

Analysis of antioxidant and antimicrobial potential of Copper oxide nanoparticles from the peel of *Carica papaya* - caries prevention

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ABSTRACT

Carica papaya commonly known as papaya belong to the family of Caricaceae. The peel contains papain and chymopapain and other enzymes (cysteine proteinases papain, caricain and glycyl endopeptidase) which are having antioxidant and anti microbial properties. It is traditionally used to treat dental infections like gingivitis, periodontitis, dental caries, oral thrush and medical infections such as diarrhoea, allergies. In this study, papaya peel extract was used for physiochemical characterisation (UV, SEM, FTIR analysis) , anti inflammatory and anti microbial tests. The UV absorption showed a peak at 299.5 nm, SEM analysis confirmed spherical copper oxide nanoparticles. FTIR analysis showed OH groups, carbon, and water. The copper oxide nanoparticles (CuO NPs) showed high antioxidant activity at 47 μ L ascorbic acid. Antimicrobial activity was observed via a clear zone of inhibition. This study shows papaya peel reduces copper nitrate to copper oxide nanoparticles (CuO NPs) , which have shown antioxidant and antimicrobial potential.

1. INTRODUCTION

Dental caries is defined as a chronic infection which affects the dentition leading to destruction of organic matter and demineralisation of inorganic matter by organic acids produced during the bacterial fermentation of dietary carbohydrates. The microorganisms mainly associated with dental caries include *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus* species, and *Actinomyces*, which is present in our oral cavity and on food accumulation leads to dental plaque biofilms on tooth surfaces ,further leading to caries formation and if that is untreated, dental caries may lead to pulpal involvement, periapical infection, pain, and eventual tooth loss, adversely affecting oral and systemic health .

Although modern dentistry employs various antimicrobial agents, antibiotics, and restorative materials to control dental infections, their prolonged or excessive use can result in adverse effects such as antimicrobial resistance, hypersensitivity reactions, disturbance of normal oral flora, and systemic toxicity. Chemical agents like chlorhexidine are effective but are associated with side effects including tooth staining, taste alteration, and mucosal irritation. These limitations have led to increasing interest in natural therapeutic agents that are safer, cost-effective, biocompatible, and environmentally friendly. Plant-based natural products are widely explored due to their antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties, making them promising alternatives or adjuncts in dental care .

Carica papaya, commonly known as papaya, is a tropical plant widely cultivated in regions such as India, Southeast Asia, Africa, and South America. Various parts of the plant, including the fruit, seeds, leaves, and peel, contain biologically active compounds such as flavonoids, phenols, tannins, alkaloids, carotenoids, and enzymes like papain and chymopapain. These phytochemicals possess antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties. Traditionally, papaya has been used in the treatment of infections, inflammation, digestive disorders, and skin conditions. The peel, often discarded as waste, is a rich source of these compounds and can be effectively utilized for biomedical applications .

Copper oxide nanoparticles (CuO NPs) have gained significant attention in biomedical and dental research due to their unique physicochemical properties and strong biological activity. CuO NPs exhibit potent antimicrobial effects by generating reactive oxygen species, disrupting microbial cell membranes, and interfering with essential cellular processes such as DNA replication and protein synthesis. Additionally, these nanoparticles demonstrate antioxidant activity by scavenging free radicals and reducing oxidative stress. Green synthesis of CuO NPs using plant extracts enhances their biocompatibility and reduces toxicity, as plant phytochemicals act as natural reducing and stabilizing agents, making them suitable for dental and medical applications .

In the present study, copper oxide nanoparticles were synthesized using *Carica papaya* peel extract through a green synthesis approach. The phytochemicals present in the papaya peel facilitate eco-friendly nanoparticle synthesis and enhance the antioxidant and antimicrobial properties of the nanoparticles. This study aims to evaluate the antioxidant and antimicrobial potential of copper oxide nanoparticles derived from *Carica papaya* peel, highlighting their potential application in dentistry for the prevention and management of dental infections and oxidative stress-related oral diseases.

