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# Punica Granatum And Elettaria Cardamomum Mediated Zinc Oxide Nanoparticles and Its Characterization Using Ultraviolet Visible Spectroscopy and Transmission Electron Microscopy.

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**ABSTRACT**
**Background:**
Nanoparticles are particles with dimension in the range of 1-100nm. They have gained great importance in the field of medical research because of their moldable physical, chemical and biological properties. They have been developed using various chemical methods, but these methods involve toxic and hazardous chemicals which cause biological risk. Hence there has been a shift towards utilizing biological methods to synthesize these nanoparticles.
**Aim:**
The aim of the study was to prepare zinc oxide nanoparticles using *Punica granatum* and *Elettaria cardamomum* and characterize it using ultraviolet visible spectroscopy and transmission electron microscopy.
**Materials and methods:**
The methodology involved collection and preparation of plant extract, followed by synthesis of zinc oxide nanoparticles. Characterization of nanoparticles using UV spectroscopy and Transmission electron microscopy.
**Results:**
The result of the current study has shown that Zinc oxide nanoparticles were formed in clumps. Majority of them were spherical in shape and few different shapes were also seen. The size of the nanoparticles ranged from 2 to 100 nm
**Conclusion:**
The current study is the first of its kind to have used the combined reducing property of *Punica granatum* and *Elettaria cardamomum* to synthesize zinc oxide nanoparticles. The nanoparticles thus formed were stable and can be used for further studies.

**Keywords**: Green synthesis, cardamom, pomegranate, zinc oxide nanoparticles.

## Introduction:

Nanoparticles are particles with dimension in the range of 1-100nm. They have gained great importance in the field of medical research because of their moldable physical, chemical and biological properties which enhance their performance over the bulk counterparts(1). The various types of nanoparticles are carbon based nanoparticles, metal nanoparticles, ceramic nanoparticles, polymeric nanoparticles, semiconductor nanoparticles and lipid based nanoparticles(2). Zinc oxide, a metal nanoparticle has been widely used in various studies because of their large bandwidth and high exciton binding energy. It has potential applications like antibacterial, antifungal, anti-diabetic, anti-inflammatory, wound healing, antioxidant and optic properties(3). They have been developed using various chemical methods like sol–gel, hydrothermal, spray pyrolysis, microwave-assisted techniques, chemical vapor deposition, ultrasonic condition and precipitation methods(4). But these methods involve toxic and hazardous chemicals which cause biological risk, hence there has been a shift towards utilizing biological methods to synthesize these nanoparticles.

Various plants, fungus, bacteria and algae have been employed for the synthesis of zinc oxide nanoparticles (3). But, plants are the preferred biological source as they help in the production of stable nanoparticles of varying size and shape(5). The phytochemicals present in plants help in reducing the metal ions and metal oxides to metal nanoparticles(6). Plant parts like roots, leaves, stem, seeds and fruit have been utilized. In the current study a combination of extract from the fruit of *Punica granatum* and seeds of *Elettaria cardamomum* was used to prepare zinc

oxide nanoparticles, which were then characterized using ultraviolet visible spectroscopy and transmission electron microscopy.
The study was carried out to prepare zinc oxide nanoparticles using *Punica granatum* and *Elettaria cardamomum* and characterize it using ultraviolet visible spectroscopy and transmission electron microscopy.

**Materials and Methods:**

**Collection and Preparation of Plant Extract:**

Fruit of *Punica granatum* and seeds of *Elettaria cardamomum* were collected from Chennai. 50gms of the collected fruit pods from *Punica granatum* were washed 3–4 times using distilled water, then crushed using mortar and pestle. The crushed mixture was added to 100ml of distilled water and boiled for 5–10 min at 60–70°C (Figure 1). The solution was filtered using Whatman No.1 filter paper. The filtered extract was collected and stored in 4° for further use. 2gms of the seeds of *Elettaria cardamomum* was washed 3–4 times using distilled water and dried in shade for 7–14 days. The well-dried seeds were made into a powder using mortar and pestle. The powder was dissolved in 100ml of distilled water and boiled for 5–10 mins at 60–70°C (Figure 1). The solution was also filtered using Whatman No.1 filter paper. Both the filtered extracts were mixed together and then boiled for 5 – 10 mins at 60–70°C and stored in 4° for further use.

