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Article **Biomedical Science**

BIO-SYNTHESIS OF CuONPs USING *Punica granatum* PEEL EXTRACT AND EVALUATION OF ANTIMICROBIAL ACTIVITY

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ABSTRACT

Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering. Therefore, much effort is being made towards exploiting natural resources and implementing biological synthesis methods with proven advantages, such as being environmentally friendly, easy to scale up, and cost-effective; thus, the green production of nanoparticles using biological resources has great potential. The biological route of synthesizing nanoparticles has many advantages, such as the stable production of nanoparticles with controlled sizes and shapes, the lack of subsequent complex chemical synthesis, the lack of toxic contaminants, and the ability for rapid synthesis using numerous medicinal plants. Overall, *Punica granatum* peel have biomolecules and bioreducing agents such as phytochemical that provide a versatile, economical, and eco-friendly method to fabricate CuONPs. The synthesized CuONPs using *Punica granatum* peel profound effect of antimicrobial growth and may use in the biomedical and industrial fields.

Keywords: *Punica granatum* peel, Phytochemicals, Nanoparticles, Antimicrobial activity

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INTRODUCTION

Nanotechnology focuses on the development of novel, functional materials at an atomic, molecular, and macromolecular scale at sizes ranging from 1 to 100 nm. In recent years, studies related to the synthesis of nanomaterials and nanoparticles with unique and desired properties have attracted growing attention due to their small size, multifucntionality, surface tailorability, and biocompatibility. These features have led to increasing numbers of applications in which nanomaterials for various branches of science, industry, and daily life have emerged; however their use in biotechnology is of particular importance. This rapidly growing, cross-disciplinary field of science gives the possibility to design and develop novel solutions that include application of multifunctional nanoparticles in biomedicine, cancer diagnosis, the food industry, or heterogenous catalysts as well as support for biocatalysts. The widespread application of nanomaterials acts as a driving force for research and development of nanomaterials-based systems in biotechnology.

Green synthesis of nanoparticles aims at minimizing generated waste and implementing sustainable processes. In recent years, green processes using mild reaction conditions and nontoxic precursors have been emphasized in the development of nanotechnology for promoting environmental sustainability. The green synthesis of nanoparticles refers to three main conditions: environmental solvents, good reducing agents, and harmless material for stabilization (Jadoun *et al.*, 2021). Green synthesized metallic nanoparticles are gaining attention because of biologically active plant secondary metabolites (Hemlata, Meena *et al.*, 2020), microbes (Ghosh *et al.*, 2021), and animal secondary metabolites (Karnan *et al.*, 2023b; 2023c and 2023d) that help in green synthesis. Plant-extracted metabolites that can act as reducing and stabilizing agents for the synthesis of nanoparticles are highly dependent on plant species, plant parts (root, stem, leaf, seed, etc.). Nanoparticles (NPs) have various considerable implications, especially in agriculture, biomedical engineering, and environmental remediation techniques, making it critical to evaluate their role in the environment and plant species.

The aim of present training is to synthesis, characterization of metallic nanoparticle using *Punica granatum* (மாதுளை/Pomegranate) peel extract and evaluation of *in vitro* antimicrobial activity.

MATERIALS AND METHODS

Collection of plant materials

The plant materials were collected from Thanjavur on May 2025. The collected plant parts were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture.

Preparation of extract and Screening of phytochemicals

1 gram of the powder of *Punica granatum* peel were transferred in to different conical flask (250ml). The conical flask containing 50ml of aqueous solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984).

Synthesis of copper oxide nanoparticles using plant extract

Synthesis of Copper oxide nanoparticles using *Punica granatum* peel aqueous extract (Ghidan *et al.*, 2016). The copper acetate monohydrate (0.03M) and stirred magnetically for 5 min at room temperature. Afterwards, added drop wise *Punica granatum* peel extract under stirring as soon as the extract comes in contact with copper ions to change the colour from blue to green color. After 10 min, the formation of water soluble monodispersed copper oxide nanoparticles were observed Characterization of Nanoparticles.

Determination of antimicrobial activity

Antibiogram was done by well diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007).

