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Terminalia Chebula Kernels Functionalized Copper Oxide Nanoparticles For Enhanced Biocidal Activity And Photocatalytic Methylene Blue Degradation.

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Abstract

Biogenic synthesis of nanoparticles is a chemical free, environment-friendly, non-toxic and sustainable approach. This green route for nanoparticle synthesis plays a pivotal role in reducing the deteriorating effects of the conventional chemical methods of nanomaterial synthesis. The present investigation demonstrates the one-pot, rapid, green synthesis of bio-entity capped copper oxide nanoparticles using *Terminalia Chebula* kernels as a bio-reducing agent and study of its antimicrobial properties and effect on the photocatalytic degradation of methylene blue dye which is found in effluents and is a major cause for environmental problems. The XRD results confirmed the formation of monoclinic crystalline copper oxide nanoparticles with an average crystallite size of 20nm. The UV-Visible spectroscopic studies showed characteristic absorption peak of copper oxide nanoparticles between 250-300nm. Fourier transform infrared spectroscopy and thermo gravimetric analysis confirmed the presence of tannins from seed extract. Scanning electron microscopic images confirmed the formation of spherical nanoparticles. The antimicrobial activity of biogenic copper oxide nanoparticles increased with increase in their concentrations against *E.Coli* and *Staphylococcus aureus*. Furthermore, the photocatalytic activity of the biogenic copper oxide nanoparticles showed 98% degradation of the dye under direct sunlight irradiation for 150 minutes which could a very efficient and cost –effective bioremediation as well. This article reports the role of biofunctionalized copper oxide nanoparticles in exhibiting a synergistic efficacy on the biological and photocatalytic activity leading to better bioremediation results in water treatment methods. In line with the presented comprehensive results , the biofunctionalized copper nanoparticles from T. Chebula have the potential in improving the biological and photocatylic activity owing to the presence of surface capped biomoleculaes synergistical complementing the copper oxide nanoparticles in its biological and photocatalytic activity.

Keywords: Biogenic, Copper oxide nanoparticles, Terminalia Chebula seeds, Tannin Antimicrobial activity, Photocatalytic degradation, Methylene Blue.

1. Introduction

The demand for clean water has been increasing at an alarming rate with the increase in the population growth and industrial requirements. The shortage of clean water is a serious issue due to the continuous contamination of water resources majorly due to several human activities. The presence of pathogens, hazardous chemicals are still a major concern in the utilization of clean water. Although there are several methods in the purification of wastewater, an effective method is the implementation of nanoparticles for a rapid and efficient waste water treatment. These nanoparticles possess unique physical, chemical and biological characteristics due to their increased surface to volume ratio when compared to the bulk scale. On that note, Copper oxide nanoparticles have found immense application in the treatment of contaminated water[1].Apart from water disinfection , these nanoparticles are known to remove organic dyes from water [2].Further,these copper oxide nanoparticles when bio-synthesized can further be efficient in terms of their antimicrobial and catalytic properties[3]. The green synthesized nanoparticles could show synergistic effect in the removal of harmful microbes as well as other toxic organic dyes. The microbial contamination of water caused by E.Coli ,Coliforms , staphylococcus aureus can cause serious health ailments and thus it is necessary to combat the microbial contamination in water . Hence these biogenic copper oxide nanoparticles possessing the antimicrobial properties of bioentites from the plant extract can ameliorate the prevention of microbial contamination. There are reports of seed extracts of Luffa cylindrica used in water disinfection[4].Several phytochemicals have been used as flocculant agent to improve the water quality[5].Similarly seed extract of Vigna unguiculata and Parkinsonia aculeate have been studied for water purification[6].Furthermore, several biological agents can also be used for the removal of organics dyes and toxic metals from contaminated water. Methylene blue dye from contaminated water has been removed by reusable nanomaterial and plant biomass composites and pericarp of corn, alfalfa, and agave bagasse wastes [7,8]. Hyperaccumulator plants have been studied for removal of toxic metals like cadmium[9].Hence when copper oxide nanoparticles are green synthesized and biofuntionalized using such bioentities , they can potentially help in an efficient water treatment process.

The green synthesis method plays a pivotal role in catering to a sustainable technology in the synthesis of cost-effective, non–toxic, environment friendly nanoparticles with improved biological compatibility[10]. These biogenic nanoparticles are synthesized from a variety of naturally available materials ranging from plants to micro organismal sources. Albeit, synthesis of nanoparticles using microbes as reducing agents is considered as an effective bio factory for synthesis, slow rate of synthesis, the lack of understanding in the complexity and the underlying mechanism of synthesis still poses a challenge. On the other hand synthesis of nanoparticles derived from natural sources like plants or their parts is a one–pot synthesis method that is simpler and biocompatible[11]. Also in these biological methods, the bioentities present in the extracts act as both reducing agent and capping agent resulting in the formation of stable nanoparticles[12-16] .

In this context, biogenic copper oxide nanoparticles synthesis using Tabernaemontana divaricate leaf extract[12], Aloe barbadensis[13], Acalypha indica[17], Tinospora cordifolia[18],carica papaya[19], Calotropis gigantean[20], Gloriosa superb leaves[21], have already been reported. These Copper oxide nanoparticles are well known for their antimicrobial and anticancer properties [17][22].They play an important role in medical field in which they act as an antimicrobial agent and also as an antioxidant [18][13]. Copper oxide nanoparticles are economical than silver nanoparticles and are easily miscible with polymers[23].It is relatively stable at room temperature[24].These nanoparticles find numerous applications ranging from antimicrobial, photocatalytic dye degradation, dye sensitized solar cells applications and water treatment,etc.,[12][17][19][20][25].

The present work aims at the biogenic synthesis of copper oxide nanoparticles using Terminalia Chebula plant seeds. Terminalia chebula Retzius (family Combretaceae) is a flowering evergreen tree with upto 30m in height and widely spread branches and a brown rounded crown. It has been used in the traditional system of medicine since ages because of the presence of polyphenols, flavanoids and steroids as major constituents .T. chebula contains tannins, chebulic acid, glycosides, sugar, triterpenoids, steroids and good quantity of phosphoric acid[26].

