TFC), and Total Saponin Content (TSC) provided a quantitative assessment of the phytochemicals responsible for the reducing, capping, antioxidant, and antidiabetic potential of the plant extracts. Considerable variation was observed among the different solvent extracts, indicating the influence of solvent polarity on the extraction efficiency of bioactive compounds.[23]

3.4.1 Total Phenolic Content (TPC)

The total phenolic content of the different root extracts was estimated using the Folin–Ciocalteu method and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract. The quantitative analysis demonstrated that the concentration of phenolic compounds varied among the solvent extracts. The methanolic extract exhibited the highest phenolic content, followed by the ethanolic extract, whereas the aqueous extract contained comparatively lower amounts of phenolic constituents.

The higher phenolic concentration observed in the methanolic extract indicates its superior extraction efficiency for polyphenolic compounds. Phenolic constituents are recognized for their ability to donate electrons and hydrogen atoms, thereby facilitating the reduction of zinc ions during the biosynthesis of zinc oxide nanoparticles. These compounds also contribute significantly to the antioxidant activity of the synthesized nanoparticles.

Table 7: Total phenolic content of different root extracts

Plant Part	Solvent	Absorbance (765 nm)	Total Phenolic Content (mg GAE/g extract)
Root	Ethanol	0.333 ± 0.003	132.00
Root	Methanol	0.089 ± 0.005	4.73
Root	Aqueous	0.385 ± 0.001	270.00

3.4.2 Total Flavonoid Content (TFC)

The total flavonoid content was determined using the aluminium chloride colorimetric method and expressed as rutin equivalents (RE) per gram of dry extract. The flavonoid concentration differed among the three solvent extracts, with the methanolic extract exhibiting the highest flavonoid content, followed by the ethanolic and aqueous extracts.

Flavonoids are important secondary metabolites that participate in the reduction and stabilization of metal ions during nanoparticle synthesis. The comparatively higher flavonoid content in the methanolic extract suggests its greater potential for facilitating the formation of stable zinc oxide nanoparticles. In addition, flavonoids are known to possess strong antioxidant and enzyme inhibitory activities, which may contribute to the biological properties of the synthesized nanoparticles.[24]

Table 8: Total flavonoid content of different root extracts

Plant Part	Solvent	Absorbance (510 nm)	Total Flavonoid Content (mg RE/g extract)
Root	Ethanol	0.224 ± 0.001	125.00
Root	Methanol	0.0967 ± 0.002	3.50
Root	Aqueous	0.362 ± 0.001	191.83

3.4.3 Total Saponin Content (TSC)

The total saponin content was quantified using the standard gravimetric procedure. The analysis revealed noticeable differences in saponin concentration among the three extracts. The methanolic extract contained the highest concentration of saponins, while the ethanolic extract exhibited moderate levels and the aqueous extract showed comparatively lower concentrations.

Saponins serve as natural stabilizing agents during nanoparticle synthesis and contribute to several pharmacological activities, including antioxidant, antidiabetic, and anti-inflammatory effects. Their presence in appreciable quantities further supports the suitability of *Clitoria ternatea* root extract for green synthesis of ZnO NPs.

Table 9: Total saponin content of different root extracts

Plant Part	Solvent	Absorbance (544 nm)	Total Saponin Content (mg DE/g extract)
Root	Ethanol	0.045 ± 0.003	35.56
Root	Methanol	0.091 ± 0.001	48.00
Root	Aqueous	0.169 ± 0.001	64.89

3.4.4 Comparative Phytochemical Profile

A comparative evaluation of the quantitative phytochemical analysis demonstrated that the methanolic extract consistently contained higher concentrations of phenolics, flavonoids, and saponins than the ethanolic and aqueous extracts. The superior phytochemical profile of the methanolic extract indicates its enhanced extraction efficiency and suggests its greater reducing and stabilizing capability during the biosynthesis of zinc oxide nanoparticles.

The abundance of these secondary metabolites is expected to enhance nanoparticle formation by acting as natural reducing agents and capping molecules while simultaneously contributing to the antioxidant and antidiabetic properties of the synthesized nanoparticles.

