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Invitro DPPH activity and cytotoxicity analysis of zinc oxide nanoparticles (ZNONPS) from *Mangifera Indica L. Anacardiaceae* leaves extract

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***Abstract*---**The experiment was analysed to characterize the nanoparticles from *MangiferaIndica L. Anacardiacea*and tested its toxicity, DPPH during the year 2018 to 2022. Nanoparticles are important aspect in nanotechnology because it has very distinctive features. Especially Zinc Oxide nanoparticles (ZnOnps) have more benefits as shown in recent research because to their extensive bandwidth and high exciton required energy and they may have “antibacterial, antifungal, anti-diabetic, anti-inflammatory, wound healing, antioxidant properties. Green synthesis approaches use chemicals that are relatively pollutant-free. It promotes the use of environmentally friendly and safe solvents like water and natural extracts. Mango leaves have a wide range of value since they are known to contain a variety of phytochemical, biological and pharmacological qualities. Hence ZnOnps was synthesized from mango leaves extract and it was characterized using SEM, EDAX, Zeta Potential and UV- visible spec with standard procedure. And its antioxidant activity and cytotoxicity also determined for its quality.

***Keywords*---**Antioxidants, Cyto toxicity, Food technology and Nano particles.

Introduction

Nanotechnology is the science and technology involved in the design, synthesis, characterisation and use of materials and devices with the smallest functional organization in at least one dimension on the nanoscale (one billion of a metre) (Saini et al., 2010). Nanotechnology collaboration in food and agriculture systems aims to improve food safety and handling, plant ability to absorb nutrients, flavour and nutrition delivery techniques, infection detection, food functioning, environmental protection, and storage and distribution cost effectiveness. Biotechnology, chemistry, physics and engineering all benefit from it since it improves the possibility for transformation. Nanotechnology is employed in agriculture, food processing, medicine, biology, biochemistry, wastewater, water treatment, flooring materials, machinery and new electronic gadgets and processes (Rashidi*et al.,* 2011).

Nanotechnology is important in the formation and application of nanoscale materials. Nanoparticles have a significant surface area and high due to their nanoscale dimension, and consequently have very distinctive features. Zinc Oxide nanoparticles (ZnOnps) have been the subject of recent research because to their extensive bandwidth and high excitonrequired energy and they may have antibacterial, antifungal, anti-diabetic, anti-inflammatory, wound healing, antioxidant and optical characteristics (Agarwal*et al.,* 2017). Nanomaterials possess a variety of uses due to their size and form and they have long been a major topic in both basic and applied sciences. Zinc nanoparticles, among other nanoparticles, are efficient semiconductors with high optical clearness and luminescence in the UV-Visible (V-Vis) range. Due to their exceptional chemical and thermal resistance, these nanoparticles have become extremely important in recent years (Fakhari*et al.,* 2019).

The FDA classified Nano-ZnO as a GRAS (generally recognized as safe) substance because it permits zinc to be absorbed efficiently by the body. (US Food and Drug Administration).ZnOnps are considered to be an economical and less lethal material than other metal Oxide nanoparticles (Melk*et al.,* 2021). The chemicals utilised in nanoparticles synthesis and stabilization is hazardous and result in non-eco-friendly by products. Plant based nanoparticles production is included in green synthesis Processes. For the synthesis of nanostructures, green synthesis approaches use chemicals that are relatively pollutant-free. It promotes the use of environmentally friendly and safe solvents like water and natural extracts. Green chemistry decreases risk at the source, and it is better used to prevent waste than to treat or clean up waste that has already occurred(Sabir*et al.,* 2014).