The major components of T.chebula fruits are polyphenols(32%-34%) like chebulagic acid , gallic acid, ellagic acid, chebulinic acid ,anthraquinones , flavanols and also contains aminoacids like succinic acid[27]. The presence of these constituents are responsible for the antibacterial, antifungal, antiviral, carcinogenic, antioxidant, hypolipidemic, hepatoprotective, cardioprotective and antidiabetic activity[27]. Antibacterial activities of T. chebula have already been reported[28-30]. It is effective in inhibiting, Clostridium perfringens, Escherichia coli, Helicobactor pylori, Xanthomonas campestrispv. Citri and Salmonella [31-34] .A water extract of T. chebula was found to have an antifungal activity on the strains of C.albicans,A.niger.[35]. These polyphenols apart from possessing pharmaceutical characteristics, also have good catalytic activity too. Phytoconstituents like polyphenols are finding significant application in the photodegradation of dyes that are major contaminants in effluents . Studies have shown that the polyphenol complexes from various plant sources like eucalyptus, melaleuca, rosemary, diosma and sage leaves [36], tea and coffee[37] can be used in the effective photodegradation of dyes and can be a potential candidate in bioremediation.

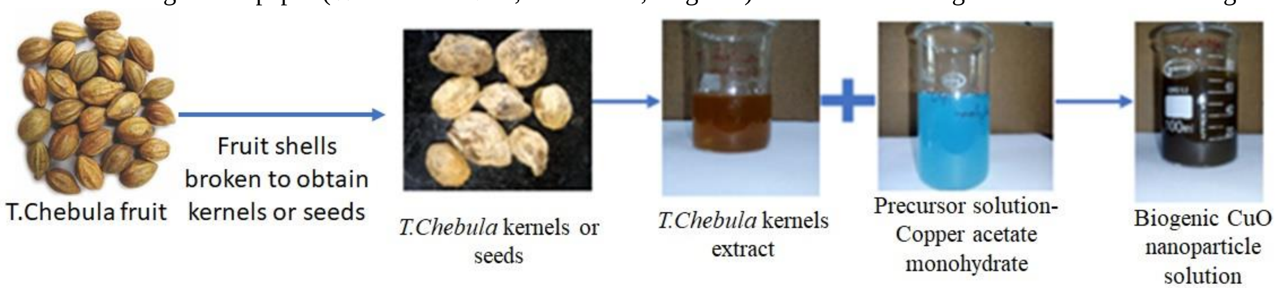
Hence this investigation involves the synthesis of copper oxide nanoparticles for the first time from the seed extract of Terminalis chebula in a rapid, simple and eco-friendly method and its effective utilization in the photocatalytic degradation of methylene blue dye. These have been characterized for the presence of various functional groups from the extract. The bio-entity functionalized nanoparticles possess antimicrobial and catalytic activity which can essentially be a potential application in the field of bioremediation.

2. Materials and methods

Terminalia chebula fruits were procured from the herbal stores, Chennai, India. Copper acetate monohydrate and Methylene Blue (MB) were of analytical grade obtained from Sigma–Aldrich Chemicals, Chennai. The microbial cultures were obtained from King Institute of Preventive Medicine and Research, Chennai, Government of Tamilnadu. Cultures were maintained on Nutrient agar at 37^{o} C at 150 rpm shaking for 24 h.

2.1. Green synthesis of copper oxide nanoparticles using Terminalia chebula seed extract

Terminalia Chebula fruits were broken manually and the seeds inside them were washed thoroughly using distilled water. Seeds were boiled with 500 ml of de-ionized water for 10 min and allowed to cool at room temperature. Finally, the extract was filtered through filter paper (Whatman No. 42, Maidstone, England) and stored in refrigerator for further investigation.



Copper acetate monohydrate was used as an originator for copper nanoparticles. Analytical grade Copper acetate monohydrate solution was prepared using de-ionized water. 0.25 M of solution was prepared in 30 ml of de-ionized water. The precursor solution was kept under continuous stirring at 400rpm using a magnetic stirrer and 30ml of seed extract was added to the solution at room temperature. Immediately on addition of the extract the solution turned to blackish –brown colour with the formation of precipitate. This mixture of the solution was kept under vigorous stirring at 500rpm for 30minutes. At the end of this step, a blackish-brown colour product was obtained as shown in figure 1. Solid product was washed twice with de-ionized water and dried at 80^{o}C for 8 hr. Final dried powder was stored in properly labelled containers and used for further analysis.

Fig.1 Synthesis of biogenic copper oxide nanoparticles using *T.Chebula* kernel or seed extract.

2.2. Characterization of copper oxide nanoparticles

The aqueous copper oxide nanoparticles and their optical properties were characterized by UV absorption spectra (UV-8000 Shimadzu). The purity and grain size of synthesized copper oxide nanoparticles were identified by X-ray diffraction (Perkin–Elmer spectrum) Cu-Kα radiations (k = 0.15406 nm) at the step size of 0.02^{o} in the range from 20^{o} to 80^{o}. Fourier Transform Infrared (FTIR) analysis was performed using FTIR spectrometer (ABB Bomem MB 3000) in the wavenumber range of 4000 to 400 cm^{-1} to determine the presence of bio-entities on the surface of the nanoparticles. The percentage weight loss of as-synthesized copper oxide nanoparticles was studied in the temperature range of 30–600^{o} C with a heating rate of 10 C/min in argon atmosphere, using Thermo gravimetric analyzer (TGA), (Mettler Toledo, Switzerland).The nanoparticles morphology was characterized by using scanning electron microscopy (Carl Zeiss SUPRA 55).

2.3. Determination of antimicrobial activity of copper oxide nanoparticles by the well-diffusion method

Antimicrobial activities of the green synthesized copper oxide nanoparticles were investigated using *E.Coli* and *Staphylococcus aureus* pathogens by Kirby Bauer disk diffusion method (modified).100 ml of microbial culture broth was spread using sterile L-rod on Muller Hinton agar plates. The spread plates were allowed to stand for10 min. The wells (5 mm size) were created on plates using sterile gel core. Five different concentrations (1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml) of copper oxide nanoparticles suspension and positive control (50µg/ml) (Chloramphenicol) were prepared. The different concentration nanoparticles solutions and positive control were poured into wells on two plates each for E.Coli and S.aureus plates with the help of micropipette. All the plates were kept for incubation at 37^{o}C for 24 hr. After incubation, the zone of inhibition was measured. Each screening test was conducted with three replicates and the data presented as mean ± SE (standard error of the mean).

