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Original Article

Pharmacognostic Approach to the Green Synthesis and Pharmacological Evaluation of Copper Sulphide Nanoparticles Using Aqueous Extract of *Catharanthus roseus* Leaves

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ABSTRACT

Background: Our study focused on the green synthesis of copper (II) complexes using the leaf extract of *Catharanthus roseus*. The phytochemical screening of the extract revealed the presence of flavonoids, tannins, glycosides, saponins, and alkaloids, which are believed to function as natural reducing, stabilizing, and capping agents in the synthesis process. These copper complexes were intended for use in antimicrobial dusting powders for wound healing applications. **Materials and Methods:** Multiple techniques were used to analyze the biosynthesized copper (II) complexes, including X-ray Diffraction (XRD), ultraviolet-visible (UV-vis) spectroscopy, Fourier-Transform Infrared Spectroscopy (FTIR), and Differential Scanning Calorimetry (DSC). Results showed that the average size of the particles was 3.4 nm. Surface plasmon resonance peaks were identified at 312 and 330 nm for the biosynthesized copper complex and at 393 and 369 nm for the pure plant extract. The synthesized copper complex and plant extract were incorporated into a dusting powder formulation. Several physicochemical characteristics of the powder were studied, including particle size, surface area, bulk density, tapped density, angle of repose, Carr's index, Hausner's ratio, and volume. The well diffusion technique was used to investigate the antibacterial effectiveness against *Staphylococcus aureus* and *Escherichia coli*. Plates inoculated with bacterial cultures were incubated at 37°C for 48 hr. **Results:** The copper complex remained stable over a six-month period, showing no agglomeration. The copper-enriched dusting powder demonstrated significant antibacterial activity, producing notable zones of inhibition compared to the plant extract formulation. The formulation displayed high permeability, rapid subcutaneous penetration, enhanced microcirculation, and accelerated histolytic tissue regeneration. **Conclusion:** The green-synthesized copper (II) complex derived from *Catharanthus roseus* exhibits promising antibacterial properties and enhanced wound healing potential. The copper-enriched dusting powder outperformed the pure plant extract formulation in antimicrobial activity and formulation characteristics, indicating its potential application in managing chronic wounds and preventing infections in medical and surgical contexts.

Keywords: Copper complex, Green Synthesis, Dusting powder, Spectroscopic characterization, Antimicrobial Activity.

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INTRODUCTION

An increasing number of bacteria are developing resistance to many drugs at a rapid pace, making antimicrobial resistance an urgent issue on a worldwide scale. We must immediately begin to address the critical "issue of microbial resistance to antibiotics" by finding new bioactive molecules to target. There are still therapeutic preparations derived from crude substances that come from plants that grow in nature. A number of metal complexes have shown promise in combating germs, fungi, and cancer, sparking renewed interest in this area.

There has been a lot of recent curiosity on the potential therapeutic uses of metal complexes. In addition to the very effective platinum-based medicines,[1] other metal compounds

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including ruthenium complexes and titanium have shown promise as chemotherapeutic agents. There has been an inquiry of copper-based medications due to the suggestion that they may be less toxic than metallo pharmaceuticals,[2] which is a concern due to the toxicity of these drugs. A variety of pharmacological effects, including antibacterial ones, have been shown for copper compounds.[3-5] The compound has antifungal, antiviral, anticancer, and anti-inflammatory properties.[6-13] In many cases, the biological activity of copper complexes is higher than that of the parent ligand alone.[14] According to Ainscough.[15] Figure 1 displays a plant overview.

The pharmacological and toxicological characteristics of many medications may be altered when they are delivered as metallic complexes botanical Classification of *Catharanthus roseus* plant show in Table 1. Given the multitude of low molecular weight copper complexes that have shown efficacy against many illnesses, including TB, rheumatoid arthritis, gastric ulcers, and malignancies, Cu^{2+} is perhaps the most researched cation in this regard.[16-18] Biological functions including oxidation, electron transport, and oxygen conveyance rely on complexes of transition metals with amino acids in peptides and proteins. Enzymes form compounds with divalent metal ions in these processes due to their very specialized active sites. When left unbound or bound to transition metals, thiosemicarbazide and its substituted derivatives disrupt the normal operation of several biological processes. It is often believed that trace metal sequestration is the mechanism by which metal-free N, N-chelating bases exhibit bioactivity, with the resultant metal complexes serving as the true active species.[16-26]

Two essential steps in the process of gene mutation and cancer development in living organisms are DNA binding and cleavage. Extensive research has shown that metal complexes have an innate ability to target DNA.[27] Furthermore, the DNA-interacting schiff base metal complexes have been the subject of much research throughout the last few decades.[28] These coordination compounds were good candidates for anti-tumor medicines, photo cleavers, and DNA secondary structure probes because of their site-specific binding capabilities and various fold uses in cancer treatment.[29,30] Copper complexes of 1, 10-phenanthroline are among the most powerful metal-based nucleases; these complexes have found widespread use in the mapping of DNA binding sites for drugs and proteins. For the purpose of understanding DNA structure as well.[31] in Molecular oxygen and a reducing agent are necessary for DNA degradation by thiosemicarbazone Cu (II) complexes. Here, we detail the environmentally friendly production and analysis of a dusting powder antibacterial that is copper-enriched and contains thiourea as a ligand. We next use the well diffusion technique to test its efficacy against a variety of bacteria, including *E. coli* and *P. aeruginosa*.

MATERIALS AND METHODS

Materials

The copper chloride, ethanol, and thiosemicarbazide hydrochloride were all acquired from Merck specialty Private limited. Hi-media Laboratories Private Limited supplied the microbiology culture medium, agarose, and DPPH. All of the chemicals used in the copper synthesis were of analytical quality.

Collection of plant material

In January 2023, the *Catharanthus roseus* leaves were gathered from the medicinal garden of ITM University in Gwalior, India (Figure 1). A topic expert verified the plant species and submitted the voucher specimen (ITM/09/23) to the Chemistry Department of the School of Sciences at ITM University in Gwalior, Madhya Pradesh, India. Preparation of *Catharanthus roseus* extract.

