GREEN SYNTHESIS OF COPPER NANOPARTICLES MEDIATED FROM Coffee arabica SEEDS EXTRACT

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ABSTRACT

The goal of this study is to synthesize copper nanoparticles (CuNPs) using Coffee arabica extract at room temperature using simple methods, characterize nanoparticle generation, and explore the impact of extract concentration on the production of copper nanoparticles, followed by a qualitative phytochemical screening of coffee extract metabolites and CuNPs toxicity demonstration. To characterize nanoparticle generation, color change, and UV-visible spectrophotometer were explored, analyzing by the Fourier Transform Infra-Red (FTIR) spectroscopy detected the availability of major functional groups, and the X-ray diffractometer demonstrated nanoparticles size. Additionally, CuNPs safety was demonstrated by hemolysis assays. The results revealed that the Coffee arabica beans solution shifted copper ions into copper nanoparticles at room temperature within 15 min of reaction time, and also registered peak absorbance at 262 nm in 3 days and 30 days to conform synthesis and stability CuNPs. In addition, FTIR analyses demonstrated the corresponding bonds (-OH, C=C, and C-H) that are responsible for the formation of CuNPs. Furthermore, the XRD results reported CuNPs to have a monoclinic crystalline shape with a mean crystallite size of 16.3 nm, CuNPs were found to be harmless in hemolytic experiments. Thus, this method can be used for optimum rapid synthesis of copper nanoparticles.

Keywords: Copper Nanoparticles, Coffee arabica, Green Synthesis.

INTRODUCTION

Nanomaterials are now considered as a new discipline in modern science and technology, nanotechnology achieved widespread attention due to its numerous applications in most parts of life, including nanoelectronics, nanomedicine, and agricultural fertilizers. Nanomaterials are essentially based on nanoparticle manufacturing, which comprises two approaches: breakdown and buildup. These ways are often handled in the physical, chemical, and biological formation of nanoparticles. The bottom-up approach is commonly used in chemical and biological synthesis, whereas the breakdown method is utilized in physical production.¹ Physical methods are limited due to their high cost, environmental contamination, difficulty of use, etc. A toxic chemical method is harmful to the environment, but the synthesis of nanoparticles by plants and microorganisms is considered clean, eco-friendly, easy, nil-energy, and low-expense. Hence, the botanical herals are selected more frequently to produce nanoparticles.² Bio-mediated nanoparticles are a build-up process that involves three stages, selection of reaction media, reduction, and stabilization.³ To build nanoparticles, phytochemical compounds such as vitamins, amino acids, proteins, polyphenols, flavones, terpenoids, polysaccharides, alkaloids, tannins, and saponins are working as antioxidant, reducing, and capping agents.⁵ Literature review indicate that CuNPs are preferred to other metals nanoparticles as a promising strong candidate for the future because the copper is a strong oxidizing agent, have a large melting degree as well as good conductor for electricity and a limited chemometer, its shape and size are small, and consider relatively cheap.⁶
The use of biological materials to produce copper nanoparticles are biochemistry method that utilizes the organic plant substance as a reduction agent to synthesize NPs. The metal NPs induction is as plain as combining metal ions with plant extract. Recently, copper nanoparticles were created using simple biological methods by many scholars.\textsuperscript{7–17} Green synthesis includes certain critical features for creating stable and better-characterized nanoparticles, such as the best plant selection, optimal reaction conditions, and characterization techniques. Choosing the ideal plant for green synthesis must be attention to its detoxifying and heavy metal deposition capabilities, as well as reaction variables like time, heat, pH, and light.\textsuperscript{18} The shape, amount, and size of nanoparticles are determined by optimum physical parameters like dark, heat, pH, plant metabolites, and the number of metal atoms. These parameters may help researchers to get the best way of nanoparticle fabrication and predict the architectural shape of nanoparticles which results in various forms such as rectangles, circles, squares, pentagons, polygons, and others.\textsuperscript{9} The widespread practice of copper nanoparticles in medicinal and industrial sectors encourage us to produce copper nanoparticles from the extract of \textit{Coffee arabica}, also known as Mokha coffee, which comes from the famous Yemeni port city named Mokha. \textit{Coffee arabica} is classified within the Rubiaceae family which contains chemical components such as alkaloids, flavonoids, glycosides, saponins, terpenes, proteins, carbohydrates, and lipids. The coffee plant also has a variety of metabolites such as fructose, asparagine, palmitic acid, linolenic acid, caffeine, trigonelline, polyphenols, choline, cafestol, kahweol, flavonoids, alkyl ester, and melanoidins.\textsuperscript{19–22} Multiple studies have found that coffee is high in pharmacologically active compounds, particularly polyphenols, which have anti-inflammatory, antibacterial, antiviral, antioxidant, and hepatoprotective properties. Coffee also includes powerful antioxidants such as chlorogenic, caffeic, and ferulic acids, as well as kahweol. This is leading to protecting the cells from injury, reducing inflammation, and increasing immunity.\textsuperscript{23–26} In this work, we proposed a simple approach for producing CuNPs from \textit{Coffee arabica} at room temperature, which will add a new method in the green synthesis nanoparticle field, to use it in many applications in life. We used a multi-concentration of coffee extract to explore the optimal condition for the synthesis CuNPs. We also demonstrated CuNPs toxicity by hemolysis experiment, and characterize it using UV-Vis spectrophotometer, XRD, and FTIR spectroscopy methods.