2.4 Photocatalytic degradation of Methylene Blue dye by biogenic copper oxide nanoparticles

The photocatalytic activity of biogenic copper oxide nanoparticles is determined using the aqueous solution of Methylene Blue dye. The degradation of dye solution using the synthesized copper oxide nanoparticles under direct sunlight irradiation is studied.100ml of 10^{-4}M of dye solution was prepared and 10mg of the synthesized biogenic copper oxide nanoparticles added to it and stirred magnetically for 30 minutes at room temperature. A control was prepared and maintained at the same experimental conditions. The dye solution was then exposed to direct sunlight on a hot sunny day between 11:30 am to 2:00pm with an atmospheric temperature of 33 – 37^{o}C . The degradation studies were carried out using the UV-Visible spectroscopy at regular intervals of 30 min,60 min,90 min,120 min,150 min of regular intervals of exposure times.

3. Results and discussion

The T.Chebula seed extract immediately reduced copper acetate monohydrate solution to copper oxide nanoparticles on addition to it and was visually found by the colour change of the precursor solution from blue to dark brown colour confirming the formation of the nanoparticles.

3.1 X-Ray diffraction characterization of copper oxide nanoparticles

The XRD analysis of green synthesized copper oxide nanoparticles is shown in figure 2. The XRD pattern revealed the orientation and crystalline nature of copper oxide nanoparticles. Peak position with 2θ values of 32.65^o,35.62^o,38.94^o,48.81^o,53.55^o,61.63^o,66.38^o,68.28^o,72.56^o,75.29^o are indexed as (110),(-111),(111),(-202),(020),(202),(-113),(022),(220),(-312),and (203) which are in good agreement with those of powder CuO obtained from the International Centre for Diffraction Data (ICDD) card No 801916. The peak positions also confirmed the crystalline monoclinic structure of CuO. Further, no other impurity peak was observed in the XRD pattern, showing the single phase sample formation. The crystalline size was calculated using the Scherrer formula, D = 0.9 λ/β cosθ, where λ is the wavelength of X-ray radiation, β is the full width at half maximum (FWHM) of the peaks at the diffracting angle θ. The average crystallite size calculated by Scherrer formula was found to be 20 nm.

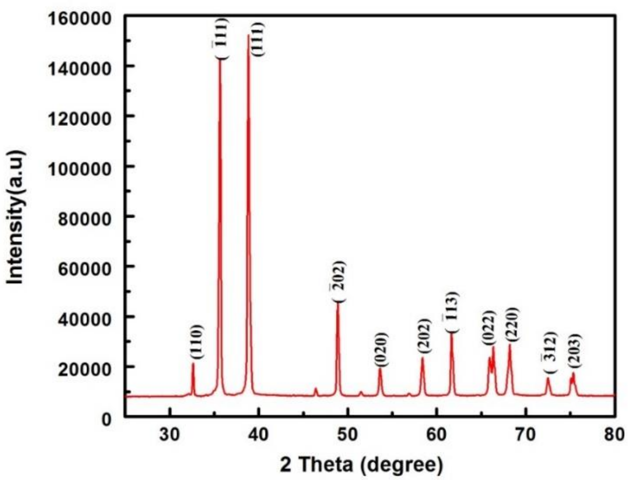


Fig.2 X-Ray Diffraction pattern of T. chebula mediated copper oxide nanoparticles.

3.2 .Fourier transform infrared spectroscopy analysis

FTIR spectroscopy analysis was carried out to find the biomolecules that were bound specifically on the copper oxide nanoparticles surface. Figure 3 shows the FTIR spectra of green synthesized copper oxide nanoparticles coated with the bioentities present in the extract obtained from Terminalis Chebula. A strong peak at 3426 cm^{-1} can be attributed to the presence of hydrogen bonded O-H groups of alcohols and phenols and the presence of amines N-H of amide. The small sharp peaks at 2926cm^{-1} and 2853 cm^{-1} are assigned to C-H stretches of alkanes. The peaks at 1586 cm^{-1} and 1422 cm^{-1} indicate the C-C- stretches of aromatic compounds. The peak at 1340 cm^{-1} corresponds to the presence of nitro compounds (N-O symmetric stretch) and peaks at 1194 cm^{-1} and 1067 cm^{-1} are attributed to C-O stretches of alcohols and C-N stretches for aliphatic amines.Further, the peaks at 766cm^{-1} and 611cm^{-1} signify the presence of N-H stretches of primary, secondary amines and C-H, -C≡ C-H bends of alkynes. The above functional groups are constituents of T.Chebula extract like hydrolysable tannins, chebulagic acid, gallic acid, chebulinic acid, ellagic acid, anthroquinones , flavanols and amino acids like succinic acid[27].The various polyphenols containing highly acidic hydrogen atoms could be the reason for the strong reducing activity of the T.Chebula extract. Hence the presence of various functional groups gives sufficient correlation for the functionalization of bioentites like tannins, polyphenols, flavonoids and amino acids present in the extract of Terminalis Chebula and their rapid and strong reducing potential in the synthesis of stable copper oxide nanoparticles.

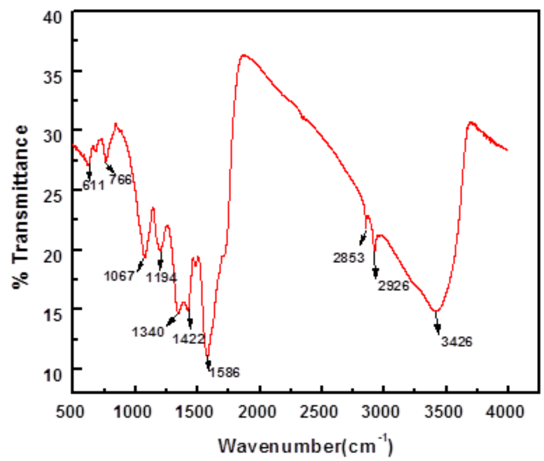


Fig.3 FT-IR spectra of T. chebula mediated copper oxide nanoparticles

3.3 UV-Visible spectroscopy

The addition of T.Chebula extract to copper acetate monohydrate solution resulted in colour change of the solution from blue to blackish-brown due to the formation of copper oxide nanoparticles. The excitation of surface plasmon vibrations with the copper oxide nanoparticles causes this change in colour. The UV-Visible spectrum of copper oxide nanoparticles obtained using Terminalia chebula is shown in figure 4. Nanoparticles synthesized using *Terminalia chebula seed* extract exhibit an absorbance maxima between 250-300 nm due to the excitation of SPR oscillations of copper oxide nanoparticles. The SPR is a characteristic of copper oxide nanoparticles, thus, confirming its formation. The relatively large intensity of the SPR peak for these nanoparticles synthesized using *T.Chebula* extract indicates the enhanced reducing power of the extract.