3.5 Green Synthesis of Zinc Oxide Nanoparticles

The biosynthesis of zinc oxide nanoparticles was successfully achieved using the root extract of *Clitoria ternatea* as a reducing and stabilizing agent. During the synthesis process, gradual changes in the reaction mixture were observed, indicating the formation of zinc oxide nanoparticles. Initially, the reaction solution appeared clear; however, upon continuous stirring and controlled heating, a noticeable colour change accompanied by the formation of a white precipitate was observed.[8]

The appearance of the precipitate indicated the successful reduction of zinc ions and subsequent formation of zinc oxide nanoparticles. The synthesized nanoparticles were separated by centrifugation, repeatedly washed with distilled water and ethanol to remove unreacted constituents, and finally dried under controlled conditions to obtain purified nanoparticle powder.

The successful synthesis demonstrated the efficiency of *Clitoria ternatea* root extract as an environmentally friendly reducing and capping agent for the production of ZnO nanoparticles without the use of hazardous chemical reducing agents.[9]

3.6 Characterization of Zinc Oxide Nanoparticles

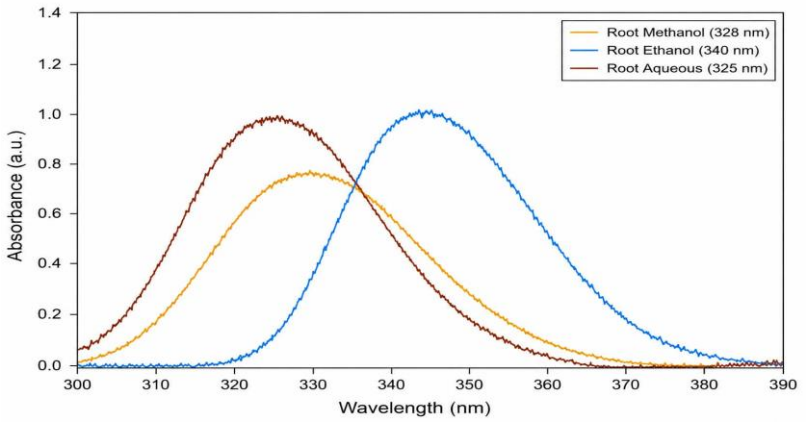
The synthesized zinc oxide nanoparticles were characterized using various analytical techniques to confirm their optical properties, functional groups, crystalline nature, surface morphology, particle size, and elemental composition. Characterization was performed using UV–Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX) analysis.[13]

3.6.1 UV–Visible Spectroscopy

The formation of zinc oxide nanoparticles was initially confirmed by UV–Visible spectroscopic analysis. The synthesized nanoparticles exhibited a characteristic absorption peak within the ultraviolet region, confirming the successful formation of ZnO nanoparticles. The observed absorption peak corresponds to the intrinsic band-gap absorption of zinc oxide and is consistent with previously reported biosynthesized ZnO nanoparticles.[14]

The sharp absorption peak indicated uniform nanoparticle formation and suggested the successful reduction of zinc ions by phytochemicals present in the root extract. The absence of additional broad peaks further confirmed the purity and stability of the synthesized nanoparticles.

Figure 2. UV–Visible Spectroscopy Analysis



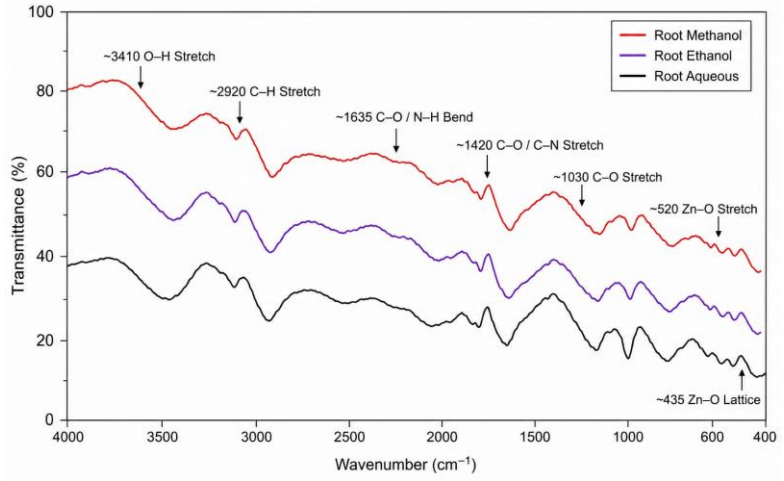
3.6.2 Fourier Transform Infrared (FTIR) Analysis

FTIR spectroscopy was performed to identify the functional groups involved in the reduction and stabilization of zinc oxide nanoparticles. The FTIR spectrum exhibited several characteristic absorption bands corresponding to hydroxyl, carbonyl, alkane, aromatic, and zinc–oxygen functional groups.