\textbf{EXPERIMENTAL}

\textbf{Materials and Methods}

All of the chemicals used, such as copper sulphate, methanol, phosphate buffer then reagents of Benedict, Biuret, Phenols, Dragendorff, Braemer, Shinoda, Liebermann, and Frothing, were prepared from the highest purity materials (Merck Company, Germany). The equipment used in the study, included the centrifuge magnetic stirrer, volumetric flasks, graduated cylinder, funnel, glass vial tubes, pH- meter, oven, and sensitive balance.

\textbf{Preparation of Extract}

The \textit{Coffee arabica} seeds were brought from Yemen's Taiz farms (kind of high quality), after grinding the seeds twenty grams of \textit{Coffee arabica} powder were weighed and put in a flask with distilled water (100 mL) and then heated for half an hour on a hot plate with a magnetic stirrer at 80°C and stored in the fridge. After 24 hours, using a vacuum pump the combination was separated by Whatman filter paper No.11. The clear brown extract solution was kept in the fridge for additional tests.

\textbf{Synthesis of CuNPs}

After that, the filtered coffee extract was combined with 40 mM of copper sulfate pentahydrate (\text{CuSO}_4\cdot 5\text{H}_2\text{O}) solution and then kept overnight at room temperature. Using a centrifuge (HERMLE Z 366 K1) at 12,000 rpm for 15 minutes at 4°C, CuNPs were collected after rinsing it three times with distilled water. The pellet was suspended in deionized water and then dried in an oven at 80°C for 18 hours to get powdered copper nanoparticles.\textsuperscript{27}

\textbf{Effect of Concentration on the Production of Copper Nanoparticles}

To explore the optimum concentration ratio for the synthesis of copper nanoparticles. Temperature, pH, and time factors were fixed. Extract of \textit{Coffee arabica} and copper sulphate solution were combined in the following ratios: (1:1; 1:2; 1:5; 1:10; 2:1; 5:1, and 10:1) with constant other factors, then were synthesized CuNPs as was mentioned.\textsuperscript{9}

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CuNPs Characterization
The change in color of the reaction mixture was the first indicator, that supports the reduction of metallic salts into nanoparticles.

UV-Vis Spectrophotometer
The copper nanoparticles were characterized using a UV-Vi’s Spectrophotometer (UV-2600, Shimadzu Europa GmbH). At three days after synthesis, CuNPs were surveyed from 200 nm to 800 nm and the values were recorded, also the survey was repeated after 30 days to approve the stabilization.

Fourier Transform Infrared Spectroscopic (FTIR)
The FTIR spectral device (IR Prestige-21, Shimadzu Europa GmbH) was employed to study the dried sample of copper nanoparticles and coffee extract with 4 cm$^{-1}$ and 64 scans, the analysis was performed with KBr pellets.