Mango (Mangiferaindica L.), a species of the Anacardiaceae family, has been deemed one of the most historically significant and economically important tropical fruit crops I the world. Minerals such as nitrogen, potassium, phosphorus, iron,sodium, calcium, magnesium and vitamins can be found in mango leaves (A,B,E and C). Mangiferin, phenolic acids, benzophenones, and other antioxidants such as flavonoids, carotenoids,quercetin, isoquercetin, ascorbic acid and tocopherols are the most active biological constituents in mango leaves. Mango leaves have a wide range of value since they are known to contain a variety of phytochemical, biological and pharmacological qualities such as antimicrobial, antioxidant, anti – diabetic, anti – tumor and immunological modulatory actions(*Kumar et al.,* 2021). In this present study (2021) we examined to synthesis of ZnNPs, their characterization, in vitro radical scavenging activity and cytotoxicity effect of synthesized nanoparticles.

Materials and Methods

Plant Material

Mangiferaindica L. Anacardiaceae leaves were collected and the plant certification was confirmed by Plant Survey of India, Coimbatore "(Authentication No: BSI/SRC/5/23/2019/Tech./188)".

Preparation of Plant Extract

The shade dried leaves of *Mangiferaindica* was powdered .The leaf powder was extracted using distilled water to prepare absolute aqueous extract overnight at room temperature in shaker incubator(Petchi*et al.,* 2011)

Synthesis of ZnOnps

The method proposed by Santhosh Kumar *et al.,* (2017) was used for the separation of zinc oxide nanoparticles from the *Mangiferaindica L. Anacardiaceae* leaves and the detailed method is given in fig 1.

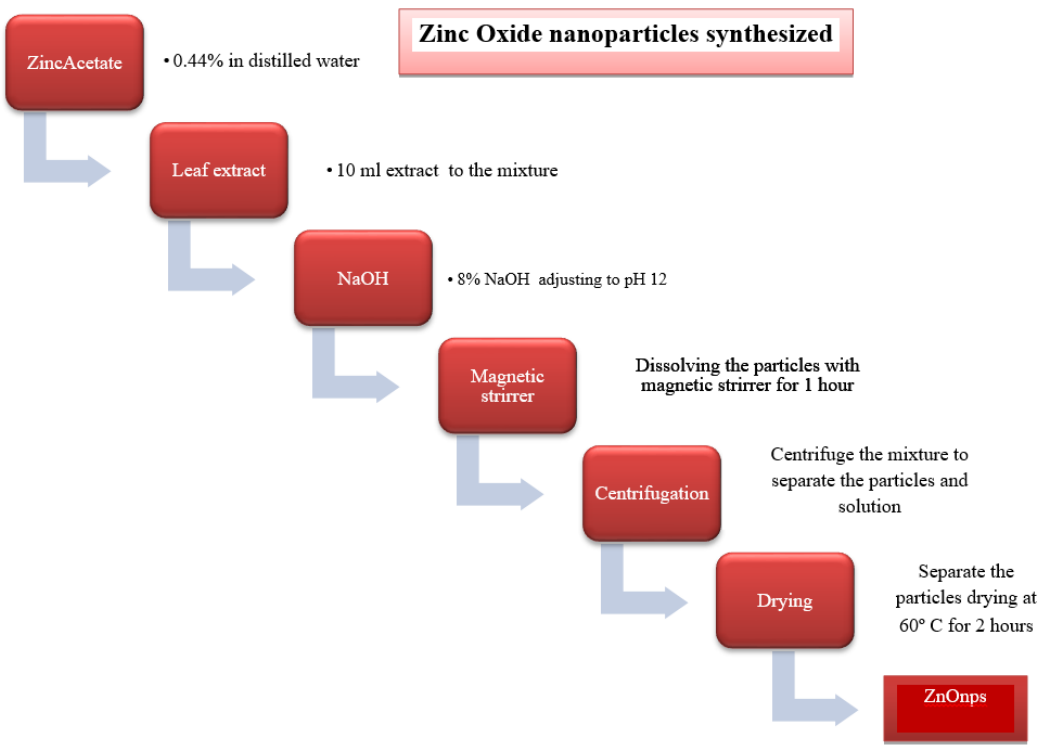


Figure: 1 Biosynthesis of *Mangiferaindica L. Anacardiacea*zinc oxide nanoparticles

Characterization of zinc oxide nanoparticles (ZnOnps)

Synthesized ZnONps were characterized based on UV absorption spectrum with the wavelength range of 250 – 800nm. SEM analysis showed the surface morphology of the nanoparticles. Nanoparticles were popped on a carbon coated copper grid for EDAX analysis. Stability of the ZnONps was characterized by zeta potential peaks.

