



## SYNERGISTIC EFFECTS OF *CARICA PAPAYA* SEED MEDIATED SILVER NANOCOMPOSITES

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### ABSTRACT

As a novel study, the current work focussed about green synthesised ZnO nanocomposites by using *Carica* ripened seed regions. Synthesised nanocomposites are imperilled to characterization, antimicrobial and antioxidant studies. UV, FTIR, DLS and SEM studies revealed the band gap energies, active compound, morphology and surface charge of the green synthesised ZnO Nanocomposites. Antimicrobial results revealed significant growth inhibition activities on gram positive, gram negative and fungal species. Antioxidant studies also revealed the reducing and scavenging activities of the tested green ZnO nanocomposites. Our studies concluded that *Carica* seed based green synthesised ZnO nanocomposites showed an enhanced compatible result which can be used as a conventional alternative source for toxic chemical-based nanocomposites.

**Keywords:** *Carica* seeds, ZnO Nanocomposites, SEM, Antioxidant activity, Antimicrobial activity.

### INTRODUCTION

Nanoparticles (NPs) are minuscule particles with improved thermal conductivity, chemical stability, non-linear optical performance, and catalytic reactivity due to their extremely small surface area to volume ratio (de Jesus *et al.*, 2023). Nanomaterials has unique features as high surface and volume ratio due to their size (1 to 100nm) which contains high surface reactivity (Geonmonond *et al.*, 2018; Suganthi *et al.*, 2019). Our ecosystem are polluted with various chemicals (Shereena *et al.*, 2025; Mahmoodha Parveen *et al.*, 2019a, 2019b) which develops the interest in researchers to develop alternative methods. According to Hosseingholian *et al.* (2023), the green synthesis process is a suitable alternative to traditional methods due to its affordability, non-toxicity, biodegradability, and

environmental sustainability (Dogaroglu *et al.*, 2024). Green nanocomposites are the safe materials with elite properties and also considered as an eco-friendly, biocompatible, bio-safe, cost effective and less toxic substances (Rosi and Mirkin, 2005; Sabir *et al.*, 2014; Raghupathi *et al.*, 2011). Due to the increased microbial resistance towards the drugs, the identification of enhanced and improved antimicrobial agents i.e. phytobased is essential for the present situation (Romero *et al.*, 2005; Boucher *et al.*, 2009).

Zinc oxide (ZnO) nanocomposites is a metallic oxide compound which has remarkable properties such as antimicrobial, anticorrosive (Siyانبola *et al.*, 2013; Okitsu *et al.*, 2007), photocatalytic, optical, electrical (Vijayakumar *et al.*, 2018; Yedurkar *et al.*, 2016), magnetic

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and piezoelectric sensors (Teodore 2006; Wang *et al.*, 2008). Nowadays, ZnO Nanocomposites is a promising materials with various applications such as wound healing, anti-cancerous, antioxidant, anti-inflammatory, gene gun and drug delivery system. are used for environmental remediation process, pharmaceutical, rubber and ceramic industries (Iravani 2011; Duan *et al.*, 2015; Bala *et al.*, 2015). So eventually, green synthesised ZnO Nanocomposites are considered as an effective, fast and eco-friendly process (Gunalana *et al.*, 2012).

Various researchers are studied about the ethnobotanical importance of *Carica papaya* plant parts as anti-ameobic, anti-helminthic, anti-microbial and rich with antioxidants (Okeniyi *et al.*, 2007; Bamisayer *et al.*, 2013; Maisarah *et al.*, 2013; Tiwari *et al.*, 2011). The objective of the study is *Carica papaya* seed based ZnO Nanocomposites synthesis. Characterization studies are performed for the size and morphology of the ZnO Nanocomposites identification. *In vitro* antimicrobial and antioxidant properties also evaluated for the green synthesised ZnO Nanocomposites.