2.MATERIALS AND METHODS:

2.1. Chemicals

The *Carica papaya* peel extract was collected from Chennai District (Tamil Nadu, India). The species were identified and authenticated at the Department of Centre for Advanced Study in Botany, University of Madras, Chennai, India. All chemicals and reagents used for this research work were purchased from Sigma Chemical Company St. Louis, MO, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA.

2.2. Synthesis of Copper oxide nanoparticles using the peel of *Carica papaya*:

Fresh papaya peel is thoroughly washed and finely cut to increase surface area for the green synthesis of copper oxide nanoparticles. About 10 ml of the moist peel is mixed with 25 ml of distilled water and shaken for 45 minutes to extract bioactive compounds like flavonoids and phenolics, which act as natural reducing and stabilizing agents. On filtration, a clear extract of about 3 ml of copper nitrate solution is added to initiate nanoparticle formation. The mixture is stirred gently and color change occurs which indicates the reduction of copper ions and then this reaction mixture is centrifuged at 3000 rpm for 10 minutes to separate the copper oxide nanoparticles (pellet) from the supernatant, which is further discarded. Further the pellet is washed with distilled water and centrifuged again to purify the nanoparticles and unreacted compounds and impurities were removed. The final pellet is then dried overnight at 50°C, removing the moisture and stabilizing the nanoparticles and once it is dried, it is carefully collected and stored in airtight containers to prevent contamination. These dried copper oxide nanoparticles are used for characterization and are also tested for their antioxidant and antimicrobial properties which plays a major role in medical and dental application.

2.3. Physico Chemical Characterisation

Scanning Electron Microscopy (SEM) provides high-resolution images to confirm the size, shape, and surface morphology of copper oxide nanoparticles (CuO NPs), which is crucial since smaller, uniform particles with larger surface areas exhibit enhanced antioxidant and antimicrobial activities. Ultraviolet-Visible (UV-Vis) spectroscopy is an analytical technique used to measure the absorbance of ultraviolet or visible light of a substance and help to confirm the formation of nanoparticles and is commonly used to monitor the reduction of copper ions and synthesize the nanoparticles by observing characteristic absorption peaks. Fourier Transform Infrared Spectroscopy (FTIR) is a technique used to identify the functional groups and molecular bonds present on the surface of nanoparticles. The FTIR helps to confirm the role of plant-derived phytochemicals in reducing and stabilizing the nanoparticles and also reveals characteristic absorption bands that correspond to functional groups such as hydroxyl (-OH), carbonyl (C=O), and amine (-NH) groups, which are commonly found in flavonoids, phenols, and proteins from the plant extract.

2.4. Anti-microbial Assay:

The antimicrobial assay was done using the agar well diffusion method where the fresh microorganism culture such as *S. aureus*, *E. coli*, *Enterococcus faecalis* which are bacterial species and *Candida albicans* which is a fungal species were grown and were spread uniformly onto Mueller-Hinton Agar (MHA) for bacteria species and Sabouraud Dextrose Agar (SDA) for fungi species using swabs method to create a lawn of microorganisms. Further the sterile cork borers were then used to create wells by punching into the agar surface. Then two different concentrations such as 25 µL and 100 µL of the green-synthesized magnesium oxide (CuO) nanoparticles from *Carica papaya* peel extract were pipetted into the wells and then the standard antibiotic disc was placed at the center of each plate as a positive control and the plates were then incubated at 37°C for 24 hours for bacterial strains and about 28–30°C for fungal strains. After incubation, zones of inhibition were noted around the wells which indicates antimicrobial activity. The diameter of these zones of inhibition were measured to evaluate the efficiency of the CuO nanoparticles in comparison with the standard antibiotic.

