



## **Cassia oleoresin Mediated Synthesis of Magnesium Oxide Nanoparticles and Brine Shrimp Lethality Assay**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author GA has managed the literature searches and wrote the manuscript. Author SR has designed the study, wrote the protocol and authors AR and DE managed the analyses of the study. All authors read and approved the final manuscript.*

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### **ABSTRACT**

*Cassia oleoresin* is an extract isolated from dried barks of *Cinnamomum cassia* Blume (family Lauracea). The plant has been reported to have anti-diabetic, anti-oxidant, anti-hypertriglyceridemic effect, mainly due to its phytochemical constituents such as phenolic and volatile compounds. Cinnamon also helps in arthritis, fibromyalgia and psoriasis. The aim of this study was to prepare magnesium oxide nanoparticles using *Cassia oleoresin* and to evaluate the cytotoxic effect on Brine shrimp. The magnesium oxide nanoparticle was prepared from magnesium chloride and *Cassia oleoresin* and was confirmed by UV- Visible Spectroscopy and morphology was confirmed by TEM. Brine shrimps lethality bioassay was carried out to investigate the cytotoxicity of *Cassia oleoresin* mediated magnesium oxide nanoparticles. Ten brine shrimp nauplii were placed in each well of the Eliza plate and filled with 5  $\mu$ L, 10  $\mu$ L, 15  $\mu$ L, 20  $\mu$ L, 25  $\mu$ L of *Cassia oleoresin* mediated magnesium oxide nanoparticles. After 24 hours of incubation, the wells were observed and the

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number of surviving brine shrimp nauplii were counted to assess the cytotoxicity. The UV -Visible spectroscopy showed a peak at 400 nm and TEM analysis showed a particle size of 70 nm. After 24 hours incubation of the brine shrimps in the nanoparticle solution, all 10 brine shrimps survived in 5 $\mu$ L and 10  $\mu$ L concentrations. 3 brine shrimps nauplii survived in 15 $\mu$ L conc. 1 brine shrimp nauplii survived in 20 $\mu$ L and 25 $\mu$ L concentrations each. Within the limits of this study it can be concluded that at low concentrations the prepared nanoparticle was safe and may be used for biomedical application.

**Keywords:** *Magnesium nanoparticles; cinnamon bark; cassia oleoresin; brine shrimps; cytotoxicity; nanotoxicology.*

## 1. INTRODUCTION

Nanobiotechnology, a branch of nanoscience has been playing a decisive role in the 21st century in deciphering diverse tribulations particularly in the fields of medicine and electronics and farming. Nanoparticles are a special group of materials with a broad range of applications. Nanoscience poses a basic scientific challenge as it requires a control over the connections between atoms. All physiochemical methods of nanoparticle synthesis have inherent limitations to a certain level which impose an important obstacle in the maturation of this science. The possibility of using biological materials for nanoparticle synthesis has appeared as the most efficient, easier and environment friendly approach. Even though chemical synthesis with vitamin C, physical synthesis with UV and ionizing radiation, are easy and efficient, stability is easy to achieve with nanotechnology using organic compounds [1]. Nanomaterials have shown unique and considerably changed physical, chemical, and biological properties compared to their bulk counterparts. Although physical and chemical methods are more popular for nanoparticle synthesis, the use of toxic compounds limits their use. Indeed, over the past many years, plants, algae, fungi, bacteria, and viruses have been used for production of metal nanoparticles [2].

Recently plant and plant materials have become potential sources for the synthesis of metallic nanoparticles. Green synthesis of metallic nanoparticles from plants has been an interesting aspect as the process is eco-friendly and non-toxic [3]. A number of researchers have reported on synthesis of metallic nanoparticles including silver, gold, titanium dioxide tungsten oxide, and copper oxide using different plant materials [4]. Due to the amenability to biological functionalization, the biological nanoparticles are finding important applications in the field of medicine [5]. The bactericidal potential of metal

based nanoparticles has led to its incorporation in cosmetics, health-related and industrial products [6]. Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years [7]. The discovery, development and clinical use of antimicrobials during the 20th century have substantially reduced mortality from bacterial, fungal, viral and parasitic infections. An antimicrobial kills the microorganisms or inhibits their growth. Zinc oxide nanoparticles were explored for their antimicrobial activity [8]. The use of higher plants and their preparation to treat infectious and non-infectious disease is an age old practice and was the only method available in the past [9]. Though natural sources such as plant phytochemicals and extracts have been used to cure many forms of ailments for many centuries, the scientific basis of different natural sources for their possible medicinal property is a recent origin [10]. The increase of antibiotic resistance microorganisms triggered the search of new materials through diverse sources including investigations with plants. Higher plants can serve as both potential antimicrobial crude drugs and a source of new anti-infective agents.