The formulation of the green synthesis of copper-inserted antibacterial dusting powder was accomplished using an aqueous extract of *Catharanthus roseus* leaves. The therapeutic properties of *Catharanthus roseus*, found in its leaves, include antioxidant, anti-ulcer, anti-diarrheal, antibacterial, anti-diabetic, anti-diarrheal, and anti-cancer properties. The freshly picked leaves were rinsed in Double Distilled Water (DDW) to get rid of any dirt or other impurities, then finely chopped and dried in the shade. To prepare the biosynthesized Copper (II) complex, 15 g of leaf powder were dissolved in 100 mL of distilled water. The combination was then left to shake for two days on an orbital shaker. After filtering the mixture using Whatman filter paper (No. 41), it was kept in the refrigerator are shows in Figure 2.

Phytochemical analysis of leaf extract

Aqueous extracts of *Catharanthus roseus* plant leaves were submitted to qualitative screening to elucidate the presence of significant phytochemicals. In this screening, we looked for phytochemicals including steroids, tannins, cardiac glycosides, flavonoids, and saponins in the leaf extract using a specific

Figure 1: *Catharanthus roseus* plant.

functional group test. According to Table 2, the *Catharanthus roseus* plant's leaves were analyzed using the standard procedure for qualitative phytochemical analysis in order to determine the presence of key phytochemicals.

Synthesis of Copper (II) complex from *Catharanthus roseus* leaf extract

The bioactive agents-copper complexes were made by slowly adding a solution of *Catharanthus roseus* leaves (1 mmol in 5 mL methanol) and a methanolic solution of thiourea (1 mmol in 10 mL methanol) while stirring continuously. The copper salts solution was 1mmol in 10 mL of methanol. Following this, the reaction mixture was refluxed for three to 6 hr. It was a very concentrated solution. Filtered and rinsed with cold ethanol and diethyl ether, the precipitated product was then dried in a vacuum desiccator over anhydrous calcium chloride shows in Figure 3. We used UV-vis, FT-IR, XRD, DSC, molar conductance, and elemental analysis methods to analyze the complex structures. Following acid breakdown, complexometric titration was used to ascertain the complex's copper concentration are shows in Table 3.

Formulation of Antimicrobial dusting powder

This paper presents a formula for a sterile medical dusting powder and an antimicrobial dusting powder that is enhanced with copper (II) complex. The formula is prepared by combining talc, starch, zinc oxide, and an appropriate ratio of biosynthesized copper (II) complex with other ingredients shows in Figure 4. In order to cure burns and wounds, especially chronic ones, prevent infections in wounds and implants, and fight infections in medical and surgical equipment, a specially made dusting powder has been developed. This powder is capable of suppressing broad-spectrum bacterial strains.

In vitro antioxidant activity of complex

Following Hataro's approach with ascorbic acid as a standard, the Free radical scavenging assay (FRSA) on copper complex was carried out using a solution of stable free radical DPPH (0.04% w/v) at concentrations of 200, 400, 600, 800, and 1000 µg/mL. The findings were averaged after each sample was run three times. The findings are shown as a percentage as the ratio of the reduction in DPPH absorption at 517 nm when the copper complex is present to the absorption at 517 nm when the complex is absent, as measured by UV-vis spectrophotometry. In order to determine the DPPH free radical scavenging assay's % scavenging activity, the following equation was used:

$$\% \text{ free radical scavenging activity (FRSA)} = A1\text{-}A2 / A0 \times 100$$

Where A_1=Absorbance of a blank sample,

A_2=Absorbance of a test sample,

A_0=Absorbance of a positive control.

In vitro anti-inflammatory activity of compounds via Albumin denaturation method

We used the prevention of albumin denaturation process, as reported by Mizushima *et al*., (1968) and Sakat *et al*., (2010) with appropriate modifications, to study the *in vitro* anti-inflammatory efficacy of the green produced copper (II) complex. To keep the pH of the reaction mixture neutral, the following ingredients were added to the plant extract: copper complex, 1% bovine serum albumin in water, and the rest of the ingredients were stirred together. After that, the sample was incubated at 37°C for 20 min, heated to 50°C for 20 min, cooled, and then its turbidity was measured at 660nm using a UV-vis spectrophotometer.

Table 1: Classification of *Catharanthus roseus*.

Domain	Eukarya: eukaryotes
Kingdom	Plantae
Subkingdom	*Tracheobionta*: Vascular Plants
Division	*Magnoliophyta*: Flowering Plants
Class	*Magnoliopsida*: Dicotyledons
Order	Gentianales
Family	Apocynaceae
Tribe	Vinceae
Genus	*Catharanthus* G. Don
Specific epithet	*Roseus* (Linnaeus) G. Don
Botanical name	*Catharanthus roseus* (Linnaeus) G. Don

Table 2: Phytochemical analysis of *Catharanthus roseus* leaf extract.

Sl. No.	Phytochemical Test	Result
1	Flavonoid	+
2	Saponins	+
3	Glycosides	+
4	Tannins	+
5	Terpenoids	+
6	Alkaloids	+
7	Phenolic group	-
8	Steroids	-
9	Anthocyanin	-
10	Anthraquinone	-

Table 3: Percentage inhibition of proteinase activity of aqueous extract of *Catharanthus roseus* leaves, and its green synthesised copper (II) complex.

Sl. No.	Compound	Concentration In µg/mL	Absorption at 210 nm	Percentage inhibition
1	Positive control	100	0.733	0%
2	Aspirin drug (Standard)	100	0.2166	70.53%
3	Aqueous extract of *Catharanthus roseus* leaves	100	0.526	60%
4	Green synthesized copper (II) complex	100	0.725	69.12%

Figure 2: Preparation of *Catharanthus roseus* leaf extract.

