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GREEN SYNTHESIS OF COPPER NANOPARTICLES BY USING FLOWERS OF *PONGAMIA GLABRA* AND EVALUATION OF ITS FLUORIDE REMOVAL EFFICACY IN GROUND WATER: SPECIAL REFERENCE TO TIRUPATTUR REGION

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ABSTARCT

Dental fluorosis is one of the predominant health problems among peoples consume fluoride contaminated well water in rural areas of Tirupattur district, Tamil Nadu. Hence, present study was aimed to synthesis the copper nanoparticles (CuNPs) by using flower extract of *Pongamia glabra* and evaluation of its fluoride removal efficacy in ground water collected from regions near Tirupattur, because CuNPs attained much attention by their diverse applications. CuNPs were synthesised by molar ratio method and structure and morphology of the CuNPs were characterized by various spectral studies such as UV, FT-IR, SEM and DLS. Agar well diffusion method was followed to analyse the antimicrobial effect of CuNPs. IS 3025 (Part 60) 2008 method was followed to measure the fluoride quantity in collected ground waters. The UV-Vis measurement showed the peak at 340-360 nm, whereas FTIR characteristic peaks of metal-oxygen (Cu–O) were confirmed in the range 506 cm^{-1} and Cu–O–H bonds led to bending absorptions in the region 875 cm^{-1}. The reduction (3450 cm^{-1} to 3424 cm^{-1}) in the absorption band reveals the formation of CuNPs. The synthesized CuNPs possess the spherical shapes and high content of copper as confirmed by SEM. The synthesised copper nanoparticles have relatively good antimicrobial property by inhibiting the wide spectrum of microbes such as several pathogenic Gram positive and Gram-negative bacteria as well as the growth of some fungi.

After treatment with CuNPs in the concentration of 1 mg/100ml, the high fluoride content in the ground water reduced significantly by the reduction of 85-95 % levels. The results revealed that CuNPs can be used as a potent source to reduce the fluoride content in drinking ground water; thereby we may suppress the effects of dental fluorosis in the affected regions.

Keywords: *Dental fluorosis; copper nanoparticles; Pongamia glabra; spectral studies; antimicrobial property.*

1. INTRODUCTION

Nearly 85% of India's population depends on groundwater for drinking; groundwater quality is a significant issue that needs to be addressed individually. Without a thorough risk evaluation, groundwater in India was taken for granted to be generally safe and often utilized for drinking. This view runs counter to several studies that show how different anthropogenic and geo-genic factors may contaminate groundwater. One of the main issues that is becoming increasingly evident and has been demonstrated to have negative consequences on all continents in the world is fluoride pollution of groundwater. Numerous studies have brought attention to the detrimental effects of fluoride on human health, including skeletal and dental fluorosis, which has major socioeconomic repercussions. Fluoride pollution of groundwater is defined as concentrations of fluoride beyond 1.5 mg/L produced by either geogenic or anthropogenic means. Fluoride levels above WHO standard limits were observed worldwide, with the majority of the higher concentrations being restricted to arid and semi-arid regions. The WHO's maximum permissible limit of fluoride in drinking water meets the national standard limit and is limited to 1.5 mg/L.

Depending on the dosage found, fluoride in drinking water can impact human health in a range of ways. As stated by Raju (2017), minimal levels of fluoride in drinking water (0.4 to 1.5 mg/L) encourage normal bone mineralization and tooth enamel calcification; however, there is a risk of dental fluorosis between 1.5 and 4 mg/L, skeletal fluorosis between 4 and 10 mg/L, and advanced fluorosis (crippling fluorosis) above 10 mg/L [Wei *et al.,* 2019]. Studies carried out in a number of countries have demonstrated that 65% of endemic fluorosis occurrences are caused by drinking water compromised with fluoride. Furthermore, there have been reports of additional negative effects, which include cancer, neurological and digestive disorders triggered on by the stomach's production of hydrofluoric acid (HF), decreased hemoglobin levels, lowered immunity, respiratory and urinary disorders, female sterility, and Alzheimer's syndrome [Rashid *et al.,* 2010].

Thus, there is a need to create an economical and environmentally benign technique for removing or lowering the amount of fluoride in groundwater. Due to their potential uses in water purification, biosynthesized nanoparticles (NPs) have garnered a lot of attention lately. Various plant species and their byproducts have been effectively employed in the production of various green nanoparticles, including those of zinc oxide, platinum, palladium, silver, cobalt, magnetic, and gold.

