Biofabricated Copper/Gelatin Nanocomposite from *Pithecellobium dulce* Leaves: Effective Catalyst for Dye Degradation

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Abstract
Pithecellobium dulce is a plant of many uses which has a versatile role in the traditional system of medicine. In the present study, antimicrobial activity and photocatalytic activity of copper nanocomposite were checked. Copper nanoparticle was synthesized using aqueous extract of Pithecellobium dulce and the gelatin hydrogel was prepared. The structure of gelatin hydrogel and synthesized copper nanoparticle was characterized by Scanning electron microscopy and Fourier transform infrared spectroscopy. Antimicrobial activity of copper nanoparticle was checked by well diffusion method. The prepared gelatin hydrogel copper nanocomposite was checked for its effect against dye degradation. Synthesised copper nanoparticle was found more effective against B. subtilis than towards E. coli and K. pneumonia.

Photocatalytic dye degradation of Cu/hydrogel composite was 25% and 95% against congo red and methylene blue dye respectively. Copper gelatin nanocomposite showed better photocatalytic decolourization of methylene blue dye and followed pseudo first order kinetics. The results of this study will be helpful in understanding the mechanism of dye degradation through composite materials and will help in the development of an efficient technique for degradation and decolourization of dye existing in industrial wastewater.

Keywords: Copper nanoparticle, Gelatin hydrogel, Copper nanocomposite, Photocatalytic degradation.

Introduction

Hydrogels are water soluble polymers having ability to hold water content. Due to its water holding capacity, it is highly used to simulate natural living tissue than any other non-natural biomaterial. When compared with other biomaterials, hydrogels are notable for its increased biocompatibility, appropriate porous structure etc. But the mechanical property of the hydrogel is the required focus to improve its utilization in various fields[5]. Hydrogels are either from natural polymers or from synthetic polymers. Also hydrogels based on their cross-linking are differentiated as reverse or physical hydrogel and chemically cross-linked hydrogel. Various methods reported for physically cross-linked hydrogels include hydrogen bonding, ionic interaction, freeze thawing etc. Similarly, methods like grafting, condensation reaction, enzymatic reaction etc. are followed in chemical cross-linked hydrogel preparation[25]. Hydrogels are having applications in different fields like medical (drug delivery, wound healing, vaccine etc.), environmental (retaining water) and domestic (creams, perfumes, water beads for plants etc.)[3]. Gelatin based hydrogels are commonly from scale, bones and different parts of animal source. Major benefit of gelatin hydrogel is its high carbon content, biocompatibility and biodegradability.

Gelatin is the degradative product of collagen. Based on the processing method followed for gelatin formation from collagen, 2 types of gelatin are common namely: type A (acid treatment) and type B (alkali treatment). Gelatin based hydrogels have applications in the manufacture of contact lens, scaffolds in tissue engineering and drug delivery system[17]. Nanocomposites have gained importance in various field due to their high surface area to volume ratio, mechanical strength, stiffness, thermal conductivity etc. Gelatin based copper nanocomposite was reported by Bakravi et al[4] as an effective drug delivery agent. Thakur et al[26] review report also highlighted the gelatin based nanocomposites in water purification and in various other biomedical application.

Pithecellobium dulce is commonly called as Manila Tamarind. All the parts of this plant are found to be utilized in cooking and also for treating various ailments[11]. *Pithecellobium dulce* is one among the various plants to be notable for its medicinal property. Roots, fruits, bark and leaves of this plant were studied for their antioxidant, antimicrobial, anti-inflammatory activity etc.[15] Nanoparticles have wide application in drug delivery, disease treatment and various biological functions. All the properties of nanoparticles depend on its size, shape, composition surface etc. Leaves of *Pithecellobium dulce* were used in various nanoparticle synthesis like gold[8], silver[12], zinc oxide[18], titanium dioxide[10] and Carbon nanodots[13]. In this present study, copper nanocomposite of gelatin was checked for its photocatalytic degradation activity.

Material and Methods

Exraction of *Pithecellobium dulce* leaves: *Pithecellobium dulce* leaves were collected, dried and grinded into powder. 25 g of leaf powder was mixed with 250 ml distilled water, then the extraction (cold maceration) was carried for a period of 5 days period by occasional shaking and was then filtered.

The filtrate was allowed to dry by evaporating the solvent[9]. Extract obtained (PdAE) was stored for further use.