## MATERIALS AND METHODS

### Synthesis of green ZnO Nanocomposites

Fresh *Carica papaya* is purchased from the botanical garden and was stored in the Microbiology Laboratory, KIRND (Tiruchirappalli, Tamil Nadu). The seeds are collected and dried for 5 days (30°C) under shade. The dried seeds are pulverised. For the aqueous extracts, 1gm of seed powder mixed with deionized water (100ml) and kept in water bath (60min, 60°C) (Lateef *et al.* 2015). The suspension centrifuged (20min, 4000rpm) and the supernatant collected and used for further studies. Zinc oxide (CAS 1314-13-2, mol. wt. 81.39) are purchased from Sigma-Aldrich. By downstream process (Rathika *et al.*, 2023), the *C. papaya* mediated ZnO nanocomposites are synthesized and used for this study.

### Characterization studies

Potassium bromide pellets-based spectrometry analyser (FTIR SHIMADZU 8400) and UV spectroscopy (Shimadzu UV-2450) are able to identify the tested samples functional or active groups and bandgap which are essential for the molecular analysis. Synthesised compound structure are predicted through Gemini 500 (Zeiss) field emission scanning electron microscopy (SEM) along with DLS.

### *In vitro* Antimicrobial activity

For the antimicrobial assay, *Klebsiella pneumonia*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Aspergillus niger* are procured from the Dept. of Microbiology, KIRND private limited, Tiruchirappalli. By using agar well diffusion method (Rajasekar *et al.* 2013), the antimicrobial activity of the green synthesised ZnO Nanocomposites are analysed. Nutrient agar media is prepared and poured into the petriplates and inoculated with respective microbes (concentration  $10^4$  cells/ml) by

spreading plate method. Three different (50, 100 & 200µg/ml) concentrations of green synthesised ZnO Nanocomposites are poured into three wells, positive control (Fungi: Clotrimazole; Bacteria: Tetracycline) and negative control (Aqueous) also maintained. Plates are incubated for 24h at 37°C and the inhibition zone is measured (in mm).

### *In vitro* Antioxidant activity

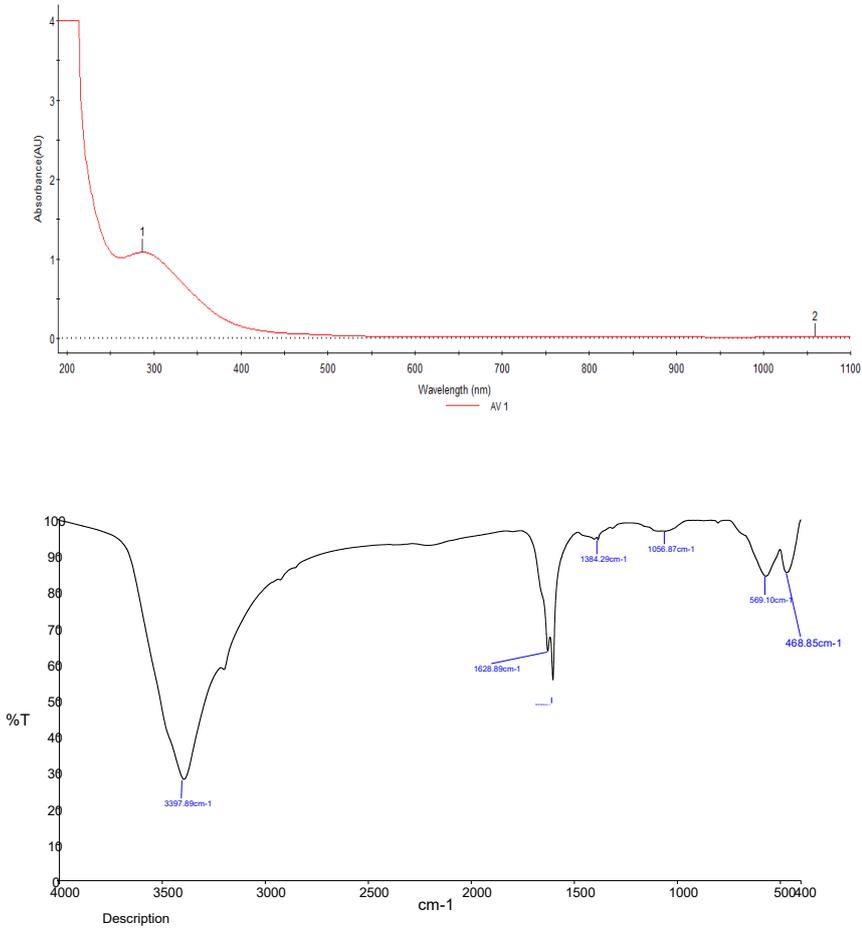
Antioxidant properties of the green synthesised ZnO nanocomposites are studied by *in vitro* DPPH, hydroxyl, reducing power and hydrogen peroxide analysis based on the protocol reported by Jeeva *et al.* (2011), Anzabi (2018) and Gocer *et al.* (2012) respectively. Absorbance of the solution read at 517 nm (Shimadzu UV-2450).