The broad absorption band observed in the higher wavenumber region indicated O–H stretching vibrations associated with phenolic compounds and alcohols. Additional peaks corresponded to C–H, C=O, C=C, and C–O stretching vibrations, confirming the presence of various phytochemicals adsorbed on the nanoparticle surface. The characteristic Zn–O stretching vibration observed in the lower wavenumber region confirmed the successful synthesis of zinc oxide nanoparticles.[15]

These observations suggest that naturally occurring phytochemicals present in *Clitoria ternatea* root extract functioned both as reducing agents and as stabilizing molecules during nanoparticle formation.

Figure 3. FTIR spectrum of synthesized ZnO nanoparticles.



3.6.3 X-ray Diffraction (XRD) Analysis

The crystalline nature of the biosynthesized zinc oxide nanoparticles was investigated using X-ray diffraction (XRD) analysis. The diffraction pattern exhibited several well-defined and sharp diffraction peaks, confirming the successful formation of crystalline ZnO nanoparticles. The characteristic diffraction peaks corresponded to the standard hexagonal wurtzite crystal structure of zinc oxide and showed good agreement with the standard JCPDS reference data.

The sharpness and intensity of the diffraction peaks indicate the high crystallinity and purity of the synthesized nanoparticles. No additional diffraction peaks corresponding to impurity phases were

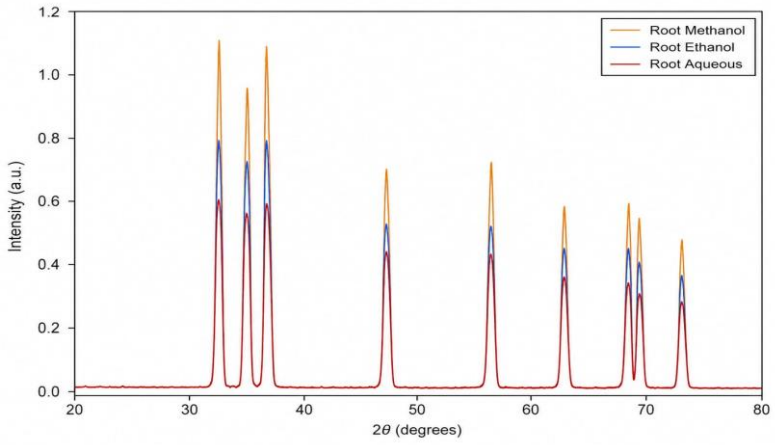
observed, suggesting that the biosynthesized ZnO nanoparticles were free from undesirable crystalline contaminants. The average crystallite size was determined using the Debye–Scherrer equation, confirming the nanoscale dimensions of the synthesized particles.[2]

The XRD analysis further validated the effectiveness of the green synthesis approach using *Clitoria ternatea* root extract for producing highly crystalline zinc oxide nanoparticles suitable for biological applications.

Table 10: XRD peak positions and crystallographic planes of synthesized ZnO nanoparticles

Sample	2θ (°)	FWHM (β)	Crystallite Size (nm)	Remarks
Root – Methanol ZnO NPs	36.4	0.345	24.0 nm	Strongest nucleation → significantly smaller crystallites
Root – Ethanol ZnO NPs	36.2	0.260	31.8 nm	Moderate crystallite reduction
Root – Aqueous ZnO NPs	36.5	0.370	22.4 nm	Most phytochemical–rich root extract → smallest crystallites

Figure 4. X-ray diffraction pattern of biosynthesized ZnO nanoparticles.



