Green Synthesis and Characterization of Zinc Oxide Nanoparticles from the Leaf Extract of *Pongamia Pinnata*

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**ABSTRACT**
The present work describes the synthesis of zinc nanoparticles using Pongamia pinnata leaf extract. The preparation of zinc nanoparticles by using pongamia pinnata leaf extract has desired quality with low cost and conventional method. The pongamia pinnata leaf extract was mixed with zinc nitrate hexahydrate solution by stirring and heating 60°C-80°C and the reduction reaction was studied by observing the colour change. The preliminary screening test confirms the presence of flavonoid and polyphenols. The zinc nanoparticles were characterized by UV-visible spectrometer, X-ray diffraction (XRD), Fourier Transform infrared (FTIR). The XRD exhibit the Crystalline in nature. FTIR confirms the presence of functional groups of stabilizer pongamia pinnata in capping the zinc nano particles. ZnO nano particles exhibits absorption peak at 370nm. Therefore, the study reveals an efficient, ecofriendly and simple method for the green synthesis of ZnO nanoparticles using green synthetic approach.

**Key words:** Green synthesis, Pongamia pinnata, zinc nanoparticles, zinc nitrate hexahydrate.

**INTRODUCTION**
Nanotechnology is one of the most dynamic fields in modern advanced materials science and engineering (1). The nanoparticles (NPs) have gained significant interest because of their very small size (1-100nm) with huge surface to volume ratio that cause both physical and chemical variations in their properties such as catalytic activity, thermal and electrical conductivity and biological properties compared to bulk of the same chemical composition (2, 3). Metal oxide nanoparticles have been receiving considerable attention for their potential applications in optoelectronics, Nano sensors, nano devices, information storage and catalysis (4). The inorganic NPs such as Silver, Gold, Copper, CuO, TiO₂ and ZnO have profound antibacterial activities. Among the inorganic NPS ZnO NPS are particularly interests because they can be prepared easily inexpensive and safe material for human beings and animals. They are extensively used in the formation of health care products (5). ZnO NPs has entered the scientific spotlight for its semiconducting properties, unique antibacterial, anti-fungal, wound healing and UV filtering properties, high catalytic and photochemical activity (6). Several methods are available for the synthesis ZnO NPs namely, wet chemical method, biosynthesis, chemical micro emulsion, hydrothermal, sol-gel, vapor phase process, microwave assisted combustion and Sonocatalysis method (7). Now a day increasing awareness of green chemistry and other biological processes have led to the development of an eco-friendly approach of the synthesis of NPs (8). The metal oxide nanoparticles synthesized by various biological systems such as plants (7), bacteria (9), fungus (10) and other similar organisms (11) have been reporters earlier. Plant extracts provide a biological synthesis route of several metallic nanoparticles, which are more eco-friendly and allows a controlled synthesis with well-defined size and shape of nanoparticles (12). Recently researchers have been discovering the possibilities of preparing nanomaterial in aqueous medium with the help of stabilizing, capping or hydrolytic agents (13).
Pongamia pinnata contains a wide range of biological active compounds such as being rich in flavonoids, terpenoids, phenols, saponins and vitamins. Pinnata was chosen because of its functional properties like anti-inflammatory, antioxidant, and anti-fungal antimicrobial (14). Furthermore, the importance of usage of natural, renewable and low cost material Pongamia pinnata could be able to produce the metal oxide nanoparticles with aqueous medium by avoiding the presence of hazardous substance and toxic solvents.

The present investigation has aimed to develop a simple green and chemical method for formation of ZnO NPs using aqueous leaf extract of Pongamia pinnata leaf act as a reducing, capping and stabilizing agent. The primary screening test, functional group, optical and structural properties was analyzed by standard characterization techniques.

MATERIALS AND METHODS
Pongamia pinnata leaves were collected form Nagapattinam, Tamil Nadu, India. Zinc nitrate hexahydrate (Zn(NO₃)₂ . 6H₂O) were purchased from Merck, India Pt. Ltd.

Preparation of Extract
The collected leaves were thoroughly washed in running water and dried at room temperature in dust free condition. Dried leaves were crushed into finest powder. 5g of the powder were boiled with 50ml of water and extracted under reflux condition at 100 °C for 5 hours. During the procedure of boiling, a light brown colored solution was formed and which was cool at room temperature, after that, the brown colored extract was filtered with whatman filter paper and stored in the refrigerator.