Antioxidant Activity by DPPH method

"The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams with slight modifications. 0.5 ml of 0.1mM DPPH solution in methanol was mixed with*Mangiferaindica L. Anacardeaceae* extract solution of varying concentrations (100, 200, 300,400,500 µg/ml). Corresponding blank sample was prepared and Quercetin (100-500µg/ml) was used as reference standard. Mixer of 0.5ml methanol and 0.5ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 520nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula".

$$\textbf{Inhibition \% = C-S/C×100}$$

Where, is C - Control S - Sample

Cytotoxic brine shrimp assay

"The given sample was weighed and dissolved in 25ml doubly distilled water to get solution of different concentration (250, 500,1000,1500,2000 µg/ml). 30 shrimps Introduced into the sample solution of various concentration (250, 500, 1000, 1500, 2000 µg/ml). The movement of shrimp is monitored at intervals of 1, 2, 4, 8, 24 hours. Blank solution: 30 shrimps in fresh distilled water.

Positive control: Potassium dichromate (1mg/ml).
The mortality of shrimp is calculated after 24 hours. 30 shrimps were added to 25ml of the sample solution. The mortality of the shrimps was monitored as that of blank and positive control. After 24 hours of incubation survivors were counted and calculation was done using Abbots formula (*Muhammad et al.,* 2012) given below.

$$\% \text{ Death} = (\text{Sample} - \text{control/control}) \times 100$$

Lethal Dose (LD_{50})was determined through calculation method.

Results and Discussion
UV – visible analysis:

The samples's absorption spectrum was measured between 250 and 800nm (Fig-2). For the confirmation of nano – sized particles, UV – visible absorption spectroscopy is commonly utilized. The formation of zinc oxide nanoparticles from *Mangiferaindica .L anacardiaceae*extract was confirmed by a high absorption peak at 250nm wavelength.

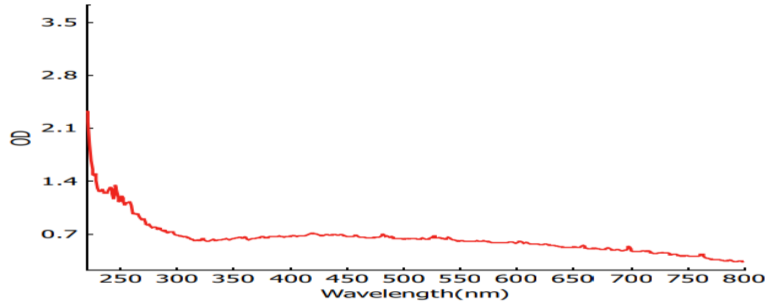


Figure: 2 UV – visible spectrum of ZnOnps

SEM and EDX analysis

The morphology of nanostructures was investigated using SEM (Fig 3A, 3B). EDAX analysis was used to investigate the signal distinctive peak of only zinc and oxygen (Fig 3C). The presence of zinc (zn) and chromium (Cr).

