# 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 being viewed as fundamental building blocks of this technology. Butterfly-pea flower tea commonly known as Blue Tea is a caffeine free or herbal tea made from infusion of the flower petals of the Clitoria ternatea plant. Plant extracts may act both as reducing agents and stabilising agents in the synthesis of nanoparticles.

**Aim**: The aim of the study is to assess the characterisation of the copper nanoparticles obtained from blue tea.

**Materials and Methods:** The blue tea mediated copper nanoparticles were synthesised using the green synthesis method. Morphological characters like the shape and size of the obtained green synthesized copper nanoparticles were observed by transmission electron microscope.

**Results**: The results confirmed that the synthesised blue tea extract mediated nanoparticles are eco-friendly, good and non toxic. TEM images showed that the copper nanoparticles were well dispersed, crystalline in nature. Copper nanoparticles are spherical in nature. The particles were of the size 5-10 microns in size.The TEM image shows that nanoparticles are not combined but are separated by equal interspace between the particles, which was confirmed by microscopy visualising under the higher resolution.

**Conclusion**: In this study, a simple, biological and low-cost approach was done for the preparation of copper nanoparticles using blue tea. Thus the synthesized copper nanoparticles can be subjected to various other biological activities such as antibacterial, antifungal and cytotoxic evaluation to know the efficiency of these nanoparticles.



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

## INTRODUCTION:

The nanoparticles (NPs) are being viewed as fundamental building blocks of this technology(1). Metal nanoparticles are extensively used in various electrochemical, electro analytical and bio-electrochemical applications owing to their extraordinary electron catalytic activity(2). Copper nanoparticles (CuNPs) are of great interest due to their extraordinary properties which includes high surface-to-volume ratio, high yield strength, ductility, hardness, flexibility, and rigidity.

CuNPs show high catalytic, antibacterial, antioxidant, and antifungal activities along with cytotoxicity and anticancer properties in many different applications(3). In this study, a simple, biological and low-cost approach was done for the preparation of copper nanoparticles using blue tea(4).Thus the synthesis copper nanoparticles can be subjected to the various other biological activities such as Antibacterial, Antifungal, Cytotoxic evaluation to know the efficiency of these nanoparticles. Hence 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 both as reducing agents and stabilizing agents in the synthesis of nanoparticles(6). 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.The biosynthetic pathway of nanoparticles preparation potentially eliminates the toxicity and makes the nanoparticles more biocompatible(7). Among the various biosynthetic approaches, the plant extracts has advantages such as 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 responsible for synthesis of nanoparticles are terpenoids, flavones, ketones, aldehydes amines etc(9).The green synthesis of copper nanoparticles is an eco-friendly method and 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 synthesise and characterise blue tea mediated copper nanoparticles using transmission electron microscope (TEM).



## MATERIALS AND METHODS:

### Preparation of blue tea extract:

A sample of blue Tea powder is taken and measured accurately to 1g to which 100mL of distilled water is added and boiled for 15-20 minutes at 60-70 degrees and the obtained extract is cooled for sometime, then the solution is 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

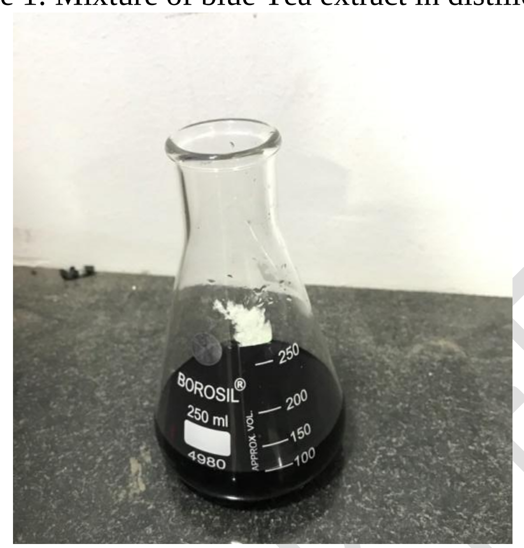


Figure 2: Concentrated extract of Blue Tea extract

**Synthesis of Copper Nanoparticles:**

Synthesis of Copper Nanoparticles is done biologically using blue tea. 20mM of copper sulphate is added to the obtained extract. The colour change was observed visually and photographed. The solution is 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.