Determination of total phenolic content: Total phenol content was determined by pipetting 1ml of Folin-Ciocalteu reagent (which was prepared in the ratio of 1:10 with water) and 1 ml of the extract. After 3 min, to the above mixture, 1ml of saturated sodium carbonate (7%) was added and final volume was made up to 10 ml by adding deionised distilled water. Then the content in the tube was kept for 120 min at room temperature in the dark. The absorbance was measured at 760 nm against the blank. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g of dry extract.

Determination of Flavonoid: Flavonoid content in the extract was determined by mixing 500µl of the extract with 1ml of aluminium chloride (10%), same volume of potassium acetate and 2.5ml of distilled water. Quercetin was used as standard and the absorbance was measured at 415nm. Flavonoid content was expressed as mg of QE/g of dry extract[23].

Green synthesis of copper nanoparticles: Copper nanoparticle was synthesized by using 1millimolar copper sulphate as precursor. 10ml of aqueous extract of *P.dulce* was mixed with 100ml of the copper precursor and mixed well. Wait for colour change (24 hours). Then the synthesised nanoparticle (PdCu) was characterized by UV-spectroscopy, FTIR and SEM analysis.

Hydrogel (Hg) preparation: 5g of gelatin was mixed with 500ml of distilled water and stirred for 30 mins using a magnetic stirrer until it was completely dissolved to form hydrogel solution (Hg) at 50°C [4].

Preparation of copper hydrogel nanocomposite: Nanocomposite gelatin hydrogel (PdCuHg) was prepared by mixing the copper nanoparticle with concentration of 0.09g of copper sulphate into 100 ml of Hg solution. Without adding copper nanoparticle was maintained as control (Hg). All were mixed for 30 mins using a magnetic stirrer[4].

UV-Spectroscopy and FTIR analysis: UV- absorption of synthesised copper nanoparticle was observed and FTIR analysis was recorded using KBr pellets in the range of 400–4000 cm^{-1}. In FTIR analysis, functional groups of the synthesized nanoparticle and gelatin hydrogel were studied[1].

Scanning electron microscope (SEM): SEM analysis is the commonly used procedure to analyse the morphology of nanoparticles and nanostructures. Synthesised copper nanoparticle and gelatin hydrogel morphology were analysed by using SEM[22].

Antimicrobial activity of Well diffusion method: The present study scientifically validates the antimicrobial potential of the copper nanoparticle synthesized using *Pithecellobium dulce,* gelatin hydrogel and copper gelatin nanocomposite. The nutrient agar media were prepared and poured into the Petri plate. Then the bacterial lawn culture was made with 24hrs old culture of *B. subtilis, E. coli, K. pneumonia* and *B. cereus.* Four wells were punctured in each plate. Add copper nanocomposite (50 μg/ml, 75μg/ml and 100 μg/ml) and control well was also maintained. The plates were incubated at room temperature. Next day the zone of inhibition was measured and recorded[19].

Photocatalytic degradation of dye: Photocatalytic activity of copper gelatin nanocomposite was determined by adding the prepared nanocomposite to MB (Methylene Blue-100microgram/ml) and CR (Congo red- 100microgram/ml) dye and kept under sunlight for a period of 6 hours. Initial and final readings of absorbance of both congo red and methylene blue (Azo dyes) at 300-650nm and 400-750nm respectively were recorded. Control was maintained for both the dyes by without adding nanocomposite and catalyst was removed by centrifugation. Percentage of photocatalytic degradation was calculated[16].

Results and Discussion

In this present study, bioactive compound analysis of aqueous extract of *P. dulce* leaves was carried out by standard protocol as mentioned by Trease and Evans[27] who showed the presence of alkaloids, flavonoids, terpenoids, phenolic compounds, tannins and glycosides whereas steroids and saponins were not observed (Table 1). Vargas-Madriz et al[28] review report of *P.dulce* reveals that leaves, seeds and aril are the commonly analysed parts for their phytocompounds and various biological activities.

It was found that the phytochemical content reported in different parts of the plant is dependent on the lab environment, solvent used, method of drying and method of extraction. Review report on *P.dulce* by Vargas-Madriz et al[28] also provided information that the aqueous extract of the plant leaves studied was mostly showing the presence of phenolic compounds like tannins, flavonoids. In this current study, the leaves extract of *P. dulce* also showed similar phytochemicals presence. Qualitative and quantitative analysis of bioactive compounds of aqueous extract of leaves was carried out to check the association of bioactive compounds in nanoparticle synthesis. Total phenolic and flavonoids analysis showed 5.13±0.09 mg/g equivalents of gallic acid and 2.52±0.11 mg/g equivalents of quercetin respectively. Aqueous leaf and bark[20] extract of *P.dulce* was reported for silver nanoparticle[21] whereas leaf extract alone was checked for gold nanoparticle synthesis[29]. Bark of *P.dulce* was used for zinc oxide nanoparticle synthesis but there is no report related to copper nanoparticle and so in this study, copper nanoparticle synthesis was focused. UV-visible spectroscopy study of the synthesized copper nanoparticle for a period of 24 hour (0^{th}, 3^{rd}, 6^{th}, 18^{th} and 24^{th} hour) showed maximum peak at 560nm on the 6^{th} hour duration (Figures 1 and 2).