X-Ray Diffraction Analysing
X-Ray Diffraction Spectroscopy (Shimadzu XRD-7000) was used to examine the crystallinity, phase content, phase purification, and size of nanoparticles. XRD studies of the prepared nanoparticles were measured between 10 and 80 degrees.

Analyses Copper Metal
The stability and purity of CuNPs were examined by calculation of copper ions from synthesized CuNPs using an Atomic Absorption Spectrometry (AAS) (Buck Scientific, USA, Model 205), with a bandpass of 0.7 nm, lamp current of 1.5 mA, and wavelength of 324.7 nm. After combining 1 mg of CuNPs with 5 mL of de-ionized water, the mixture was kept at room temperature for 30 days. CuNPs were separated from de-ionized water by centrifuging it for five minutes at 12,000 rpm. The supernatant was later used to determine the amount of Cu ions. A standard solution containing 5 ppm copper was prepared according to standard methods.

Phytochemical Screening – Qualitative Analysis
For the recognition of active substances in *Coffee arabica*, qualitative phytochemical screening was performed using basic phytochemical analytical techniques. 28

Hemolysis Study of Copper Nanoparticles
The toxic effect was measured using the same procedure as in a previous research. 29 Human blood (5 mL) was drawn and placed in a tube containing EDTA as an anticoagulant. The blood sample was centrifuged at 1000 rpm for 5 minutes. The plasma was removed, and the cells were rinsed three times in a sterilized phosphate buffer solution (PBS). Then 0.5 mL suspension cells were combined with 1 mL of the nanoparticles at concentrations of (200, 100, 50, 25, 12.5, and 6.25 mg/L). The mixture was left to stand for one hour at 37 °C. After sedimentation, the free hemoglobin in the supernatant was quantified using a digital spectrophotometer (Milton Roy Spectronic 20D) at 540 nm. For the negative and positive control, phosphate buffer solution (PBS) was the negative control, and 1% HCl was the positive control. The % hemolysis of the samples was estimated using the following equation:

\[
\% \text{ Cytotoxic} = 100 \times \frac{(SA - NCA)}{(PCA - NCA)}
\]

Where, SA = Sample Absorbance, PCA = Positive Control Absorbance, and NCA = Negative Control Absorbance.

RESULTS AND DISCUSSION

Synthesis of CuNPs
*Coffee arabica* extract and copper sulphate solution were quickly converted into CuNPs, and the color change was visible to the naked eye. The appearance of the extract was shifted from brown to green immediately due to surface plasmon vibrations, then after 30 min transformed into a clear yellow-green color (Fig.-1) The colonization and agglomeration sediment of solution with yellow-green supernatant indicate the reduction of phytochemical compounds present in the plant extract, likewise, in other studies.
the color changing and agglomeration were observed when CuNPs was synthesized using extracts of *Punica granatum*, *Ageratum houstonianum*, aqueous garlic and *Ocimum basilicum*.30–33

**Effect of Concentrations on the Synthesis of Copper Nanoparticles**

For the best result to increase synthesis nanoparticles, the concentration rate of *Coffee arabica* extract to CuSO$_4$ solution was adjusted. Each concentration produced varying amounts of synthesized CuNPs (Table-1). The amount of 71.2 mg was harvested at a concentration ratio of (1:1). The scholars mentioned that the synthesis of nanoparticles is affected by time factor, for example, color changes can occur within just as 10 minutes in Citrus limon fruit extract, 30 minutes in *Aloe vera* flower extract, 1 hour in *Asparagus adscendens* leaves extract, and 48 hours in *Allium sativum* extract.9,27,34 The compound type in the extract is critical in the production and stability of CuNPs, also CuNPs size, and shape are influenced by the concentrations of phytochemicals and used metal ions. For example, the optimum formation of CuNPs was found with lemon extract with 1mM CuSO$_4$ (1:10), *Myrtus communis* leaves extract with 1mM CuSO$_4$ (1:1), *Jatropha curcas* leaf extract with 3mM CuCl (1:4) and *Fortunella margarita* plant extract with 1mM CuSO$_4$ (1:2).9,36–38 The pH is a significant factor to control the synthesis CuNPs. The number of hydrogen ions in the extract might cause inactivity of active substances, leading to a change in the amount of the formed nanoparticles. According to a former paperwork recommendation, in our study we used an acidic medium (pH 4) likewise, a study on the synthesis of CuNPs by *Azadirachta indica* leaves stated that the optimum conditions are pH 6. Another investigation into the production of copper nanoparticles with *Piper retrofractum* extract concluded that the optimum reaction is an acidic medium, but other scholars recorded pH 7 as the best condition for the fabrication of CuNPs.39,40