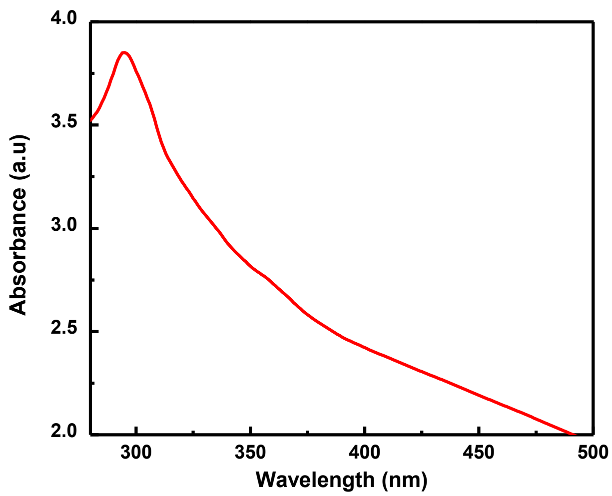


Fig.4 UV-Visible spectrum of copper oxide nanoparticles obtained using Terminalia Chebula seed extract

3.4. Thermo gravimetric Studies

The thermal decomposition of bio entity capped copper oxide nanoparticles is studied and the weight loss % and the heat flow curves of synthesized copper oxide nanoparticles are shown in the below figure 5. In the Fig.5 (i), there is continuous weight loss observed during heating of the copper oxide nanoparticles from 30-800ºC. The major weight loss% exhibited by the synthesized nanoparticles is found to be 30.5% in the temperature range of 150 -500ºC.

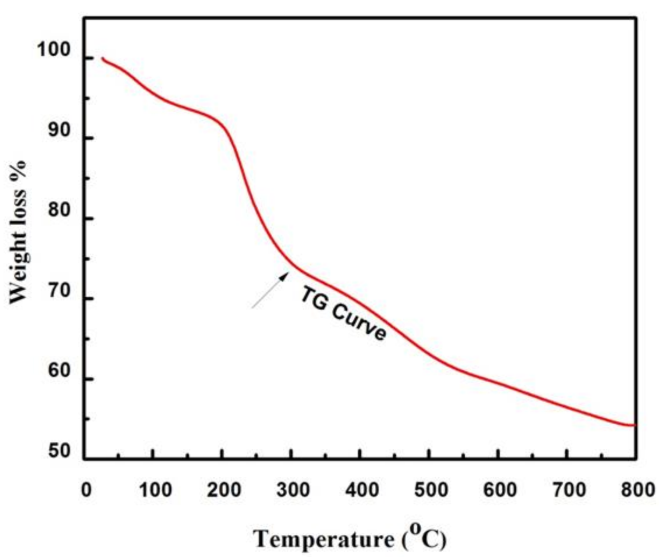


Fig.5. (i) Weight loss of Copper oxide nanoparticles synthesized using Terminalia Chebula extract.

The weight loss may be attributed to the decomposition of the bioentites coated on the synthesized copper oxide nanoparticles, namely chebulagic acid, gallic acid, ellagic acid, chebulinic acid, anthroquinones, flavanols and amino acids and hence this could be the reason for the weight loss of about 30.5% due to their thermal instability. Hence the thermal decomposition studies can be used for yet another confirmation that the copper oxide nanoparticles are capped with theraupetically useful bioentities from the extract.The heat flow curve of copper oxide nanoparticles using Terminalia Chebula extract as shown in fig.5(ii) exhibits a broad exothermic peak at around 250ºC which lies within the single step reduction temperature range for weight loss. This exothermic peak can be attributed to the crystallization of nanoparticles [38]

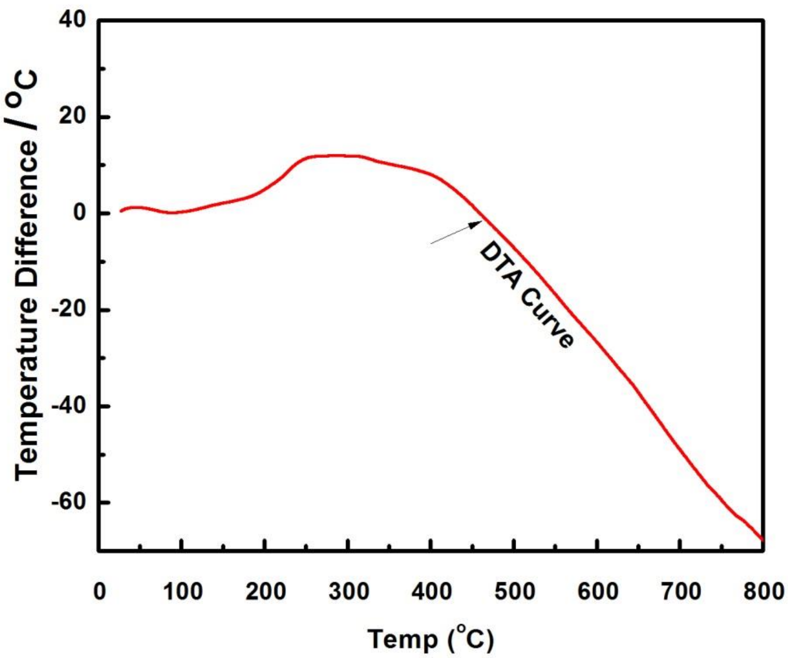


Fig.5 ii) Heat flow curves of Copper oxide nanoparticles synthesized using Terminalia Chebula extract.

3.5. Scanning electron Microscopic studies

Morphology of synthesized copper oxide nanoparticles was characterized by SEM analysis. The samples were placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. The SEM images of copper oxide nanoparticles are shown in figure 6.It can be viewed that the copper oxide nanoparticles formed are well dispersed and evenly distributed in all directions. SEM images show very clearly that most of the particles are monodispersed, spherical and very little agglomeration can be noticed.

