Analysis of the UV-VIS Spectra and FWHM for Biogenic Synthesis of Copper Oxide Nanoparticles Using *Piper Betel L.* Leaves Extract

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Abstract. Currently, studies on green synthesis of copper oxide nanoparticles (CuO NPs) applying plant extracts as reducing agents are developing rapidly. This methodology is very cost-effective and eco-friendly. *Piper Betel L.* is one of the medicinal plants used for the biosynthesis of CuO NPs. In our study, the biosynthesis of CuO NPs was conducted using different volume ratios of the plant extract and $CuSO_4$ with increasing reaction times. According to the outcomes of the phytochemical test, flavonoids, tannins, phenols, terpenoids, and saponins are present in betel leaf extract. These phytochemicals act as a reducing agent, reducing Cu^{2+} to Cu^0. Color change was observed from the blue solution to a deep greenish brown with increasing the reaction time. From UV-Visible spectra, the highest wavelength was observed at 292nm, which proves the formation of copper oxide nanoparticles. Due to surface plasmon resonance (SPR), these spectra got sharper with increasing reaction time and different volume ratios of plant extract and $CuSO_4$.The particular shape and size of CuO NPs are indicated by the formation of multiple absorption peaks in the UV-Visible spectra. These green-synthesized copper nanoparticles show antimicrobial and anticancer properties for human health. They also contain antioxidants to reduce oxidative stress. Due to their small size, they are widely used to prepare nanomedicine to control drug release.

Keywords: Copper oxide nanoparticles, Betel leaves, Green synthesis, Volume ratio

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1 Introduction

Piper Betel L. referred to as betel leaf, is an aromatic climber plant in the Piperaceae family, one of the popular medicinal plants. The leaves are looking glossy and heart-shaped, chewed for their stimulant effects [1]. These plants are widely grown throughout South and Southeast Asia. Betel leaves contain different bioactive compounds like phenols, alkaloids, and flavonoids, which help to show antibacterial, antioxidant, and anti-inflammatory characteristics [1]. Phenolic components of the leaves indicated activity against the obligatory oral anaerobes that cause halitosis [2]. Due to the inherent therapeutic potential, these leaves are used in the formation of nanoparticles in green synthesis methods and various applications of herbal medicines [3].

Nanotechnology is the process of modifying materials at the atomic level to achieve special features that can be appropriately controlled for the intended applications.In current days, the combination of biology and nanotechnology has the potential to transform the fields of health and medicine and solve several biomedical problems [3]. This combination is known as nanobiotechnology. This advanced technology is used for imaging, sensing, gene delivery systems, artificial implants, and other medicinal applications etc. According to their enhanced ability, new medications prepared from nanoparticles of metals, polymers, or ceramics can combat human pathogens, such as bacteria and cancer diseases [4]. Mostly, metal nanoparticles are prepared from reagents, but there is a growing need to create ecologically safe nanoparticle synthesis methods to prevent toxicity levels. Preparation of nanoparticles from plant extracts, enzymes, or microorganisms is known as the green synthesis. In current days, this technique is more advisable as it is eco-friendly [4]. However, this process does not require a long time or complex maintenance of cell cultures, etc. [5].

Copper oxide nanoparticles (CuO NPs) are a nanoscale substance of copper (II) oxide that exhibits various physicochemical properties, including a high surface area-to-volume ratio, intense catalytic activity, and antibacterial properties. Betel leaf-derived copper oxide nanoparticles contain antioxidants and target mitochondria to reduce oxidative stress in cells. This process triggers apoptosis in tumor cells and helps to stop unwanted tumor growth in different organs. These nanoparticles are utilized to treat prostate, eye, breast, liver, kidney, and lung tumors [6,7]. In the current days, these nanoparticles are widely used in nanomedicine to control drug release and targeted therapies.

