Green chemistry method prepared effective copper nanoparticles by lemon flower (citrus) extract and its anti-microbial activity

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Green chemistry method is a toxic less Development of metal nanoparticle towards eco-friendly biosynthesis process for various applications. In the present investigation, we fabricated the Copper Nanoparticles using a lemon plant and its family name RUTACEAE). Copper sulphate (CuSO\textsubscript{4}) was used as precursor for the formation of copper Nanoparticles by using flower extract of lemon. The physico chemical characterization of copper nanoparticle were analysed by XRD, SEM and DRS study. The SEM results show that the copper Nanoparticles are aggregated cross rectangle/spherical shape morphology. The optical characterization was carried out using UV – Vis analysis. The results are showed that the optimum concentration of flower extract is important for the synthesis of copper nanoparticles. The as prepared Copper nanoparticle have the efficient ability to inhibit the growth of various pathogenic microorganisms.

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1. Introduction

Fabrication of Nanoparticles from natural resources is the field of growing interest among scientist and researchers. The nanoparticle such as silver and gold particle produced by herbal extract and other toxin free solvents have developed as potential candidate for green chemistry and biomedical industries. Copper Nanoparticle are already proven as excellent component with respect to their excellent physical and chemical properties [1-3]. Metal nanoparticle produced by metal ions are believed to constitute a main role in toxicity. Copper NPs increase of copper ion in the oxidation state balance of the redox pair could produce the reactive oxygen species and further induces cell death. Until now, various studies reported the toxicity behavior of copper-based NPs. Toxicity characteristics of copper nanoparticle are due to partially by dissolution ability in the respective aqueous medium [2-4]. The design of advanced nanoparticle synthesis for antifouling and antimicrobial properties is driven by a wave of scientific research interest covering window of applications. Despite the various application of bulk copper in various industry example optics, electronics, building materials. The use of copper nanoparticles is restricted by copper inherent instability under normal atmosphere conditions, which results in prone to oxidation [5]. The wet chemical method to produce copper NPs is long established approach and the reduction of copper salts such as CuSO\textsubscript{4}, copper (II) acetyl acetone, CuCl\textsubscript{2}, or copper nitrate. Reducing agents used in the past are toxic in nature and it create polluting side products. An alternated approach is reveres micellar method, which involves the oil-in water microemulsion by adding as surfactant to polar and nonpolar solvents. Copper oxide

CuO NPs are more stable than Ag\textsuperscript{0} and Cu\textsuperscript{0} NPs, which are sensitive to atmospheric oxygen and sunlight. Even for copper and copper oxide NPs, the mechanism of their biocidal action has been related to the induction of oxidative stress, the pathway of bacteria and viruses, towards DNA degradation and its protection capacity. Based on previous reports, Cu NPs and CuO NPs exert different action on microbes. In particular, Cu NPs are more attractive towards interact with in the
membrane of bacteria due to maintain its integrity. On the other hand, CuO NPs tend to penetrate the membrane of bacteria with respect to nanosized and inside the membrane it releases copper nanoparticle to create toxins to kill the microbes [6-9]. Copper Nanoparticles have wide applications as catalyst in chemical industries and heat transfer and coating material for antimicrobial action. Due to high volume to surface ration values of Copper nanoparticles are very reactive in surface reactions [10] and increase their antimicrobial actions. Colloidal form of copper particles has been already reported as efficient antimicrobial agent for food materials protection and healthcare water purification process in the past decades [11]. Coppers nanoparticles have created much attention towards wound dressings and biocidal properties [12, 13]. In literature, the various synthesis method has reported for Cu nanoparticles such as vapor deposition [14], electrochemical reduction [15], thermal decomposition, room temperature synthesis using hydrazine hydrate and starch, chemical reduction of copper metal salt precursor [16]. In recent time, the green chemistry synthesis of Cu nanoparticles was achieved by using microorganisms [17] and plant extract [18]. Copper nanoparticles show potential antimicrobial effects against infectious organisms such as Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Vibrio cholerae Bacillus subtilis, Vibrio cholerae and Syphilis typhus, [19, 20].