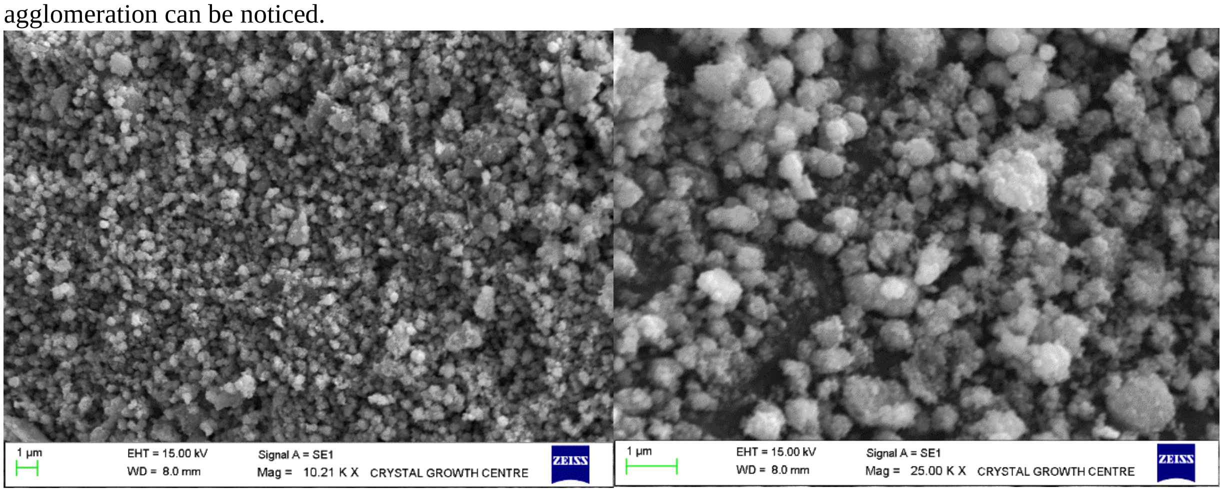


Fig .6 Scanning electron microscopic images of biogenic copper oxide nanoparticles

3.6. Antimicrobial studies

The fig.7 shows Mueller Hinton Agar (MHA) plates seeded and incubated with E.coli and Staphylococcus aureus along with copper oxide nanoparticles synthesized using the T.Chebula extract. Chloramphenicol is used as the positive control. The positive control at the center of the seeded plate showed a very strong zone of inhibition. Copper oxide nanoparticles at five different concentrations, 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml, 62.5 µg/ml , were incubated in the MHA plates.

The maximum zone of inhibition was found for copper oxide nanoparticles with 1000µg/ml concentration against both the microorganisms. The following table shows the measurements of zone of inhibitions at various concentrations of biogenic copper oxide nanoparticles against the two different microorganisms, E.Coli and Staphylococcus aureus. As the concentration increases the antimicrobial activity of the nanoparticles also increases against both the micro-organisms. It is evident from the Table.1 that S.aureus is less susceptible to CuO nanoparticles when compared to E.coli which is similar to the earlier report by H.R.Naika et al.[21]. There are several mechanisms proposed for antimicrobial activity of copper oxide nanoparticles.

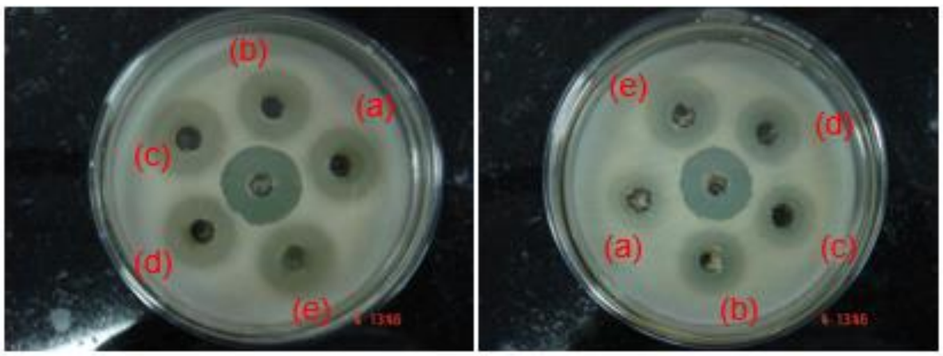


Fig.7 Antimicrobial activity using copper oxide nanoparticles biosynthesized from Terminalia Chebula at five different concentration (a)62.5µg/ml, (b)125µg/ml,(c)250µg/ml, (d)500µg/ml, (e)1000µg/ml against (A) E.Coli, (B)Staphylococcus Aureus

S.No	**Microorganisms**	**Zone of inhibition in mm**				
		1000µg	**500µg**	**250µg**	**125µg**	**62.5µg**
1	E.Coli	7	6.4±0.16	6.2	6.1±0.36	5
2	Staphylococcus Aureus	5±0.26	4	3.6±0.11	3.45	3

Table.1. Zone of inhibition (in mm) at five different concentrations of biogenic copper oxide nanoparticles against E.Coli and Staphylococcus aureus.

Copper oxide nanoparticles are known to kill the microorganisms using a contact killing mechanism in which the bacteria suffers damage to the cell envelope then followed by an independent pathway of each copper oxide nanoparticles [30]. Another mechanism proposed is that the copper ions may get attached to the negatively charged bacterial cell wall and rupture it causing protein denaturation and cell death[40].There are reports for similar mechanism by Nawaz et.al [41] also. Apart from their own unique property of antimicrobial active ,such green synthesis method facilitates in enhancing the properties of nanoparticles by coating these particles using the therapeutically important bioentities present in the extract[15].

4. Possible mechanism of reduction of copper acetate monohydrate to copper oxide nanoparticles by Tannins

The major phytoconstituents of the seed are hydrolysable tannins(polyphenols) namely chebulagic acid, gallic acid, ellagic acid, chebulinic acid[27].These are known for their astringent, antioxidant and many other biogenic properties. They are reported to be very efficient in acting as a strong reducing agent for the synthesis of nanoparticles[42]. The role of these polyphenols in the synthesis of silver nanoparticles has already been reported[43,44].