2.5. Anti oxidant assay :

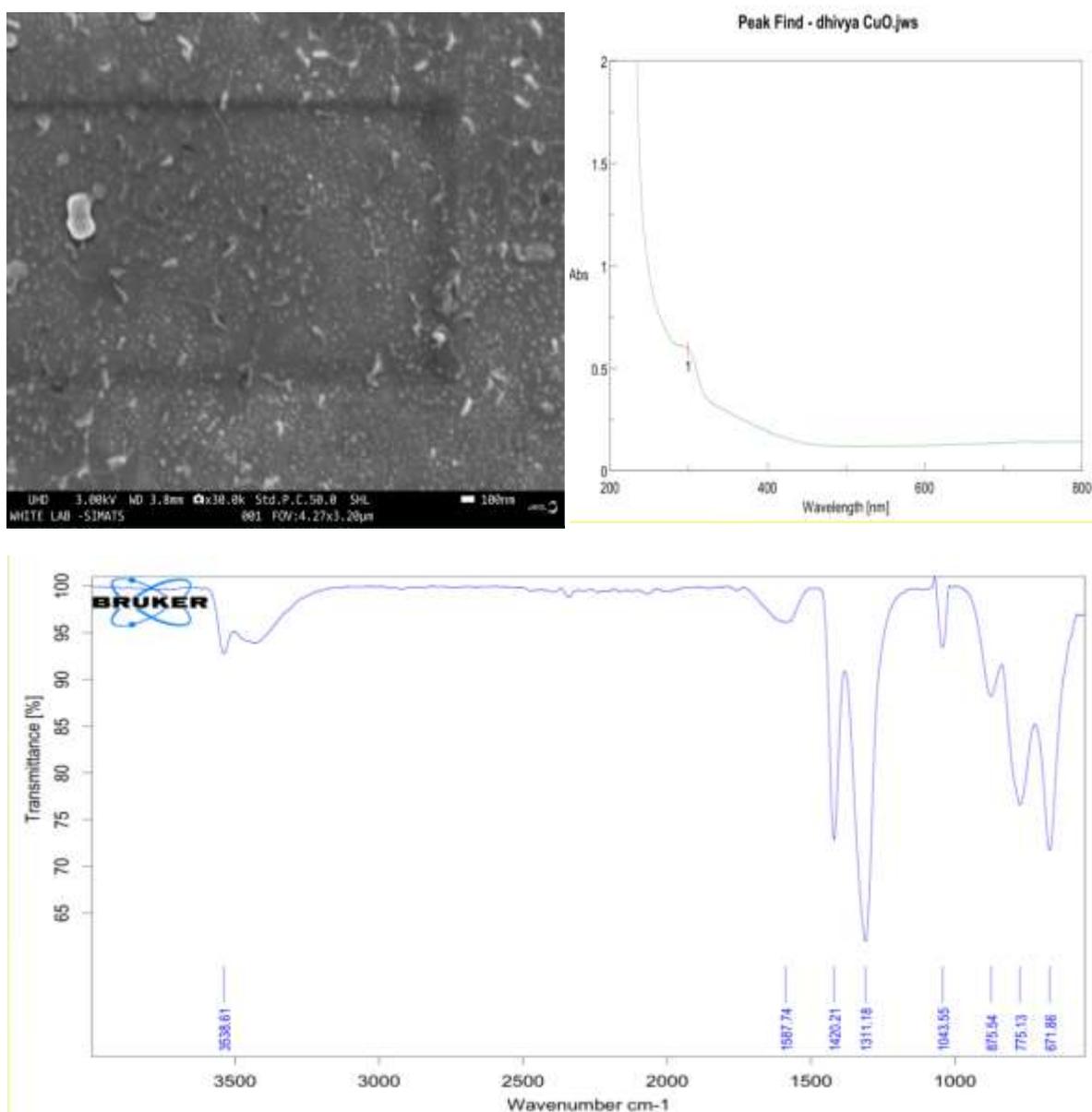
This assay is done using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method. It is used due to its effectiveness in measuring radical-scavenging activity. DPPH is a stable free radical. It also donates electrons or hydrogen atoms to DPPH by reducing it to a colorless or pale yellow compound when antioxidants are present. After which a known concentration of the synthesized CuO nanoparticles is mixed with the DPPH solution and incubated in the dark at room temperature for about 30 minutes and after incubation, the absorbance is measured using a UV-Visible spectrophotometer and compared with a control (DPPH solution without nanoparticles). The formula $[(A_0 - A_1) / A_0] \times 100$ shows that A_0 stands for the absorbance of the control and A_1 is the absorbance of the test sample where high percentage of scavenging shows stronger antioxidant activity. Henceforth the nanoparticles can be effectively used in biomedical applications to prevent oxidative stress-related cellular damage.

