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Green Synthesized Zinc Oxide Nanoparticles from *Elettaria cardamomum* and its Characterization Image Processing and Antiproliferative Activity

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Abstract: Breast cancer is the second commonest cancer with increasing incidence in the world. One of the most important metal oxide nanoparticles, Zinc oxide nanoparticles are employed in various fields due to their specific physical and chemical properties. The present study investigated the anticancer activities of zinc oxide nanoparticles synthesized by using *Elettaria cardamomum* (seed pod). *Elettaria cardamomum* commonly known as Cardamom is a perennial herb belonging to the ginger family, Zingiberaceae. The synthesized nanoparticle was characterized using UV-VIS, XRD and SEM analysis. The green synthesized zinc oxide nanoparticles were tested for its anticancer potential against MCF7 cell lines.

Keywords: Zinc oxide nanoparticles, Breast cancer, MCF7, *Elettaria cardamomum*

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Introduction

Cancer, a condition of uncontrolled cell differentiation, has usually been treated by chemotherapy, radiation and surgery during the past several decades (Smalley and Herlyn, 2006). These therapies are certainly efficacious in the destruction of cancer cells, but, alongside that, they come with the cost of an increasing rate of adverse consequences due to unselective effects directed towards normal cells as well. These therapies are now gradually becoming outdated in cancer treatment due to the development of nanomedicine, targeted drug delivery and multi-target inhibitors (Chavez *et al.*, 2009).

Nanomedicine, with its advanced imaging and therapeutic capabilities, has the potential for early detection of cancer and cancer treatment. The major aspects of nanomedicine comprises inorganic nanoparticles. Many inorganic nanoparticles conjugated with anti-cancerous drugs or bioactive molecules (Peptides, proteins, DNA, etc.) have already been approved by the U.S. Food and Drug Administration (FDA) and European markets (Bisht and Rayamajhi, 2016).

Inorganic nanoparticles themselves show selective cytotoxicity towards cancer cells. Inorganic nanoparticles such as iron oxide

nanoparticles, copper oxide nanoparticles, silica nanoparticles, titanium dioxide nanoparticles, cerium oxide nanoparticles, zinc oxide nanoparticles, etc., are being widely researched and used for anticancer therapy (Na *et al.*, 2009). Zinc oxide nanoparticles as one of the most important metal oxide nanoparticles, are popularly employed in various fields due to their peculiar physical and chemical properties. Zinc oxide nanoparticles are firstly applied in the rubber industry as they can provide waterproof of the rubber composite, improve performance of high polymer in their toughness and intensity and antiaging, and other functions. Because of the strong UV absorption properties of zinc oxide, they are increasingly used in personal care products, such as cosmetic and sunscreen (Xiang and Xiao, 2020).

In addition, zinc oxide nanoparticles have superior antibacterial, antimicrobial, and excellent UV blocking properties. Therefore, in the textile industry, the finished fabrics by adding zinc oxide nanoparticles, exhibited the attractive functions of ultraviolet and visible light resistance, anti-bacteria and deodorant (Jiang *et al.*, 2018). It is generally known that zinc as an essential trace elements extensively exists in all body tissues, including the brain, muscle, bone, skin, and so on. As the main component of various enzyme systems, zinc takes part in body's metabolism and plays crucial roles in proteins and nucleic acid synthesis, hematopoiesis and neurogenesis. The development of green processes for the synthesis of nanoparticles have been evolving into an important branch of nanotechnology as green nanotechnology which deals with the safe and eco-friendly methods for nanomaterials fabrication and is considered as an alternative for the conventional physical and chemical methods (Prabhakaran *et al.*, 2011). Green nanotechnology is gaining importance due to the elimination of harmful reagents and provides the effective synthesis of expected products in an economical manner. Noble metallic nanoparticles such as silver, gold and platinum are widely applied in medicinal applications (Rai *et al.*, 2015).

There is a growing need to develop an environmentally friendly process for the synthesis of nanoparticles that does not employ toxic chemicals. Thus, synthesizing nanoparticles by biological means, which has the advantages of non-toxicity, reproducibility in production, easy scaling up and well defined morphology, has become a new trend in nanoparticles production. In particular, microorganisms and plants have been used as a new resources with considerable potential for synthesizing nanoparticles (Westphal-Settele *et al.*, 2018). In the case of biological methods, nanoparticles synthesis using plants extract is the most adopted method, because it is eco-friendly, it can act as a source of several metabolites, it is much safer to handle, and easily available (Sorbiun *et al.*, 2018).