In the synthesis of copper oxide nanoparticles, Cu^{2+} ions can form intermediate complex with the hydroxyl groups of the hydrolysable tannins which subsequently undergoes oxidation to form quinone forms and leads to the formation of copper(cupric) oxide particles(black coloured) as shown in the figure 1.The presence of antioxidant reductants in the polyphenolic extract of T.Chebula seed causes the reduction of Cu^{2+} to CuO nanoparticles indicating that it has a strong reducing power. A similar mechanism has been reported by Jebakumar e.t al for the synthesis of silver nanoparticles by tannins[45].Apart from acting as a strong reducing agent, one of the important biogenic properties recently reported about polyphenols is their ability to use for the prevention of prostate cancer. The polyphenols present in green tea and pomegranate are reported to be anti-cancerous[46]. Hence this essentially proves its breakthrough applications in therapeutics and drug delivery in near future. The mechanism of formation of the nanoparticles is shown below in the figure 8.

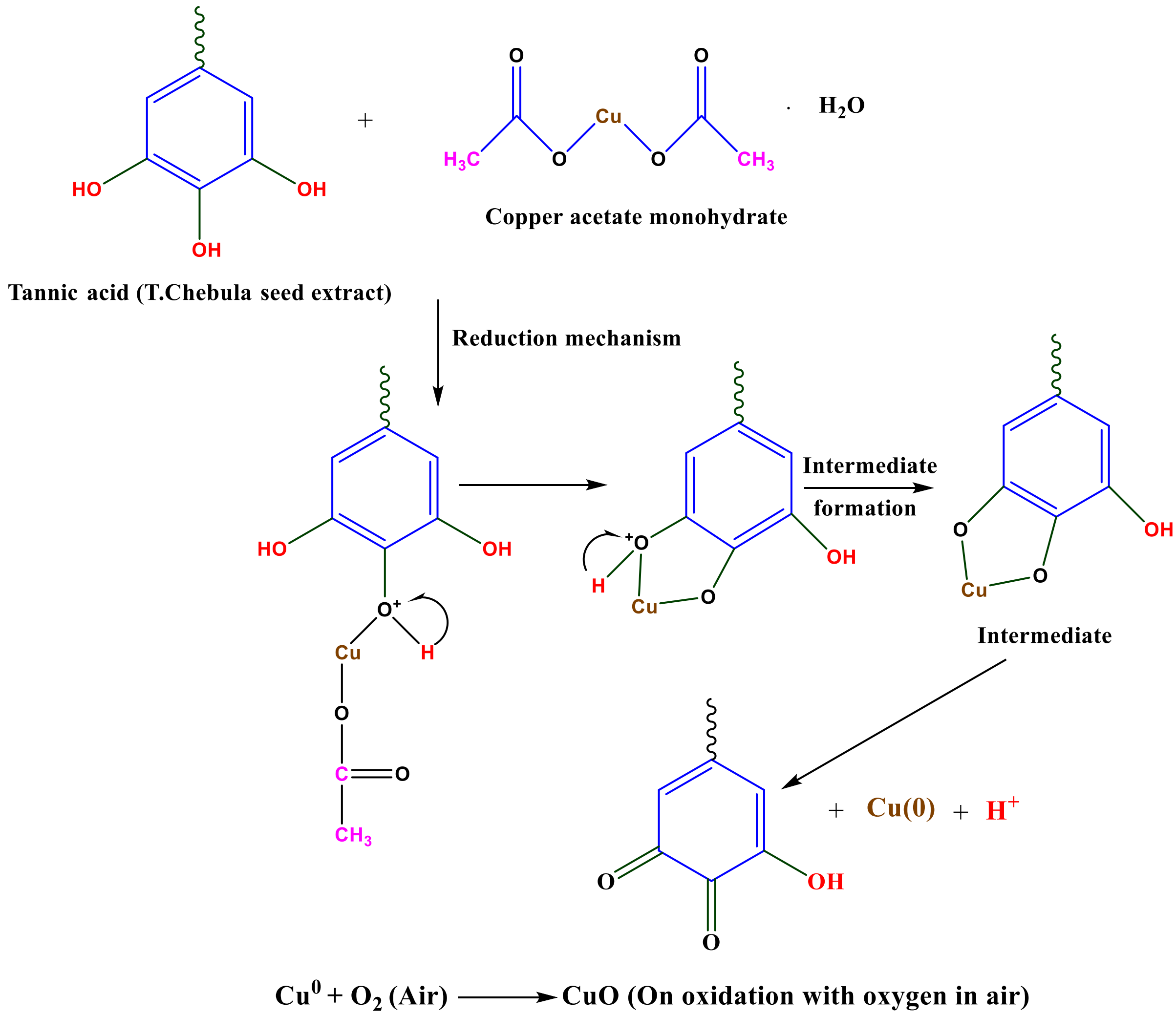


Fig.8. Mechanism of formation of copper oxide nanoparticles

5. Photocatalytic activity of biogenic copper oxide nanoparticles

The photocatalytic activity of the biosynthesized CuO nanoparticles was evaluated by the degradation of methylene blue dye under solar irradiation. Dye degradation was visually confirmed by the gradual change in the color of the dye solution from deep blue to colorless.. The characteristic absorption peak for methylene blue was noticed at 659 nm as shown in figure 9. There was no colour change observed in the control sample during solar irradiation. UV spectra of the photocatalytic degradation of methylene blue dye showed significant decrease in the peak intensity starting form 30 min to 150 minutes of exposure to sunlight. The decrease in the peak intensity (at 659nm) during 2.30 hrs of exposure in sunlight is also shown in the figure 9.

The variation of dye concentration with degradation time is shown in Fig 10. The dye degradation (%) was calculated using the following equation.

$$\text{Dye degradation (\%)} = [(C_0 - Ct / C0)] \times 100$$

Where C_0 is the initial concentration of the methylene blue solution and C_t is the concentration of the dye solution after t hours of exposure in solar irradiation. As the concentration is directly proportional to the absorbance value, all the dye concentrations were measured by taking the value of absorbance at 659 nm in the UV–Vis spectra measurements.

A control experiment was carried out to confirm the photocatalytic activity of the synthesized nanoparticles . In the absence of the nanoparticles , when exposed to sunlight ,the dye showed no degradation . Similarly, dye showed almost negligible degradation when placed in the dark in the presence of NPs. Figure 11 depicts the degradation capability of the synthesized copper oxide nanoparticles for MB, which reached to 98%. The degradation percentage is found to be 98% in 150 minutes and can be said to be an efficient and cost effective route for dye degradation.