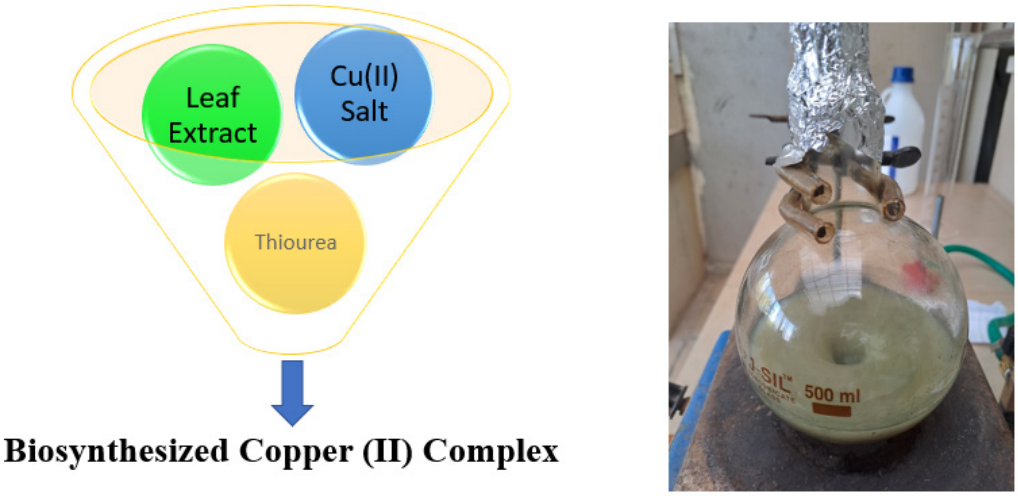

Figure 3: Green synthesis of Copper (II) complex from aqueous extract of *Catharanthus roseus* leaves.

In vitro anti-bacterial activity of compounds

The agar well diffusion technique was used to evaluate the dusting powder's *in vitro* antibacterial efficacy against *E. coli* and *Pseudomonas aeruginosa*, 2 g-negative bacteria. The powder was composed of aqueous extract of *Catharanthus roseus* leaves and a copper complex. For 24 hr at 37°C, the bacterial strains were subculture in agar medium. As a control, we used dusting powder devoid of copper complex; as a positive control, we employed conventional antibiotic streptomycin. We measured and documented the inhibitory zones. Dissolved in DMSO were the two dusting powder compositions. Two independent investigations employing DMSO as a negative control demonstrated that the solvent had no influence on the biological screening when tested against various bacterial strains are shows in Tables 4 and 5.

RESULTS AND DISCUSSION

The current study pertains to a formulation of antimicrobial dusting powder that is enhanced with copper. This powder may block infections caused by a wide range of microorganisms, has a high sterilizing impact, is very permeable, and has long-lasting antimicrobial properties. Additionally, it promotes wound healing. Improved microcirculation of wound surrounding tissue, effective activation and promotion of histiocytic growth, accelerated wound healing, reduced inflammation and cicatrix generation, and promotion of a long-lasting antibiotic effect

Table 4: **The Trolox equivalent antacid activities of compounds.**

Concentration (mg/mL)	DPPH activity (IC_{50} µg/mL)			
	Aqueous extract of *Catharanthus roseus* leaves	Green synthesized Copper (II) complex	Quercetin compound (Positive control)	Ascorbic acid (Standard substance)
200	1.84	2.86	1.70	3.46
400	2.86	4.86	3.70	6.92
600	3.88	6.86	5.70	10.38
800	4.90	8.86	7.70	13.84
1000	5.92	10.86	9.70	17.30

Table 5: ***In vitro*** **antibacterial efficacy of compounds.**

Sl. No.	Concentration (mg/mL) in DMSO	*E. coli*		*Pseudomonas aeruginosa*	
		Aqueous extract of *Catharanthus roseus leaves*	Green synthesised Copper (II) Complex	Aqueous extract of *Catharanthus roseus leaves*	Green synthesized Copper (II) Complex
1	10 mg/mL	2 mm	3 mm	2.5 mm	3 mm
2	20 mg/mL	2.5 mm	3.5 mm	4 mm	4.5 mm
3	40 mg /mL	2 mm	3 mm	4 mm	4.5 mm
4	60 mg/mL	1 mm	2 mm	5 mm	5.5 mm
5	80 mg/mL	3 mm	4 mm	6 mm	6.5 mm
6	100 mg/mL	4.5 mm	6 mm	6.5 mm	7.5 mm
7	Positive control (Streptomycin antibiotic)	10 mm	9 mm	15 mm	12 mm
8	Negative control (DMSO solvent)	6 mm	6 mm	6 mm	6 mm

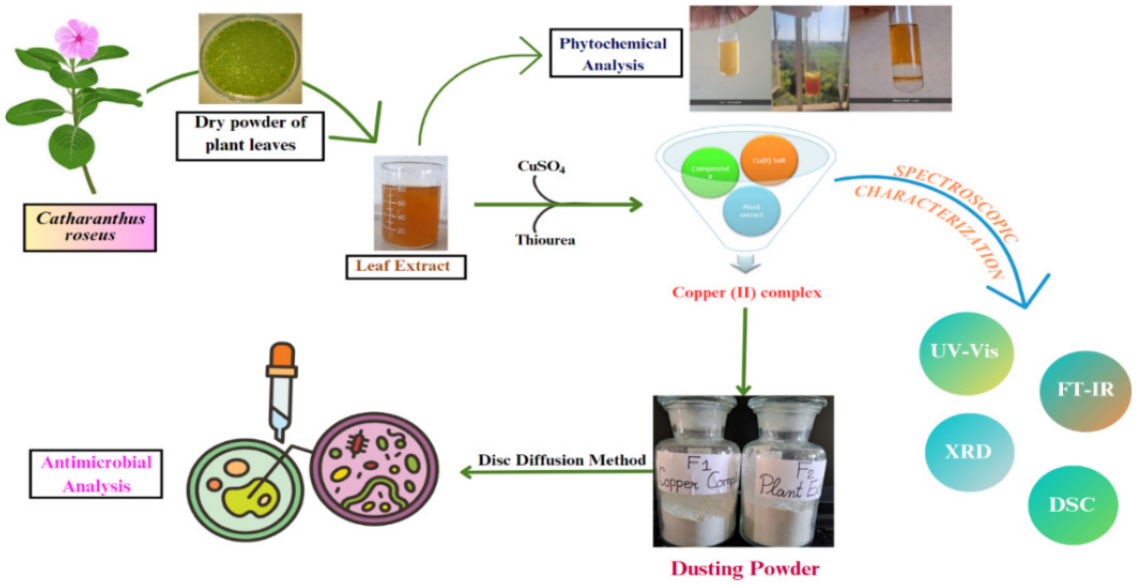

Figure 5: Possible mechanism for the green synthesis of copper (II) complex from plant extract.

are all benefits of the specially formulated antibacterial dusting powder. A protective coating of copper ions, oriented outward, forms on sick skin and slowly releases copper ions into the human body, giving it a long-lasting antibacterial action.