Synthesis of Zinc Oxide Nanoparticles
About 2g of Zinc nitrate hexahydrate crystals were mixed with 20ml of Pongamia Pinnata leaf extract under constant stirring using magnetic stirrer. After complete dissolution of the mixture, the solution was boiled at 60°-80°C by using magnetic stirrer until the formation of deep colored paste. The paste was heated in furnace at 400°C for 2 hours. The obtained light yellow colored powder was used for the further studies.

Characterization Techniques
Photochemical Screening Tests

The preliminary screening test was used to find the secondary components presence in the leaf extract according to the standard methods (15).

Test for Saponins
Fourth Test: 1ml of extract was slowly added to 2-3 ml of double distilled water. Then the mixture was shaken vigorously. Finally the formation of foam confirms the presence of saponins in the leaf extract (15, 16).

Test for Alkaloids
A 3ml of concentrated extract was taken in the test tube and 1 ml of hydrochloric acid (HCl) was added to the extract. Then the mixture was heated gently for 20 min and cool down to room temperature. Finally, the obtained mixture was filtered using filter paper. the filtrate was used following test.

Hager’s test: presence of alkaloids confirmed by the obtained yellow colour precipitate when 1ml of extract was treated with Hager’s agent.

Test for Proteins
Xanthoproteic test: 2 ml of extract was treated with few ml of nitric (HNO₃) acid changes the colour of solution in to yellow indicates presence of proteins.

Test for Flavonoids
a) Alkaline regent test: formation of intense yellow colour when the extract was treated with 10ml NaOH solution indicates the presence of flavonoids.

b) Zn test: 2 ml of extract treated with Zn dust and concentrated HCl changes the solution as red colour indicates the presence of flavonoids.

c) Lead acetate test: 2ml of leaf extract was treated with the few drops of lead acetate solution. formation of yellow colour precipitate indicates the presence of flavonoids.

Test for Phenol
Ferric chloride test: 2ml of leaf extract was treated with four drops of alcoholic ferric chloride (FeCl₃) solution. Changes in this solution as bluish black confirms the presence of phenolic compounds.

Test for Cardial Glycosides
Keller killani test:2ml of leaf extract was treated with 2ml of glacial acetic acid containing the drop of FeCl₃ a brown coloured formation indicates the presence of cardial glycosides.

Test for Carbohydrate
1 ml of leaf extract was dissolved gradually in 5 ml of double distilled water and filtered. This filtrate was used for the following test.
Benedict's test: 2ml of filtrate was treated with Benedict's reagent and heated gently. Formation of orange red precipitate indicates the presence of carbohydrate.

**Test for Steroids**
2ml of leaf extract was taken in at test tube and dissolved with 10ml of chloroform equal volume of concentrated H₂SO₄ acid was added to the mixture through the side wall of the test tube. Steroid was confirmed by the changes in the upper layer of the solution as red and H₂SO₄ acid layer as yellow with green fluorescence.

**FTIR Analysis**
The Fourier transform infrared (FTIR) investigation is carried out using PERKIN ELMER Spectrometer in the range 400-4000cm⁻¹. The functional group were identified using the peak assignments.

**UV-Visible Spectroscopy Analysis**
UV Spectrometer was carried out using PERKIN ELMER (Lambda 35 model) spectrometer in the range 190nm to 1100nm.

**XRD Analysis**
X-ray diffraction was carried out using XPERT PRO diffractometer (40kV, 30mA) with Cu Kα radiation (λ = 1.54060Å) at 20 angle.

**RESULT AND DISCUSSION**

**Photochemical Screening Analysis**
Pongamia piñata plant extract is used as potential substitute and reducing agent due to the combination of its bio-components such as alkaloids, polyphenolics, proteins, and polysaccharides (17). As shown in table, phenols and flavonoids are the major chemical constituents of the essential extracts obtained from pongamia pinnata leaf extract. Many reports have specified that phenols and flavonoids are involved in the bio reduction, formation and stabilization of metal and metal oxide nanoparticles (18, 19) presence of OH groups in phenol and flavonoids are the responsible for reducing zinc nitrate into ZnO NPs. In the present study, phenols and flavonoids in the aqueous leaf extract bind the surface of Zinc in Zinc nitrate to activate the formation of ZnO NPs and also control the size. The OH groups from the phenol and flavonoids compounds can act as a capping agent (20). Phenols and flavonoids are secondary metabolites that are almost present in all medicinal plant have been reported to serve as a bio reductant of metallic ions in aqueous medium and exhibit a wide range of biological activities including antioxidant and anti-carcinogenic activity (21).