RESULTS AND DISCUSSION

Phytochemicals screening

Phytochemicals are mainly plant extracts (essential oils, oleoresins and flavonoids) and their active principles. The main mode of action consists in controlling pathogen contamination and modulating the intestinal microbiota, although it has also been demonstrated to exhibit **antimicrobial, antiviral**, anticoccidial, fungicidal and/or antioxidant properties. The most common phytochemicals include polyphenols, carotenoids, flavonoids, coumarins, indoles, isoflavones, lignans, organosulfures, catechins, phenolic acids, stilbenoids, isothiocyanates, saponins, procyanidins, phenylpropanoids, anthraquinones, ginsenosides, and so on (Zhao *et al.* 2018a). Plants are the basis for the development of modern drugs for many years in daily life to treat diseases all over the world. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides, many pharmaceutical drugs and also used phytochemicals mediated metallic nanoparticles synthesis. Table 1 shows the presence of secondary metabolites in *Punica granatum* peel aqueous extract and significant amounts of total phenol (216.80 mg/gm), and flavonoids (120.00 mg/gm) were repented. Histochemical behavior of *Punica granatum* peel powder were summarized in figure 2 and in this results further confirmed the presence of phytochemicals.

Table 1: Qualitative analysis of phytochemicals in *Punica granatum* peel aqueous extract

S. No	Phytochemicals	Extract
1	Saponin	++
2	Flavonoids	++
3	Steroids	++
4	Terpenoids	++
5	Alkaloids	+
6	Polyphenol	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

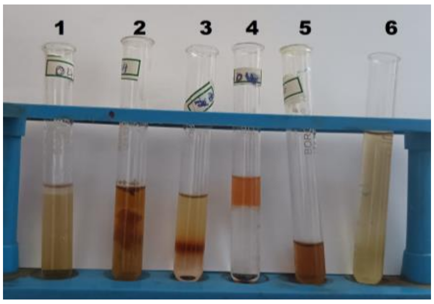
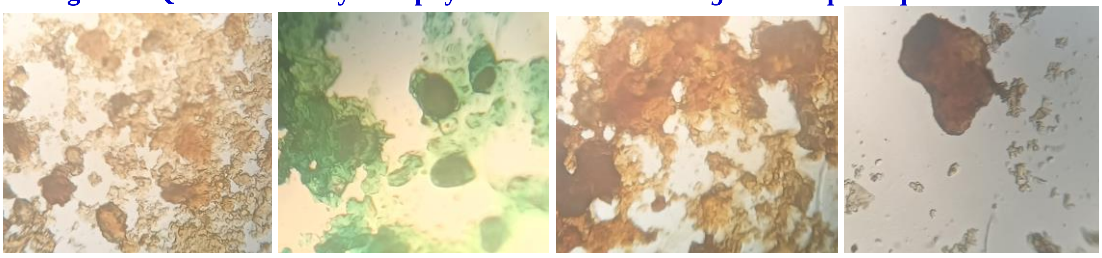


Figure 1: Qualitative analysis of phytochemicals in *Punica granatum* peel aqueous extract



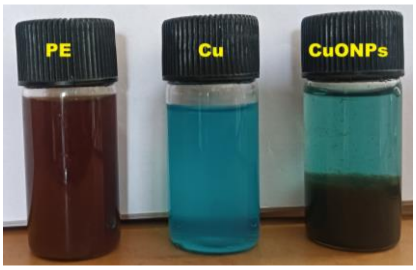
Flavonoids **Polyphenol** **Terpenoids** **Alkaloids**

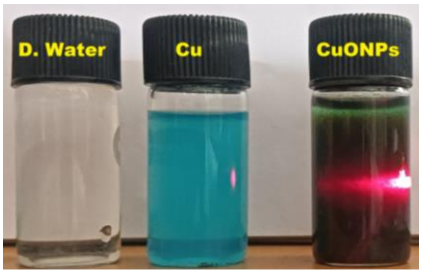
Figure 2: Histochemical examine the *Punica granatum* peel

Green synthesis of NPs

Plant extract is mixed with metal precursor solutions to synthesis of nanoparticle at different reaction conditions (Mittal *et al*., 2013). The parameters determining the conditions of the plant leaf extract (such as types of phytochemicals, phytochemical concentration, metal salt concentration, pH, and temperature) are admitted to control the rate of nanoparticle formation as well as their yield and stability (Dwivedi and Gopal, 2010). Therefore, plant extracts are considered to be an excellent and benign source for metal as well as metal oxide nanoparticle synthesis. Additionally, plant extract play a dual role by acting as both reducing and stabilizing agents in nanoparticles synthesis process to facilitate nanoparticles synthesis (Malik *et al*., 2014). The composition of the plant extract is also an important factor in nanoparticle synthesis, for example different plants comprise varying concentration levels of phytochemicals (Mukunthan *et al*., 2012). The main phytochemicals present in plants are flavones, terpenoids, sugars, ketones, aldehydes, carboxylic acids, and amides, which are responsible for bio reduction of nanoparticles (Prathna, 2010).