Breast cancer is a public health problem as it is the second commonest cancer with increasing incidence (1 in 8 women aged 45-55) in the world. It is the second most common cause of death after lung cancer in the west. Among women, breast cancer is the leading cause of cancer deaths and the most common cancer worldwide. Globally, 1.3 million new cases of breast cancer are diagnosed and approximately 465,000 deaths are recorded annually (Lorenzo *et al.*, 2007).

The exact cause of cancer is unidentified. Although the genetic aspects is involved in five to ten per cent of cancers, other reasons including poor diet, certain infection, lack of physical activity, obesity, the use of tobacco and pollution may also directly or indirectly influence the activity of crucial genes that could lead cancer development. In the last few years, scientists have used a new pathway for treating cancer, dependent on the concept of nanotechnology. Nanotechnology is a science based on the technique and tools from diverse disciplines, including biology, chemistry, engineering and medicine. This field could critically improve the drug bioavailability, and in turn reduce the toxicity associated with the high doses that are typically required for optimum response and ability to transport the substances on a specific organ

(Doody *et al.*, 2000).

Cardamom, belonging to the family of Zingiberaceae, is obtained from the seeds of *Elettaria cardamomum* Maton and it is mostly cultivated in southern India, Sri Lanka, Tanzania and Guatemala. The genus consists of about six species. Only *Elettaria cardamomum* Maton occurs in India and this is the only economically important species. It is highly valued from ancient times, because of its very pleasant aroma and taste and its referred as the “Queen of spices” (Sengupta and Bhattacharjee, 2009).

The major use of cardamom is culinary purpose for flavoring food. It is also used in medicine as an aromatic stimulant, carminative and flavoring agents. Cardamom was used by ancient Greek and Roman and also recommended by the Apicius, a famous Roman empire to counteract over indulgence. Cardamom is often named as the third most expensive spice in the world (after saffron and vanilla) (Halpern and Weverka, 2002).

According to Ayurveda cardamom is used against heart disease, kidney disease, urinary disease, bacterial infection, teeth infection, pulmonary tuberculosis, asthma, food poisoning, eyelid inflammation, digestive disorders, sore throat, colds, bladder disease, snake bite, scorpion bite and constipation. Keeping the above point in mind we have decided to synthesize zinc oxide nanoparticles using *Elettaria cardamomum* and also to assess its anticancer activity.

Materials and Methods

Preparation of Aqueous Seed Pod Extract:

100 g of fresh *Elettaria cardamomum* (L) Maton seed pod (Fig. 1) were washed with running tap water followed by Milli-Q water. The seed pod were subjected to air drying under the shade. After drying they were ground by an electrical mixer until they became a powder. Then the powdered sample was stored in cool place. The powdered sample 10 g was mixed with 100 ml Milli-Q water and then boiled at 70 °C for 8 min.

The extract was allowed to cool at room temperature, filtered through Whatman No. 1 filter paper, and the filtrate was stored for further experimental use (Babu and Ashok, 2021).



Fig. 1: *Elettaria cardamomum* (EC) seed pod.

Quantitative Phytochemical Analysis (Ashok and Babu, 2021):

Test for alkaloids (Mayer's Test):

To the extract, 2 ml of Mayer's reagent was added; formation of reddish brown precipitate indicates the presence of alkaloids

Test for saponins:

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

Test for tannins:

To the extract, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins

Test for cardiac glycosides (Keller-Killani test):

To 1 ml of the extracts added 2 ml of glacial acetic acid containing a drop of FeCl₃. Equal volume of conc. H₂SO₄ was added from the sides of the tube. A brown colour ring indicates the presence of cardiac glycosides.

Test for flavonoids (Alkaline reagent test):

Extract was treated with 10% NaOH solution;

formation of intense yellow colour indicates presence of flavonoids.

Test for phenols (Lead acetate test):

The extract was taken and 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds

Test for steroids:

1 ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated H_2SO_4 was added from the sides of test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroids.

Test for terpenoids (Salkowski test):

5 ml of extract was mixed with 2 ml of chloroform, and concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for Quinones:

The extract was treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of Quinones.

