BLUE TEA MEDIATED SYNTHESIS AND CHARACTERISATION OF COPPER NANOPARTICLES: AN IN VITRO STUDY

Running Title: Synthesis and characterisation of copper nanoparticles using blue tea

ABSTRACT:

Background: Nanoparticles (NPs) are often regarded as essential components of nanotechnology. Butterfly-pea flower tea, often known as Blue Tea, is a caffeine-free or herbal tea produced from an infusion of the *Clitoria ternatea* plant's flower petals. In the creation of nanoparticles, plant extracts may act as reducing and stabilizing agents.

Aim: The study's goal was to evaluate the characterization of copper nanoparticles derived from blue tea.

Materials and Methods: The green synthesis approach was used to make the blue tea mediated copper nanoparticles. UV spectrometry and transmission electron microscopy were used to examine morphological characteristics such as form and size of the green produced copper nanoparticles.

Results: The results revealed that the nanoparticles mediated by blue tea extract were eco-friendly, excellent, and non-toxic. Copper nanoparticles were well disseminated and crystalline in nature, according to TEM pictures. Copper nanoparticles were spherical in shape. The particles were 5-10 µl in size. The TEM image showed that nanoparticles were not mixed, but were separated by equal interspaces between them, as validated by microscope visualization at a greater resolution.

Conclusion: Using blue tea, a simple, biological, and low-cost method for the manufacture of copper nanoparticles was developed in this study. To determine the efficacy of these nanoparticles, the generated copper nanoparticles can be exposed to several biological activities such as antibacterial, antifungal, and cytotoxic evaluation.

Keywords: Butterfly pea; *Citoria ternatea*; Green synthesis; Innovative; Nanoparticles.

INTRODUCTION:

Nanoparticles (NPs) are thought to be the basic building blocks of nanotechnology (1) due to their exceptional electron catalytic activity, metal nanoparticles are widely used in a variety of electrochemical, electro analytical and bio-electrochemical applications (2). CuNPs (copper nanoparticles) are fascinating because of their unique features, which include a high surface-to-volume ratio, high yield strength, ductility, hardness, flexibility, and stiffness.

In a variety of applications, CuNPs showed excellent catalytic, antibacterial, antioxidant, and antifungal activity, as well as cytotoxicity and anticancer effects (3). The creation of copper nanoparticles using blue tea was done in this study in a simple, biological, and low-cost manner (4). To determine the efficacy of these nanoparticles, the synthesized copper nanoparticles can be exposed to several biological activities such as antibacterial, antifungal, and cytotoxic evaluation. Therefore they can be used in daily purposes as a substitute for conventional products and help in the betterment of life (5)

Plant extracts are very useful as they may act as reducing and stabilizing agents for the synthesis of nanoparticles (6). The plant extract mentioned in the study, *Clitoria ternatea*, is commonly known as butterfly pea, which is a perennial herbaceous plant from the Fabaceae family. Butterfly-pea flower tea commonly known as Blue Tea is a caffeine free or herbal tea, beverage made from infusion of the flower petals of the *Clitoria ternatea* plant. These plants have been widely used in traditional medicine, particularly as a supplement to enhance cognitive functions and alleviate symptoms of numerous ailments including fever, inflammation, pain, and diabetes. The novel anthocyanins from C. *ternatea* were termed as “ternatins” which render *C. ternatea* flowers with their vivid blue color were isolated. In addition to it there were abundant unique anthocyanins alongside other secondary metabolites in *C. ternatea* which makes the plant an ideal source of natural additives that can enhance the appearance and nutritive values of consumer products for various pharmacological activities.