### Statistical Analysis

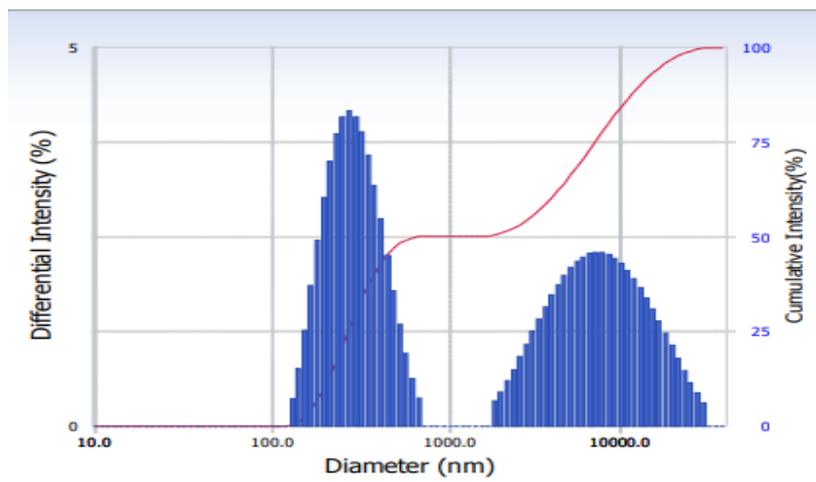
Results are interpreted by SPSS (7.0 version). Least significant differences between the mean±SD values the tested groups are compared by using Duncan post hoc testing. By using regression, the IC50 values are predicted.

