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Biosynthesis of copper nanoparticles; its characterization and efficacy against human pathogenic bacterium

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ABSTRACT

Synthesis of nano-material with the controlled size and shape is the prime concern of research in nanotechnology. Recently, biosynthesis of metallic nano-particles has gained popularity owing to its eco-friendliness. Use of natural plant extracts in the preparation of nano-particles provides advancement over chemical and physical methods, due to its t is cost effectiveness and environment friendly nature. In the present work, copper nano-particles have been synthesized with simple and green technique by using *Ocimum sanctum* plant leaf extract as reducer as well as stabilizer. The size and shape of synthesized nano-particles were pigeonholed by particle size analyzer and SEM, further were characterized by EDS and FT-IR spectral technique for their elemental and chemical composition. The experimental results showed copper nano-particles having Z-average diameter of 122.7 nm with higher stability and significantly higher antibacterial activities.

1. Introduction

Owing to the unique physical and chemical properties, metal nano-particles have potential applications in industries like electronic, chemical, pharmaceutical, etc. [1–6]. Nano-particles can be synthesized using various chemical, physical and biological methods. Among these methods, chemical synthesis method is found to be easy and cost effective but some of them use toxic raw materials. It has been reported by many researchers that, as biosynthesis of nano-particles is free from toxic chemicals it is more suitable for the biological application of nano-particles [7–12].

Various methods of synthesizing metal nano-particles using microorganisms, plant extracts and other natural bio-materials have been reported. Plant extracts provide a better way for nano-particle synthesis as they are free from toxic chemicals and are natural capping agents as well. Plant extracts also reduces the cost of synthesis in comparison to microorganism synthesis, as cost involved for isolation and culture media enhancement is eliminated. Biomolecules as reducing agents are found to have a significant advantage over their counterparts as protecting agents [13–18]. *Ocimum sanctum* linn commonly known as *Tulsi* is a widely and easily available plant throughout India [19,20]. Leaves of a mature tulsi plant contain bright yellow coloured volatile oil with pleasant smell. The oil content in the *Tulsi* leaves, which has medicinal properties, depends upon the place of cultivation and season of its collection. The oil is collected by steam distillation method from the leaves and flowering tops. It contains eugenol, carvacrol and eugenol methyl ether. It also contains caryophyline, ursolic acid, aspignigenium, luteolin, spigenium-7, glucuronid, orientin and molludistin. ß-ursolic acid from the ursan group is reported to inhibit the growth of several strains of Staphylococci [21–26].

Copper is well known for possessing an inhibitory effect towards many bacterial strains and microorganisms commonly present in medical and industrial processes. Earlier we have reported the chemical reduction method to prepare copper nano-particles [27] and their effect on physic-mechanical and antimicrobial properties of cotton textiles [28]. In this work, an attempt has been made to synthesize copper nano-particles using methanolic extract of *Tulsi* (*O. sanctum*) leaves. The size and shape of the synthesized particles was analyzed by particle size analyzer and SEM. The prepared particles were elementally analyzed by EDS and FTIR. Finally, the copper nano-particles were tested for their efficiency against human pathogens.

2. Materials and methods

All the chemicals and solvents used for the synthesis were of analytical reagent grade and procured from SD Fine Chem Ltd. (SDFCL) in India. The samples were prepared by using fresh

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double-distilled water throughout the process. Green mature leaves of *O. sanctum* (*Tulsi*) abundantly available in nature were collected without causing any appreciable damage to the parent plant. The leaves were collected in the month of February from South Gujarat region in India.

2.1. Preparation of leaf extract

O. sanctum leaves were thoroughly washed in double distilled water. These leaves were fast dried at 110 °C in an oven and crushed to make powder. 10 gm of the powder was added to 100 ml methanol and stirred at 250 rpm for 1 h to make pure extract. The extract was then filtered using Whatman's No. 1 filter paper (Particle retention pore size 11 μm). The filtrate was collected in a clean and dried container and stored for further use without any purification or analysis.

2.2. Preparation of copper nano sol

100 ml solution of 1×10^{-3} M $CuSO_4$ was slowly reduced by drop-wise addition of 15 ml methanolic extract of *O. sanctum* leaves under atmospheric conditions. During the process of reaction, the solution mixture was stirred vigorously. The process was carried out for 10 min at room temperature. After the incubation period, the solution was filtered thrice using Whatman's No. 1 filter paper. The filtered solution was stored for further use.