There are several methods for synthesis of nanoparticels which includes metal vapour deposition, electrochemical reduction, thermal decomposition, chemical reduction etc from which chemical reduction is the highly preferred method among all other methods as it is simple and economical. The chemical reduction methods use separate reducing and stabilizing agents which are known to generate copper nanoparticles with controlled size and shape. The morphology and size of the copper nanoparticles were investigated and evaluated using the Scanning Electron Microscope and Transmission Electron Microscope The transmission electron microscope was employed to characterize the size, shape and morphology of the prepared copper nanoparticles. The SEM is used mainly to generate high-resolution images of shapes of objects ,to reveal the spatial variations in the chemical compositions external morphology (texture), chemical composition, and crystalline structure of the generated nanoparticles. The biosynthetic pathway for the preparation of nanoparticles potentially eliminates the toxicity and makes the nanoparticles more biocompatible (7). Among the various biosynthetic approaches, the plant extracts has many advantages which includes easily available, safe to handle, possess a broad viability of metabolites, eliminating the cumbersome process such as maintaining the cell culture and extraction and separation can be easily scaled up for the large-scale synthesis of nanoparticles using biosynthesis(8). The main

phytochemicals which are responsible for the synthesis of nanoparticles are terpenoids, flavones, ketenes, aldehydes amines etc(9).The green synthesis of copper nanoparticles is an eco-friendly procedure which uses natural solvent that requires no toxic solvents and no dangerous material for the environment(10) (11–13)(14–17) (18,19).

Our team has extensive knowledge and research experience that has translated into high quality publications.(20)(21)(22)(23)(24)(25)(26)(27)(28)(29)(30–39) The aim of the study was to synthesize and characterize blue tea mediated copper nanoparticles using UV spectrometry transmission electron microscope (TEM).

MATERIALS AND METHODS:

Preparation of blue tea extract:

A sample of blue tea powder was taken and measured accurately to 1g to which 100mL of distilled water was added and boiled for 15-20 minutes at 60-70 degrees and the obtained extract was cooled for some time, then the solution was filtered by using whatman no.10 filter paper. The filtered extract was collected and stored in the refrigerator for further use.

Figure 1: Mixture of blue tea extract in distilled water

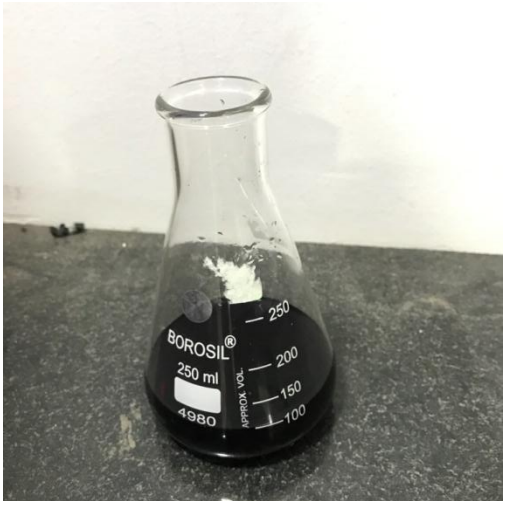

Figure 2: Concentrated extract of Blue tea extract

Synthesis of Copper Nanoparticles:

Synthesis of copper nanoparticles was done biologically using blue tea. 20mM of copper sulphate was added to the obtained extract. The colour change was observed visually and photographed. The solution was kept in a magnetic stirrer for nanoparticle synthesis. The reaction mixture of copper sulphate and blue tea was centrifuged at 8,000xg for 10 minutes. The resulting pellet was washed three times with distilled water and filtered and the supernatant so formed was collected.

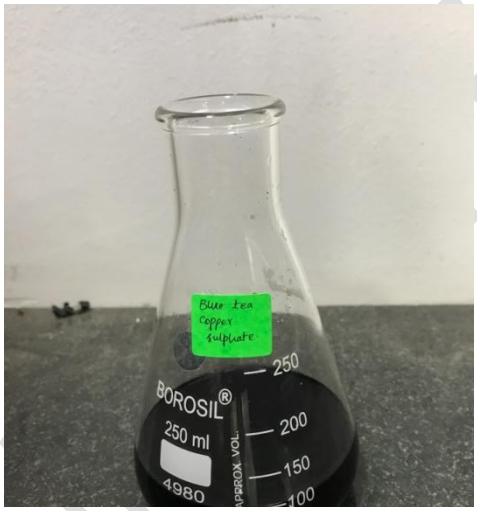

Figure 3: Mixture of copper sulphate and Blue tea

Figure 4: blue tea mediated $CuSO_4$

UV spectrometric analysis of synthesized nanoparticle:

Spectrometric analysis was evaluated by UV-visible spectroscopy. The biologically reduced solution mixture was scanned by Shimazdu, Lambda UV mini-1240 instrument operated at a resolution of 1 nm. The UV-visible analysis was performed in the absorption wavelength of 200 to 700 nm periodically for one hour to observe rapid reduction of copper nanoparticles and the results were recorded for the graphical analysis.