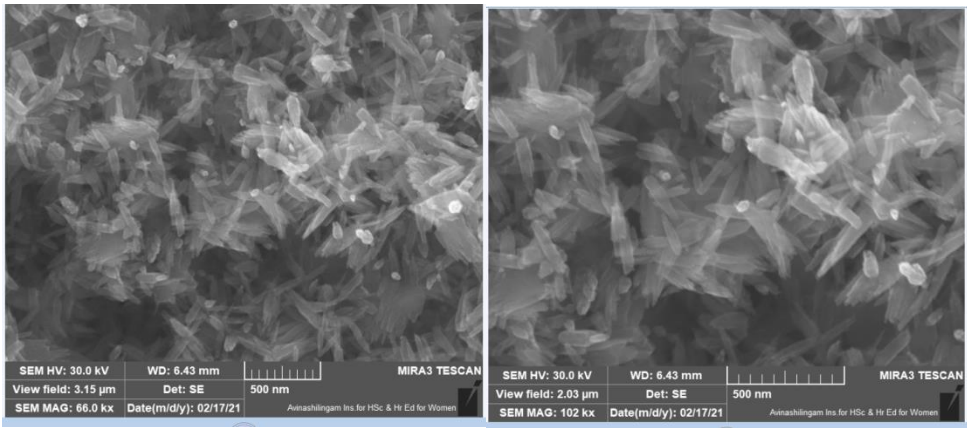


Figure: 3A SEM image of ZnOnps

Elemental mapping of ZnOnps

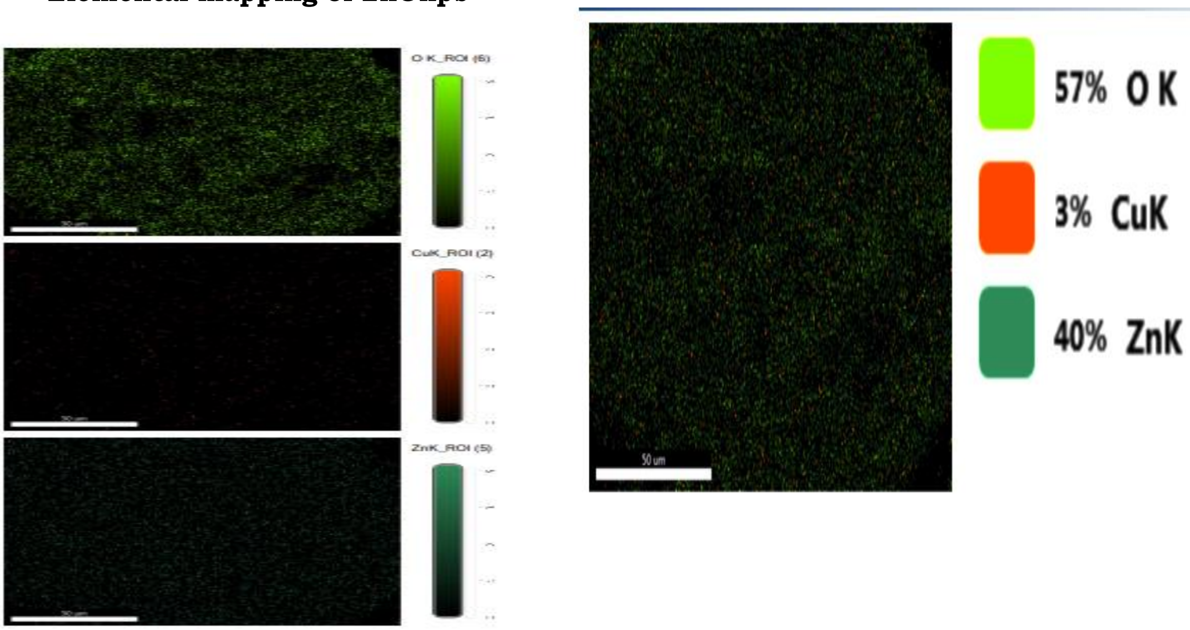
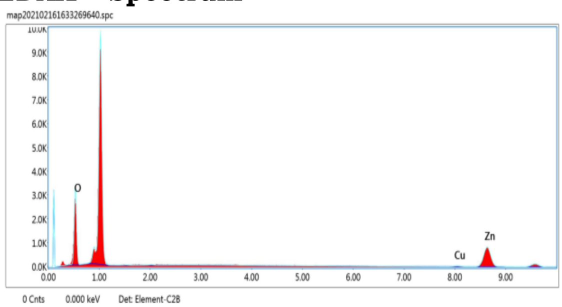