Table 1
Bioactive compound analysis of aqueous extract of *Pithecellobium dulce* leaves

Alkaloid	+
Terpenoids	+
Steroids	-
Phenolic compounds	+
Flavonoids	+
Glycosides	+
Tannins	+
Saponins	-

\+ positive, - negative

Bioactive compounds of plant extracts play important role in regulating the size and shape of nanoparticles. The reactive groups of flavonoids are involved in nanoparticle green synthesis. Other bioactive compounds like alkaloids, terpenoids, curcumin also showed reduction property in copper nanoparticle synthesis[6]. Phenolic compounds present in plant extracts are found to function as reducing agent in nanoparticle synthesis[14].

FTIR analysis showed the presence of functional groups like alcohol, alkenes, alkyl halide and alkanes (Figure 3). SEM analysis of PdCu nanoparticle showed spherical morphology (Figure 4). Functional groups present PdAE extract might play important role in nanoparticle synthesis. Review report by Gawande et al[7] on copper and copper based nanoparticles reported that the commonly used precursor includes copper sulphate and copper chloride. Copper is known for its ready reaction with other metals, polymers and ceramics. Copper based nanoparticles are found to have photocatalytic property.

Al-Hakkani[2] review study on copper nanoparticle and its applications reported that green synthesized copper nanoparticle showed peak in the range between 560-590nm, mostly spherical shaped and exhibited antimicrobial, anticancer activity and dye degradation effect.

Synthesised copper nanoparticle was then incorporated into gelatin hydrogel (Figure 5). Gelatin copper nanocomposite SEM analysis showed the copper nanoparticle fabrication into the gelatin hydrogel (Figure 6). Gelatin based hydrogels are found to have wider applications in different fields because of their biocompatibility, non-toxic and cost effective role. Natural polymer gelatin based hydrogel are utilized in agricultural field, food industry, waste water treatment, heavy metals removal etc.[17]

Copper nanoparticle (PdCu) was showing concentration dependent antimicrobial activity. Among the 3 microorganism assessed, *B. subtilis* was found to be highly sensitive (10mm±0.3) to the synthesized copper nanoparticles whereas *E. coli* and *K. pneumonia* showed equal zone of inhibition (5mm±0.5) (Figure 7a and b). Various research studies proved the effectiveness of gelatin hydrogel in the removal of organic inorganic contaminants of water. As both copper and gelatin hydrogel have wide applications, the dye degradtion property of gelatin copper nanocomposite (PdCuHg) was checked. It was found that the copper gelatin nanocomposite (PdCuHg) was more effective against methylene blue than towards congo red and it may be due to the sustainable release of copper (Figure 8a and b).

Methylene blue is utilized in diagnosing and treating certain diseases, in textile industry, food industry and so on. But usage of higher level than the allowed amount, it is found to be toxic and also the continuous release of the same from the textile industries is identified as the major source that pollutes water. Various methods of removal of methylene blue before its release in to the environment are almost followed everywhere but the drawback is that the methylene blue is found to be highly light stable, heat stable and non-biodegradable.

The current research focused in utilizing gelatin copper nanocomposite as effective catalyst in methylene blue degradation. Dye degradation results also revealed the potential of degradation. At the same time, congo red degradation was found less with copper gelatin hydrogel (PdCuHg). Further study by varying the concentration of copper nanoparticle or by changing the other parameters will be helpful in improving degradation property.

