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Research Article

Biogenic Synthesis of Copper Nanoparticles Using Hydroethanolic Flower Extract of *Tabernaemontana divaricata* and Assessment of Antibacterial Properties on Cotton Fabric

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Abstract

Background: Green synthesis of metallic nanoparticles using medicinal plant extracts has emerged as an eco-friendly and sustainable approach for developing bioactive nanomaterials with potential biomedical and textile applications. Tabernaemontana divaricata is a medicinal plant rich in phytochemicals that can act as reducing and stabilizing agents during nanoparticle synthesis.

Objective: To synthesize copper nanoparticles (CuNPs) using the hydroethanolic flower extract of Tabernaemontana divaricata, characterize the synthesized nanoparticles, and evaluate their antioxidant and antibacterial properties on cotton fabric.

Materials and Methods: Copper nanoparticles were synthesized through a green synthesis approach using hydroethanolic flower extract of Tabernaemontana divaricata. The synthesized CuNPs were characterized using UV–Visible spectroscopy, FTIR, SEM, and XRD analysis. Qualitative phytochemical screening was performed to identify bioactive constituents. Antioxidant activity was assessed by DPPH radical scavenging, FRAP, and Hydrogen Peroxide scavenging assays. Cotton fabrics were coated with CuNPs using the dip-coating method and evaluated for antibacterial activity against Escherichia coli and Bacillus subtilis, including wash durability studies.

Results: Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, proteins, carbohydrates, saponins, and starch. UV–Visible spectroscopy confirmed nanoparticle formation through characteristic surface plasmon resonance. FTIR analysis revealed the presence of hydroxyl and other functional groups involved in nanoparticle stabilization. SEM images showed predominantly spherical nanoparticles, while XRD analysis confirmed their crystalline nature. The synthesized CuNPs exhibited concentration-dependent antioxidant activity in DPPH, FRAP, and hydrogen peroxide scavenging assays. CuNP-coated cotton fabrics demonstrated effective antibacterial activity against both E. coli and B. subtilis and retained considerable antimicrobial efficacy after washing cycles.

Conclusion: The study successfully demonstrated the green synthesis of copper nanoparticles using Tabernaemontana divaricata flower extract. The synthesized CuNPs exhibited significant antioxidant and antibacterial activities, and the nanoparticle-coated cotton fabric showed durable antimicrobial properties, highlighting its potential application in medical textiles and healthcare products.

Keywords: Tabernaemontana divaricata, Copper Nanoparticles, Green Synthesis, Antioxidant Activity, Antibacterial Activity, Cotton Fabric, Medical Textiles.

1. INTRODUCTION

Medicinal plants have long been recognized as valuable sources of bioactive compounds and have been extensively utilized in traditional medicine for the prevention and treatment of various diseases [1,2]. The growing demand for eco-friendly and sustainable technologies has led to increased interest in the green synthesis of nanoparticles using plant extracts. Compared with conventional physical and chemical methods, green synthesis offers several advantages, including low cost, environmental compatibility, reduced toxicity, and enhanced biocompatibility [3,4].

Among different metallic nanoparticles, copper nanoparticles (CuNPs) have attracted considerable attention due to their remarkable antimicrobial, antioxidant, catalytic, and biomedical properties [5,6]. In addition, copper is more economical and readily available than noble metals such as silver and gold, making it suitable for large-scale industrial and medical applications [7]. Tabernaemontana divaricata, a medicinal plant belonging to the Apocynaceae family, is rich in phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, and glycosides, which can serve as reducing and stabilizing agents in nanoparticle synthesis [8,9].

The present study focuses on the biogenic synthesis of copper nanoparticles using the hydroethanolic flower

extract of T. divaricata and their characterization through UV–Visible spectroscopy, FTIR, SEM, and XRD analyses. Furthermore, the antioxidant activity and antibacterial efficacy of CuNP-treated cotton fabrics against pathogenic bacteria were evaluated to explore their potential applications in healthcare and antimicrobial textile products [10,11].



Fig 1 : *Tabernaemontana divaricata*

2. METHODS AND MATERIALS

2.1 Plant Material Collection

Fresh flowers of Tabernaemontana divaricata were collected from Coimbatore, Tamil Nadu, India. The plant material was authenticated and washed thoroughly with distilled water to remove adhering impurities. The flowers were shade-dried at room temperature and pulverized into fine powder for further experimental studies [12,13].