**Table-1: Impact of Various Factors on the Generation of CuNPs**

<table>
<thead>
<tr>
<th>Concentration Percentage</th>
<th>pH (CuSO$_4$.5H$_2$O)</th>
<th>Temperature (°C)</th>
<th>Time (days)</th>
<th>Yield NPs (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>4</td>
<td>30</td>
<td>3</td>
<td>29.4</td>
</tr>
<tr>
<td>1:5</td>
<td>4</td>
<td>30</td>
<td>3</td>
<td>31.4</td>
</tr>
<tr>
<td>1:2</td>
<td>4</td>
<td>30</td>
<td>3</td>
<td>39.3</td>
</tr>
<tr>
<td>1:1</td>
<td>4</td>
<td>30</td>
<td>3</td>
<td>71.2</td>
</tr>
<tr>
<td>2:1</td>
<td>4</td>
<td>30</td>
<td>3</td>
<td>34.4</td>
</tr>
<tr>
<td>5:1</td>
<td>4</td>
<td>30</td>
<td>3</td>
<td>28.4</td>
</tr>
<tr>
<td>10:1</td>
<td>4</td>
<td>30</td>
<td>3</td>
<td>29.3</td>
</tr>
</tbody>
</table>

**CuNPs Characterization by UV-Visible Spectrophotometer**

The surface plasmon resonance (SPR) of CuNPs was measured between 200 and 800 nm using a spectrophotometer (UV- 2600, Shimadzu Europa GmbH). CuNPs have an absorption peak at 264 nm in concentration (1:1, 2:1, and 10:1), the high absorbance (1.240) was recorded at (1:1) concentration. Peaks at 347 and 348 nm are also observed in volumes of (2:1 and 10:1, respectively). This spectrum shows the presence of copper metal and supports the previous result which mentioned that optimal conditions for the synthesis CuNPs are concentration (1:1). Copper oxide nanoparticles typically absorb between 250 to 360 nanometers and copper nanoparticles display absorbance around 550-700 nm.41 In this work, UV-Vis spectrum at 264 nm and 564 nm supports the reduction of copper ions and synthesis of copper nanoparticles (Fig.-2) [Cu$^{++}$ + 2e- (from biomolecules) → Cu + O$_2$ (air) → CuO].
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The spectrum of nanoparticles ranged from ultraviolet UV to visible spectrum, also a broad crest at longer wavelengths often suggests an increase in particle size, whereas a narrow crest at shorter wavelengths indicates the creation of smaller CuNPs. In our study, the sharp peak, which happened in the UV zone agrees with another scholar whose were listed the copper SPR band at 305, 260, 380, and 357 nm.\(^{42-45}\) Also, our results recorded in the visible zone are similar to other researchers who mentioned that the copper SPR band occurred at 550 nm and 588 nm.\(^{46}\) On the other hand, *Coffee arabica* extract was recorded absorbance at 234, 281, and 337 nm, near to our result, a scholar stated that caffeine and chlorogenic acid absorbs at around 274 nm.\(^{47}\)

**Stability Nanoparticles**

Stability NPs are the principle of green fabrication methods. Capping a substance’s phytochemicals aid in the stabilization of NPs for more time, whereas chemical synthesis processes cause them to degrade and settle within 24 hours also generating NPs of large-size.\(^{27}\) In this work, after 30 days from synthesis CuNPs, peaks were found at 265, 262, 257, 256, and 250 nm with increasing absorbance values and disappeared other peaks compare to 3 days (Fig.-3). This supports the stability and small size of copper nanoparticles in the solution. The narrow bands confirm the formation of CuNPs and indicate that the biological molecule in the coffee extract has a dual function of stabilization and formation of CuNPs.\(^{48}\) The stability of copper nanoparticles after thirty-day from synthesis was confirmed, as well as agglomeration was noted. Likewise, one of the studies reported that CuNPs have a high tendency to aggregate in ultrapure water.\(^{49}\)