This study investigated green synthesis of copper oxide nanoparticles from *Piper betel L.* extract with different volume ratios. Specific volume ratio of Cu^{2+} ions and extracts is one of the factors influencing the reaction rate, size, and shape of CuO NPs developed by biological methods [8,9]. These specific volume ratios of plant extract and copper sulfate solution were chosen on the basis of a previous study and earlier optimization [9]. To determine the optimum ratio, different volumes of extract and precursor ($CuSO_4$) ratios were prepared. This methodology is consistent and helps to find a sharp UV-Vis peak. Formation of CuO NPs can be confirmed by observing the color change from blue solution to a deep greenish brown [10-13]. The size, stability, and rate of reaction of the CuO NPs were determined by analyzing the absorption peak in the UV-Vis spectrum.

2 Materials and Methods

2.1 Sample preparation -

Fresh betel leaves were collected from the local market. Collected leaves were weighed and washed properly under deionized water. The process was repeated two to three times to make sure that all debris and unnecessary particles were removed from the surface. The cleaned leaves were dried in the shade for the next 2 hrs. Again, dried leaves were oven-dried at 50^0C. After the drying process, leaves were cut and carefully ground with a blender to make a powder form.

2.2 Plant extract preparation-

5 g of plant powder were added to 100 ml of distilled water in a clean, dry round-bottom flask to prepare the solution. After that, the mixture was allowed to boil for 30 mins at a regulated temperature of 80°C. The solution was then allowed to cool to room temperature after the boiling period. The cooled solution was filtered with a Whatman 41 filter paper to separate the pure solution. Finally, the prepared extract solution was kept in an airtight bottom flask and stored in a refrigerator for future use.

2.3. Different phytochemical qualitative experiment of *Piper Betel L.* leaves extract

2.3.1 Phenols test –

Ferric chloride test: 2 drops of 5% $FeCl_3$ were added to 2 ml of the extract solution. A blueish-green colored solution was found, which proves the presence of a phenolic group in the solution.

2.3.2 Saponins test –

Foam test: extra deionized water was added to the extract and shaken vigorously. Persistent foam was observed, proving the presence of saponins.

2.3.3 Flavonoids test –

Alkaline reagent test: 1ml of 2% NaOH was added to the extract, and an intense yellowish green solution was found. It has been proven that the presence of flavonoids.

2.3.4 Terpenoids Test –

Salkowski test: 2 ml of $CHCl_3$ and 1 ml of conc. H_2SO_4 was added to the extract and shaken vigorously in a test tube. Reddish brown precipitate was observed, which proves the presence of terpenoids in the solution.

2.4 Preparation of 0.1M $CuSO_4 \cdot 5H_2O$

0.245 g copper sulphate was weighed on a weighing machine. 100ml of deionized water was added to it to prepare the solution.

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2.4.1 Preparation of 100 ml 0.01(M) polyvinylpyrrolidone solution (Control study)

0.111g polyvinylpyrrolidone was measured using a weighing balance. 100ml of deionized water was added to it to prepare the solution.

2.4.2 Preparation of 100 ml (N/2) sodium borohydride solution (Control study)

0.0425 g sodium borohydride was measured using a weighing balance. 100ml of deionized water was added to it to prepare the solution.

2.5 Synthesis of copper oxide nanoparticles without plant extract (Control study)

The prepared polyvinylpyrrolidone solution was taken in a 50 ml beaker. Then, a freshly prepared copper sulfate solution was added to the same beaker. After 10 minutes of stirring, dropwise sodium borohydride ($NaBH_4$) was added to the solution, and a color change was observed.

2.6 Biosynthesis of copper oxide nanoparticle (CuO NPs)

The prepared leaf extract was added to the 0.1 M $CuSO_4{,}5H_2O$ solution under stirring conditions. Two sets of different volume ratios of leaf extract and copper sulphate solution were prepared. Set-a was prepared with volume ratios of extract: $CuSO_4$ (v/v) with a final volume of 10ml [1:9,2:8, 3:7,4:6, and 5:5]. Set-b was prepared with different volumes of extract with the same volume of $CuSO_4$ solution [1:8,2:8,3:8,4:8, and 5:8]. After that, the solutions were kept in the dark room for the duration of the CuO NPs characterization process, which was 1 hour, 24 hours, 48 hours, and 72 hours.