There are also other reports of biogenic copper oxide nanoparticles from sources like fish scales[47] and Carica papaya [48] to mention a few which have reported the presence of photocatalytic activity. Whereas this synthesis is a very rapid , cost effective method and the photocatalytic degradation of the dye is calculated to be 98% at the end of 150 minutes of sunlight irradiation of the dye which is significantly higher than the earlier reports .

Also the synthesized nanoparticles possess antibacterial activity which together with catalytic activity can be useful for effluent treatments and bioremediation. The ameliorating effect of these biogenic copper oxide nanoparticles in dye degradation can be attributed to the presence of functional moieties from the seed extract which can also enhance the catalytic activity. There are reports of polyphenols from eucalyptus, melaleuca, rosemary, diosma and sage leaves [43], tea, coffee[44] being used for catalytic degradation studies , albiet, the exact mechanism of how these extracts enhance the catalytic activity along with copper oxide nanoparticles is being studied.

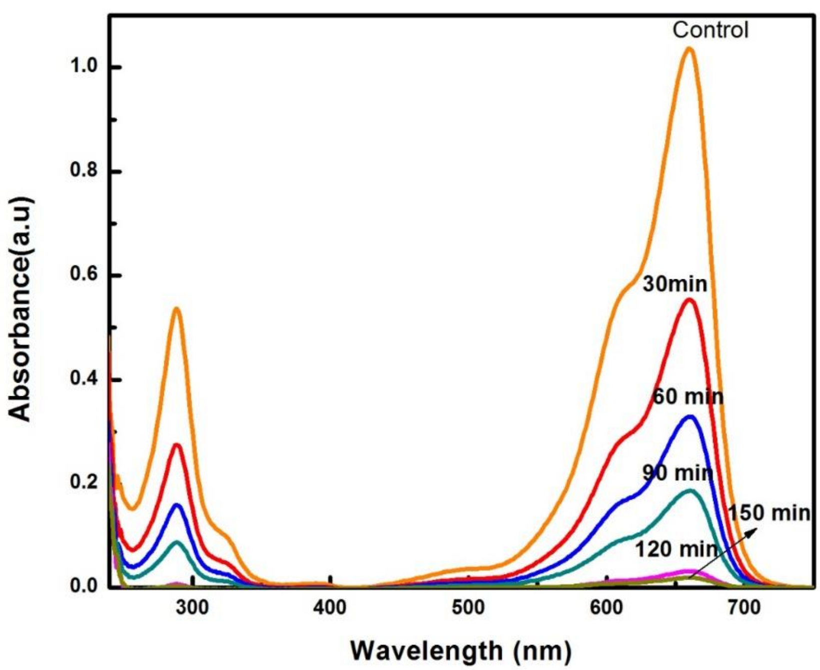


Fig.9 UV Spectra of Methylene Blue degradation using biogenic copper oxide nanoparticles.

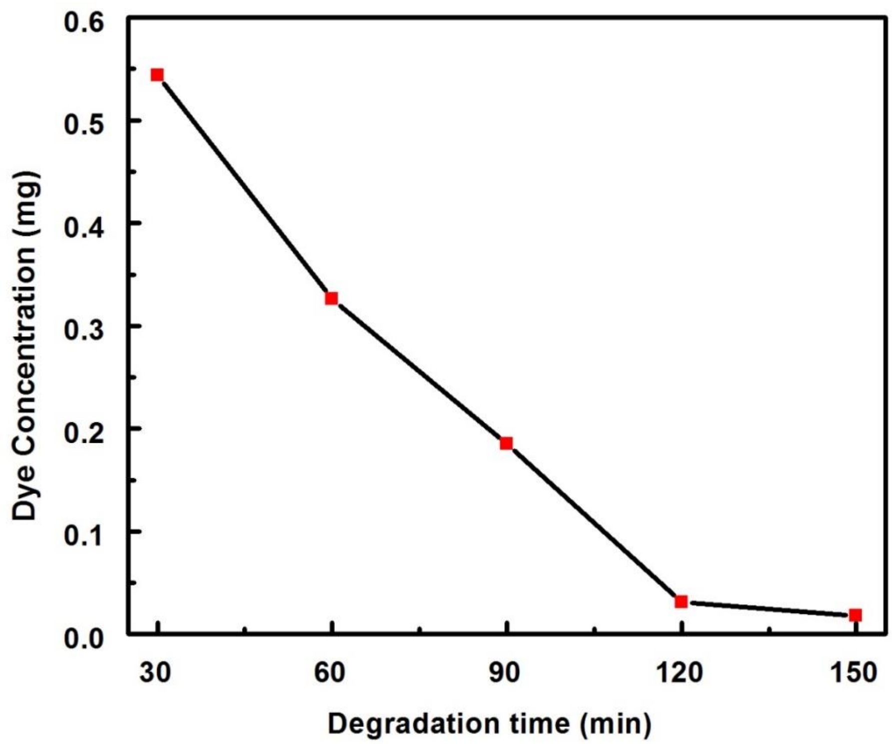


Fig.10 . Variation of Dye concentration with exposure time

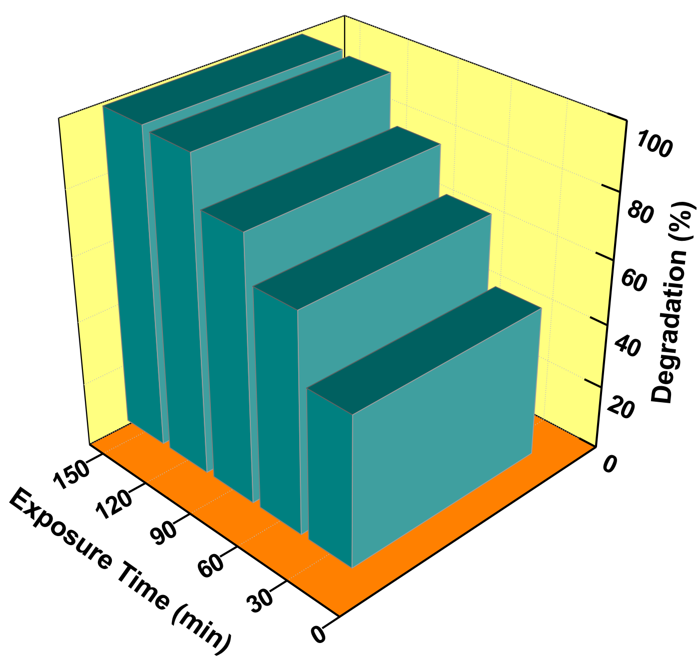


Fig.11 3D Graphical representation of degradation percentage of the dye with exposure time.

