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Green Synthesis of Copper Nanoparticles Synthesized Using Black Tea and its Antibacterial Activity Against Oral Pathogens

Research Article

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Abstract

In this current study, Copper nanoparticles were synthesized through green route using black tea as a reducing agent. The green synthesized copper nanoparticles were characterized using UV-visible spectroscopy. And the synthesized nanoparticles was subjected to test its efficiency in biomedical applications such as antioxidant and antimicrobial activity. The UV results confirms the synthesis of copper nanoparticles by representing maximum absorption peak around 350-500nm. The antibacterial activity results showed effective potential of black tea mediated copper nanoparticles.

Keywords: Copper Nanoparticles; Black Tea; Green Synthesis; Oral Pathogens.

Introduction

Copper (Cu) is an essential mineral and transition metal with atomic number 29, known since old occasions. It is a significant follow component for most living beings in all realms. In human biological system, copper assumes job as a cofactor for various chemicals, for example, Cu/Zn-superoxide dismutase, cytochrome c oxidase, tyrosinase, ceruloplasmin and different proteins, essential for respiration ,iron transport and metabolism, cell development, and hemostasis [1, 2].

Past ten to twenty years are the hour of fast advancement in nanotechnology and nanomedicine. Term nanotechnology by and large alludes to science and physical science of 1–100 nm measured particles. Decrease of size has opened additional opportunities for utilization of metallic components and their mixes in medication. Cations of metal can be complexed with multi-part macromolecular ligands, so the subsequent concoction develops can beat constraints in circulation, bioavailability and restricting explicitness of basic compounds [3-6]. Biocidal properties of copper have been known since antiquated occasions and possess antibacterial, antifungal, molluscicidal, nematocidal, antiviral properties [7, 23-25]. Copper has efficient antimicrobial properties. Copper-silver electrolytic ionization frameworks are utilized in numerous medical clinics to diminish number of *Legionella* dwelling in boiling water pipes. Metals and amalgams utilized in orthopedic implants can be doped with copper particles, in request to decrease danger of disease after prosthetic medical procedure [8, 19-22].

As per World Health Organization, 80% of the population in developing countries relies upon traditional medication to fix their wellbeing needs [10]. At this moment, scientific system is focusing on the bioactive mixes, compound structure, and pharmacological ability of various plant species to convey drugs with minor side effects [9-11].

In traditional physical and chemical techniques, the reducing and

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stabilizing agents involved in the production of nanoparticles convey a danger of lethality to the earth and to the cell. In the green synthesis technique wherein nanoparticles with biocompatibility are made, these agents are ordinarily present in the employed natural living beings [12].

In worldwide, the most consumed beverage is Tea. Tea is prepared from the dried leaves of the evergreen plant Camellia sinensis. Green tea is increasingly famous in Japan and China though, black tea is consumed around the world [13]. Studies reveals that black tea component contain anti-inflammatory, antioxidant, antiviral, and against cancer causing properties [14-16].

The present study stands remarkable because it deals with the biosynthesis of copper oxide nanoparticles with black tea extract (*Camellia sinensis*) as reducing agent. The aim of this study is to test and reveal the antibacterial, antioxidant, anti-inflammatory and cytotoxic effect of the synthesized black tea intervened copper oxide nanoparticles.

Materials and Methods

The chemicals used in this study such as Copper sulphate, Mueller Hinton agar, Mueller Hinton broth, acid were purchased from Hi-media laboratories Pvt. Ltd, India. DPPH from Sigma Aldrich and the bacterial cultures such as *Staphylococcus aureus, Streptococcus mutans, Enterococcus faecaliswere* isolated and collected from Saveetha dental college and hospital, SIMATS, Poonamallee, Tamilnadu, India.

Preparation of Plant Extract

Black tea powder *(Camellia sinensis*) was bought at a supermarket near Poonamallee. To set up the extract, 1g of black tea powder was dissolved in 100ml of distilled water and boiled at 60-80°C for 10 minutes using a heating mantle. The boiled extract was filtered using Whatmann No.1 filter paper. The filtrates were stored in 5°C for further experiments.