2.7 Copper oxide nanoparticles characterization with UV-Visible spectra

The characterization of copper oxide nanoparticles was performed using a UV-Visible spectrophotometer. Color changes were noticed at reaction times of 0mins, 30mins,1hr, 90mins, 2hrs, 24hrs ,48hrs and 96 hrs. The absorption peak was observed between 200-800nm with increasing reaction time. The solution was diluted by taking 1 ml, and then distilled water was added to fill the rest of the cuvette to measure the spectrum. The collected data were presented in the form of tables and graphs. Graphs are plotted with the help of Origin software.

3 Results and Discussion

Performing different phytochemical qualitative tests of betel leaves extract flavonoids, terpenoids, phenols, and saponins are found as a reducing agent. Color changes proved their presence (Table 1). The hydroxyl groups(-OH) are known to exist in several flavonoid compounds. These hydroxyl groups play an important role in reducing Cu^{2+} to Cu^0 and decreasing the Cu^{2+} ion also.

Table 1. Phytochemicals test for betel leaf extract

Phytochemicals	Observation	Outcomes
Flavonoids	Yellowish green solution	Positive
Phenols	Blueish green colored solution	Positive
Saponins	Persistent foam	Positive
Terpenoids	Reddish brown precipitate	Positive

These phytochemicals play a vital role in the biosynthesis of copper oxide nanoparticles.

3.1 UV-Visible spectra analysis

3.1.1 Control study

Before adding plant extract, a control experiment was performed using only copper sulfate solution. Polyvinylpyrrolidone (PVP-k30) was used to stabilize the nanoparticle formation. Sodium borohydride($NaBH_4$) was used as a reducing agent. After adding it to the copper sulfate solution, a color change was found from deep blue to raw yellow. In UV-Vis spectra, a sharp peak was measured at (λ_{max}) 290 nm. **Fig.1** represents the graph found as the outcome of the experiment.

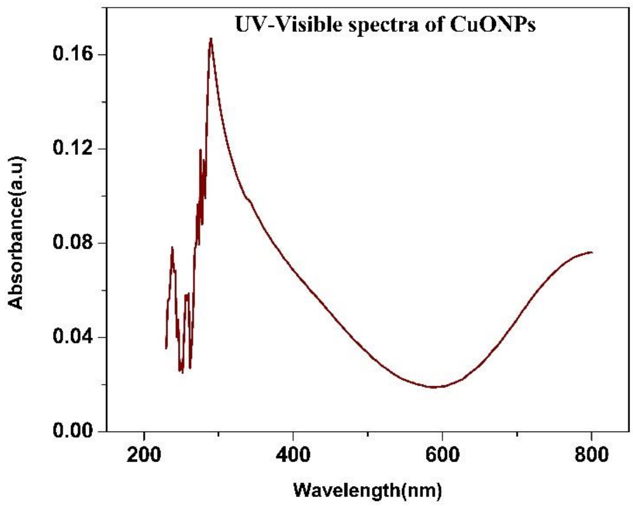

Fig. 1. UV-Visible spectra of the control experiment using only $CuSO_4$.

3.1.2 Kinetic study using plant extract

After adding plant extract to the copper sulfate solution, at 0 minutes, no sharp absorption peak was found for all the solutions. The first peak was observed 30 minutes after the reaction began. The peak was measured within 200-800 nm. **Fig. 2** represents the changes in the absorption peak with increasing reaction time. The highest peak for the copper oxide nanoparticle was observed near 270-320 nm. For every reaction, λ_{max} is observed at 288 nm.