**Synthesis of ZnO NPs:**

0.861g of zinc sulfate is dissolved in 50 ml of double-distilled water. 50ml of the plant extracts of *Punica granatum* and *Elettaria cardamomum* were added with the metal solution and made into 100 ml solution. The color change was observed visually and photographed every 2 hours. The solution was placed in a magnetic stirrer for NPs synthesis for 72 hours (Figure 2).

**Characterization of ZnO NPs using ultraviolet visible spectroscopy**

The synthesized NPs solution is preliminarily characterized using ultraviolet (UV)-visible spectroscopy. Three milliliter of the solution are taken in the cuvette and scanned in double-beam UV-visible spectrophotometer from 300 nm to 700 nm wavelength. The results were recorded for the graphical analysis (Figure 3).

**Preparation of NPs Powder for Transmission Electron Microscopy**

The NPs solution is centrifuged using Lark refrigerated centrifuge. The ZnO NPs solution is centrifuged at 8000 rpm for 10 min and the pellet is collected and washed with distilled water twice. The final purified pellet is collected and dried at 100–150°C for 2/24 h, and finally, the NPs powder is collected and stored in an airtight Eppendorf tube (Figure 4). The shape and size distributions of synthesized ZnO nanoparticles were characterized using transmission electron microscope (TEM, TECNAI). The analysis was performed by sonication the liquid sample of ZnO NPs for 10 min using citizen digital ultrasonic bath before placing it on the carbon-coated copper grid.

**Result and Discussion:**

The result of the current study has shown that Zinc oxide nanoparticles were formed in clumps. Majority of them were spherical in shape and few different shapes were also seen. The size of the nanoparticles ranged from 2 to 100 nm (Figure 5).
Siti Nur amalina et al in their study biosynthesized zinc oxide nanoparticles using the fruit peel of *Punica granatum* and found that they were mostly spherical and hexagonal in shape with size ranging from 32.98 nm to 81.84 nm(7). *Punica granatum* contains an abundance of phytochemical compounds that play an important role as reducing and stabilizing agent for the successful yield of zinc oxide nanoparticles(8) Punicalagin and gallic acid are the main contributors towards reducing zinc ions in the aqueous solution to stable zinc atoms and the formation of nanoparticles(9)
Biosynthesis of zinc oxide nanoparticles have also been carried out using *Elettaria cardamomum.* In a study by Shinjini Pal et al it was observed that the synthesized nanoparticles were rod shaped and 177+/-2.3 in size(10). The ability to form nanoparticles by green cardamom is owed to the high amount of flavonoids which have great reducing capacity.(11)
Fruit mediated zinc oxide nanoparticles have been prepared using the peel of other fruits like tomato, orange, grapefruit, lemon etc (12). The morphology of these bio inspired zinc oxide nanoparticles was predominantly found to be polyhedral in shape. The formation of these nanoparticles is attributed to the polyphenols present in the peel of the fruits(13). Other studies have also found that the fruit of lemon has a good reducing ability, which helps in the formation of zinc oxide nanoparticles. The nanoparticles thus formed were 20-200nm in size with varied widely in shape from cuboid, hexagonal prism, thin rods, near-spheroid, and irregular shapes(14)
The nanoparticles thus formed is aided by citric acid, which is known for it potent reducing property.
The fruit of tomato was also used to synthesis zinc oxide nanoparticles. The fruit was found to contain terpenes, flavonoids, saponins, tannins, glycosides and alkaloids. These substances had great reducing and stabilizing potential and hence enabled the formation of zinc oxide nanoparticles form zinc nitrate(15) .Thus numerous studies are currently

being undertaken to synthesis nanoparticles using green methods inorder to counteract the negative effects of chemical methods. The current study was the first of its kind to use *Punica granatum* and *Elettaria cardamomum* to synthesize the nanoparticles. The reducing property of both the fruits was exploited inorder to prepare the nanoparticles.
Further studies can be carried out to find the antioxidant, antibacterial, antifungal and anti -inflammatory activity of these green synthesized zinc oxide nanoparticles.

**Conclusion:**
The current study is the first of its kind to have used the combined reducing property of *Punica granatum* and *Elettaria cardamomum* to synthesize zinc oxide nanoparticles. The nanoparticles thus formed were stable and can be used for further studies.