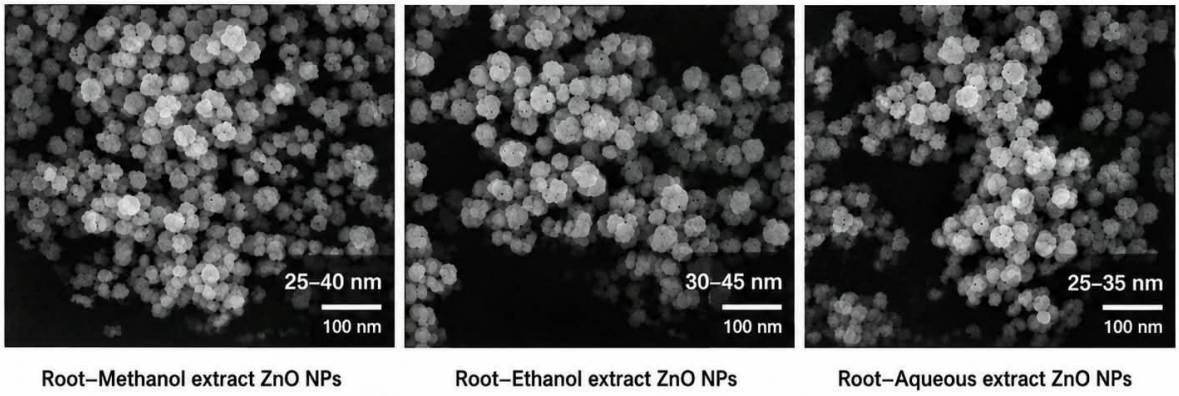
3.6.4 Scanning Electron Microscopy (SEM) Analysis

The surface morphology and particle size of the synthesized zinc oxide nanoparticles were examined using Scanning Electron Microscopy (SEM). The SEM micrographs revealed that the nanoparticles possessed a predominantly spherical to nearly spherical morphology with slight aggregation. The aggregation observed in certain regions may be attributed to the high surface energy of nanoparticles and intermolecular interactions among phytochemical capping agents.

The nanoparticles appeared relatively uniform in distribution with rough surface characteristics. The observed particle size remained within the nanometre range, confirming the successful synthesis of nano-sized ZnO NPs through the green synthesis approach. The presence of phytochemicals from the root extract effectively prevented excessive particle growth and contributed to the stabilization of the nanoparticles during synthesis.[12]

The SEM observations confirmed that the biosynthesized ZnO nanoparticles possessed suitable morphological characteristics for subsequent antioxidant and antidiabetic investigations.

Figure 5. SEM micrograph showing the surface morphology of biosynthesized ZnO nanoparticles.



3.7 Evaluation of Antidiabetic Activity

The antidiabetic potential of the biosynthesized zinc oxide nanoparticles was evaluated through **α-amylase inhibitory activity** and the **glucose diffusion inhibition assay**. The results demonstrated concentration-dependent inhibition in both experimental models, indicating the ability of the synthesized nanoparticles to regulate carbohydrate digestion and glucose transport.

3.7.1 α-Amylase Inhibitory Activity

The inhibitory effect of the biosynthesized zinc oxide nanoparticles against the α-amylase enzyme was evaluated at different concentrations and compared with the standard antidiabetic drug. The results revealed that enzyme inhibition increased progressively with increasing concentration of the nanoparticles, demonstrating a clear dose-dependent response.[22]

Among the tested samples, the nanoparticles synthesized using *Clitoria ternatea* root extract exhibited significant α-amylase inhibitory activity. The inhibition percentage increased steadily across all tested concentrations, indicating effective suppression of enzymatic starch hydrolysis. Although the standard drug exhibited comparatively higher inhibitory activity, the biosynthesized nanoparticles demonstrated appreciable antidiabetic potential, suggesting that the phytochemical-coated ZnO nanoparticles may contribute to delayed carbohydrate digestion and reduced postprandial glucose absorption.

The observed enzyme inhibition may be attributed to the synergistic effect of zinc oxide nanoparticles and phytoconstituents such as phenolics, flavonoids, and saponins adsorbed on the nanoparticle surface.

3.7.2 Glucose Diffusion Inhibition Assay

The glucose diffusion inhibition assay was carried out to evaluate the ability of the biosynthesized zinc oxide nanoparticles to retard glucose movement across the dialysis membrane, thereby simulating delayed intestinal glucose absorption. The results indicated that the synthesized nanoparticles effectively reduced glucose diffusion in a concentration-dependent manner.