An 80 mL solution of copper sulfate was mixed with 20 mL of *Catharanthus roseus* leaf extract in a 1:2 v/v ratio. The mixture was stirred continuously for 24 hr at room temperature in order to biosynthesize a copper complex from plant extract. The presence of phytochemicals in the leaf extract caused a change in color of the solution mixture from blue to green throughout the reaction, which was further validated by UV-vis spectroscopy. This change in coloration indicated the creation of a copper (II) complex. Biosynthesized copper complex electronic spectra are generated by stimulating the Surface Plasmon Resonance (SPR) phenomenon.[30,32-35] The *Catharanthus roseus* leaf extract contains tannins, saponins, phenols and alkaloids phytochemicals that serve as capping, reducing and stabilizing agents for green synthesis of the copper complex and they may also be responsible for the stability of biosynthesized Copper (II) complex.[36] The aggregation of copper (II) complex was not observed six months after the synthesis. The possible mechanism of the synthesis of the copper complex is shown in Figure 5. In the UV-vis spectrum, the green synthesized copper complex showed absorption peaks at 330 and 312 nm, but no peaks appeared for the copper salt or the plant extract solution as shown in Figure 6.

UV-visible Spectroscopic Analysis

At room temperature, the green synthesized copper complex's optical characteristics were assessed using a UV-visible spectrophotometer. Absorption is most pronounced between 200 and 700 nm. By comparing the absorption bands at 369 and 393 nm for plant extract and biosynthesized copper (II) complex, we can see that the former forms a high-quality complex, whereas the latter uses bands at 312 and 330 nm. The complex formation-induced surface plasmon resonance event also produced an additional signal at 556 nm. When bioactive compounds were coordinated with Cu^{2+} ions, the electronic spectra changed from 393 nm to 312 nm. The coordinated copper (II) complex underwent the bathochromic shift as a result of the shifting of the plant extract's electronic spectra, which was caused by the back bonding of d electrons from Cu^{2+} ions to the unoccupied d orbitals of functional groups in the extract. Functional groups in the plant extract had their bond energies reduced due to d-electrons back bonding. This allowed the aqueous *Catharanthus roseus* leaf extract to produce a stable Copper (II) complex.

FT-IR Analysis

One of the most trustworthy and precise ways to determine if complexes include functional groups is to conduct FT-IR spectroscopy studies of the produced compounds. FT-IR spectra of plant extract showed that phytochemicals present in leaf extract showed keto-enol isomerism and exist in equilibrium condition for stability of complexes. In the ketonic form phytochemicals were coordinated with copper ions in a divalent oxidation state via an azomethine bond (>C=N). The coordination capacity of phytochemicals causes a bathochromic shift in UV-visible spectroscopy and a change in the infrared frequency of copper (II) complexes from 1640 to 1680 cm-1. Tables 6 and 7 show the FT-IR spectra of the copper (II) complex and plant extract,

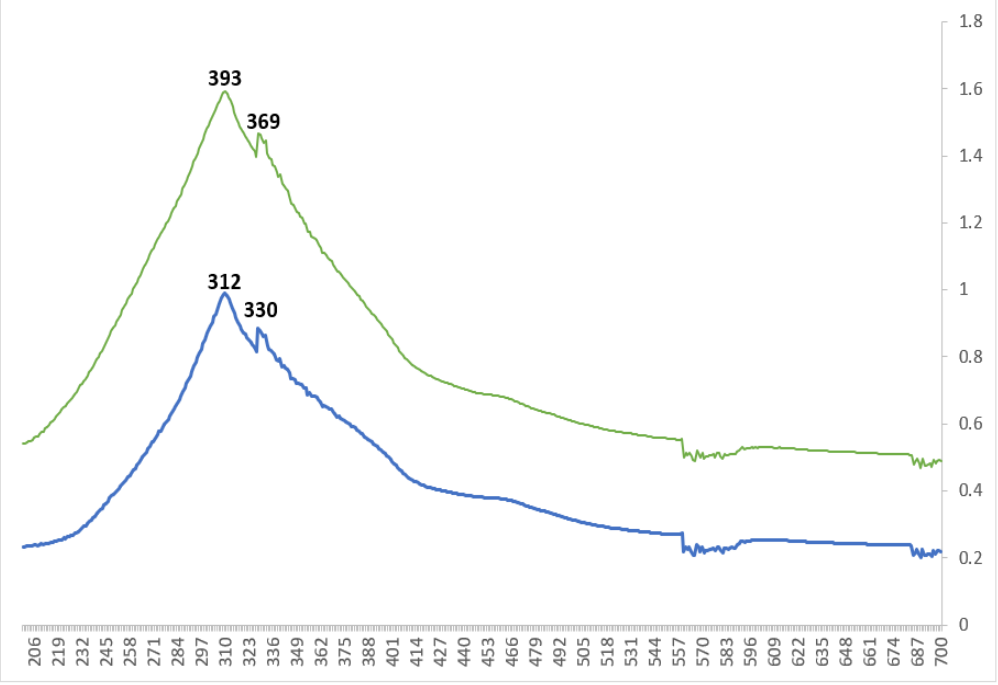

Figure 6: UV-vis spectral analysis of plant extract and its biosynthesized copper complex.