2.2 Preparation of Hydroethanolic Flower Extract

The dried flower powder was extracted using hydroethanol (70:30 ethanol) by maceration method. The extract was filtered through Whatman No.1 filter paper and concentrated under reduced pressure. The concentrated extract was stored at 4°C until use for nanoparticle synthesis and phytochemical analysis [14,15].

2.3 Green Synthesis of Copper Nanoparticles

Copper nanoparticles (CuNPs) were synthesized using the hydroethanolic flower extract as a reducing and stabilizing agent. Copper nitrate solution was mixed with the extract under continuous stirring. The formation of CuNPs was visually confirmed by the color change of the reaction mixture from pale brown to bluish-green, indicating the reduction of Cu^{2+} ions into copper nanoparticles [16,17].

2.4 Characterization of Copper Nanoparticles

The synthesized nanoparticles were characterized using different analytical techniques. UV–Visible spectroscopy was employed to monitor surface plasmon resonance and nanoparticle formation. Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups involved in nanoparticle stabilization. Scanning Electron Microscopy (SEM) was performed to determine particle morphology and size, while X-Ray Diffraction (XRD) analysis confirmed the crystalline nature of the synthesized CuNPs [18,19].

2.5 Phytochemical Analysis

Qualitative phytochemical screening of the hydroethanolic flower extract was carried out using standard protocols to detect carbohydrates, proteins, alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, and starch. These phytoconstituents play an important role in nanoparticle synthesis and biological activity [20,21].

2.6 Antioxidant Assays

2.6.1 DPPH Radical Scavenging Assay

The free radical scavenging activity of the synthesized CuNPs was determined using the DPPH assay. The reduction in absorbance at 517 nm was measured and expressed as percentage inhibition [22].

2.6.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of the nanoparticles was evaluated by measuring the conversion of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which reflects antioxidant capacity [23].

2.6.3 Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide scavenging activity was determined by assessing the ability of the synthesized nanoparticles to neutralize H_2O_2 radicals under in vitro conditions [24].

2.7 Fabric Treatment

2.7.1 Scouring

Cotton fabrics were scoured using a standard detergent solution to remove impurities, oils, and waxes present on the fabric surface before nanoparticle treatment [25].

2.7.2 Dip-Coating Method

The scoured cotton fabrics were immersed in varying concentrations of CuNP suspension for 24 h. The treated fabrics were washed with distilled water and dried in a hot-air oven at 37°C for 10 min to ensure proper fixation of nanoparticles onto the fabric surface [25,26].

2.8 Wash Durability Test

The durability of the CuNP coating on cotton fabric was evaluated through repeated washing cycles using detergent solution. The antibacterial activity of the treated fabric was assessed after each wash cycle to determine coating stability [26].

2.9 Antibacterial Activity

The antibacterial efficacy of untreated and CuNP-treated cotton fabrics was evaluated against Escherichia coli and Bacillus subtilis using the agar diffusion method. The inoculated Mueller–Hinton Agar plates were incubated at 37°C for 24 h, and the zones of inhibition were measured to determine antibacterial effectiveness [27].

3. RESULTS AND DISCUSSION

3.1 SYNTHESIS OF COPPER NANOPARTICLES

The aqueous extract of *Tabernaemontana divaricata* was initially pale brown in color before the addition of

the copper nitrate solution. Upon mixing with the copper nitrate solution, the reaction mixture gradually changed to a bluish-green color, indicating the formation of copper nanoparticles. This visible color change suggests the reduction of copper ions by the bioactive compounds present in the plant extract and serves as a preliminary confirmation of nanoparticle synthesis. The phytochemicals in the extract act as both reducing and stabilizing agents during the green synthesis process. thesis of Copper Nanoparticles [28].