Characterization of prepared copper Nanoparticles:

The synthesized Cu NPs were characterized using TEM (Transmission Electron Microscope). The morphological analysis of the particles was done with TEM. A sample of Cu NPs was loaded on a carbon-coated copper grid, followed by solvent evaporation at room temperature for an hour. The TEM micrograph images were recorded on Zeiss- EM10C instrument on carbon coated copper grids with an accelerating voltage of 80 KV. The clear microscopic views were observed and documented in different ranges of magnifications.

RESULTS:

The synthesized copper nanoparticles were predicted through the visual observation of solution colour change from light purple to dark purplish blue colour. The colour change signifies the presence of copper nanoparticles which was thereby confirmed by the UV-Visible spectrophotometer.

Copper nanoparticles were successfully synthesized using blue tea extract after being subjected to continuous heat and stirring. The purple reaction mixture slowly changed to a thick purplish blue suspension after several minutes of the reaction. The development of intense purplish blue in colour owing to the surface plasmon resonance confirmed the synthesis of the copper nanoparticles. Colour changes of the reaction mixture 240 minutes after the bioreduction process, which were recorded by UV-visible spectrophotometer. UV–Visible readings were recorded in the wavelength range of 200 - 600nm. The absorption formed in the reaction media has an absorbance peak at 570nm. The surface plasmon resonance absorbance was very sensitive to size and shape of the

particles. It was observed that the SPR bands were located at the range 570 nm which is the characteristic absorption peak for copper nanoparticles in this study. (Figure 5)

The centrifuged substrate was then subjected for the TEM analysis for the characterization so as to determine the size, shape and distribution of nanoparticles. TEM images showed that particles were well dispersed, crystalline in nature as shown in the figure below; Copper nanoparticles were spherical in nature. The particle size was ranging from 5 to 10 microns in size. The TEM image showed that nanoparticles were not combined but were separated by equal interspaces between the particles, which were confirmed by microscopy visualizing under the higher resolution. This image explains that the copper nanoparticles are bounded with the phytochemicals of the plant extract. (Figure 6)

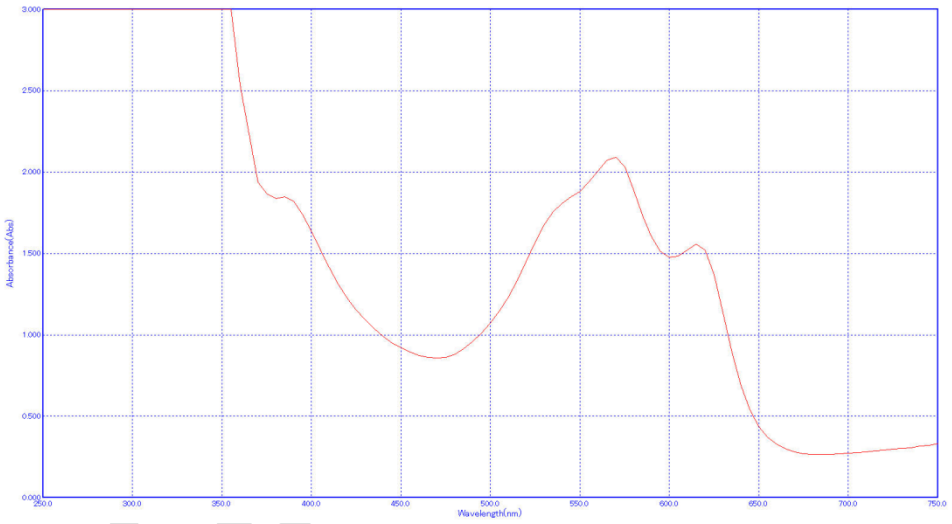

Figure 5: UV-Visible Spectrophotometer's image depicting the synthesis of copper nanoparticles using blue tea extract. The X axis represents the wavelength (nm) and the Y axis represents absorbance (Abs). The UV visible spectra of the copper nanoparticles showed a peak of 570 nm.