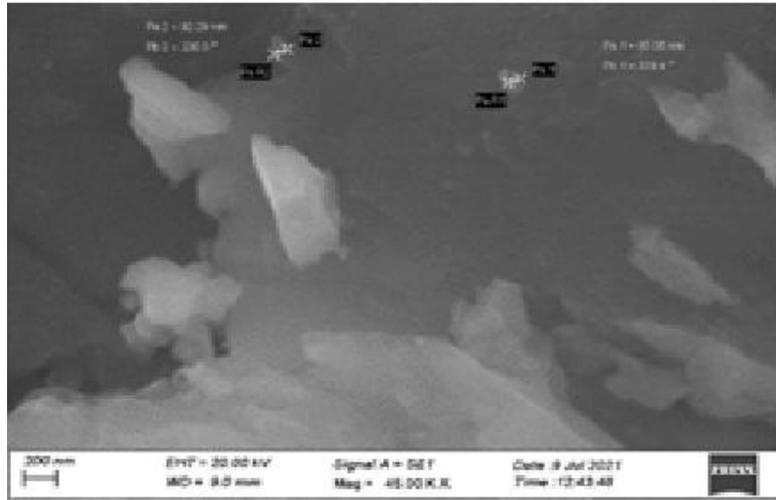
## RESULTS AND DISCUSSION

During green synthesis of ZnO Nanocomposites, the chemical colour changed into pale yellowish white precipitate which represented the reduction of reactants (Zinc nitrate) into the product ZnO nanocomposites. Green synthesised ZnO Nanocomposites UV spectrum is ranged between 200 to 1100nm. Two peaks were observed at 287.30 and 1059.20nm (Fig. 1) and their respective absorbance were 1.087 and 0.021. The direct band gap energies are measured as 4.32 and 1.17eV respectively. UV spectral analysis (Figure 1) revealed the presence of alkyne (C-H), (C=C), alkane (CH<sub>3</sub>) bending and (C-I) for their respective peaks at 3397, 1628, 1384 and 569cm<sup>-1</sup> respectively. SEM analysis revealed the aggregated hexagonal particles and DLS also proved the size of the ZnO Nanocomposites as ~30nm (Figure 2). Treated groups showed significant results against the treated microbes in all the concentrations (Table 1). The maximum inhibition zone was observed against gram positive *Bacillus subtilis* (17.01±0.03mm) treated with 50µg/ml green synthesised ZnO nanocomposites. ZnO Nanocomposites treated group growth inhibition results are similar to their respective positive control growth inhibition. The significant antioxidant potential of *Carica* seed based green synthesised ZnO nanocomposites was evaluated. Based on the mean results for each nanocomposite concentration, the linear regression was predicted for the four antioxidant parameters. Based on regression equation (Y=MX+C), the DPPH radical scavenging assay predicted as IC50 4.71µg/mL. Similarly, IC50 values for hydroxyl, FRAP and hydrogen peroxide assay are evaluated as 6.99, 12.92 and 0.61 µg/mL (Figure 3).