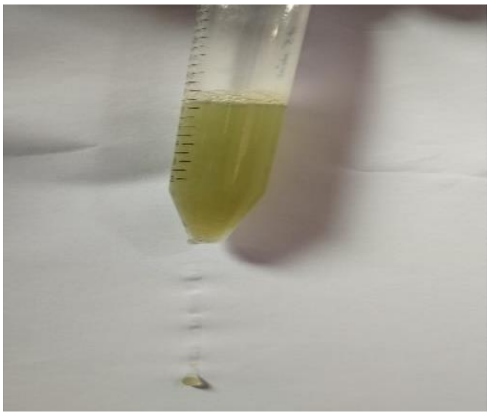

Figure 1: Synthesised Copper nanoparticle using *P.dulce* aqueous extract

Conclusion

Green synthesis of copper nanoparticle is effective and environmentally beneficial process. In this research work, aqueous extract of *Pithecellobium dulce* was used for copper

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nanoparticle synthesis and characterization was done by UV-Spectroscopy, FTIR and SEM analysis. Nanocomposite was prepared by mixing copper nanoparticle and gelatin. Prepared copper nanocomposites of *P.dulce* showed effective antimicrobial and dye degradation property and it could be utilized in biomedical field and in textile industry for waste water treatment.

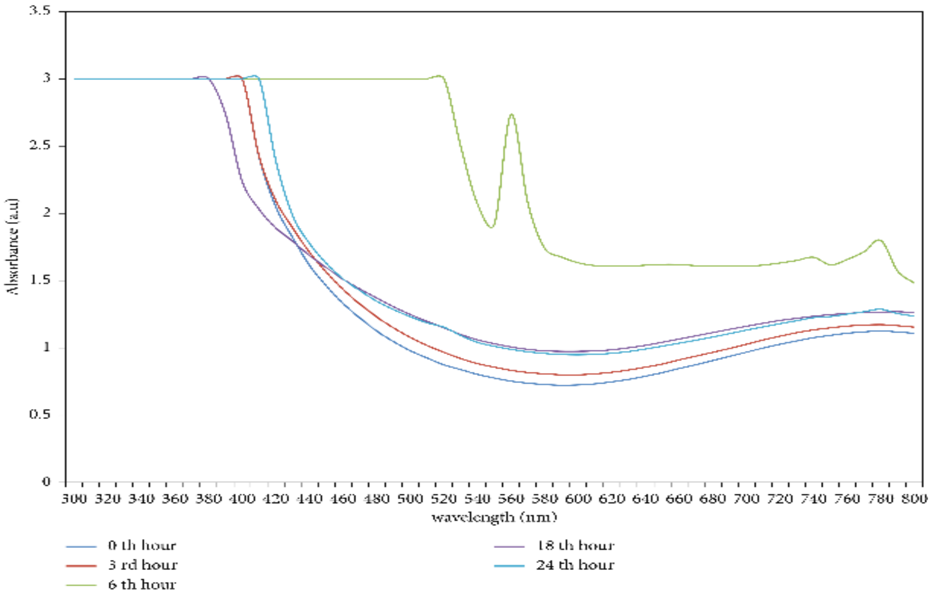


Figure 2: UV Spectroscopy of Copper nanoparticle