3. RESULTS AND DISCUSSION:

3.1. CuO NPs physicochemical properties:

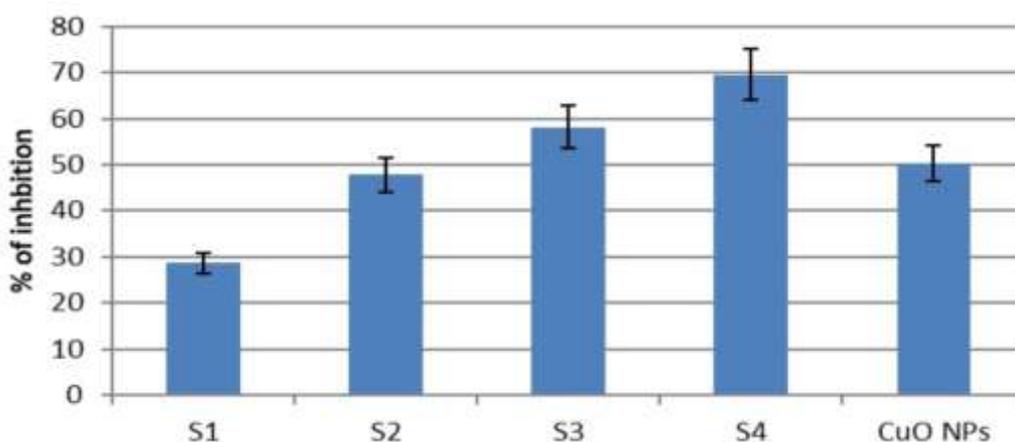
The biosynthesis of CuO nanoparticles using *Carica papaya* peel extract is visually, clearly seen by the conversion from a turbid mixture to a clear supernatant and a dense, dark pellet, culminating in a fine, uniform black powder characteristic

of phase-pure CuO nanoparticles. SEM analysis reveals predominantly spherical, well-dispersed nanoparticles with minimum agglomeration, attributed to phytochemicals in the plant extract that act as reducing and stabilizing agent and this green synthesis matches with studies focused on optimizing physical parameters like precursor concentration and temperature, which improve nanoparticle crystallinity and morphology through controlled thermal and chemical means rather than biological capping[22]. UV-Vis spectroscopy shows CuO formation where there is a sharp surface plasmon resonance peak near 270 nm and that matches with antioxidant potential by enabling reactive oxygen species (ROS) scavenging. FTIR analysis supports these findings, showing broad hydroxyl ($-OH$) bands around $3400-3500\text{ cm}^{-1}$. The peaks for aromatic and carbonyl groups are involved in reduction and stabilization, while decreased wavenumber Cu–O vibrations confirm nanoparticle formation. Compared to other studies, additional functional groups such as C–N and C–H stretching appear, showing the importance of diverse biomolecules contributing to synthesis and bioactivity and together these characterization techniques affirm that plant phytochemicals play a crucial dual role as eco-friendly reducing and capping agents, producing biocompatible, stable CuO nanoparticles with enhanced functional properties[23].



Physicochemical characterization of CuO NPs. (a) SEM image, (b) UV–v spectrum and (c) FT-IR spectra.