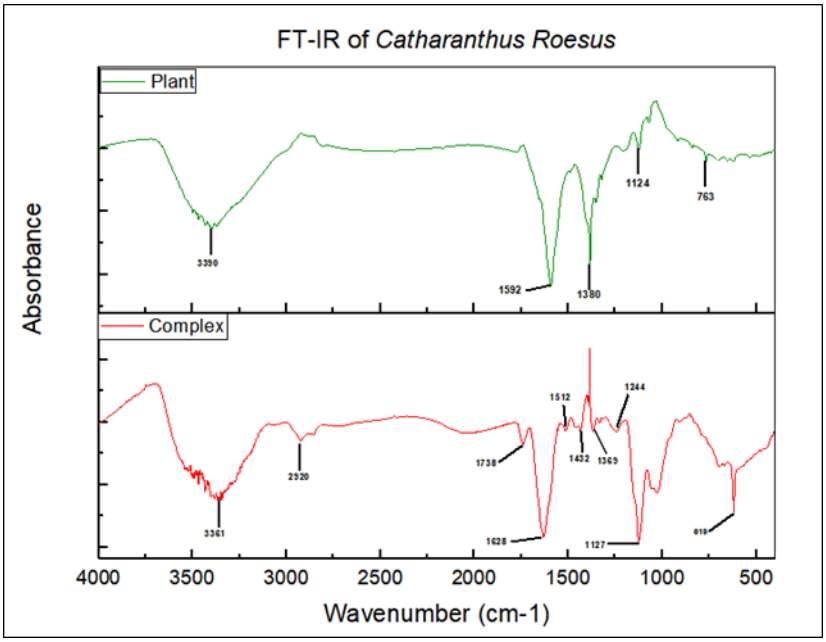

Figure 7: FTIR of plant extract and its green synthesized copper complex.

respectively, as shown in Figure 7. A description of the possible functional groups, the number of waves, and the main peaks. The FT-IR spectrum data further demonstrates that the reduction and stabilization of the copper complex are caused by the phytochemicals or functional groups of *Catharanthus roseus* leaf extract.

Various functional groups included in plant extracts are responsible for the absorption bands seen in leaf extract at 3390, 1592, 1380, 1124, and 763 nm, respectively. All at once, Absorption bands for the green synthesized Copper (II) complex at 3361, 2920, 1738, 1638, 1512, 1432, 1369, 1244, 1127 and 710 cm^{-1} is responsible for the stability of the copper (II) complex that was green synthesized and may correlate to the functional groups of O-H, C-H, C=N, C-O, N-O, and C-S.

Powder X-ray Diffraction Analysis of compounds

The crystalline phase of the green synthesized copper complex was characterized by XRD Analysis. The XRD method is a crucial crystallographic tool for determining if a material is crystalline whether made from traditional chemical processes or natural phytochemicals. A Bruker Axis D8 phase X-ray Diffractometer (XRD) from the CIF Laboratory at Jiwaji University in Gwalior (M.P.) was used to determine the structure and particle size of the copper complex. It seems that the complex has a deformed octahedral structure, since the diffraction intensities ranged from 5 to 800 at a 2θ angle. Corroborating with the values of the JCPDS card (card no. 895899) (Figure 8), five distinct peaks were noted at 2θ angles of 16.26°, 32.40°, 39.83°, 49.98°, and 53.45°. These peaks correspond to the h, k, and l values of the reflections from (110), (111), (210), (800), and (713), respectively.

Table 6: Composition of Antibacterial dusting powder.

Ingredients	Formula 1	Formula 2
Aqueous extract of *Catharanthus roseus* leaves	2	-
Biosynthesized Copper (II) complex	-	2
Zinc Oxide	14.8	14.8
Starch	25	25
Talc	60	60

The Scherrer formula is used for the calculation of particles size of green synthesized Copper (II) complex. The formula is:

$$D = 0.9 \lambda / \beta.\text{Cos } \theta$$

Where,

λ= wavelength of X-ray which is equal to 1.54 A°.

On the (III) plane, the average crystalline size of the green synthesized copper (II) complex, which was determined by B= Full width at half maximum at the θ angle, was 14±1 nm.

The reflection of the XRD spectral peaks is small and sharp and indicates the crystalline size of the copper (II) complex. In this research work, the Copper (II) complex was synthesized from the pure fresh leaf extract of *Catharanthus roseus* plant that's why no additional peaks of impurities are observed in the XRD spectra plant extract and it's Copper (II) Complex (Figure 9).

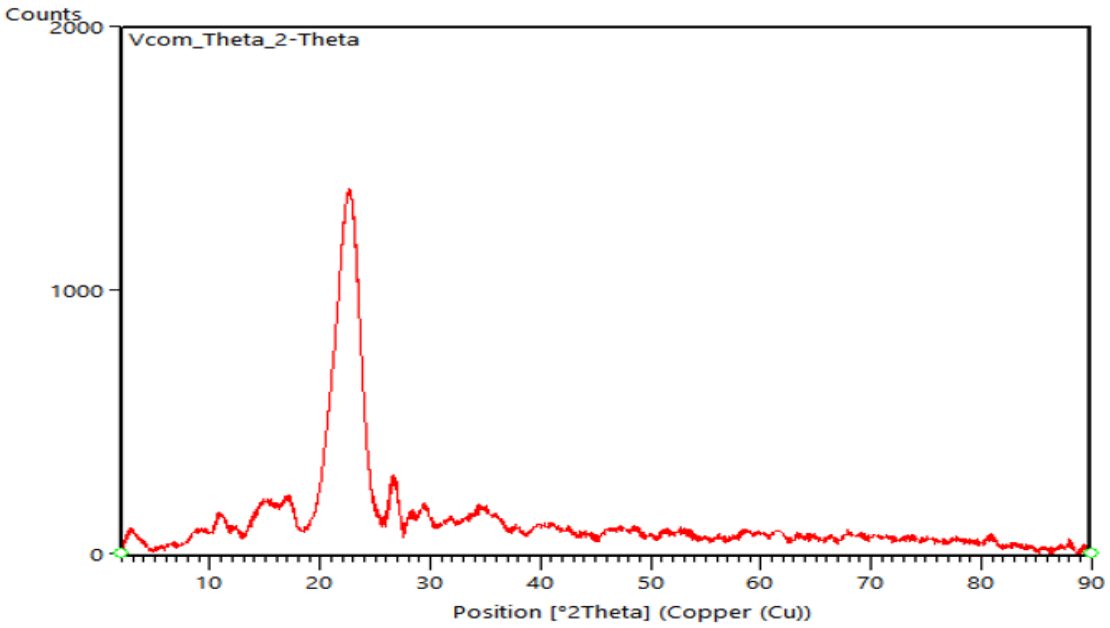

Figure 8: XRD of the biosynthesized copper complex obtained from Aqueous extract of *Catharanthus roseus* leaves.