**Infrared Spectroscopy using the Fourier Transform (FTIR)**

The FTIR spectrum of CuNPs and *Coffee arabica* powder was explained in (Fig.-4). Although there are minor differences in spectral patterns and intensity among CuNPs and *Coffee arabica* extract, the functional groups are generally comparable. The spectrum of 3435, 2860, 2931, 1747–1388, and 1172 cm\(^{-1}\) are attributed to the band OH, CH\(_2\), CH, C=O, and COC groups, respectively, that matched alkaloids, polyphenols, terpenoids, tannins, flavonoids, and other compounds. Peaks at 1023 cm\(^{-1}\) are caused by the aromatic C-H group. Another notable peak can be seen between 500 and 800 cm\(^{-1}\), which points to the C-Cl band of alkyl halides, alkanes, and flavonoid molecules as the stabilizing component of CuNPs. The synthesis of CuNPs was illustrated by the sharp peaks at 632 and 1105 cm\(^{-1}\), similar outcomes using different plant extracts were documented in other studies.\(^{34,50,51}\) Other results registered the FTIR spectrum of copper sulfate pentahydrate with strong peaks at 1153, 981, and 450-658 cm\(^{-1}\), which was marked by the linking of sulfur to oxygen (S=O) in the Cu\(_2\)SO\(_4\).5H\(_2\)O salt.\(^{52}\) According to the results presented here may CuNPs are attracted to the phenols and flavonoids that are found in the *C. arabica* extract and helps in their stability. Many researchers have suggested that macromolecules such as proteins may attach to NPs, especially groups of amine and sulfhydryl, and also mentioned that these compounds cover the NPs surface as a capping agent.\(^{39,40,53,54}\)

**X-ray Diffraction Analysing**

The crystalline property of the CuNPs is determined based on XRD analysis. Figure-5 shows peaks at 20 of 21.39, 24.04, 28.07, 29.35, 31.25, 42.38, 44.00, 51.13, 64.28, and 77.53 corresponding to (100), (75),
(88), (54), (79), (46), (50), (50), (50), and (58) planes, respectively. Based on the XRD spectrum and the International Centre for Diffraction data map (JCPDS NO: 01-410-5040 and 01-080-1148), CuONPs and CuNPs have a monoclinic structure and are crystalline.

Using the Debye-Scherer equation, the mean particle size of CuNPs and CuONPs was found about ~16.3 nm. The crystallization size of less than 100 nm is indicating the nanocrystalline nature of the synthesized CuNPs. Additionally, an XRD analysis revealed other impurity peaks that might be related to precipitates of the capping agent for the plant extract. Similar outcomes were obtained from the synthesized CuNPs by another plant extract.
Fig.-5: XRD Patterns of Cu nanoparticles using *Coffee arabica* Extract

**Analyses Copper Metal**
The liberation of copper ions from synthesized CuNPs after 30 days was calculated (Fig.-6) and was low value (0.32 ppm). According to these results, almost all copper sulfate pentahydrate (CuSO$_4$.5H$_2$O) was transformed into copper nanoparticles and coated by coffee phytochemicals. The finding of the present study confirmed the stability of copper nanoparticles and this is agreed with a scholar who produced copper nanoparticles using *Wrightia tinctoria* extract.57

Fig.-6: Copper Ion Release Assay after 30 days from CuNPs Synthesis by *Coffee arabica* extract, Amount is Mean ± SD (Assay Repeated Three Times)