Test for proteins (Ninhydrin test):

The extract was taken and few drops of freshly prepared Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Biosynthesis of Zinc Oxide Nanoparticles:

15 ml extract of *Elettaria cardamomum* was added to 2.195 g of zinc acetate dihydrate dissolved in 35 ml of distilled water (overall concentration 200 mM). The reaction mixture was kept on magnetic stirrer for 6 h. After 6 h, 2 M NaOH (4 g of NaOH pellet in 50 ml of Milli-Q water) was added to the solution and it was placed in incubator at 60 C on magnetic stirrer for overnight. White mixture was centrifuged at 14000 rpm for 15 min. Precipitate was subjected to washing with alcohol and

distilled water three times each. Precipitate was dried in an incubator at 40 C and fine powder was prepared with the help of ceramic pestle and mortar (Fig. 2). Fine powder was used for characterization with SEM, XRD, UV-Vis (Meulenkamp, 1998).



Fig. 2: Synthesis of zinc oxide nanoparticles from extract of *Elettaria cardamomum* (seed pod).

Characterization of ZnO nanoparticles:

UV-Vis spectroscopy:

UV-visible Spectroscopy as an analytical tool was used to examine the optical properties of nano-sized particles. Synthesized Zinc oxide nanoparticles were scanned in UV region of the electromagnetic wave around 250-800 nm.

X-ray diffraction:

Washed and dried sample of zinc oxide nanoparticles was used for XRD analysis using X-ray powder diffractometer model D8, at the wavelength of 1.5418 angstrom within the 2 theta range of 20-80° and 1 sec/step speed of scan were maintained while operating at 35 KV.

SEM analysis:

The structural morphology of zinc oxide nanoparticles was carried out using SEM (JSM-6510 LV; JEOL, TOKYO, JAPAN). To avoid charging the mechanism during SEM measurement, the powder samples were coated by gold sputtering.

Cytotoxicity Assay:

Human breast carcinoma cell line (MCF7) were procured from NCCS. Stock cells were cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), Penicillin (100 IU/ml),

Streptomycin (100 if/ml) in an humidified atmosphere of 5% CO₂ at 37 C until confluent. The cell was dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The viability of the cells was checked and centrifuged. Further 50,000 cells/well was seeded in a 96 well plate and incubated for 24 h at 37 C, 5% CO₂ incubator. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10⁵ cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000 cells/well) was added (Ghate *et al.*, 2013).

After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentration of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37 C for 24 h in 5% CO₂ atmosphere. After incubation the test solution in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37 C in 5% CO₂ atmosphere. The supernatant was removed and 100 µl of DMSO were added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drugs needed to inhibit cell growth by 50% (IC₅₀) values is generated from the dose response curves for each cell line.

$$\% \text{ Viable cells} = \frac{\text{Total number of viable cells/ml of aliquot}}{\text{Total number of cells /ml of aliquot}} \times 100$$

Results and Discussion

Phytochemical Analysis:

The phytochemical test analysis is necessary to check the presence of compounds, such as flavonoids, polyphenols, tannins etc. Phytochemical compounds such as flavonoids, tannins, aromatic compounds or secondary metabolites act as defence mechanism against many microorganisms. The therapeutic properties of medicinal plants are perhaps due to the

presence of various secondary metabolites such as flavonoids, alkaloids, tannins, saponins and phenolic compounds (Table 1).

Characterization of Zinc Oxide Nanoparticles:

X-ray Diffraction Analysis:

Sharp diffraction peaks observed in the case of the synthesized Zinc oxide nanoparticles sample indicated a decent crystallinity of the sample and effect of environmental conditions maintained during the synthesis procedure is shown in Figure 3. These plane values are matched with the hexagonal wurtzite structure of zinc oxide.

UV- visible:

The characteristic peak of green Zinc oxide nanoparticles was observed around 350 nm which is shown in Figure 4. A high value of excitation binding energy was responsible for the obtained peak at room temperature. This result confirmed the presence of zinc oxide nanoparticles synthesized from *Elettaria Cardamomum*.

SEM Analysis:

The morphology of zinc oxide nanoparticles was studied with the help of SEM. The SEM results of these samples are shown in Figure 5 which show the distinctive and abundant flower shaped Zinc oxide nanoparticles. The observations illustrated that there were huge hexagonal arrays of zinc oxide nanoparticles assimilated to form flower shaped bundles.