| S.No | Test                                           | Result  |
|------|-----------------------------------------------|---------|
| 1    | Saponins- forth test                          | Negative|
| 2    | Alkaloids- Hager’s test                       | Positive|
| 3    | Protein-Xanthoproteic test                    | Negative|
| 4    | Flavonoids-                                   | Positive|
| 5    | Alkaline reagent test                         | Positive|
| 6    | Zn test                                       | Positive|
| 7    | Lead acetate test                             | Positive|
| 8    | Cardialglycosides- keller-killani test        | Positive|
| 9    | Carbohydrate- benedict’s test                 | Positive|
| 10   | Terpenoids-salkowski test                     | Positive|
| 11   | Phenol- ferric chloride test                  | Positive|
| 12   | Steroids                                      | Positive|

**UV Spectroscopy**
The optical property of synthesized ZnONPs was studied by UV-Visible spectroscopy and shown in fig.1. The absorption peak observed at 370 nm confirms the formation ZnONPs by bio synthesis route (22). The peak 226 nm corresponds to the extract of Pongamia pinnata. The strong absorption of the ZnO particles in the UV region proves the applicability of this product in various medical applications such as sun-screen protectors or as antiseptic in ointments (23).
**FTIR analysis**

The FTIR spectrum was recorded for the determination of possible functional groups which leads to formation of ZnO NPs. The broad stretch at 3436 cm\(^{-1}\) and 3418 cm\(^{-1}\) shows the presence of O-H stretch and hydrogen bonded groups in alcohol or phenolic or water molecules in extract. The peak at 1384 cm\(^{-1}\) indicates the asymmetric stretching vibration of nitrate ions. The absorption peaks at 1634 cm\(^{-1}\) and 1631 cm\(^{-1}\) indicates the stretching vibration of C=O hydroxyl or carboxyl groups on the surface of the sample. The vibrations peak between 400 cm\(^{-1}\)-600 cm\(^{-1}\) regions denoted by metal oxides. The peak present in the range 2426 cm\(^{-1}\) indicates the free carbonyl group. The stabilization and capping agent of synthesized ZnONPs may be due to the coordination of ZnONPs with OH and C=O groups.

![Figure 1: UV-Vis Spectrum of (PLE) leaf extract and (PZN) ZnO nanoparticles.](image1)

![Figure 2: FTIR Spectrum of (PLE) leaf extract and (PZN) ZnO nanoparticles](image2)
XRD Analysis
The XRD pattern of green synthesized ZnONPs and it confirms the hexagonal structure. From the XRD pattern the noticeable reflection planes are 110, 002,101,102,110,103,200,112,201,202 and corresponding diffraction angle 31.84°, 34.26°, 36.25°, 47.48°, 56.47°, 62.80°, 66.35°, 67.71°, 69.10°, 76.83°. The obtained peaks matches with the JCPDS No: 36-1451 confirms the ZnO hexagonal phase without addition of any impurities (24). The sharp and narrow diffraction peaks are indicates the pure crystalline nature of the sample. The diffraction peak maximum observed at the 101 and the crystalline size was calculated by using Scherer formula.

\[ D = \frac{0.94\lambda}{\beta \cos \theta} \]

Where, D is the crystalline size, \( \lambda \) is the X-Ray wavelength, \( \beta \) is the full width half maximum of the peak, the average crystalline size was of the sample was found to be 45nm.

![Position [°2Theta] (Copper (Cu))](image)

**Figure 3: Powder XRD Pattern of ZnO Nanoparticles.**

CONCLUSION
This methods are easily available starting materials are inexpensive and procedure is easy to carry out in any laboratory, use of toxic reagent is avoided and pollution free, here we report eco-friendly synthesis of ZnO NPs using Pongamia piñata leaf extract. The preliminary screening test confirms the presence of flavonoids and phenols. The formation of ZnONPs was indicated by the observation of color change and also it was revealed by a peak observed at 370 nm in UV-Vis spectroscopy. In FTIR confirm the presence of OH and C=O stretching. The XRD analysis confirmed that the obtained NPs were Crystalline and Hexagonal structure.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interests regarding the study or this article.
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