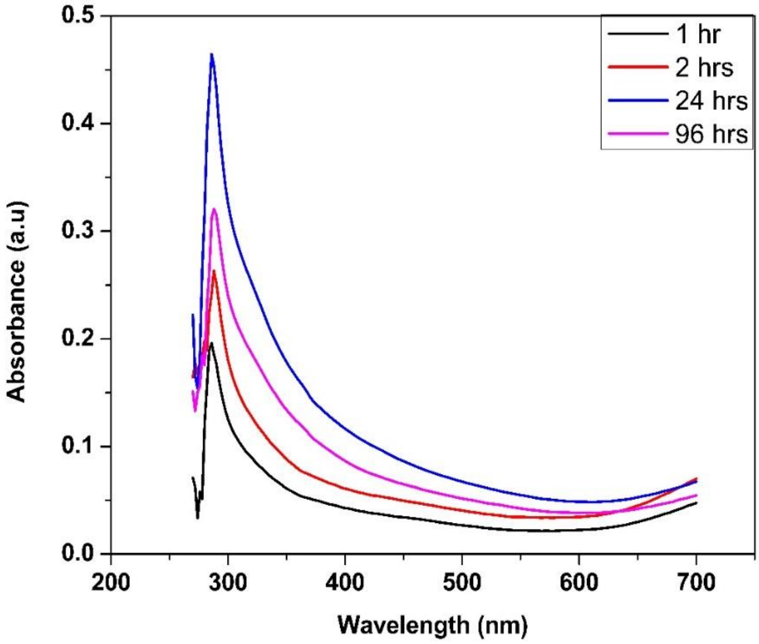

Fig. 2. Changes of absorption peak with increasing reaction

For different volume ratios of betel leaf extract and copper sulphate solution were measured using UV-Vis spectra. Two sets are prepared. In set-a, the total volume of the solution was 10ml [1:9, 2:8, 3:7, 4:6, and 5:5]. Absorption peak was observed for 1hr, 24 hrs., and 72 hrs. In **Fig.3,** the first graph shows the peaks observed for the reaction time in 1hr. With increasing volume of the extract sharpness of the peak was observed. The second graph shows the peaks found for the reaction time in 24 hrs., and the third one depicts the peaks for 72 hrs. The highest absorption peak for all solutions was found at 288nm, which strongly proved the formation of copper oxide nanoparticles. Increased absorption intensity demonstrates that the quantity of CuO NPs generated in the solution increases with the acceleration of the reaction rate.

In set a, it is observed that the volume ratio of extract and copper sulfate solution (3:7) shows absorption peaks at 0.281, 0.419, and 0.296 with an increase in reaction time from 1 hr to 24 hrs and 72 hrs. The volume ratio of extract and copper sulfate solution (4:6) shows the peaks 0.318, 0.507, and 0.438, respectively. For a 5:5 volume ratio of extract and copper sulphate solution, peak intensities of 0.543, 0.535, and 0.716 are observed with increasing time.