Cinnamon (*Cinnamomum cassia* Blume) is a member of the *Lauraceae* family and it is one of the most important spices and traditional herbal medicine used [11,12]. It is distributed widely in China, Madagascar, India, Srilanka, Seychelles, Vietnam and Malaysia, *Cinnamomum cassia* oil exhibits different properties such as antioxidant, anti-allergy, anticancer and antibacterial [13]. The bark of *C. cassia* is a major source of commercial raw materials based on its high essential oils and trans-cinnamaldehyde content [14] and it has been observed to contain highly desirable pharmaceutical properties. One method which has the potential to be used for evaluating the cytotoxicity of extract for potential pharmaceutical application is brine shrimp toxicity test [15,16]. This assay was initially designed to check the lethality of laboratory

invertebrate model, *Artemia salina* but it was later perceived to be an easy, fast and cost effective method to determine the acute toxicity of essential oils. Few researches have been conducted to explore this technique for evaluating cytotoxicity of different plants and spices [17].

Among the various inorganic metal oxides, magnesium oxide nanoparticles (MgO NPs) are with the advantages of being nontoxic and relatively easy to obtain. Magnesium oxide nanoparticles have been known as one of the most safe materials by the United States Food and Drug Administration [18,19]. Recent advances have led to developments with enormous potential in materials and medicines; for example, magnesium oxide nanoparticles can relieve heartburn, initiate post-activation of bone repair scaffolds and act as hyperthermia agents in cancer therapy. Recently, it was specifically found that MgO NPs have widely pronounced antibacterial activities against *Staphylococcus aureus* in culture media [20]. Previous studies demonstrated that the peptide linkages in *Pseudomonas aeruginosa* and *Escherichia coli* cell membrane were destroyed by the generation of ions on the surface of magnesium oxide nanoparticles. They can distort and damage the cell membranes of *E. coli*, resulting in the leakage of their intracellular content and eventually death. However, little is known about the antimicrobial properties of MgO NPs toward plant pathogenic bacteria and their ability to control plant disease are not yet clear. [18] Given the prospect of employing nanomaterial in agriculture, it is important to mention here that the increasing concern regarding the toxicity of MgO NPs to environmental systems cannot be ignored [21]. This study was done to assess the cytotoxicity of magnesium oxide nanoparticles prepared from *cassia oleoresin* against brine shrimps.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Magnesium Oxide Nanoparticles

99.5 mL of distilled water was taken in a conical flask, 2.0 g of Magnesium Chloride was added and mixed thoroughly. In a separate jar, 10 mL of distilled water was mixed with 500  $\mu$ L of *cassia oleoresin*. This was then mixed with the 50 m of MgCl solution and kept in the shaker until magnesium oxide nanoparticles were formed (Fig. 1A). After the formation of MgO

nanoparticles, the solution was centrifuged to collect the nanoparticles and stored in airtight container for further study.

### 2.2 Characterization of Magnesium Oxide Nanoparticles

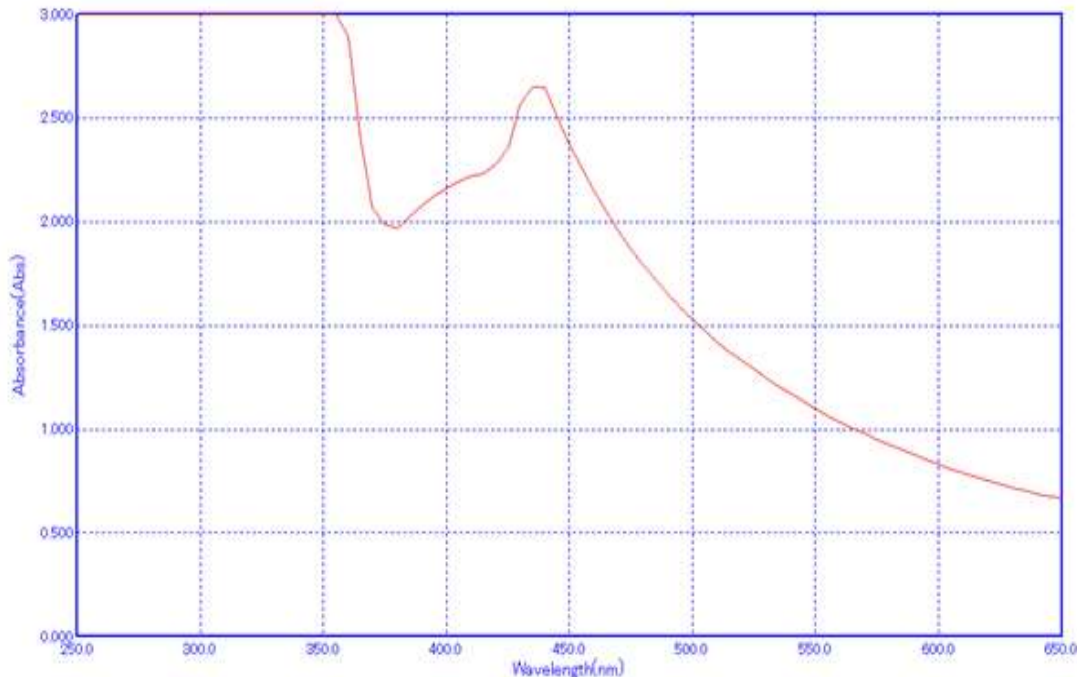
The absorbance was observed every 2 hours by UV- Visible spectroscopy (Fig. 1B) to confirm the formation of MgO NPs formed from *Cassia oleoresin* by scanning it between 250-600nm Transmission Electron Microscopy (TEM) images of magnesium oxide nanoparticles was taken by suspending in absolute alcohol (Fig. 2) to identify the morphological characteristics of the nanoparticles. TEM Copper grid was used for the study.