**Phytochemical Screening-Qualitative Analysis**
The phytochemical screening method is used to estimation active components and secondary metabolites in plants. Alkaloids, flavonoids, terpenoids, steroids, saponins, and phenolic compounds were tested by qualitative assays method.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Reagent</th>
<th>Findings</th>
<th>Photograph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>Benedict</td>
<td>Green color looked</td>
<td><img src="image" alt="Table-2" /></td>
</tr>
<tr>
<td>Protein’s</td>
<td>Biuret</td>
<td>Green precipitate detected</td>
<td><img src="image" alt="Table-2" /></td>
</tr>
</tbody>
</table>
According to diagnostic tests with different reagents (Table-2) summarizes the results and indicates that *Coffee arabica* extract contains compounds of proteins, carbohydrates, phenolics, tannin, flavonoid, terpenoid, steroids, alkaloids, and saponins. Alkaloids are a kind of organic compound that contains nitrogen atoms, and have a wide range of biological effects, including central nervous activation, antihypertension, analgesic, and antiparasitic. A phenolic substance is typically composed of hydroxyl groups. Phenolic acid plays several biological roles in the body, including raising blood pressure, reducing cholesterol levels, and controlling bacteria. The finding of this study revealed to the attendance of some compounds in *Coffee arabica* extract, likewise, other studies have corresponded with our result.\textsuperscript{23,58–61}

**Hemolysis Study of Copper Nanoparticles**

ISO 10993-4 states that a hemolysis rate of less than 5% indicates the test substance can be used for biomedical applications. Our study result (Fig.-7) explained that the supernatants of the tubes (100, 50, 25, 12.5, and 6.25 mg/L) and negative control, were colorless.

![Fig.-7: Toxicity Testing of Red Blood Cells Treated with CuNPs at Different Concentrations](image)

<table>
<thead>
<tr>
<th>Phenols</th>
<th>Phenols</th>
<th>blue black coloration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff</td>
<td>A reddish-brown turbidity color</td>
</tr>
<tr>
<td>Tannins</td>
<td>Braemer</td>
<td>The formation of a yellow precipitate</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda</td>
<td>Pink/yellowish color formation</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Liebermann Burchardt</td>
<td>A red-brown color denoted</td>
</tr>
<tr>
<td>steroid</td>
<td>Chloroform and conc. H_2SO_4</td>
<td>The top part is red, while the sulfuric acid layer yellow</td>
</tr>
<tr>
<td>Saponin</td>
<td>Frothing test</td>
<td>foam formation</td>
</tr>
</tbody>
</table>

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However, the supernatants of the tubes 200 mg/L and positive control were red in color, this demonstrates that the red blood cells had been destroyed. The hemolysis rate in this experiment at 100 mg/L was less than 5% (4.3%), indicating that copper can use in medical applications. Similar results by many researchers investigated the effects of CuNPs on RBCs, the majority of them observed hemolytic actions of CuNPs on RBCs of less than 5%. On the other hand, other researchers have noted that CuNPs induced damage in red blood cells when using physical synthesis methods. RBCs were found to be harmful at a concentration of 200 mg/L, but it's crucial to remember that this corresponds to a human quantity of 14 g of CuNPs for a 70 kg person. Therefore, a toxicity test by hemolytic experiment considers successful and coffee-synthesized CuNPs are safe because the damage was found only at higher concentrations, the high doses are very far to use in the biomedical field, but the data displayed are important in the knowledge of the dose impact of copper nanoparticles on RBCs.

CONCLUSION
Green fabrication of copper nanoparticles with extracted coffee beans has been a significant success. The optimal concentration for the production of CuNPs was one volume of coffee extract to one volume of 40 mM copper sulphate pentahydrate. Copper nanoparticles have been characterized with UV-Vis spectrophotometry, FTIR, and XRD measurements. After 30 days, UV spectrophotometry and atomic absorption analyses confirmed the stability of the produced copper nanoparticles. Coffee arabica extract was examined by qualitative method, and the results indicated it contains proteins, carbohydrates, phenolics, tannin, flavonoids, terpenoids, steroids, alkaloids, and saponins. The toxicity of CuNPs was determined using a human blood assay and concluded that synthesized copper nanoparticles can be used in medical applications.

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CONFLICT OF INTERESTS
The authors declare that there is no conflict of interest existed.

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