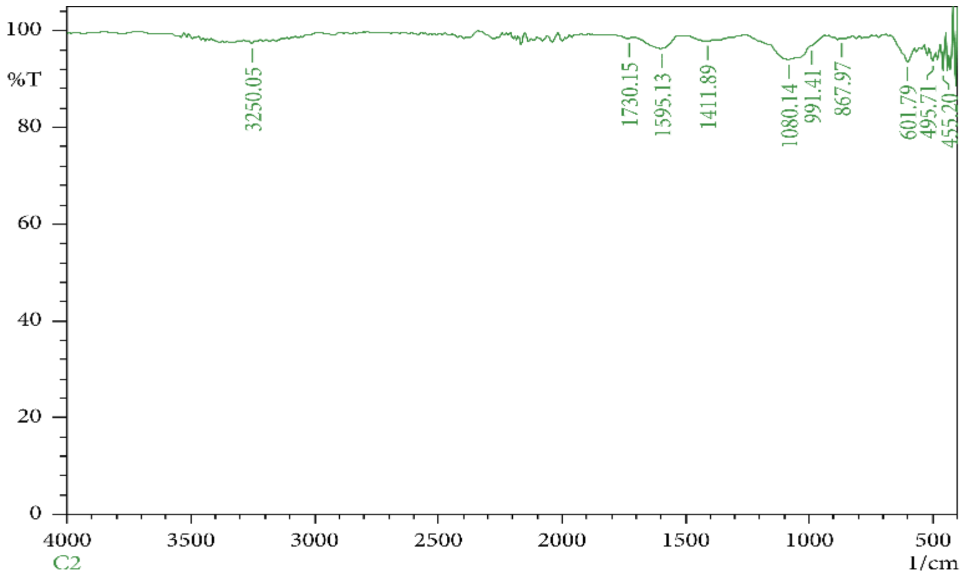


Figure 3: FTIR analysis of synthesized copper nanoparticle

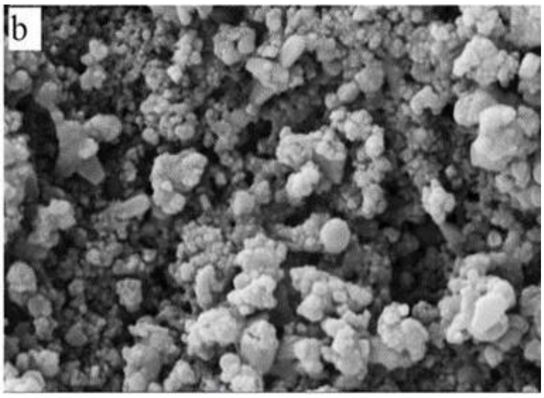


Figure 4: SEM analysis of copper nanoparticle

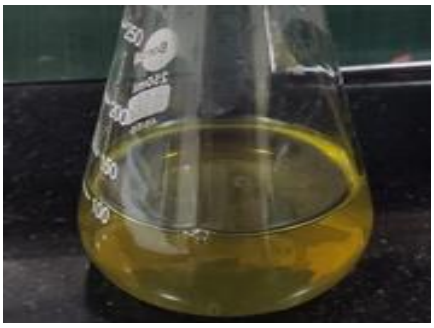


Figure 5: Gelatin Hydrogel

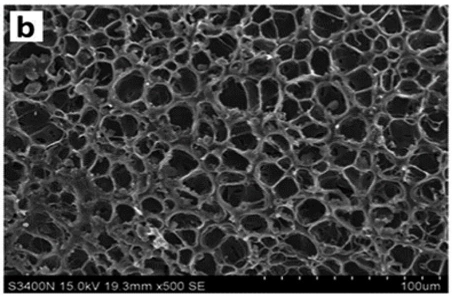
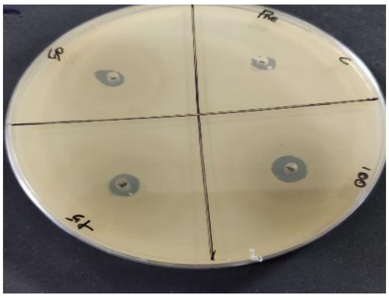
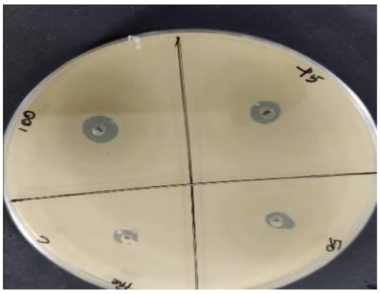


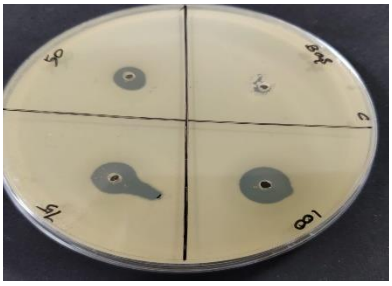
Figure 6: SEM analysis of gelatin hydrogel



a) ***B. subtilis***

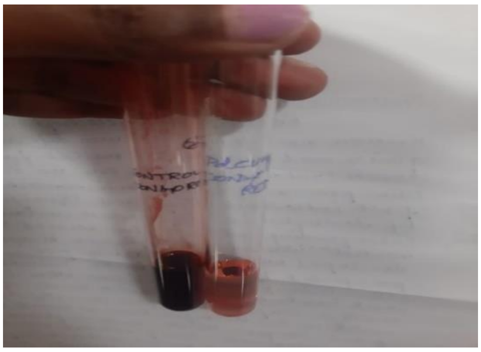


b) ***K. pneumonia***

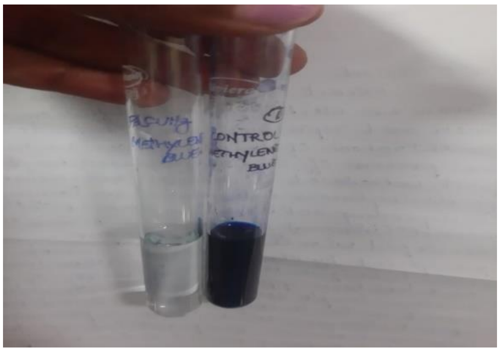


c) ***E. coli***

Figure 7: Antimicrobial activity of synthesized Copper nanoparticles



a) Congo red



b) Methylene blue

Figure 8: Dye degradation degradation of copper/gelatin nanocomposites

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