### 2.3 Brine Shrimps Lethality Assay

Brine shrimps were cultured in the laboratory. Artificial seawater was prepared by dissolving 36 g of sea salt in 1Litre of distilled water to be used for hatching. The seawater was put in a small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Brine shrimps were added into the dark side of the chamber while the lamp above the other side (regular light) will attract the hatched shrimp. Two days were allowed for the eggs to hatch and mature as brine shrimps (nauplii). After two days ten brine shrimps were placed in vials containing 5  $\mu$ L, 10  $\mu$ L, 15  $\mu$ L, 20  $\mu$ L, 25  $\mu$ L of magnesium oxide nanoparticle solution. One vial with 10 brine shrimps was kept as control and nanoparticles were not added. The vials were left uncovered under the lamp. The number of alive brine shrimp nauplii after 24hrs were counted manually by observing under dissection microscope and results were noted.

## 3. RESULTS AND DISCUSSION

The size, shape and interaction between the particles contribute to the optical properties of the nanoparticle. The absorption spectrum showed a peak at 400 nm. (Fig. 1B). The UV-Visible spectroscopy result show the bio-reduction property of *Cassia oleoresin* by the formation of magnesium oxide nanoparticles. TEM can give 2 –dimensional images to understand the morphology of the particle. In the present study, it showed spherical shape, dispersion and versatile nanoparticles. They appear to be arranged in a cluster, open and quasi-linear superstructures. They were of 70 nm in size (Fig. 2).



**Fig. 1A.** Image showing UV-Visible - spectroscopy results of Magnesium oxide nanoparticles prepared from *Cassia oleoresin*



**Fig. 1B.** Image shows preparation of magnesium oxide nanoparticles from magnesium chloride crystals using *Cassia oleoresin*

Results of brine shrimp lethality bioassay were estimated using mortality data. The study revealed that *Cassia Oleoresin* mediated magnesium nanoparticles have maximum cytotoxicity if used more than 10 $\mu$ L. After 24 hours of incubation of the brine shrimps in the nanoparticle solution, all brine shrimps survived in 5  $\mu$ L and control, 9 brine shrimps survived in 10  $\mu$ L concentrations, 3 brine shrimps survived in 15  $\mu$ L concentration and 1 brine shrimps survived in 20  $\mu$ L and 25 $\mu$ L concentrations each. (Fig. 3).

Cytotoxicity test was used to measure the degree of toxicity on certain cells [22]. The brine shrimps lethality bioassay was employed here in order to predict its suitability for pharmaceutical applications [15,23]. Generally, this brine shrimps cytotoxicity bioassay test may be used as preliminary test to investigate anti-cancer, and pesticidal activities [24]. Brine shrimps species correspond similarly to functionalities of mammalian cells. Therefore, this cytotoxicity test contributes to a significant impact on this research [16,25].