Though several studies on the synthesis of CuNPs using various plant extracts have been published, there hasn't been much research done on the use of CuNPs in the treatment of water purification. Due to their numerous uses in the fields of chemical, biological, and environmental sciences, nanomaterials are quite interesting these days [Bordbar *et al.,* 2018]. Numerous uses for the NPs were demonstrated, such as optical, electrical, thermal conductivity, antioxidant, antibacterial, and anticancer properties, as well as catalysts. Because of its catalytic, high electrical conductivity, optical, antifungal, and antibacterial qualities, CuNPs are the NPs that are receiving the most attention. Much emphasis has been paid to the synthesis of nanoparticles (NPs) because of their special physical and chemical properties, which are not shared by bulk materials [Fernández-Arias *et al.,* 2020]. Several techniques, including chemical, biological, and physical ones, were employed in recent years to synthesize nanoparticles [Van Viet *et al.,* 2016]. Researchers were drawn to the biological approach of NPs synthesis because it is straight forward, non-toxic, and environmentally favorable compared to chemical and physical methods. The biological approach of synthesizing nanoparticles (NPs) using a variety of sources, including plant extracts, bacteria, fungi, actinomycetes, yeast, and algae. Phytochemicals, which serve as reducing, capping, and stabilizing agents for the production of nanoparticles, are found in plants and include flavonoids, polyphenols, alkaloids, terpenoids, saponins, vitamins, and proteins [Sankar *et al.,* 2014]. Based on the previously mentioned data, we designed a method to synthesize copper nanoparticles using *Pongamia glabra* flowers, and evaluation of its antimicrobial and fluoride reducing potential in ground water collected from various regions of Tirupattur.

2. MATERIALS ANS METHODS:

2.1. Chemicals

Copper sulphate, Nutrient broth, Muller Hinton agar, agar-agar, Sabouraud Dextrose Agar (SDA) was purchased from Hi media, Mumbai.

2.2. Collection and Preparation of flower extract from *Pongamia glabra*

Fresh flowers of *Pongamia glabra* was collected from trees near Vaniyambadi regions, Tirupattur Dist. The flowers are then washed and shade dried for one week, following this, the dried flowers are freeze dried to obtain a constant mass which is then grind finely and extract was prepared with sterile distilled water by overnight maceration method.

2.3. Synthesis of CuNPs by using plant flower extract

The aqueous extract of *Pongamia glabra* mixed with the freshly prepared 0.2 M copper sulphate solution in a ratio of 1:10. The pH of the resultant mixture was adjusted to 7.0 by adding 1N NaOH solution. With the constant stirring, the solution is heated for at least 4 hours at 55°C until a colour change from blue to sea green is observed. The reduced solution will be further centrifuged for 15 mins at room temperature (5000 rpm), washed and stored at 4°C until further use. (Sankar R. *et al.,* 2014)

2.4. Characterization of CuNPs

Synthesized copper nanoparticles were characterized by UV visible spectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and Dynamic light scattering (DLS). (Santhoshkumar J *et al.,*2019)

2.5. Antimicrobial activity of CuNPs

2.5.1. Antibacterial activity

The pathogenic bacterial strains *Bacillus subtilis, Enterococcus faecalis, Streptococcus agalactiae, Salmonella enterica, Pseudomonas aeruginosa* and *Proteus mirabilis* were evaluated against the synthesized nanoparticles. An analysis of antibacterial activity was conducted using the conventional agar well diffusion method. Using 2% DMSO (dimethyl sulfoxide), different quantities of the CuNPs (75, 100, and 150 µg/ml) were generated. Using the spread plate approach, 10 µl of 24-hour test cultures of bacterial strains were placed onto the appropriate Mueller-Hinton agar media. The positive control was administered with vancomycin and gentamycin. After that, the plates were incubated for 24 hours at 37°C. To measure the antibacterial activity, the diameter of the zone of inhibition surrounding the well was measured.