Thermal behaviour of green synthesized copper (II) complex

The DSC analysis of the green synthesized copper (II) complex indicates that the copper (II) complex began to decompose at 69.27°C and shows the endothermic nature of the decomposition reaction. The second decomposition of the Copper (II) complex was done at 220.79°C, this was again an endothermic reaction. A comparison of the decomposition temperature of compounds showed that copper (II) complex decomposed at higher temperatures as compared to phytochemicals present in the aqueous extract of *Catharanthus roseus* leaves. Due to the formation of a coordinate bond of Cu+2 ions with phytochemicals. In the process of complex formation, a large amount of energy (-27.35 J/g) was released. So that complex became stable at room temperature for up to 6 months (Figure 10).

Evaluation of Formulated Antibacterial Dusting Powder

Angle of Repose

Get a dry, clean funnel that has a flat tip and a spherical stem (20-30 mm in diameter) and fasten it to the burette stand. Set a dry and clean piece of graph paper on a level surface underneath the funnel. Get the gap between the funnel's base and the sheet to the exact height of 2 cm. Pour the sample slowly into the funnel starting at the top and working your way down until a mound of powder develops, reaching the very bottom of the funnel. Covering around 90% of the powder, draw a circle around the stack with a pencil. To get an average reading, you need to repeat the process four times. Table 8 gives the average diameter and radius of each drawn circle.

$$\varnothing = \tan^{-1} (h/r)$$

Estimation of Volume and Density at the Tap Level

Pinch 25 g of powder (W1) and put it in a dry graduated cylinder; then, record the volume (V1) in milliliters. Fill the bulk density instrument with the sample-containing cylinder. Get the equipment set up for 100 taps and start tapping away. V2 is the volume in milliliters that the powder takes up.

The mass-to-volume ratio, or bulk density, is W/V1 g/mL.

The formula for tapped density is mass/tapped volume, which may be expressed as W1/V<2 g/mL.

The ease of index of powder flow, also known as Hausner's ratio, may be determined using the following formula:

Using the formula: Hauser's ratio = tap density / bulk density

Carr's compressibility index, which indicates the percentage of compressibility of the mix, was computed using the following formula:

Using the formula: Carr's Index= Tap Density-Bulk Density*100/Tap Density

Using a pH meter, we found out what the pH of a 1% solution of the created powder and the standard was.

To find the powder's moisture content, we heated 3 g of the material in a hot air oven set at 70°C for 1 hr (Figure 11).

Ascertaining Ash values

The total Ash value was determined by precisely measuring 2 g of powder in a silica crucible that had been fired and coated with tar. After that, the substance was heated to 400°C until it became white, which meant there was no carbon, and then it was ignited.

After that, the material is cooled in a desiccator and its total ash content is determined.

Soluble in acid as a means of detecting sand or boil contamination, the ash value is the residue that remains after treating the total ash with hydroalcoholic acid. After 5 min of boiling in 25 mL of 2 m HCl, strain the resulting residue onto ashless filter paper. Rinse with hot water, then burn the cooled mixture in desiccators and measure the weight.

Finding the Values of Extractive Operations

To determine the water-soluble extractive value, a conical flask with a glass stopper was used to hold 5 g of powder. After that, it's macerated for 18 hr in 100 mL of chloroform water. After that, it was filtered and about 25 mL of the resulting filtrate was put to a China dish. The dish was then dried in a water bath. After being dried at 105°C for 6 hr, it was chilled and weighed.

For the purpose of determining the alcohol solubility extractive values, a conical flask with a glass stopper was used to hold 5 g of powder. The next step is an 18-hr maceration in 100 mL of ethanol. After that, it was filtered and about 25 mL of the resulting filtrate was put to a China dish. The dish was then dried in a water bath. After being dried at 105°C for 6 hr, it was chilled and weighed.