2.3. Characterization of copper nano sol

Copper particles prepared by bioreduction process were analyzed in terms of their size and chemical composition. The change in colour of mixed copper sulphate and methanolic extract of *O. sanctum* leaves solution (metals with free electrons possess plasmon resonances in visible spectrum, which give rise to intense colour hydrosol indicating the formation of nano-particles) were observed and recorded using spectrophotometer interphased with computer colour matching system. Methanolic extract of *O. sanctum* leaves was analyzed for spectral transmittance in the visible wavelength range (400–700 nm). *CIE L**, *a**, *b**, c and h values were found using Spectra Scan 5100 spectrophotometer (Premier Colour Scan Instruments, India). The absorbance of nano colloidal solution was recorded using UV–vis spectrophotometer (Shimadzu UV-2450) in the wavelength range of 300 nm to 700 nm. The analysis of particle size and size distribution of the copper colloid was carried out using particle size analyzer (Malvern instrument, DTS Version 4.20. U.K.). Copper nano-particles were deposited on carbon coated aluminium sheet for examination in Scanning Electron Microscope (SEM). Image processing and analysis of the Scanning Electron Microscopy data was performed on SEM (Model JSM5610LV, Version 1.0, Jeol, Japan). The synthesized nano-particles were also elementally analyzed by SEM using Oxford-Inca software (Oxford, U.K.). The chemical composition of prepared nano sol was examined using FTIR spectrophotometer (Nicolet iS10 FT-IR Spectrometer, Thermo Scientific, Japan).

2.4. Antibacterial activity test

The antibacterial activities of the synthesized nano sol were tested against gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*). The disc diffusion method was used to screen the antimicrobial activity [29]. Stock cultures were maintained at 4 °C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube with Muller-Hinton broth (MHB) consisting of the gram positive and gram negative bacteria that were incubated without agitation for 24 h at 37 °C and 25 °C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities measuring to 2.0×10^6 colony forming units (CFU/ml) for both kinds of bacteria.

The Muller Hinton Agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 min 0.1% inoculum suspension was swabbed uniformly and allowed to dry for 5 min. A sample quantity of 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 min. The plates were then kept for incubation at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

3. Results and discussion

3.1. Formation of copper nano sol

Copper nano-particles were prepared by bioreduction of copper salt as discussed earlier. The change in colour of the copper

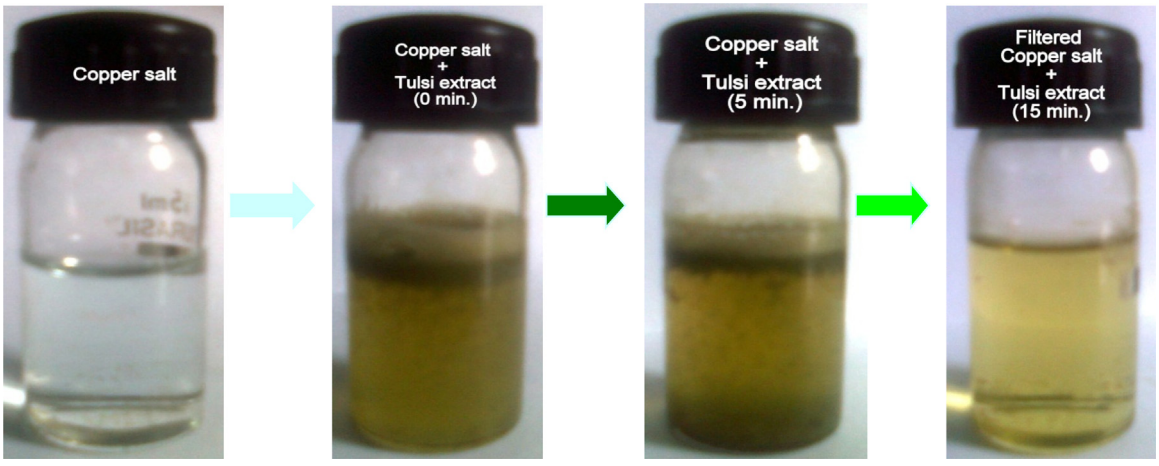

Fig. 1. Progressive colour change of copper sulphate solution during the preparation of copper nano-particles.