3.2 Characterization of Copper Nanoparticles

3.2.1 UV Visible Spectral analysis

The UV–Visible spectrum of the synthesized copper nanoparticles exhibited a characteristic absorption band, which is attributed to the surface plasmon resonance (SPR) phenomenon of copper nanoparticles. The SPR absorption arises from the collective oscillation of conduction electrons on the nanoparticle surface when exposed to incident light. The position and intensity of this absorption band are influenced by the size and distribution of the nanoparticles, with changes in particle size leading to corresponding shifts in the SPR peak. Thus, the observed absorption band provides preliminary evidence for the successful formation of copper nanoparticles [29]

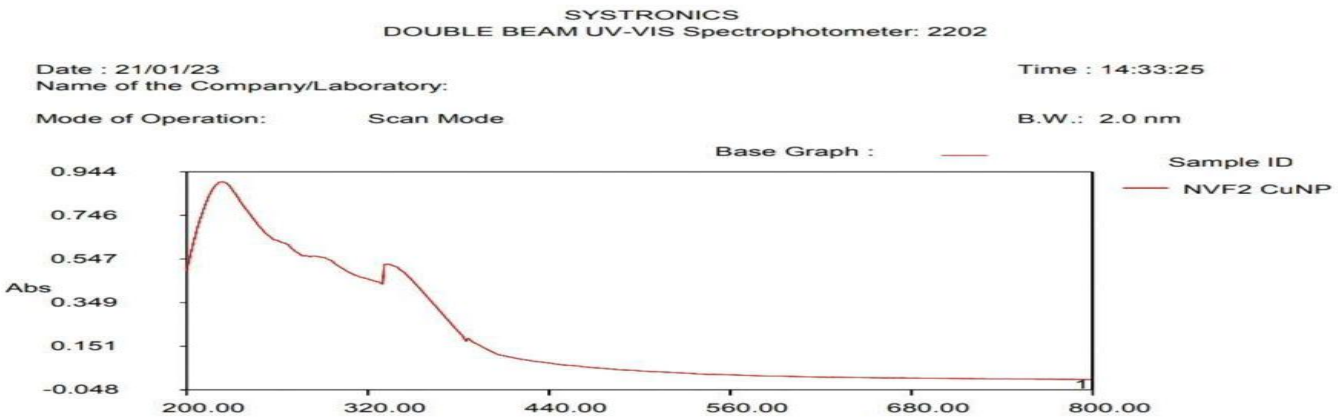
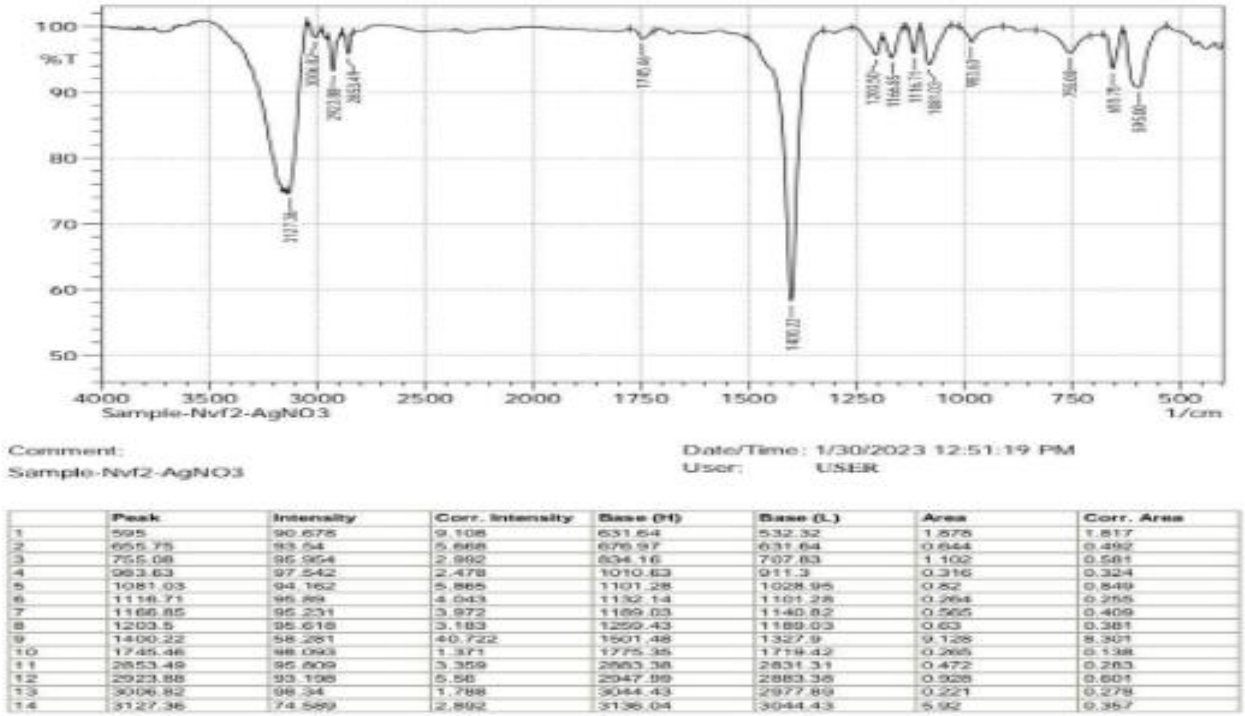