Figure: 3B Elemental mapping

EDAX – Spectrum



Element	Weight %	Atomic %
ZnK	83.02	56.28

Figure: 3C EDX for ZnOnps

Zeta Potential

The zeta potential was used to determine the stability of the ZnOnps. It was determined using 0.11 ml HCL dispersant medium and 0.1 g of ZnO in distilled water. The stability of particles with zeta potentials larger than ± 60 mv is excellent. The zetapotential of ZnOnps was found to peak at 15.8 mv (Figure 4), indicating that the biosynthesized Nano – ZnO particles were positively charged and moderately dispersed in the medium. The stability of the nanoparticles was attributed to positive zeta potential values.

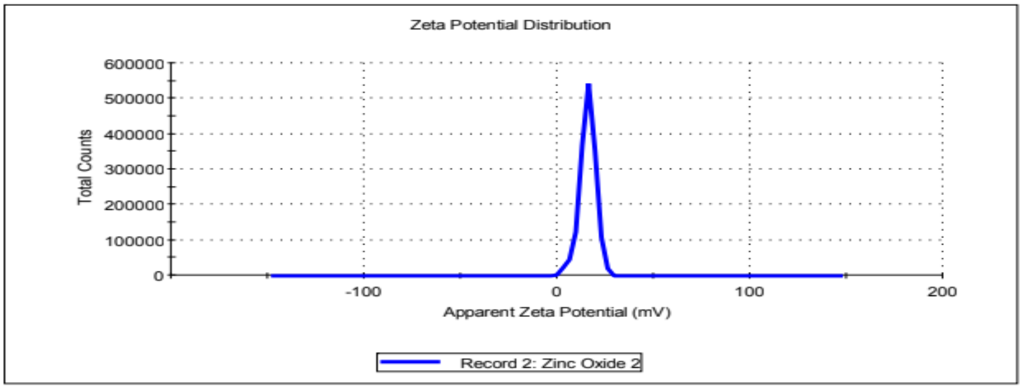


Figure: 4Zeta Potential of ZnOnps

Antioxidant activity (DPPH Assay)

When DPPH is scavenged, it becomes a stable free radical with a deep violet colour. This feature is used in the DPPH experiment to demonstrate free radical scavenging. The amount of discolouration reflects the antioxidant chemicals ability to scavenge free radicals. As the absorptionof ZnOnps increased from 100 - 500g/ml, the absorbance at 511nm decreased, indicating that ZnOnps have radical scavenging activity.