6. Mechanism of dye degradation of Methylene Blue dye using bio functionalized copper oxide nanoparticles

A possible mechanism of Methylene Blue Dye degradation using Biogenic copper oxide nanoparticles in the presence of sunlight is proposed below.

The photoexcitation of the copper oxide nanoparticles in the presence of solar irradiation causes the photocatalytic degradation of the dye in solution. The formation of hydroxyl radicals, $\cdot OH$ and superoxide radicals, $\cdot O_2^-$ play a major role in the dye degradation and are regarded as the dominant reactive species in photocatalysis. It is proposed that photoexcitation by sunlight forms an electron-hole pair on CuO photocatalyst. The excitation of the electrons present in the valence band (VB) to the conduction band (CB) of the CuO photocatalyst forms electron rich conduction band regions and the corresponding hole rich valence band regions in the photocatalyst as represented in equation 2. This creates a spatial separation of electron hole pairs.

$$CuO + h\nu \text{ (Solar radiation)} \longrightarrow CuO\ (e^-_{CB} + h^+_{VB}) \quad (1)$$

The photoexcited electron in the conduction band of CuO nanoparticles react with the oxygen molecules producing the free radicals, anionic superoxide radical, $\cdot O_2^-$ as in equation 2. Whereas the photogenerated holes in the VB of CuO nanoparticles react with water to produce $OH^\cdot$ Radical as in equations 3 and 4. These hydroxyl radical are extremely powerful reactive species in dye degradation.

$$e^-_{CB} + O_2 \longrightarrow O_2^- \quad (2)$$

$$h^+_{VB} + H_2O \longrightarrow H^+ + \cdot OH \quad (3)$$

$$h^+_{VB} + OH^- \longrightarrow \cdot OH \quad (4)$$

The hydroxyl and superoxide radicals thus formed in the above reactions efficiently possess very high oxidation potential which catalyse the degradation of RhB dye represented in Equations 5 and 6. The holes present in the valence band of the photocatalyst due to their high oxidation potential are also involved in the direct oxidation of the dye forming the reactive intermediates as shown in Equation 7. [42]

$$\cdot O_2^- + RhB \longrightarrow \text{Dye degradation products} \quad (5)$$

$$\cdot OH + + RhB \longrightarrow \text{Dye degradation products} \quad (6)$$

$$h^+_{VB} + RhB \longrightarrow \text{Reactive intermediates} \quad (7)$$

It has also been previously proposed that during oxygen radicals formation, H_2O_2 is also produced.[42] This hydrogen peroxide formation inhibits the electron hole recombination thereby increasing the hydroxyl, OH radical concentration. It is understood that H_2O_2 accept the photogenerated electrons from the conduction band leading to the charge separation and thereby forming $\cdot OH$ radicals described in the below Equations 9 and 10.

$$H_2O_2 + e^- \longrightarrow \cdot OH + OH^- \quad (8)$$

$$H_2O_2 + \cdot O_2^- \longrightarrow \cdot OH + OH^- + O_2 \quad (9)$$

Methylene Blue dye degradation using CuO nanoparticles under solar irradiation is thus facilitated by the generation of highly reactive hydroxyl and oxygen radicals in the reactions. CuO nanoparticles by themselves show photocatalytic activity, nevertheless surface modifying the nanoparticles for improved radiation absorption can be beneficial in further ameliorating the photocatalytic property. One of our previous studies has also shown that biocapping of CuO/ZnO nanocomposites using quercetin molcues can greatly improve the dye degradation efficicieny of Rhodamine B dye when compared to the uncapped nanocomposite.

The photocatalytic ability of phytogenic CuO nanocatalyst can thus be ascertained to the synergistic role extract biomolecules present on the surface of the nanoparticle together with the nanocatalyst that improves the absorption of radiation. This coupling effect enhances the degradation of Methylene blue dye much efficiently.

Earlier reports substantiate the role of phytochemicals like flavonol molecules in absorbing a wider range of radiation and can act as an electron donor in the catalytic process on photoexcitation. These photoexcited electrons in the conduction band are then transferred to the CuO catalyst facilitating Methylene Blue dye degradation adsorbed on the catalyst surface.[46]

7. Conclusion

Copper oxide nanoparticles are successfully biosynthesized in an eco-friendly, simple and very rapid method using T.Chebula seed extract. T.Chebula seed extract acts as a reducing and capping agent for the synthesis of stable biogenic copper oxide nanoparticles. Monoclinic, crystalline nanoparticles in the average size of 20nm were synthesized which was confirmed by X-Ray diffraction studies. The UV-Visible spectroscopic studies showed that the synthesized copper oxide nanoparticles had an absorption maxima between 250-200nm due to surface plasmon resonance, a characteristic of copper oxide nanoparticles. The Fourier transform infrared spectrum of biosynthesized copper oxide nanoparticles exhibits the characteristic peaks of functional groups of major constituents of the T.Chebula extract namely, chebulagic acid, gallic acid,ellagic acid, chebulinic acid, anthroquinones ,flavanols and aminoacids. The presence of bioentities on the synthesized copper oxide nanoparticles is further confirmed using thermal analytic studies,which shows the decomposition of various bioentities and weight loss of 30.5% in the temperature of 105^{o}-500^{o}C. Scanning electron microscopic(SEM) images showed that the synthesized nanoparticles are well dispersed and spherical in their shape. These biogenic copper oxide nanoparticles have good antimicrobial activity against E.Coli and S.aureus, wherein S.aureus is less susceptible to CuO nanoparticles when compared to E.coli. The synthesized copper oxide nanoparticles efficiently degrades Methylene Blue dye to 98% in 150 minutes of direct sunlight irradiation. It could be concluded that these bioentity functionalized copper oxide nanoparticles along with its antimicrobial and photocatalytic degradation properties will pave way for a cost-effective, rapid and efficient effluent treatment and environment clean-up process.

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