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**Figure 1: Figure showing the preparation of pomegranate extract from the crushed fruit pods of *Punica granatum* and cardamom extract from the crushed seeds of *Elettaria cardamomum***

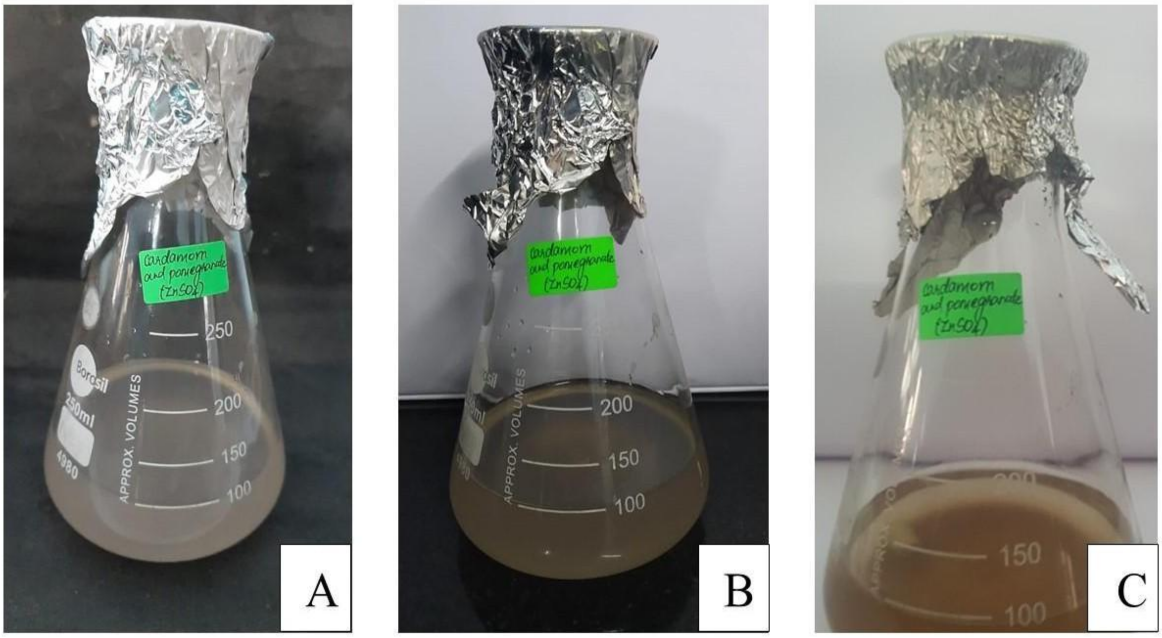


**Figure 2: Figure showing colour change observed over a period of 72 hours. A) Pale pink colour at 24 hours B) Mild colour change recorded after 48 hours C) Brown colour observed after 72 hours which correlated with the peak in the UV spectroscopy, indicating the formation of Nano particles.**

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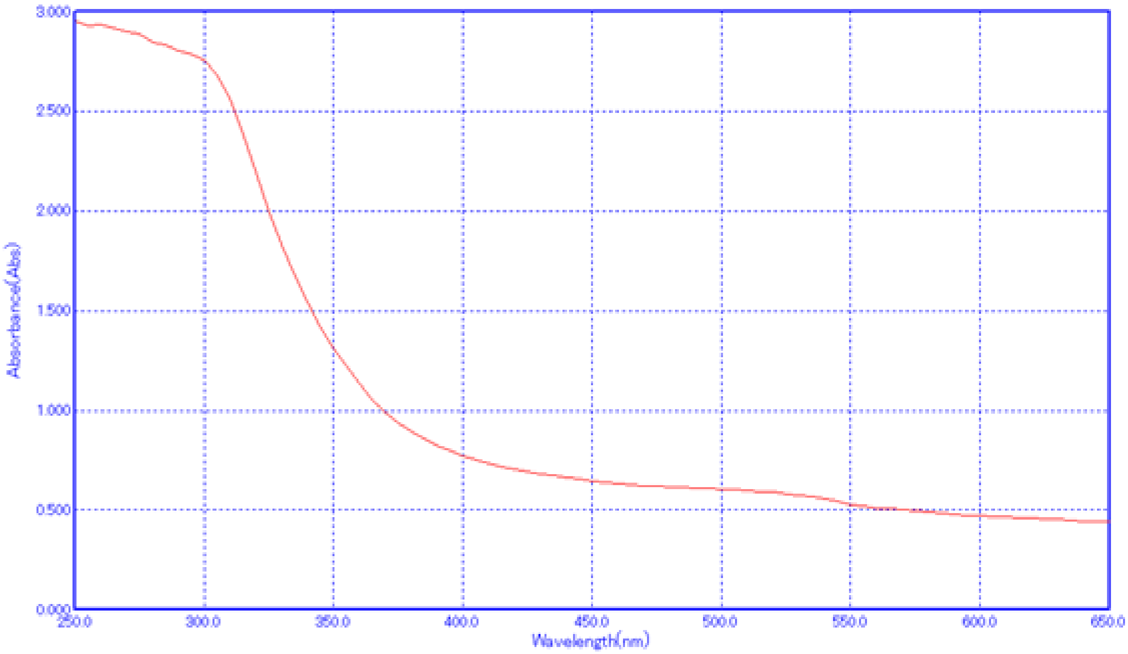