Cytotoxicity Assay:

Cytotoxicity (*in vitro*) of green Zinc oxide nanoparticles was determined using the MCF7 cell lines. MTT assay was done following exposure of cells to various concentration of zinc oxide nanoparticles (3.12, 6.25, 12.5, 25, 50, 100 µg/ml) for a time span of 24 h. It was observed that Zinc oxide nanoparticles depicted a dose dependent decrease in cell survivability (Fig. 6). The IC₅₀ value of given sample (*Elettaria cardamomum*) and standard (Cisplatin) is >100 µg/ml and 3.02 µg/ml, respectively. Thus, we can conclude that

Table 1: Preliminary phytochemical analysis of the *Elettaria cardamomum* extract

S. No.	Secondary metabolites	Aqueous extract
1	Carbohydrates	+
2	Tannins	+
3	Saponin	+
4	Flavonoids	+
5	Alkaloids	+
6	Quinones	-
7	Glycosides	-
8	Terpenoids	-
9	Triterpenoids	-
10	Phenols	+
11	Coumarins	-
12	Acids	-
13	Proteins	-
14	Anthocyanin	-
15	Cardiac glycosides	-
16	Steroids	-

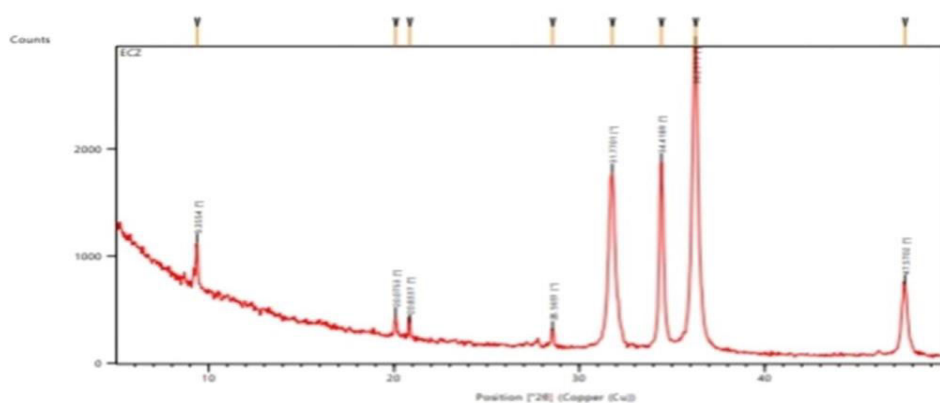


Fig 3: XRD of green synthesized Zinc oxide nanoparticles.

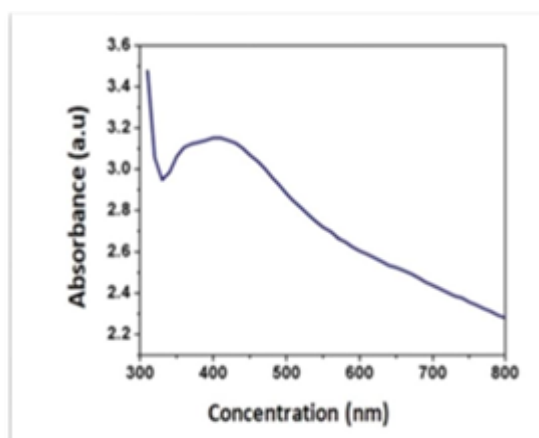


Fig. 4: Graphical representation of spectral pattern of green synthesized Zinc oxide nanoparticles using the ultra-violet range.

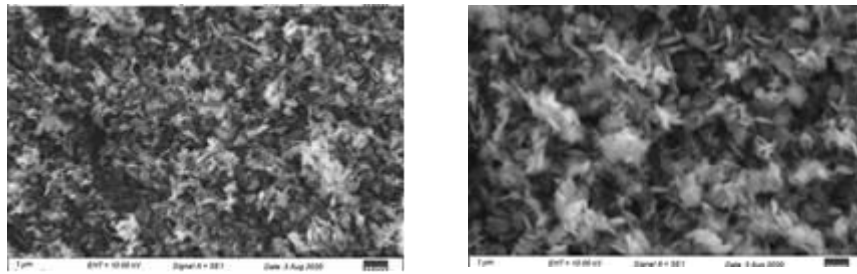


Fig. 5: SEM image of zinc oxide nanoparticles.