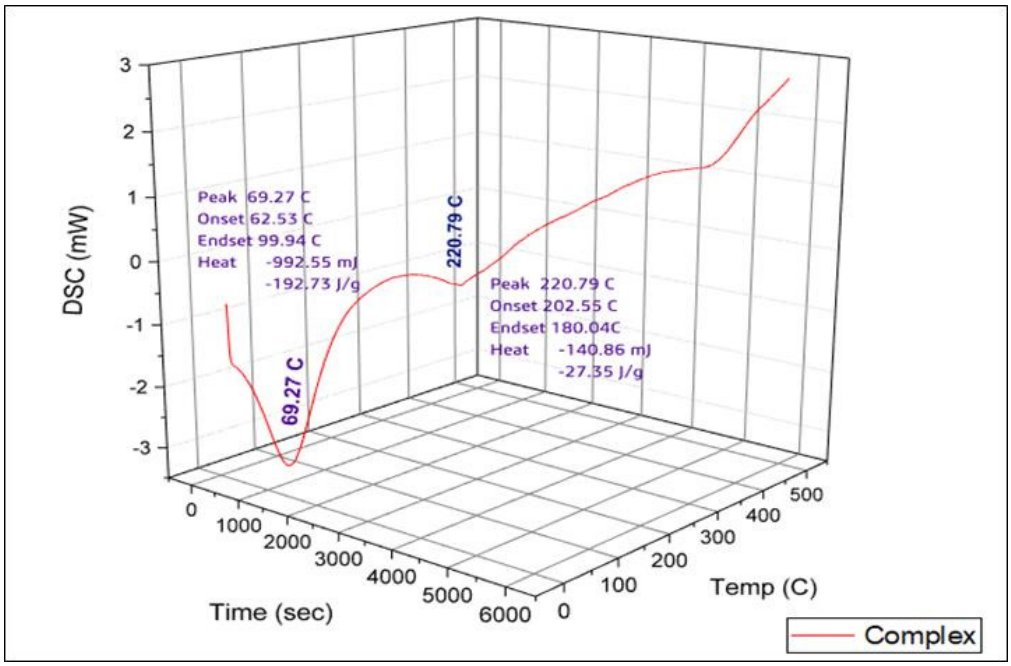

Figure 9: DSC spectra of Biosynthesized Copper (II) complex.

Table 7: Functional groups identified in the FTIR spectra of plant extract and its biosynthesized copper complex.

Sl. No.		Aqueous extract of *Catharanthus roseus leaves.*			Biosynthesized copper complex	
	Frequency range (cm^{-1})	Functional group		Frequency range (cm^{-1})	Functional group	
1	3390	O-H stretching	alcohol	3361	N-H stretching	aliphatic primary amine
2	1592	N-H bending	amine	2920	C-H stretching	alkane
3	1380	C-H bending	alkane	1738	C=O stretching	aldehyde
4	1124	C-O stretching	tertiary alcohol	1638	C=C stretching	Alkene (monosubstituted)
5	763	C-H bending	1,2,3- Trisubstituted	1512	N-O stretching	nitro compound
6	-	--	-	1432	O-H bending	carboxylic acid
7	-	-	-	1369	O-H bending	Alcohol

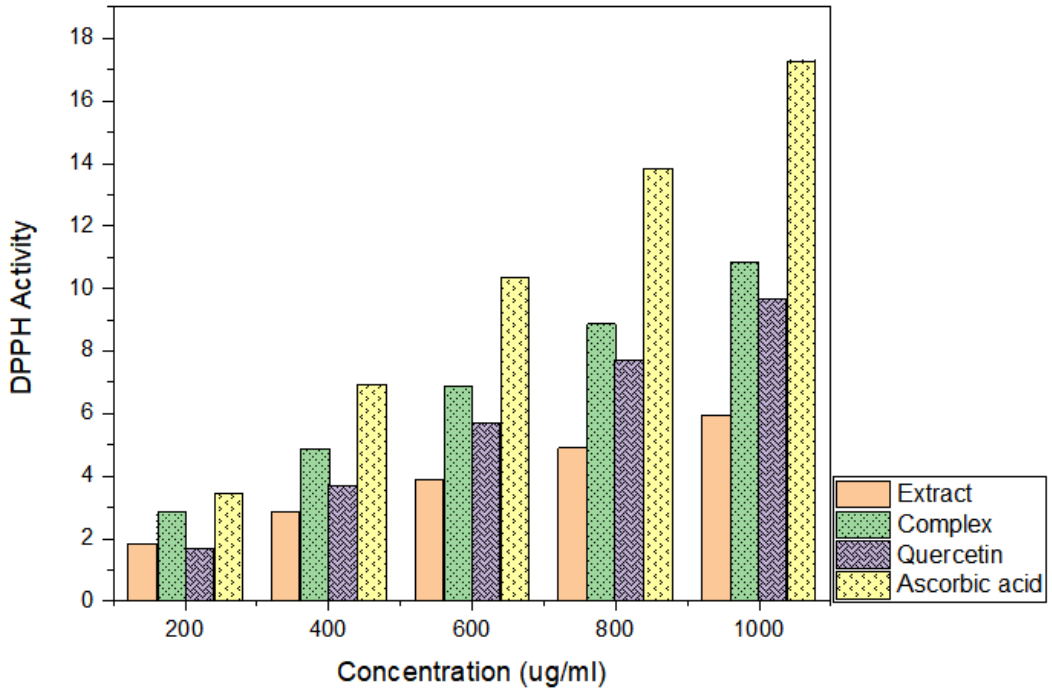

Figure 10: Antioxidant activity analysis of aqueous extract of *Catharanthus roseus* leaves and its green synthesized Copper (II) complex. Note: Trolox Equivalent antioxidant activity has been calculated from molar absorptivity by diving 1.64×10^{-3}.