Synthesis of Copper Nanoparticles Using Black Tea Extract

The aqueous extract of Black tea (*Camellia sinensis*) was used for the bio reduction of copper sulphate into copper nanoparticles. For biosynthesis of CuNP, 0.2M of Copper sulphate was dissolved in 60ml of distilled water and kept in magnetic stirrer for few minutes. To that, 40ml of filtered black tea extract (*Camellia sinensis*) was added. The solution mixture was kept in magnetic stirrer at 650-800rpm for 72 hours. The colour changes in the reaction mixture were noted. The reduction ability of copper sulphate into copper nanoparticles by black tea extract as reducing agent was confirmed using double beam UV-visible spectrophotometer at different wavelength ranges from 250-650nm.The synthesized black tea extract mediated copper nanoparticles were centrifuged at 8000rpm for 10 minutes. The obtained copper nanoparticle pellet was calcined using a hot air oven at 70°C for 2 hours and preserved in air tight vials for further use.

Characterization of Copper Nanoparticles

Double Beam UV-visible spectrophotometer (Esico Spectrophotometer, India) was utilized to view and confirm optical property of black tea mediated copper nanoparticles in the wavelength range of 300-600nm.

Determination of Antimicrobial Activity Of Copper Nanoparticles

The antimicrobial activity was determined by agar well diffusion method. Mueller Hinton Agar was prepared, sterilized using autoclave at 121°C for 15-20minutes. The sterile MHA media was poured on the surface of the sterile Petri plates and allowed for solidification. After solidification, the pathogens such as *Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis* were swabbed using sterile cotton swabs. The wells were made using a T – shaped well cutter. Among four wells per plate 3 wells were loaded with black tea extract (*Camellia sinensis*) copper pellet solution in the concentration range of 25µL,50µL,100µL (100µg/ml) and the fourth well loaded with a standard antibiotic (Amoxyrite) in the concentration of 10µg/mL.

Then the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed and measured for Zone of inhibition around the nanoparticle and antibiotic loaded wells.

Result And Discussion

Visual Observation

The visual observation and identification of colour change is a primary tool that affirms the capacity of plant extract in nanoparticle synthesis [18]. Formation of red to brown black colour could affirm the nearness of copper nanoparticles. Transformation of red to brown black colour was observed after the reaction mixture was kept under constant stirring using a magnetic stirrer at 650-800rpm at 60°C. The colour intensity present in the reaction mixture affirms the capacity of the black tea extract to lessen copper sulphate into copper nanoparticles.

Figure 1. Synthesis of copper nanoparticles using Black tea (*Camellia sinensis*) extract.

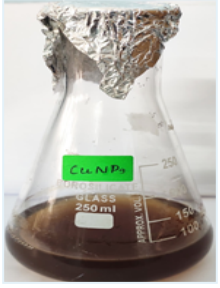


Figure 2. UV-visible spectra of black tea mediated copper nanoparticles.

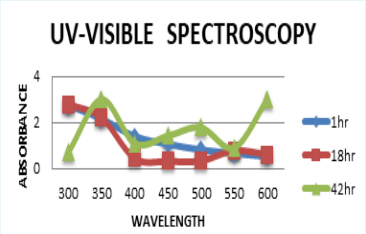
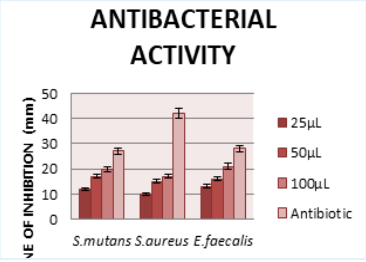


Figure 3. Antibacterial activity of black tea intervened copper oxide nanoparticles.



UV –Visible Spectroscopy

Fig 2 shows the optical property of black tea intervened copper nanoparticles that exhibits maximum absorption around 350-500nm.This intense surface plasm on resonance reveals the formation of copper oxide nanoparticles.

Antibacterial Activity

Oral pathogens such as *Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis* was used to test the antibacterial activity of the black tea mediated copper oxide nanoparticles.

Histogram shows that gram positive organism, *Streptococcus mutans* shows better antibacterial activity with zone diameter of 20mm at 100µL concentration than the other gram positive organism, *Staphylococcus aureus* which shows zone diameter of 17mm at 100µL concentration.

On other hand, gram negative organism *Enterococcus faecalis* shows higher antibacterial activity with zone diameter of 21mm at 100µL concentration than the gram positive organisms. The distinction in inhibitory action of copper oxide nanoparticles by black tea extract against gram positive and gram negative microorganisms is because of the arrangement of the cell wall [18].

Conclusion

The copper nanoparticles synthesized using green tea shows good antibacterial activity against oral pathogens. Based on this this results the copper nanoparticles maybe used in many dentistry applications and development of dental based products.

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