The percentage inhibition increased gradually with increasing nanoparticle concentration, indicating enhanced glucose entrapment and slower glucose diffusion. The results suggest that the phytochemical-coated ZnO nanoparticles may play a significant role in controlling postprandial hyperglycaemia by delaying glucose transport. The observed activity can be attributed to the combined influence of bioactive phytochemicals and zinc oxide nanoparticles, which may interact with glucose molecules or modify membrane permeability.[4]

The glucose diffusion inhibition assay further supported the antidiabetic potential of the biosynthesized nanoparticles.

3.8 Evaluation of Antioxidant Activity

DPPH Radical Scavenging Activity

The antioxidant activity of the biosynthesized zinc oxide nanoparticles was evaluated using the DPPH free radical scavenging assay. The nanoparticles exhibited considerable free radical scavenging activity, which increased with increasing concentration, indicating a dose-dependent antioxidant response.

At lower concentrations, moderate radical scavenging activity was observed, whereas higher concentrations exhibited substantially greater inhibition of DPPH radicals. The enhanced antioxidant activity is attributed to the presence of phenolic compounds, flavonoids, and other phytochemicals adsorbed onto the surface of the ZnO nanoparticles during green synthesis. These phytochemicals are capable of donating hydrogen atoms or electrons to neutralize free radicals, thereby reducing oxidative stress.[7]

Although the standard antioxidant demonstrated comparatively higher scavenging activity, the biosynthesized nanoparticles exhibited significant antioxidant potential, supporting their possible application in the management of oxidative stress-associated disorders.

4. Discussion

The present study has successfully demonstrated the green synthesis of Zinc oxide nanoparticles (ZnO NPs) using the root extract of *Clitoria ternatea*. Phytochemical screening was done, and flavonoids, phenolics, alkaloids, tannins, saponins, terpenoids, and glycosides were detected, with the methanolic extract having a relatively higher phytochemical content. The formation and characterisation of nanoparticles were confirmed by UV–Visible, FTIR, XRD, and SEM analyses. The synthesized nanoparticles showed good antidiabetic activity by inhibiting alpha-amylase and retarding the diffusion of glucose. Moreover, the DPPH assays showed promising antioxidant activity, especially for the methanol-mediated ZnO NPs. These results suggest their potential to be further explored in plant-based nanotherapeutic strategies for diabetes management.

5. Conclusion

In the present study, the methanolic, ethanolic, and aqueous root extracts of *Clitoria ternatea* have been used to successfully synthesize ZnO NPs via a green route. Based on the phytochemical screening, flavonoids, phenolics, alkaloids, tannins, saponins, and glycosides were found in the plant, with the methanolic extract having comparatively higher phytochemical content and bioactivity. The synthesized nanoparticles were confirmed to be formed, crystalline, and nano-sized by UV–Visible, FTIR, XRD, and SEM analysis. Moreover, DPPH radical scavenging and alpha-amylase inhibition assays showed good antioxidant and antidiabetic activities, especially for methanol-mediated ZnO NPs. In conclusion, the results suggest the potential of the roots of *Clitoria ternatea* for eco-friendly synthesis of ZnO nanoparticles and subsequent formulation of antidiabetic agents using plants.

6. Future Scope

The present study offers ample scope for future research on the application of nanotechnology using medicinal plants for antidiabetic drugs. In vivo experimental studies may be continued to check the

pharmacological efficacy and toxicity profile, as well as the safety of ZnO NPs prepared by roots in animal models. Experimental work can also be conducted to determine the therapeutic potential of engineered nanoparticles in diabetic patients.

To enhance understanding of the interactions of ZnO NPs with the metabolic pathways involved in diabetes, advanced molecular studies such as docking analysis, gene expression analysis, and mechanistic studies can be applied. Further studies could also concentrate on the formulation of controlled-release and targeted therapeutic applications of the *Clitoria ternatea* plant root-mediated nanoparticles, as well as on nano-drug delivery systems. Further large-scale synthesis and stability measurements could aid the development of industrial and pharmaceutical applications of these environmentally friendly nanoparticles.

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