3.2. Antioxidant effect of CuONPs



The bar graph represents the antioxidant activity of the samples S1, S2, S3, S4 and copper oxide nanoparticle (measured as percentage inhibition of free radicals), where S4 shows the highest antioxidant activity at nearly 70% followed which CuO NPs demonstrated substantial activity around 50%, suggestive of effective free radical scavenging, indicating strong antioxidant potential is attributed to the phytochemicals from *Carica papaya* peel extract that remain bound to the nanoparticle surface. The antioxidant properties of CuO NPs is directly linked to their antimicrobial action, due to production of reactive oxygen species (ROS), which can damage microbial cell membranes and interfere with essential cellular functions. Henceforth the antioxidant assay highlights not only the oxidative stress-reducing capacity of the CuO NPs but also supports their effectiveness as antimicrobial agents and this aligns with the findings reported by Mashaba et al. (2023), where green-synthesized CuO nanoparticles using *Athrixia phylicoides* extract shows strong antioxidant activity with an IC_{50} value of $10.68 \pm 0.03 \mu\text{g/mL}$ with notable antibacterial effects against *S. aureus* and *B. cereus* having MICs of 0.16 and 0.62 mg/mL, respectively. The connection between antioxidant capacity and antimicrobial activity is reinforced by the role of reactive oxygen species (ROS), which disrupts the microbial membranes and induces cytoplasmic damage, leading to cell death. While Mashaba et al. highlight the eco-friendly and non-toxic nature of biosynthesized CuO NPs, demonstrating low cytotoxicity in HEK-293 cells ($LC_{50} = 66.08 \mu\text{g/mL}$), the current findings similarly support the potential of plant-mediated CuO NPs as dual-functional agents with both antioxidant and antimicrobial efficacy, and further highlight the advantage of green synthesis routes over conventional chemical methods in producing biocompatible nanomaterials[24].

3.3. Antimicrobial activities of CuO NPs



Micro organisms	Antibiotics	25	100
Candida albicans	35mm	13mm	15mm
Staphylococcus aureus	25mm	-	12mm
Enterococcus faecalis	28mm	-	-
Eschericia coli	28mm	11mm	12mm

The table denotes the measure of diameter of zones of inhibition. The standard antibiotics control used showed significantly larger zones of inhibition across all organisms ranging from 25 mm to 35 mm in the four discs and that confirmed their strong antimicrobial activity. The CuO nanoparticles exhibited mild antimicrobial activity that increased slightly with concentration, as we see in the above table in *C. albicans*, the inhibition zones were 13 mm (25 μ L) and 15 mm (100 μ L), for *E. coli*, they were 11 mm and 12 mm, respectively. The *S. aureus* and *E. faecalis*, inhibition was only noticeable at 100 μ L (12 mm), with no observable effect at 25 μ L and this study showed mild antimicrobial properties the copper oxide nanoparticles on increasing the concentration. In contrast, Awasthi et al. (2023) reported significantly stronger antibacterial effects using morphologically distinct CuO nanostructures such as nanorods and multi-armed nanoparticles are synthesised by wet-chemical and electrochemical methods. The CuO NPs shows rapid and broad-spectrum bactericidal activity, where about 91 - 99.99% bacterial cell killing (including *B. anthracis* and *E. coli*) happens in just 30 minutes at concentrations as low as 0.5–2 mg/mL. They also achieved complete bacterial inactivation (100%) under optimized conditions and outperformed bulk CuO, demonstrating a 99.55% reduction in viable cells compared to 65.71% with the bulk counterpart. These factors attributed to the dual mechanism which involves mechanical disruption by sharp nanoparticle morphology and oxidative stress induced by Cu^{2+} ion release. While our study highlights the safer, eco-friendly synthesis and moderate antimicrobial efficiency of CuO NPs derived from *Carica papaya* peel, which underscores the critical impact of nanoparticle morphology, concentration, and synthetic method on antimicrobial performance[25].

4. CONCLUSION:

The present study successfully demonstrates the green synthesis of copper oxide nanoparticles (CuO NPs) using *Carica papaya* peel extract, offering an eco-friendly and cost-effective approach to nanoparticle production. Characterization techniques confirmed the formation, morphology, and functionalization of the nanoparticles, while biological assays revealed significant antioxidant and antimicrobial activities. The strong antioxidant potential is attributed to the phytochemicals from the papaya peel, which aid in free radical scavenging, while the antimicrobial effects against selected pathogens highlight their ability to disrupt microbial growth. Overall, the study suggests that CuO NPs synthesized through this green method have promising applications in biomedical and dental fields. Further research is needed to explore their in vivo safety, cytotoxicity, and practical therapeutic uses.

Credit authorship contribution statement

Dhivya Sarathi : Writing – original draft, Data curation, Conceptualization. Rajalakshmanan Eswaramoorthy: Investigation, Data curation. Saranya : Investigation, Data curation. Palanivel Sathishkumar: Writing– review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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