**Figure 1.** Green synthesised ZnO nanocomposites - UV and FTIR spectrum.

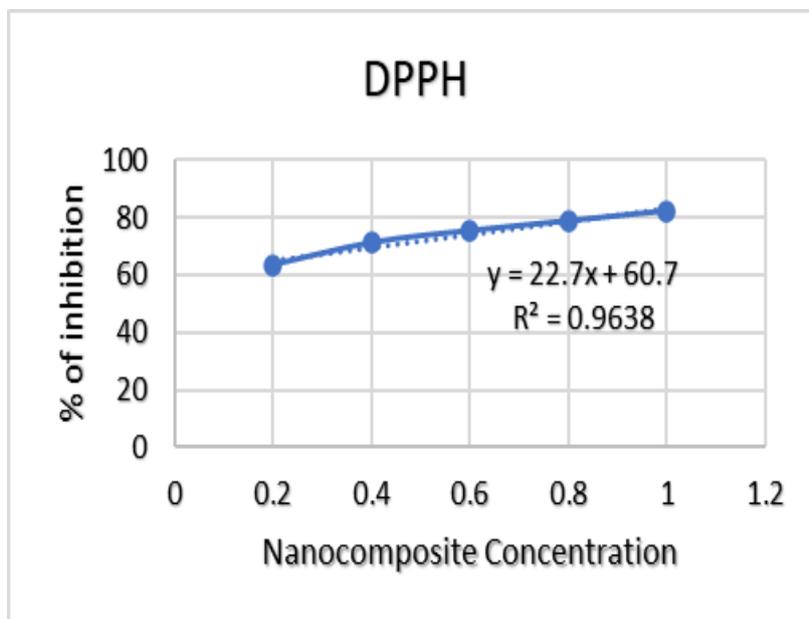


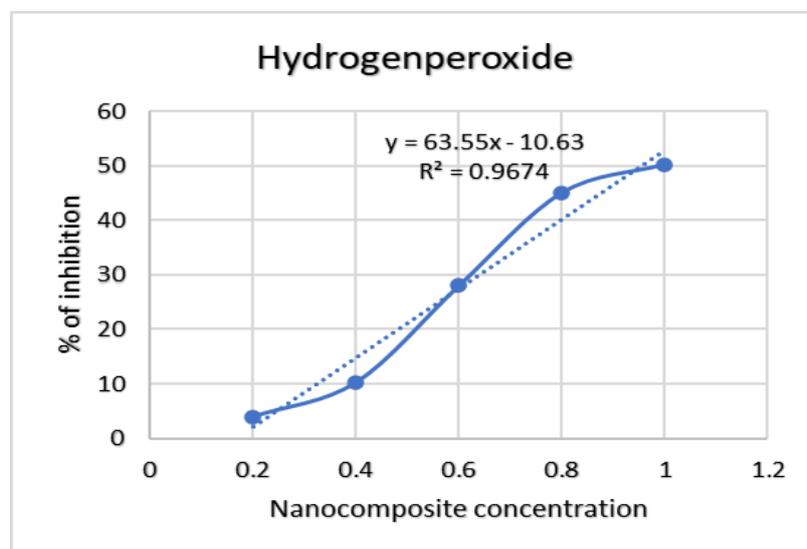
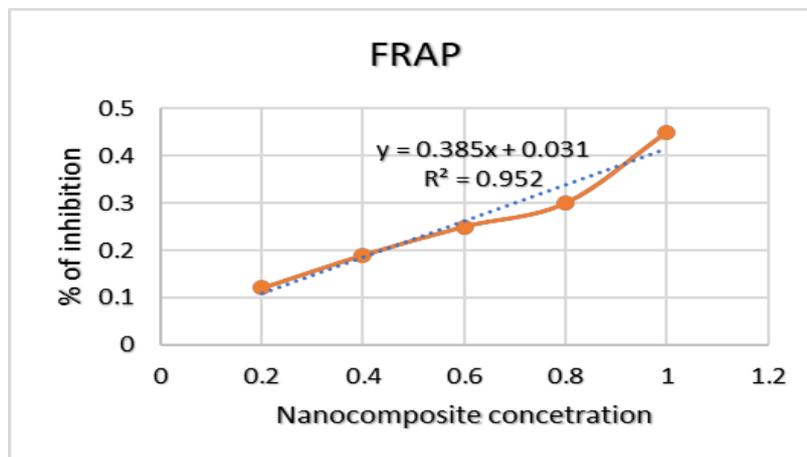
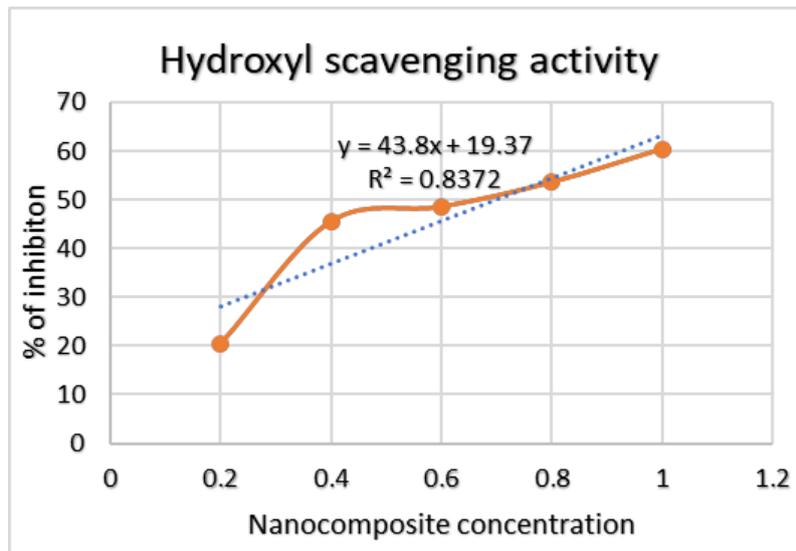


**Table 1.** Antimicrobial activity of green synthesised ZnO nanocomposites.

Microbes	PC	Green synthesised ZnO Nanocomposites		
		50 µg/ml	100µg/ml	200µg/ml
<i>Escherichia coli</i>	16.06±0.02	14.04±0.03	16.07±0.06	15.03±0.02
<i>Klebsiella pneumonia</i>	15.8±0.04	14.06±0.04	15.04±0.07	15.04±0.07
<i>Bacillus subtilis</i>	14.04±0.02	17.01±0.03	16.04±0.08	16.06±0.05
<i>Staphylococcus aureus</i>	15.08±0.04	16.04±0.07	15.09±0.01	14.02±0.01
<i>Aspergillus niger</i>	15.01±0.03	16.05±0.02	14.04±0.03	15.06±0.03