In the present study, we demonstrated the low-cost preparation method using Lemon flowers for the preparation of copper nanoparticle. The as prepared NPs have monitored and characterized by UV-VIS spectrometer. The structural morphology characterization and antimicrobial efficiency have also been explained.

2. Materials and methods

2.1. Materials
The following analytical grade materials were used without further purification: copper sulphate (CuSO₄). A.C.S. reagent (Sigma – Aldrich, 99% purity by wt).

2.2. Plant Materials
The fresh Lemon flowers were collected from the cuddalore district, Tamilnadu, India. The collected flower is washed very well with water and dried in medium dark temperature for 2 days.

2.3. Preparation of the lemon flower extract
The as collected Lemon flowers are used to prepare the aqueous extract. Lemon flowers weighing 25 gm were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed into 100 mL distilled water was added and boiled to 65°C – 70°C for half an hour. The resulting crude extracts filtered through Whatman No.1 filter paper (pore size 25µm). The filtrate was further filtered through microporous filter ( 0.6µm size filter).

2.4 Synthesis of Copper Nanoparticles
One millimole aqueous solution of copper sulphate (CuSO₄) was mixed with above prepared aqueous extract for the fabrication of copper nanoparticles. The 1:9 ratio of of lemon
flower extract and copper sulphate precursor used for the synthesis of copper Nanoparticles. For example, 10ml of lemon flower extract was added into 90ml of aqueous solution of 1mM copper sulphate. The as prepared 1:9 ratio solution is kept in magnetic stirrer for 2hours at 60°C.

2.5. CHARACTERIZATION OF COPPER NANOPARTICLES
To monitor the synthesis of copper Nanoparticles by adding lemon flower extract to study the reducing capacity of the copper ions in copper mixed extract solutions by optical characterized by using UV – Visible spectrometer (Perkin Elmer LS 45 spectrophotometer) and the absorption spectra was measured, using. The above prepared Copper sample solution is sonicated for dispersion of nanoparticle under alcohol medium followed by studied the UV – Vis spectrum.

The surface morphology of as prepared Cu Nanoparticles was characterized using SEM analysis and XRD analysis. The Scanning Electron Microscopy (SEM) used for this purpose is a (JEOL – JSM–Japan).

The antimicrobial activity of the synthesized Cu Nanoparticles is investigated against different types of pathogenic bacteria such as Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa that were cultured on agar plates added with same concentration of copper Nanoparticles by disc diffusion method [14].

3. Results and discussion
3.1. Structural Characterization and Morphology
The as prepared aqueous colloidal copper nanoparticle solution was further centrifuge to make the solid powder of copper nanoparticle. The prepared powder is analysed for crystalline structure of copper nanoparticle. The lemon flower extract method prepared copper nanoparticles are indexed and most intense peak at 2θ values of 42.3. The obtained XRD peaks are very well match with the reported JCPDS card number 01-1242 [20]. The other similar peaks are appeared together with more Intense peak due effective reactivity nanoparticle get absorb oxygen and transform in to Cu2O, which is also called as shoulder peak in XRD analysis. From XRD analysis its is very much clear that the major phase of copper nanoparticle formed by our methodology. We have also continuously monitored the formation of copper nanoparticle form from the lemon flower extract by UV-Visible spectrometer. UV-Vis analysis provides the absorption maximum for the respective nanoparticle present in the extract at trace level concentration [17].