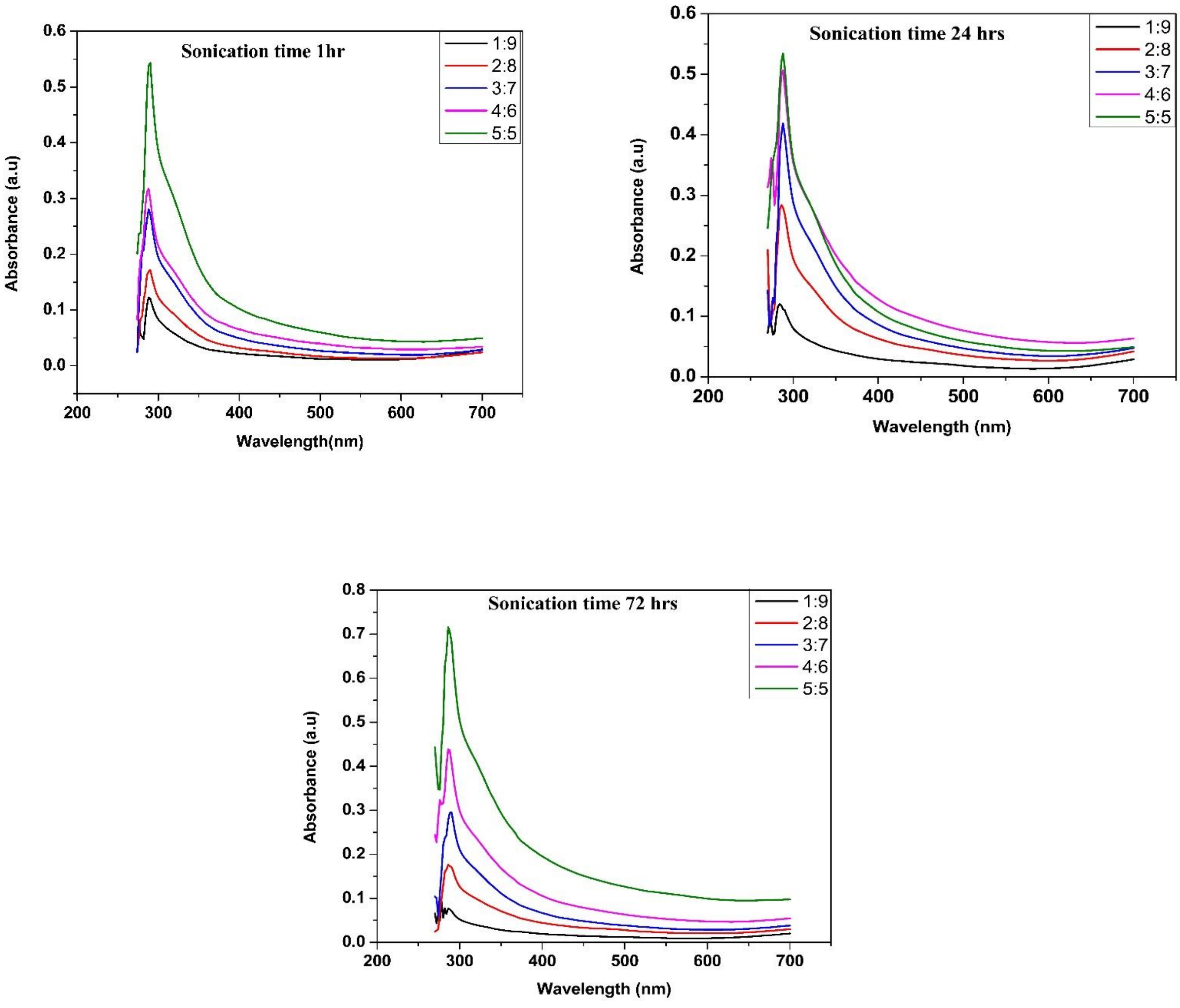

Fig. 3. Changes of absorption peak with different volume ratios prepared in Set-a. The above figure represents sonication time with 1 hr, 24 hrs., and 72 hrs.

For set b, different volume ratios of extract: copper sulphate were prepared [1:8,2:8,3:8,4:8, and 5:8]. In this case volume of copper sulphate solution was constant. The UV-Vis spectra for these solutions were also observed with increasing reaction time. For the first two sets of volume ratios, like 1:8 and 2:8, changes in peak intensity were not that much in 1 hr. But after 48 hrs. and 72 hrs., changes were quite noticeable (0.117 to 0.148 and 0.183 to 0.309), respectively. The last three cases of those solutions (3:8,4:8, and 5:8) show changes in peak intensity. These solutions show the absorption peak from 0.281 to 0.439 and 0.606, respectively. The highest absorption peak at λ_{max} =288 nm was observed for the volume ratio (5:8) of extract and copper sulphate solution. It was observed to be 0.606. **Fig.4** shows the changes in the absorption peak intensity with increasing reaction time. The first graph shows the peaks found for the reaction in 1 hr, and the second & third one show the absorption peaks observed after 48 hrs. and 72 hrs. of the reaction.