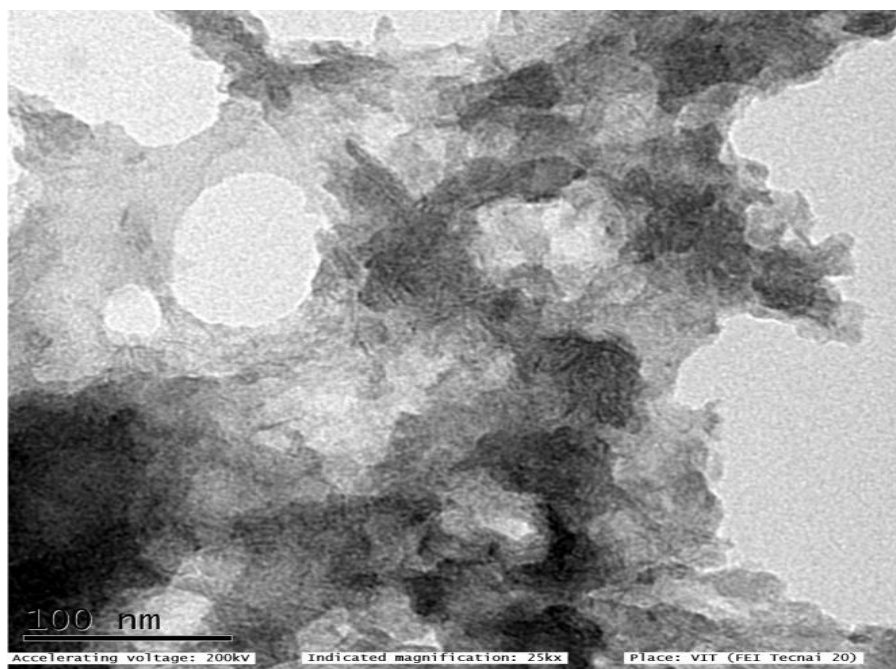


Fig. 2. Image showing Transmission Electron Microscopy (TEM) of *Cassia oleoresin* (Size 70 nm) mediated magnesium oxide nanoparticles

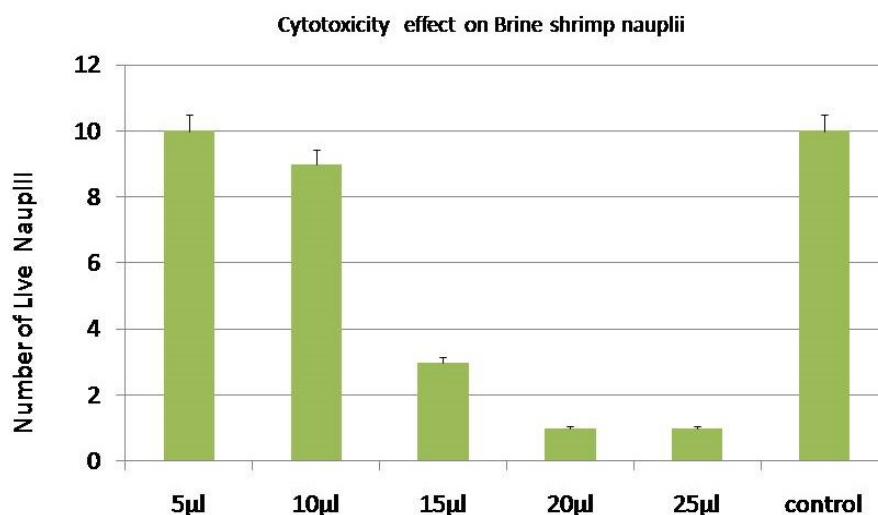


Fig. 3. Bar graph showing Cytotoxic effect of *Cassia oleoresin* mediated magnesium oxide nanoparticles

X- axis shows the concentration of nanoparticle, Y- axis shows number of brine shrimps Naupli that survived in each concentration. All brine shrimp Nauplii survived were in 5 µL and in control, survival rate decreased with the increase in concentration

The increasing popularity of using plant extract for the synthesis of nanoparticles [26-28], this study was carried out for the synthesis of Magnesium oxide nanoparticles using *Cassia*

*oleoresin*. Magnesium particles are connected to modified cell demise and expanded cytotoxicity in specific conditions. Rapid advances in the field of nanotechnology led to creation of nano-sized

particles with very different chemical and physical properties as compared to particles in their bulk form [3,29]. Nanoparticles possess diverse applications in various aspects of human life, from cosmetics and medical products to water purification and solar energy capture [30, 31]. The widespread contact and interaction of nanomaterials with human body, the potential hazard to human health and safety has become the matter of concern [32]. MgO NPs have been recognized as safe materials by the United States Food and Drug Administration (21CFR184.1431). Recent advances have led to conspicuous developments with enormous potential in materials and medicines.[33].

In this research, the cytotoxicity property was exhibited by Magnesium Oxide nanoparticles prepared from *cassia oleoresin* on brine shrimps [34]. The study showed a dose dependent cytotoxic effect that t lethality was directly proportional to the concentration of the *Cassia oleoresin* mediated magnesium oxide nanoparticles. In this study, mortality increased with increasing sample concentration which is due to the toxicity of magnesium oxide [34,35].

#### 4. CONCLUSION

The present study conclude that magnesium oxide nanoparticles prepared from *Cassia oleoresin* showed least cytotoxic effect at the lowest concentrations ,5µL and 10µL, used in the study. The nanoparticles were toxic in 15 µL, 20 µL and 25µL .Further studies must be conducted in the embryonic toxicology, animal studies to rule out the cytotoxicity of magnesium oxide nanoparticles prepared from *Cassia oleoresin*.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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