Positive control: For Bacteria- Tetracycline; For Fungi-Clotrimazole; Negative control: Aqueous (no inhibition zone)





**Figure 3.** *In vitro* Antioxidant studies of ZnO nanocomposites.

Green synthesised ZnO nanocomposites characterization results are evidenced by Vahidi *et al.* (2019), Chikkanna *et al.* (2019) and Mahvash *et al.* (2020). *Carica papaya* leaf extract was added to a ZnO–CuO nanocomposite using the hydrothermal technique, greatly improving the nanocomposites characteristics. A cubic crystal structure with an average size of 22.37 nm was verified by energy dispersive X-ray analysis. The ZnO–CuO nanocomposite exhibited characteristic vibrations at 627, 661, and 751 $\text{cm}^{-1}$ , which corresponded to stretching and vibration modes, according to the FTIR. SEM pictures revealed the presence of an irregular, cubic-like structure (Aswini *et al.*, 2024). Mansur *et al.* (2025) reported the *Ficus sycomorus* leaf extract mediated zinc oxide nanoparticles production by downstream process. The creation of ZnO Nanocomposites by the UV-Vis was demonstrated by the existence of a characteristic SPR peak at 320 nm caused by harmonic oscillation of electrons in the conduction band of the spectrum. The SEM analyses showed the presence of a spherical hexagonal wurtzite structure with an average size of 18.94 nm by XRD. Plant compound mediated nanoparticles contains numerous bioactive compounds which acts as capping and stabilizing agents for nanoparticles and also acts as capping and reducing agents against various gram positive and negative bacteria (Talbot *et al.*, 2006; Rajeshkumar *et al.*, 2019; Sergiev *et al.*, 2019; Mohamed and Akladios 2017; Rehman *et al.*, 2017). Green synthesised ZnO nanocomposites showed significant antimicrobial activities due to the activation of reactive oxygen species by hydrogen peroxide, hydroxyl radical and superoxide anion development in the microbial system (Rasmussen *et al.*, 2010; Xiong *et al.*, 2013). *Carica* leaf extract mediated ZnO–CuO nanocomposite showed strong antibacterial action by inhibiting *Klebsiella pneumonia* (17 mm) and *Staphylococcus aureus* (20 mm). The outcomes demonstrate the effectiveness of a ZnO–CuO nanocomposite generated from *Carica papaya* leaves for environmental and health issues (Aswini *et al.*, 2024). *F. sycomorus* leaf extract mediated ZnO NPs in vitro antibacterial activity were assessed as *S. aureus* (21 mm), *S. typhi* (18 mm), and *Candida albicans* (28 mm) at different concentrations of 12.50, 25.0, and 50.0 (mg/mL) (Mansur *et al.*, 2025). In general, increased ROS species by various plant metabolic pathways as well as chemically synthesised NPs leads to mutations and lethality to cells (Halliwell and Gutteridge, 1999; Yen *et al.*, 1993) whereas *Carica* seed based green ZnO NPs showed significantly increased antioxidant levels than the control. In microbial cytoplasm, ROS production resulted the gradient variation which leads to the increased membrane permeability, genomic instability and cytotoxicity (Shi *et al.*, 2014; Jiang *et al.*, 2016; Dutta *et al.*, 2013).

## CONCLUSION

This study is focused about the green synthesis of ZnO nanocomposites from *Carica papaya* seed which is an eco-friendly compound and used as a conventional and promising compound for the toxic chemical-based nanocomposites. Synthesised ZnO Nanocomposites

characterization results revealed the particle size, morphology, band gap energies and surface charges. Antimicrobial and antioxidant studies also revealed the compatibility and efficiency of *Carica papaya* seed-based green synthesised ZnO nanocomposites.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

Not applicable

## FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

## AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

## DATA AVAILABILITY

Data will be available on request

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