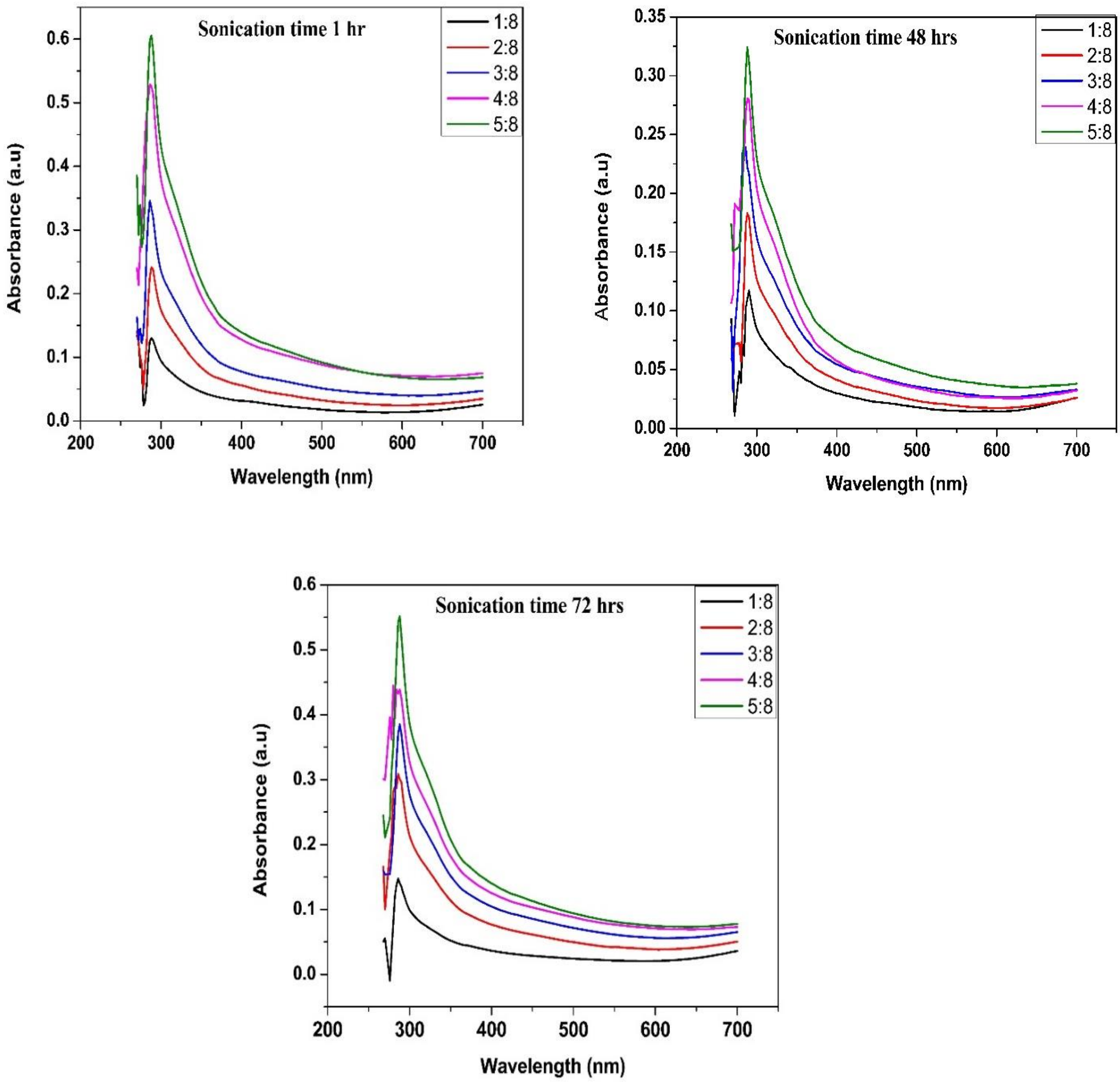

Fig. 4. Changes of absorption peak with different volume ratios prepared in Set-b. The above figure

3.2 Analysis of FWHM (Full Width Half Maximum)

The FWHM (full width at half maximum) of UV-Visible spectra provides information about the size distribution of nanoparticles and their polydispersity. A narrower FWHM indicates a more uniform nanoparticle size, while a broader FWHM suggests a more polydisperse size distribution. Therefore, the measured FWHM values offer insights into the homogeneity of the synthesized material and confirm the formation of nanoparticles [14].

The FWHM value for different volume ratios of the solution was measured. In the first case, for set a, the highest FWHM value was found to be 89.76±0.5nm. This is for the volume ratio (4:6) at 24 hrs. reaction time. Outcomes of the experimental FWHM data for set-a are shown in (Table 2**)**. Each solution was performed twice under the same conditions. All reported highest wavelength (λ_{max}) and FWHM values were calculated as the average(±) of two different measurements.