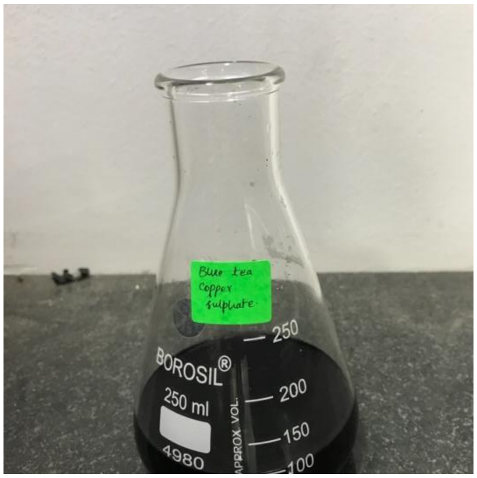


Figure 3: Mixture of Copper sulphate and Blue tea

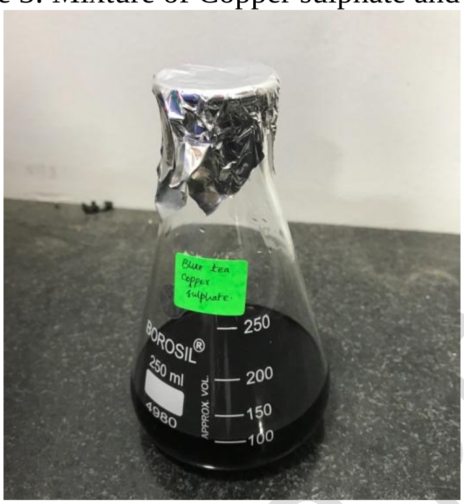


Figure 4: The final reaction with blue tea mediated CUSO4 Nanoparticles

**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.

**Characterisation of prepared Copper Nanoparticles:**

The synthesized Cu NPs were characterised using TEM (Transmission Electron Microscope). The morphological analysis of the particle 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 synthesised copper nanoparticles were predicted through 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 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 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 are 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 characterisation so as to determine the size, shape and distribution of nanoparticles. TEM images show that particles are well dispersed, crystalline in nature is 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 are not combined but are separated by equal interspace between the particles, which was 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.**

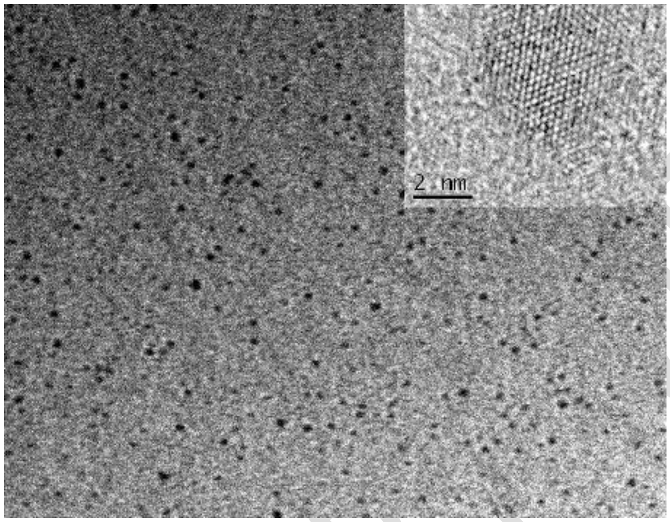


**Figure 6: TEM image which confirms the synthesis of CuNPs, which were crystalline, cubical in shape with an average size 5-10 microns.**

## DISCUSSION:

The current study was undertaken to synthesize and characterise blue tea mediated copper nanoparticles. Thereby analysing its biologic properties to be used as adjunct in medical and dental fields.

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

Similarly, Sampath M et al in 2016 synthesised 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 confirms 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 biosynthesised copper nanoparticles were crystalline,cubical 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 synthesised copper nanoparticles from coriander oleoresin extract and their morphological characteristics were analyzed using TEM (43) .The copper nanoparticles synthesised were crystalline cubical 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 characterisation 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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