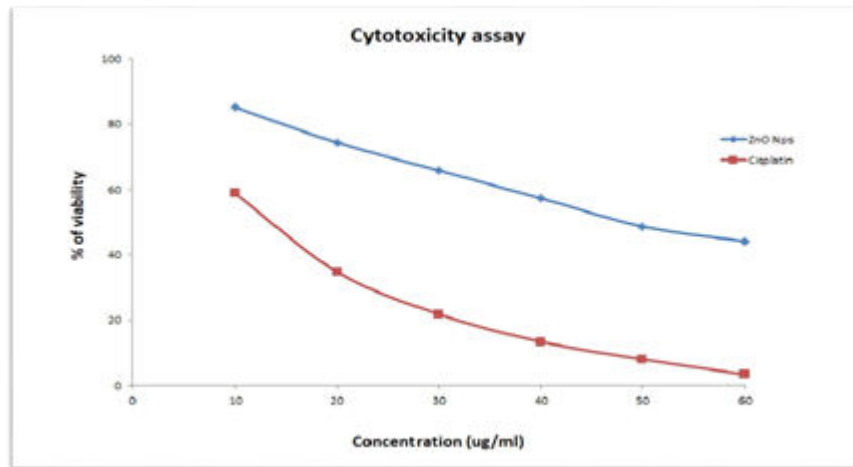


Fig. 6: Survivability of MCF7 cell lines after exposure to green zinc oxide nanoparticles.

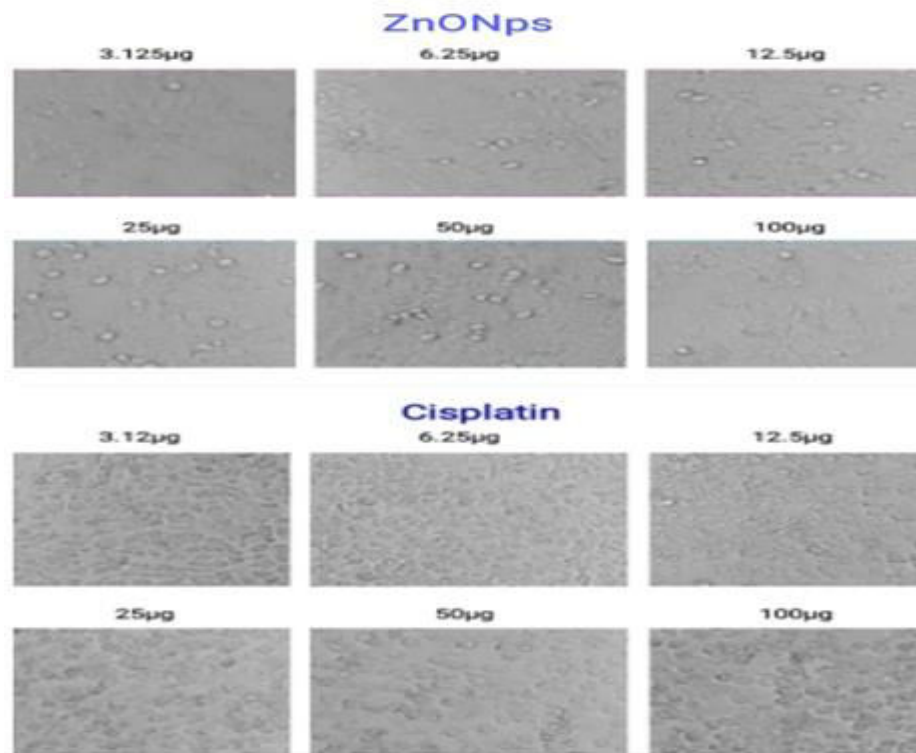


Fig. 7: Treatment with ZnONPs induce apoptosis in MCF-7 cell lines.

the synthesized Zinc oxide nanoparticles could successfully induced apoptosis that in turn resulted in the death. The *in vitro* biosynthesized zinc oxide nanoparticles was studied in MCF-7 cell lines. The 3.12 µg concentration showed 85.165702% viability. The 50 µg concentration was more effective compared to 3.12 µg, 6.25 µg, 12.5 µg and 25 µg. The concentration dependent cell viability is shown in Figure 7. It was found that its toxicity strongly depended on the concentration of green synthesized *Elettaria cardamomum* (seed pod) extract.

Conclusion

The green synthesis of zinc oxide nanoparticles using *Elettaria cardamomum* (seed pod) extract provides an effective route for eco-friendly methods of synthesis of nanoparticles. This synthesis procedure is cost-effective, less tedious, non-hazardous and eco-friendly. The formation of zinc oxide nanoparticles was confirmed by UV-vis, XRD and SEM analysis. The phytochemicals which have been identified in the plant extract would have involved in the synthesis of ZnONPs. The synthesized NPs were studied for its cytotoxic activity against breast cancer MCF7 cells. Within the limits of the study, we can say that *Elettaria Cardamomum* mediated Zinc oxide nanoparticles could be developed which has a therapeutic activity against breast cancer.

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