Table 2: Estimation of the size of CuO NPs formation in green synthesis using betel leaf extract (Set -a) {a: experimental value, b: reference}

Volume ratio	Reaction time	Highest wavelength (λ_{max})[±nm]	FWHM[±nm][a]	CuONP size(nm) [b][predicted]
3:7	1 hr.	288±1.0	62.99±0.5	30-35
	24 hrs.	288±1.0	67.45±0.9	35-40
	72 hrs.	288±1.0	51.09±0.5	40-45
4:6	1 hr.	288±0.5	48.15±0.9	45-50
	24 hrs.	288±0.5	89.76±0.5	20-25
	72 hrs.	288±0.5	54.66±0.5	40-45
5:5	1 hr.	288±1.0	59.23±0.5	45-55
	24 hrs.	286±1.0	51.61±0.2	40-45
	72 hrs.	286±1.0	53.86±0.2	40-50

The prediction of the copper oxide nanoparticles was done using FWHM data. In the above table, FWHM values of different volume ratios (3:7,4:6, and 5:5) were measured with increasing reaction time. The FWHM value of the first case with a ratio of 3:7 increased from 62.99±0.5 to 67.45±0.9 nm at 1 hr to 24 hrs. Again, the FWHM value of the second case with a ratio of 4:6 also increased from 48.15 to 89.76 nm with different reaction times. These two solutions had an increased FWHM value, which indicates the formation of polydisperse copper oxide nanoparticles. But for the last set (5:5) FWHM values decreased from 59.23±0.5 to 51.61±0.2 nm with increasing time. This indicates formation of nanoparticle size was larger than the first two cases, and they are not uniform size [13-15]. **Fig.5** represents the image of FWHM for different volume ratio of extract and copper sulfate solution for set a.

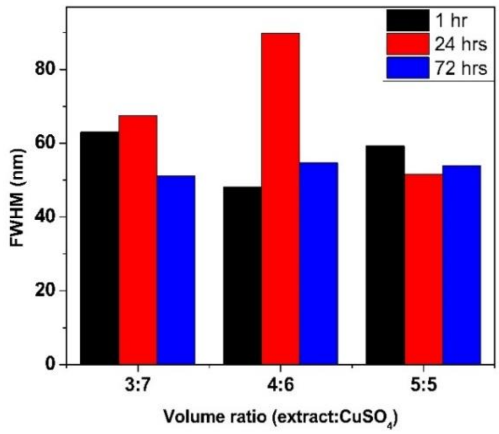

Fig. 5. Image of the FWHM value of different volume ratios (3:7,4:6, and 5:5) with increasing reaction time [set-a].

Outcomes of the experimental FWHM data for set-b are shown in Table -3. All reported highest wavelength (λ_{max}) and FWHM values were calculated as the average (±) of two different measurements.

Table 3: Estimation of the size of CuO NPs formation in green synthesis using betel leaf extract (Set -b) {a: experimental value, b: reference}

Volume ratio	Reaction time	Highest wavelength (λ_{max})[±nm]	FWHM[±nm][a]	CuONPs size(nm) [b][predicted]
3:8	1 hr.	288±1.0	44.30±0.5	50-55
	48 hrs.	286±1.0	58.01±0.9	35-40
	72 hrs.	286±1.0	52.27±0.5	40-45
4:8	1 hr.	288±1.0	63.20±0.9	30-36
	48 hrs.	288±1.0	62.87±0.5	30-40
	72 hrs.	286±1.0	91.42±0.2	20-25
5:8	1 hr.	288±1.0	70.74± 0.5	30-35
	48 hrs.	288±1.0	51.33±0.2	40-45
	72 hrs.	288±1.0	89.57±0.9	20-25

The prediction of the copper oxide nanoparticles was done using FWHM data. In the above table, FWHM values of set -b (3:8,4:8, and 5:8) were measured with increasing reaction time. The FWHM value of the first case with a ratio of 3:8 increased from 44.30±0.5 to 58.01±0.9 nm at 1 hr to 48 hrs. The FWHM value of the second case with a ratio of 4:8 decreased from 63.20 to 62.87 nm with different reaction times. But it increases up to 91.42±0.2 nm within 72 hrs. This indicates the formation of polydisperse copper oxide nanoparticles. For the last set (5:8) FWHM values increased from 70.74± 0.5 to 89.57±0.9 nm with increasing time. This indicates formation of nanoparticle size was smaller than the first two cases, and they are not uniform size [9,14,15]. **Fig. 6** represents the image of FWHM for different volume ratios of extract and copper sulfate solution for set b.