Figure 2: UV VISIBLE SPECTRAL ANALYSIS

3.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of the synthesized nanoparticles showed a characteristic peak at 3127.36 cm^{-1}, indicating the presence of hydroxyl (–OH) functional groups [30].



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	595	90.678	9.108	651.64	532.32	1.575	1.817
2	655.75	93.54	5.868	676.97	631.64	0.644	0.492
3	755.08	96.954	2.992	834.16	707.83	1.102	0.581
4	983.63	97.542	2.478	1010.63	911.3	0.316	0.324
5	1081.03	94.162	5.865	1101.28	1028.95	0.52	0.549
6	1116.71	96.894	4.043	1132.14	1101.28	0.264	0.255
7	1166.85	96.231	3.972	1169.03	1140.82	0.565	0.409
8	1203.5	95.618	3.183	1259.43	1189.03	0.63	0.381
9	1400.22	58.281	40.722	1501.48	1327.9	9.128	8.301
10	1745.46	98.093	1.371	1775.35	1719.42	0.265	0.138
11	2853.49	95.809	3.359	2863.38	2831.31	0.472	0.283
12	2923.88	93.198	5.56	2947.89	2883.38	0.928	0.601
13	3006.82	98.34	1.788	3044.43	2977.89	0.221	0.279
14	3127.36	74.589	2.892	3136.04	3044.43	5.92	0.357

Figure 3: Fourier Transform Infrared Spectroscopy (FTIR)

3.2.3 SEM Analysis (Scanning Electron Microscope)

SEM analysis revealed the successful formation of copper nanoparticles synthesized using *T. divaricata* extract. The micrographs showed aggregated clusters of nanoparticles with varying particle sizes, predominantly exhibiting a spherical morphology [31].

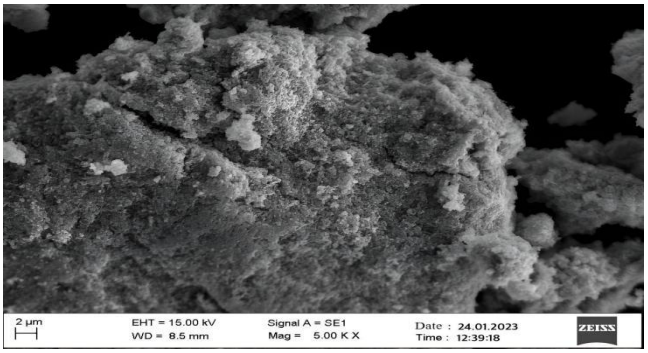


Figure 4: SEM Analysis(Scanning Electron Microscope)

3.2.4 X RAY Diffraction

X-ray diffraction (XRD) analysis further confirmed the successful synthesis of copper nanoparticles [32].

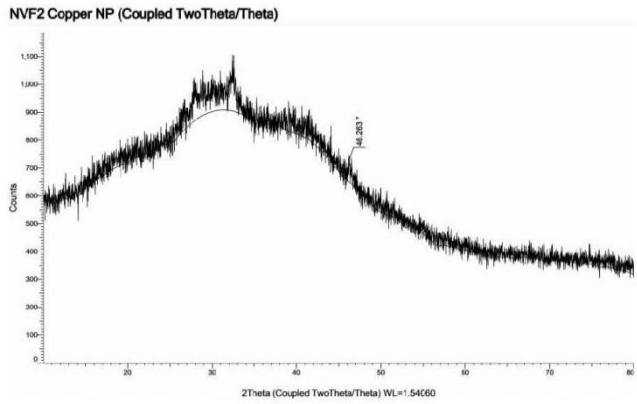


Figure 4: X RAY Diffraction

3.3 Phytochemical Analysis

Preliminary phytochemical screening of the Tabernaemontana divaricata leaf extract revealed the presence of several important bioactive constituents. Qualitative analysis showed positive results for carbohydrates (Molisch test), proteins (Ninhydrin test), alkaloids (Wagner's test), flavonoids (Lead acetate test), tannins (Ferric chloride test), terpenoids (Salkowski test), glycosides (Keller–Killiani test), saponins, and starch (Iodine test). The presence of these phytochemicals suggests that the plant is a rich source of secondary metabolites that may contribute to its reported medicinal properties, including antioxidant, antimicrobial, and anti-inflammatory activities [33]