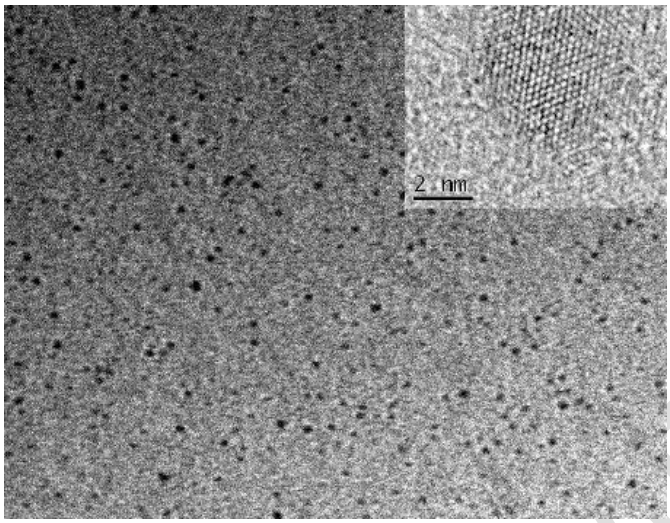

Figure 6: TEM image which confirmed

the synthesis of CuNPs, which were crystalline, spherical in shape with an average size 5-10 microns.

DISCUSSION:

The current study was undertaken to synthesize and characterize blue tea mediated copper nanoparticles which could be used as adjuncts in medical and dental fields.

Cheirmadurai K et al in 2014 synthesized copper nanoparticles using henna leaf extract and their morphological characteristics were evaluated using transmission electron microscope. The TEM analysis revealed the crystalline spherical, shape of copper nanoparticles of size 25-30 nm synthesized from henna leaf. The UV-visible spectrum showed maximum absorption at 265 nm which confirmed the presence of active compound laws one (40). The change of colour from blue to reddish brown indicated the formation of copper nanoparticles.

Similarly, Sampath M et al in 2016 synthesized copper nanoparticles using *Eclipta prostrata* leaf extract and their morphological characteristics were evaluated using TEM (41). TEM analysis revealed the crystalline face centric cubic shape of copper nanoparticles of size 23-50 nm .The UV-visible spectrum showed maximum absorption at 385 nm which confirmed the presence of copper nanoparticles

Niharika N et al in 2018 generated copper nanoparticles from *Azadirachta indica* leaves and their morphological characteristics were analyzed using UV-visible spectrophotometer. The biosynthesized copper nanoparticles were crystalline, spherical in shaped with the average size of 48nm.The highly stable copper nanoparticles obtain the maximum absorption peak at 430 nm (42).

Amir K et al in 2016 synthesized copper nanoparticles from coriander oleoresin extract and their morphological characteristics were analyzed using TEM (43) .The copper nanoparticles synthesized were crystalline spherical in shape of an average size of 20 nm. The UV-visible spectra showed a surface plasmon resonance at 560 nm.

The current study was conducted similar to the previous studies (16) (18,19) and their characterization done using transmission electron microscope revealed similar results to the articles mentioned above. However further analysis must be done to study their antibacterial, anti inflammatory ,antioxidant activities .

CONCLUSION:

In this study, a simple, biological and low-cost approach was used for the preparation of copper Nanoparticles using blue Tea(44) (45) (46) (47) (48) (49) (50) (51) (52) (53) (54) ((54,55) (56) (57). The green synthesized copper nanoparticles can be subjected to the various other biological activities such as antibacterial, antifungal, cytotoxic evaluation to know the efficiency of these nanoparticles do that they can be used as a substitute for conventional chemical products thereby reducing the cytotoxicity.

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