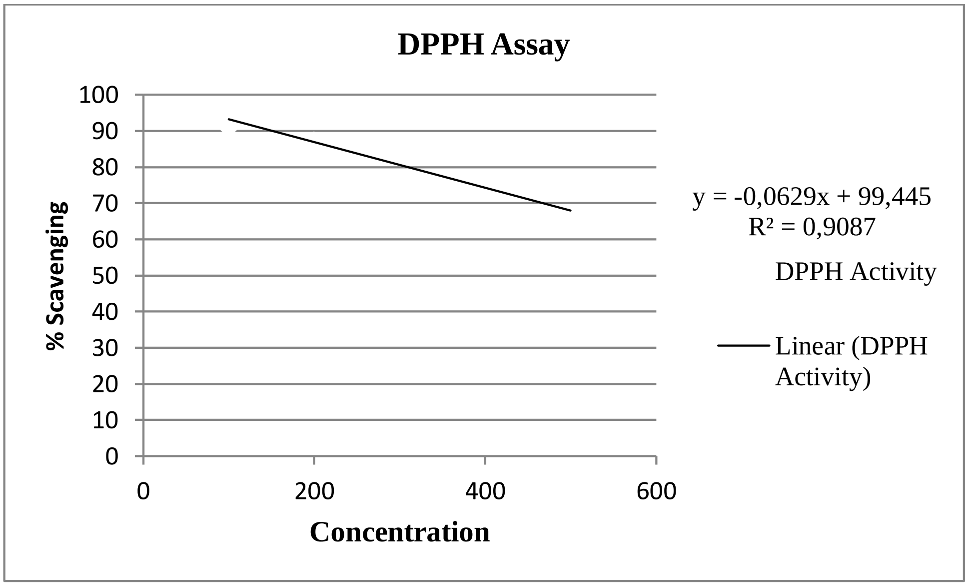


Figure: 5 Antioxidant Activity of ZnOnps

Cytotoxicity Brine Shrimp Assay

The sample was weighed and diluted in 25 ml doubly distilled water to produce solutions of various concentrations (250, 500, 1000, 1500 and 2000g/ml) Thelarvicidal efficacy of zinc oxide nanoparticles against brine shrimp was week. ZnOnps had lethality concentration of 250, 500, 1000, 1500 and 2000g/ml, respectively (Fig 5) The degree of lethality was proportional to the nanoparticle concentration. At a concentration of 500g/ml the lowest mortalities (23%) were observed. The nanoparticle concentration was directly linked to the degree of lethality. According to the findings, the mortality of brine shrimps exposed to various g/ml of ZnO was dependent on concentration. The existence of cytotoxicity was indicated by the ZnO's lethality in brine shrimps. The ZnOnps are slightly unsafe (active) if the LC50 value is less than 1000g/ml, and safe (inactive) if the LC_{50} value is larger than 1000g/ml, according to these findings (Suprajaet *al.*, 2018). This study's LC_{50} value is 659.33g/ml, indicating that the ZnOnps from Mangiferaindica L. Anacardiaceae are non – toxic.

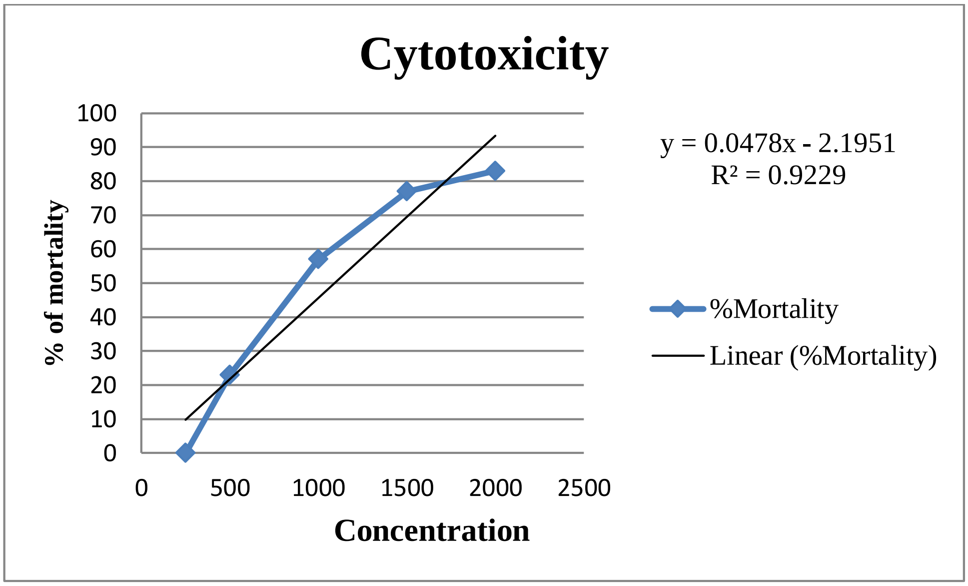


Fig: 6 Cytotoxicity of ZnOnps

Conclusion

Zinc Oxide was produced with Mangiferaindca L. Anacardiaceae and characterised with UV-visible spectroscopy, SEM, EDX and zeta potential in the current study. The cytotoxicity and antioxidant activities of ZnO nanoparticles were discovered. This study conclusively demonstrates and environmentally benign technique to the manufacture of zinc oxide nanoparticles, emphasising its anti-toxic properties as well as their potential for usage as an effective antioxidant.

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