2.5.2. Antifungal activity

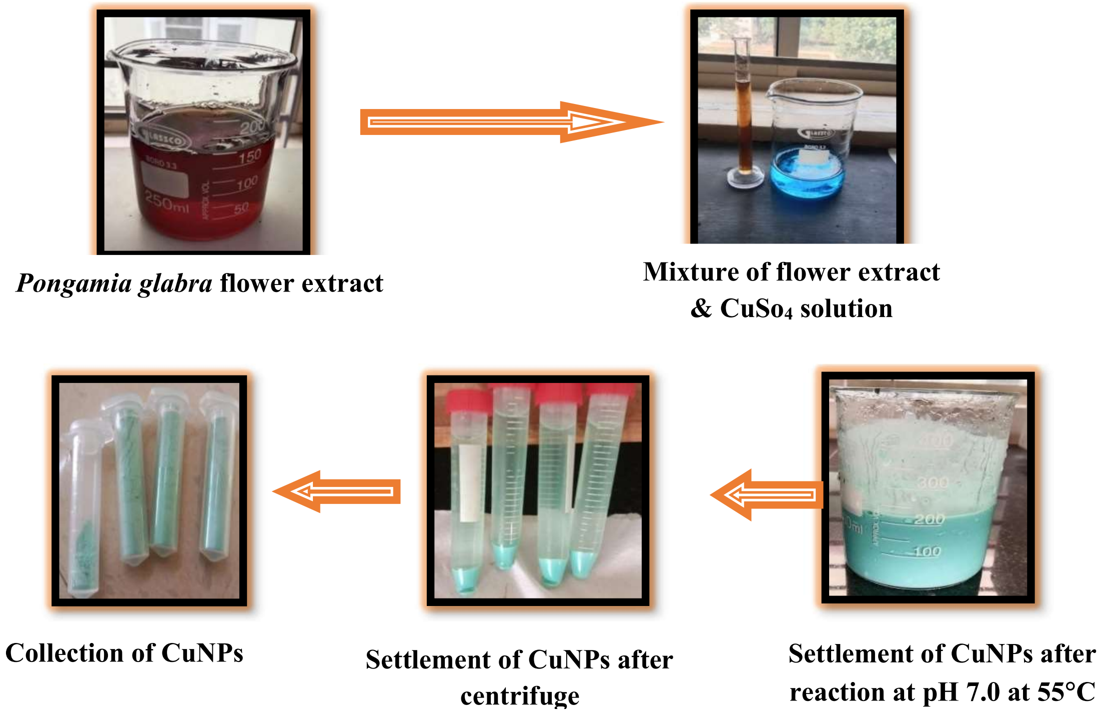
Using the conventional agar well diffusion method, antifungal activity was examined. Different concentrations of CuNPs (25, 50, 75, 100, and 150 µg/ml) were made with 2% dimethyl sulphoxide (DMSO). Using the spread plate approach, 10µl of 72-hour test cultures, *Candida albicans*, were placed onto the appropriate Sabouraud dextrose agar medium. After that, the plates were incubated for 24 hours at 37°C. By measuring the diameter of the zone of inhibition that formed around the well, the antifungal activity was examined.

2.6. Determination of fluoride removal efficacy

Fluoride reducing potential of CuNPs were evaluated by IS 3025- P 60 (2008); an electrochemical technique is directly suitable for measuring fluoride content in water samples.

3. RESULTS AND DISCUSSION:

The copper nanoparticle synthesized by molar ratio method was obtained as a sea green coloured powder after vacuum evaporation and the yield. The schematic illustration of complex synthesis is shown in scheme 1. The UV-Visible spectra of flower extract and CuNPs were depicted in Figure 1. UV–Visible analysis shows a characteristic peak around 360 nm for copper nanoparticles. The IR spectral data of plant extract and copper nanoparticle is shown in Figure 2 [IR (KBr, vcm^{-1}). In order to study the binding mode of copper with plant extract, IR spectrum of the plant extract was compared with the IR spectrum of the copper nanoparticle. (Hussain Imtiyaz *et al.,* 2015)