3.4 Antioxidant Study

3.4.1 DPPH radical scavenging Assay

The antioxidant activity of the synthesized sample was evaluated using the DPPH radical scavenging assay. The results demonstrated a concentration-dependent increase in radical scavenging activity, indicating the presence of significant antioxidant potential. As the concentration increased from 200 to 1000 µg/mL, the percentage of DPPH radical inhibition also increased, suggesting an enhanced ability to donate electrons or hydrogen atoms to neutralize free radicals. This trend confirms the antioxidant properties of the sample [34].

3.4.2 Ferric Reducing/ Antioxidant Power Assay

The ferric reducing antioxidant power (FRAP) assay demonstrated a concentration-dependent increase in reducing activity for the sample extract. As the concentration increased from 200 to 1000 µg/mL, the reducing power of the sample increased progressively from approximately 18% to 48%, whereas the standard exhibited higher activity ranging from about 40% to 85%. The results indicate that the sample possesses significant electron-donating ability and antioxidant potential by reducing Fe^{3+} to Fe^{2+} ions. Although the antioxidant activity of the sample was lower than that of the standard, a steady increase in reducing power with increasing concentration confirms its dose-dependent antioxidant efficacy [35].

3.4.3 Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging assay revealed a concentration-dependent increase in the free radical scavenging activity of the sample extract. The scavenging activity increased from approximately 4% at 200 µg/mL to 36% at 1000 µg/mL, while the standard showed higher inhibition ranging from about 9% to 47% over the same concentration range. The gradual increase in H_2O_2 scavenging activity indicates the ability of the extract to neutralize hydrogen peroxide and prevent the formation of highly reactive hydroxyl radicals. Although the activity was lower than that of the standard, the results confirm that the extract possesses notable antioxidant potential in a dose-dependent manner [36].

3.5 Antibacterial Assay

The antibacterial activity of copper nanoparticle-coated cotton fabric was evaluated against *Escherichia coli* and *Bacillus subtilis* using the disc diffusion method. After incubation at 37°C for 24 h, clear zones of inhibition were observed around the treated fabric samples, indicating effective antibacterial activity. The test organisms exhibited sensitivity towards all treated samples (WT, UT, and C), while the positive control (1 mg/mL) showed the highest inhibitory effect. The results demonstrate that the copper nanoparticle-treated cotton fabric possesses significant antibacterial activity against both Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*) bacteria [37].

3.5.1 The wash Durability test

The wash durability test was performed to evaluate the retention of antibacterial activity of the *Tabernaemontana divaricata*-mediated copper nanoparticle-coated cotton fabric after detergent washing. Even after the washing treatment, the coated fabric exhibited noticeable zones of inhibition against both *Escherichia coli* and *Bacillus subtilis*, indicating that the antibacterial coating remained effective. The results demonstrate the good durability and wash-fastness of the antimicrobial finish, confirming that the *T. divaricata* extract-mediated coating possesses sustained antibacterial activity against both Gram-negative and Gram-positive bacteria [38].

4. CONCLUSION

In conclusion, the present study successfully demonstrated the green synthesis of copper nanoparticles using the flower extract of *Tabernaemontana divaricata*. The formation and stability of the nanoparticles were confirmed through UV–Visible spectroscopy, while FTIR, SEM, and XRD analyses verified their functional groups, morphology, and crystalline nature. The synthesized copper nanoparticles exhibited significant antibacterial activity against *Escherichia coli* and *Bacillus subtilis* when applied to cotton fabric. Furthermore, the coated fabric retained its antimicrobial efficacy after washing, indicating good durability. These findings suggest that the nanocomposite cotton fabric has promising applications in medical textiles and healthcare products.

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