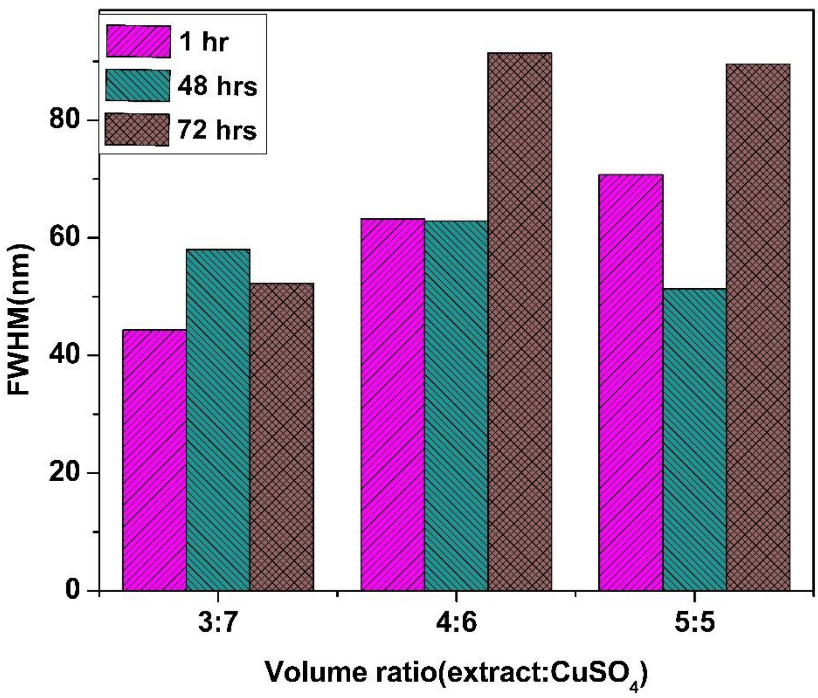

Fig. 6. Image of the FWHM value of different volume ratios (3:8,4:8 and 5:8) with increasing reaction time [set-b].

4 Conclusion

A control experiment was performed using only copper sulfate without adding the plant extract. In the presence of a strong reducing agent ($NaBH_4$), copper oxide nanoparticle formation was found. The extract of *Piper Betel L.* leaves contains different phytochemical compounds like flavonoids, phenols, terpenoids, and saponins, which help in the green synthesis of copper oxide nanoparticles. In both cases, a color change was observed, and a UV-Vis peak was found at 288 nm. Hence, it was proved that phytochemicals play an important role in reducing Cu^{2+} to Cu^0 and forming copper oxide nanoparticles. The solution containing the extract of betel leaves could produce CuO NPs with different volume ratios of two different sets [1:9,2:8,3:7,4:6, and 5:5] & [1:8,2:8,3:8,4:8, and 5:8]. The volume ratio of the betel leaf extract and 0.1 M $CuSO_4$ affects the biosynthesis time. From the observed UV-Vis absorption peak green synthesis of CuO NPs was analyzed. With increased extract and copper sulphate ratio rate of production of CuO NPs increases respectively. The highest absorption peak was observed between 280-320 nm. If we increase the extract amount from the copper sulphate solution, it may overlap the absorption peak. An excess amount of phytochemicals may cause partial nanoparticle agglomeration, which may expand and merge absorption characteristics. Another possibility is that the secondary metabolites present in the extract, including phenolics, flavonoids, and terpenoids, may absorb in a comparable spectral region and contribute extra peaks that overlap with the distinctive copper oxide absorption. Therefore, the observed peak overlap mostly comes from background absorption by secondary metabolites as well as nanoparticle agglomeration. Hence, in that case, nanoparticle formation may not be found.

5 Future Work

In this paper, the kinetic study of the UV-Vis spectra for copper oxide nanoparticles prepared from *Piper Betel L.* leaves is only highlighted. FWHM (Full width half maxima) is calculated manually. In our future work, SEM and TEM images of the nanoparticles will be collected and compared to the nanoparticle size and shape with the theoretical values. We will also perform XRD to correlate FWHM values with them. We will also perform an antimicrobial study of these nanoparticles to determine their medicinal values.

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Conflict of Interest

No conflicts of interest have been declared.

Data Availability

All sources of data for this work are available upon reasonable request.

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