**Figure 3: Graph indicating the peak in UV spectroscopy observed after 72 hours, which indicates the formation of zinc oxide nanoparticles**

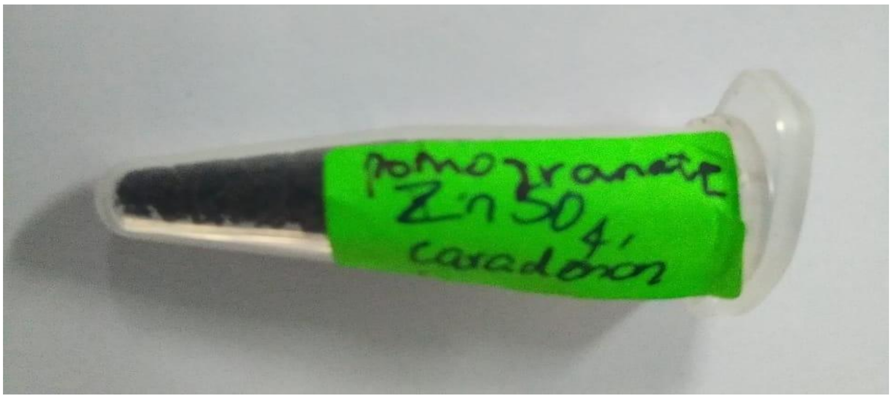

**Figure 4: Pellet collected in an Eppendorf after zinc oxide nanoparticles solution is centrifuged and then dried at 100–150°C for 24 hrs. The dried pellet is used for characterization using transmission electron microscopy.**

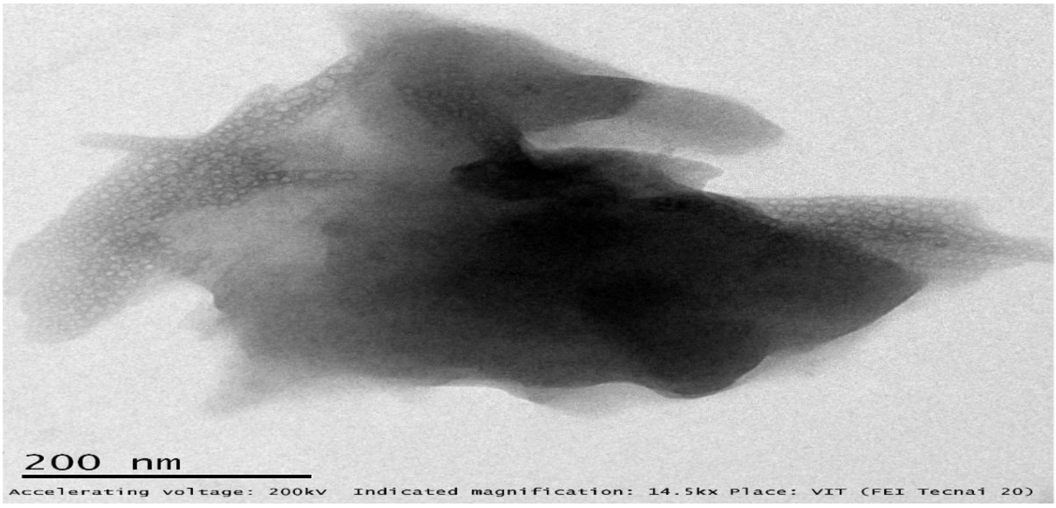

**Figure 5: Transmission electron microscopic image of Zinc oxide nanoparticles formed using *Punica granatum* and *Elettaria cardamomum***