The surface morphology and particle size of the copper nanoparticles was examined using scanning electron microscopy (SEM). Fig. 1b shows the scanning electron microscopy of copper Nanoparticles synthesizd by the plant extract of lemon flower is obtained from the proposed by bio-reduction method. The aggregated particle morphology obtained for the colloidal copper nanoparticle.
3.2. Optical characterization of copper Nanoparticles

The brown colour crystalline powder of as prepared copper nanoparticle was insoluble in water and almost in all organic solvents. Hence a UV – Visible spectrum was recorded for the powder form of copper NPs dispersed in methanol solution and it is represented in Fig: 2. UV – Visible absorption results confirmed the formation of copper Nanoparticles prepared in liquid by bio reduction method. The absorption peak observed at 220 nm is the characteristics peak of copper Nanoparticles.
3.3. Antimicrobial Activity of copper Nanoparticles

Lemon flower extract route prepared Copper Nanoparticles are further tested for antimicrobial activity. The as prepared Nps are shown effective destruction of gram positive and gram negative pathogenic bacteria.

Figure 3 shows the effective antibacterial property of copper NPs against E-coli, S-typhi, and S.aureus are showing promising results with respect to different concentration of as prepared copper nanoparticles. Among the tested pathogens, E-coli mixed copper nanoparticle shows very high efficiency and killing nature of antibacterial pathogens. After 24 hours of expressing, the zone of inhibition was measured in millimetre scale and tabulated in Table 1. The s. aureus is inhibited by the copper nanoparticle prepared from flower extract was showing effective inhibition after the addition 50 and 100 µg of sample addition.

![Antimicrobial activity of copper nanoparticles with Escherichia coli.](image)

Table 1. Microbial application of green chemistry method prepared Copper nanoparticle towards Antimicrobial applications.

| S.No | Name of the organisms    | Raw Copper salt (1mM) | Copper Nps (µg) |
|------|--------------------------|-----------------------|-----------------|
|      |                          |                       | 5   | 10   | 25     | 50 | 100 |
| 1    | S.typhii                 | -                     | 11  | 13   | 15     | 16 | 16  |
| 2    | S.mutants                | -                     | 10  | 11   | 13     | 14 | 15  |
| 3    | E.coli                   | -                     | 12  | 13   | 14     | 14 | 14  |
| 4    | S.aureus                 | -                     | -   | -    | -      | 11 | 13  |
| 5    | P.aurigenosa             | -                     | 11  | 12   | 13     | 13 |     |

The antibacterial activity assay of lemon flower aqueous extract assisted method synthesized copper nanoparticle (NP) showed the effective inhibitory action against common pathogens such as S.typhi, S.mutants, E.coli, S.aureus and P.aurigenosa (Fig.3 and Plate 2 and 3). The synthesized copper nanoparticles exhibit the higher activity at very low concentration such as 5µg, 10 µg and 15 µg against S.typhi, S.mutants, E.coli followed by P.aurigenosa and S.aureus (Table 1). There was no antibacterial activity observed in crude plant extract and copper nitrate solution. Hence, the obtained results support the use of this plant extract for the synthesis nanoparticles are worked as effective antibacterial agents against the above-mentioned pathogenic bacteria. Copper have found extensive and varied applications, from medical devices and home appliance disinfection to water treatment because of their high microbicides action against various species. Currently, CuNPs showing inhibitory activities toward several microorganisms are widely employed in the pharmaceutical industry and antiviral agents.
4. Conclusion

The present study reported the lemon flower extract route prepared copper nanoparticle for eco friendly catalytic and antimicrobial applications. The successful formation of copper nanoparticle from lemon flower extract was confirmed by UV-Vis spectrometry and XRD analysis. The structural morphology of as prepared materials shows aggregated particle morphology with spherical shapes. The optical characteristics are further confirmed for the analysis of crystalized copper nanopowder by UV-Vis analysis. The peak at absorption maximum obtained at 220 nm. The as prepared copper nanoparticle has further showed effective antimicrobial activity for pathogenic microorganisms like Escherichia coli even at usage of lower concentration of copper nanoparticle prepared from lemon flower extract. The other gram-positive bacteria’s shows the good inhibition by copper nanoparticle at higher concentration of nanoparticle addition in the diffusion medium. Hence, its clear that the as prepared green chemistry route prepared copper nanoparticle could be produced at low cost method for effective action against bacteria and viruses.

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