Aqueous extract were used to synthesis CuONPs. The copper acetate solution was incubated with *Punica granatum* peel extract to formation of green colour and brownish precipitate, whereas copper acetate solution without plant extract showed no colour change was observed. The presence of green colour in *Punica granatum* peel extract is a clear indication for CuONPs formation. (Figure 3). In the UV–Vis spectra of the reaction mixture of copper acetate solution with *Punica granatum* peel extract the peak was observed at 345nm guajavating the presence of copper oxide nanoparticles which is synthesized by *Punica granatum* peel extract (Figure 4). The synthesized CuONPs revealed the presence of alcohol, phenol, amines, aldehydes, and carboxylic acid functional groups, which, in response to reducing agents present in *Punica granatum* peel extract, resulted in the formation of phytochemical-mediated CuONPs. The Tyndall effect is the phenomenon where a beam of light is scattered by nano-particles in a colloid or a very fine suspension (Figure 3).





CuONPs synthesis **CuONPs Tyndall effect**

Figure 3: CuONPs synthesis using *Punica granatum* peel aqueous extract

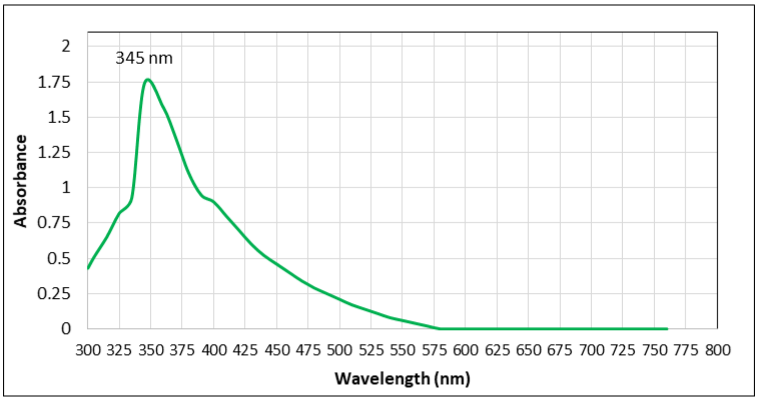
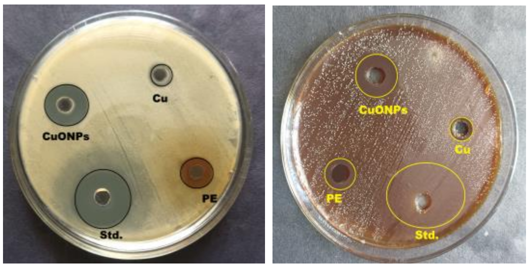


Figure 4: UV-visible characterization of CuONPs

Evaluation of antimicrobial activity of synthesized CuONPs

Pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Metallic nanoparticles have unique antimicrobial properties that make them suitable for use within medical and pharmaceutical devices to prevent the spread of infection in healthcare. Therefore, the aim of this training was synthesis of CuONPs from *Punica granatum* peel aqueous and evaluation of antimicrobial activity against *E. coli* and *Candida albicans.* In the present study evaluation of antimicrobial activity of *Punica granatum* peel aqueous extract (12.00nm and 9.50nm), copper acetate (7.00nm and 0.50nm), and CuONPs (14.00nm and 13.50nm) were evaluated, CuONPs was highly active properties were reported compare with copper acetate and peel extract against both microbial strains while bacterial standard of Chloramphenicol (24.00nm) and Fluconazole (30.00nm) for fungai, represent in figure 5.



E. coli ***Candida albicans***

Figure 5: Evaluation of antimicrobial activity of synthesized CuONPs using *Punica granatum* peel aqueous extract against microbial pathogen

SUMMARY AND CONCLUSION

Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, **biology**, physics, materials science, and engineering. Therefore, much effort is being made towards exploiting natural resources and implementing biological synthesis methods with proven advantages, such as being environmentally friendly, easy to scale up, and cost-effective; thus, the green production of nanoparticles using biological resources has great potential. The biological route of synthesizing nanoparticles has many advantages, such as the stable production of nanoparticles with controlled sizes and shapes, the lack of subsequent complex chemical synthesis, the lack of toxic contaminants, and the ability for rapid synthesis using numerous medicinal plants.

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