Scheme 1: Schematic representation of CuNPs Synthesis

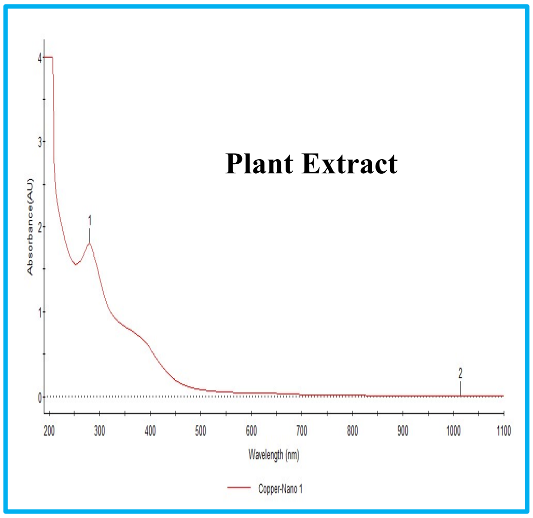


Figure 1: UV-Visible Spectra of Flower extract and CuNPs

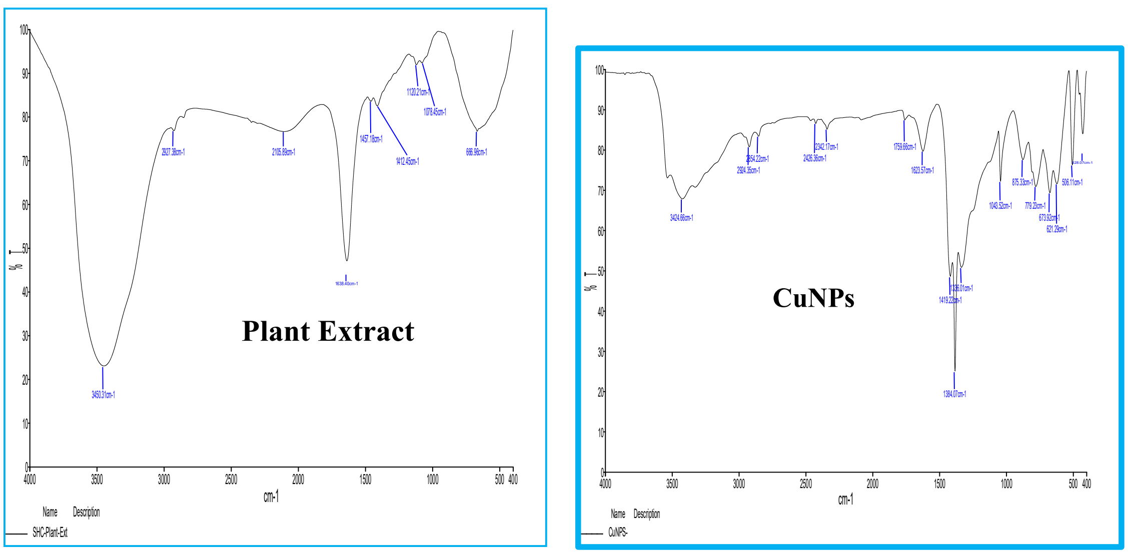


Figure 2: FT-IR Spectra of Flower Extract and CuNPs

Plant extract shows absorption bands at 3450 [–OH], 1638 (C=O)] whereas CuNPs these bands underwent a lower frequency [3421 and 1623 vcm^{-1}] after complexation, indicating the coordination of the hydroxyl group and carbonyl group with the copper ion (Shard *et al*., 2019). The nature of copper and flower extract bonding was further confirmed by a newly formed band at 506 cm$^{–1}$ in the IR spectrum of the nanoparticle which is tentatively assigned to M–O vibration. In both plant extract and CuNPs, the peaks around 2924 cm^{-1} and 1419 cm^{-1}are due the presence of C-H and S=O stretching, respectively. The morphology of CuNPs was evaluated by SEM (Figure 3). SEM analysis showed that the synthesized CuNPs were spherical and tended to form a random aggregate. (Singh Ravina *et al.,* 2011)

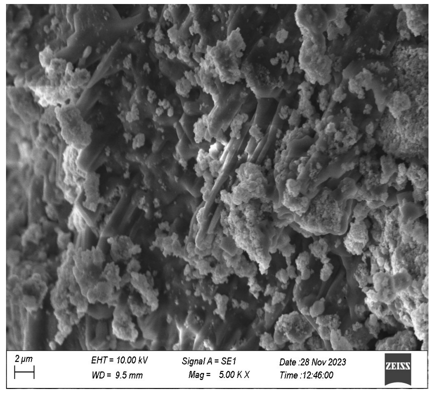


Figure 3: Scanning Electron Microscopy (SEM) of CuNPs

The antibacterial activity of CuNPs was evaluated against six bacterial stains—three of which were gram positive and three of which were gram negative—using the agar well diffusion method was depicted in Figure 4 and Table 1. *Bacillus subtilis, Enterococcus faecalis, Streptococcus agalactiae, Salmonella enterica, Pseudomonas aeruginosa* and *Proteus mirabilis* were tested to determine the antibacterial activity of synthesized nanoparticles. CuNP was tested at three different concentrations (75, 100, and 150 µg/ml) against all pathogenic bacteria. A strong zone of inhibition in the CuNP is indicated by the growing concentration gradients. The zones of inhibition for the three additional concentrations (75, 100, and 150µg/mL) of copper nanoparticles are larger than those for the positive control gentamycin and Vancomycin in all six organisms. However, the copper nanoparticles exhibit a discernible zone of inhibition, which is suitable to demonstrate its antibacterial activity. (NCCLS, 1997)

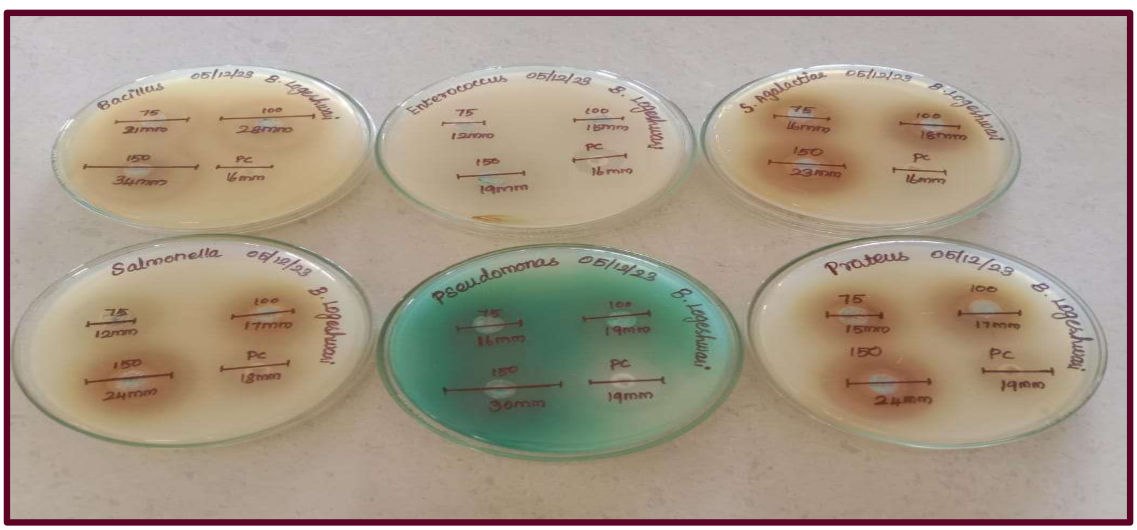


Figure 4: Antibacterial activity of CuNPs

Table 1: Antibacterial activity of CuNPs (Zone of Inhibition)

	Organisms		Concentration in µg/ml

S. No.		Positive control (mm) Vancomycin - V Gentamycin - G	75	100	150
			Zone of Inhibition (mm)		
1	*Bacillus subtilis*	V - 16	21	28	34
2	*Enterococcus faecalis*	V- 16	12	15	19
3	*Streptococcus agalactiae*	V -16	16	18	23
4	*Salmonella enteric*	G - 18	12	17	24
5	*Pseudomonas aeruginosa*	G - 19	16	19	30
6	*Proteus mirabilis*	G - 19	15	17	24

SDA agar medium was used for assessing the degree of inhibition against *Candida albicans* at various concentrations (25, 50, 75 µg/mL) and (50, 100, 100 µg/mL) in order to evaluate the fungicidal characteristics of CuNPs, which was depicted in Figure 5. The CuNPs effectively inhibit *Candida albicans* from growing. Still, when the concentration gradient rises, an increasing area of inhibition is observed. CuNPs thus provide a significant zone of inhibition. 75 µg/mL of CuNPs exhibit 26mm of zone of inhibition whereas 150 µg/mL shows 32mm inhibition zone against *Candida albicans* (Schlumbaum *et al.,* 1986)

Figure 5: Antifungal activity of CuNPs

The fluoride removal efficacy of the synthesised nanoparticles were determined by Indian standard method (IS 3025- P 60 (2008) is depicted in table 2. The flouride content of water samples collected from different regions near tirupattur were tested before and after treatment with synthesised copper nanoparticels. Before the treatment the water samples contains very high amount of flouride but after the teratment with CuNPs the level of flouride content reduced significntly. The water samples collected from Jaklgambarai and Vaniyambadi possess 6.65 g and 3.05 g of flouride in 100 ml, respectively, but after treatment there is an redution of 94% and 85% in flouride levels.

4. Conclusion

The present study reveals the formation of Nanoparticle between Copper and flower extract of Pongamia glabra and its characterization by various spectral studies like UV, FT-IR, and SEM Analysis. The findings of the present study exemplifies that the antibacterial and antifungal activity of CuNPs against various human pathogenic microbes. In addition, the synthesized Nanoparticle treatment leads to reduce the fluoride content in drinking water. These findings suggest that copper nanoparticles synthesized by using *Pongamia glabra* flowers has complimentary potency to develop as an antimicrobial drug for the treatment of various microbial infections and it can be used as a potent agent to remove the fluoride content in the regions which has high level in drinking ground water.

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