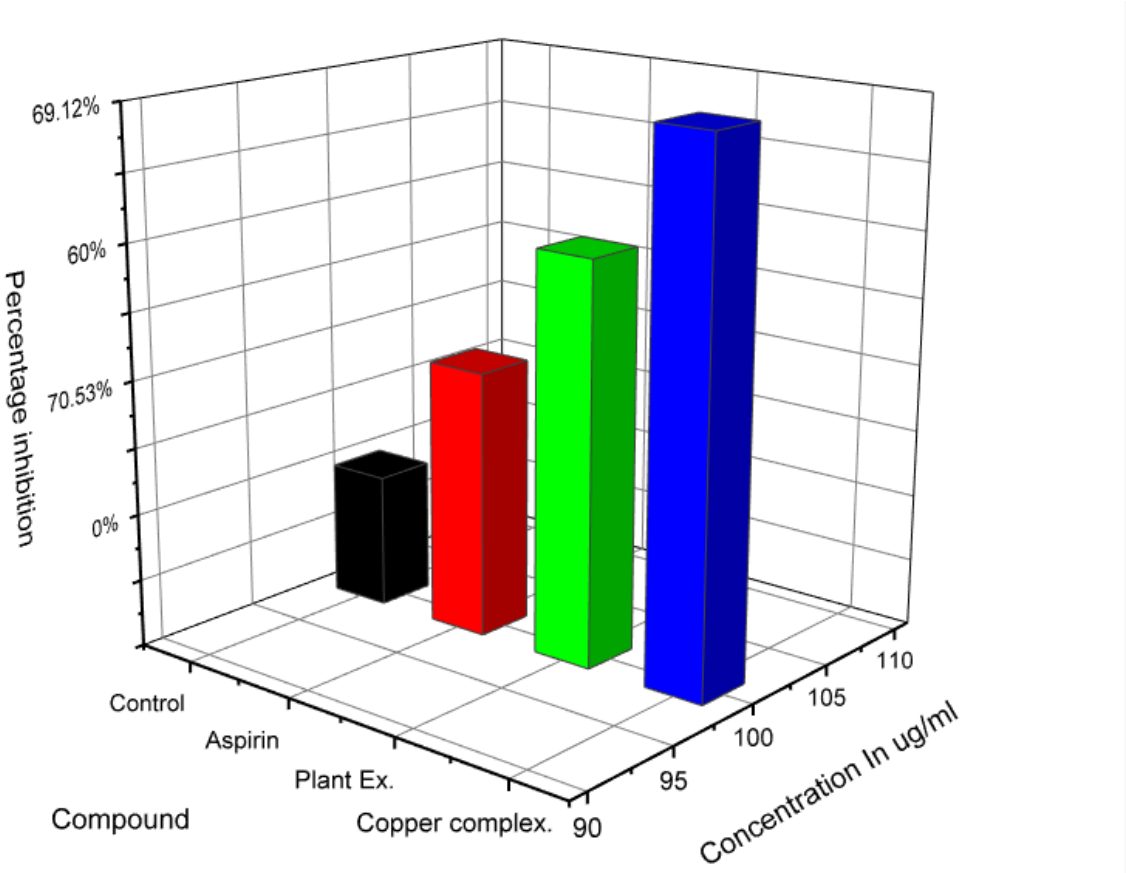

Figure 11: Anti-inflammatory activity analysis of aqueous extract of *Catharanthus roseus* leaves. And its green synthesised copper (II) complex.

Anxiety assessment

Locate and mark a one square centimeter region on the back of your left hand. The designated area was dusted with a certain amount of dusting powder and the amount of time it took was recorded. The presence of redness, swelling, or irritation was recorded at regular intervals for up to 24 hr (Figure 12).

Pharmacological activities of compounds

Antioxidant activity of compounds

We used the DPPH assay to test the antioxidant activity of a copper complex that was produced in a green way. The radical scavenging activity of chemicals, both natural and artificial, may be evaluated using DPPH, a stable free radical molecule. In terms of antioxidant activity, the green synthesized copper complex outperformed both the aqueous leaf extract of the *Catharanthus roseus* plant and the quercetin compound (positive control), but it was not as effective as the standard ascorbic acid. It is worth mentioning that the DPPH activity of the green synthesized copper (II) complex was higher than that of the plant extract. These findings align with the theoretical aspects of antioxidant activity, which emphasize the significance of the position, number of bioactive agents, and degree of conjugation within the entire molecule in determining the antioxidant activities of compounds. The quantity of phytochemicals or functional groups found in a plant extract is strongly related to the antioxidant activity of natural bioactive agents, according to herbal chemistry.

In vitro anti-inflammatory activities of compounds

When proteins are subjected to an external stressor or chemical, Denaturation happens when proteins are exposed to certain conditions, such as high temperatures, concentrated inorganic salts, organic solvents, or acids, and their tertiary and secondary structures are destroyed. When denatured, the biological activities of the majority of proteins are lost. A well-established source of inflammation is the denaturation of proteins. Based

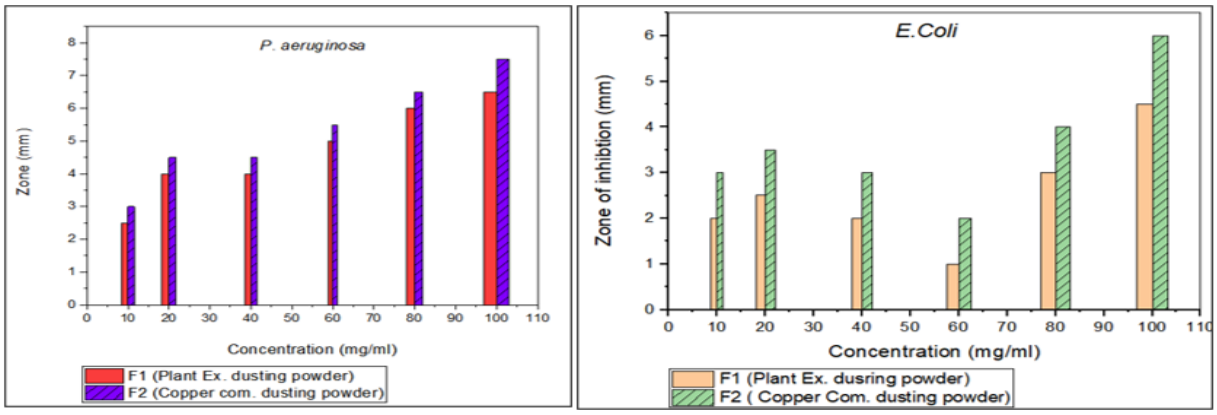

Figure 12: Antibacterial activity of Dusting powder formulations F1 and F2.

Table 8: Physical Parameters of Antibacterial dusting powder.

Sl. No.	Physical Parameters	Value			
		F_1	F_2	F_3	Standard
	Angle of repose	28.39	31.38	32.60	25.75
	Bulk density	0.50	0.48	0.58	0.53
	Tapped density	0.52	0.50	0.53	0.57
	Carr's Index	3.67%	5.50%	6.2%	7.0%
	Hausner's ration	1.01	1.06	1.08	1.07
	pH	7.2	6.9	7.0	6.4
	Moisture content	8%	6%	10%	15%
Ash value					
Total ash		16%	14%	13%	12%
	Acid insoluble ash	8%	7%	6.5%	6%
	Extractive values				
	Water soluble	3%	2.3%	2%	2%
	Alcohol soluble	4.9%	4.1%	3.2%	3%
	Irritancy test	NIL	NIL	NIL	NIL

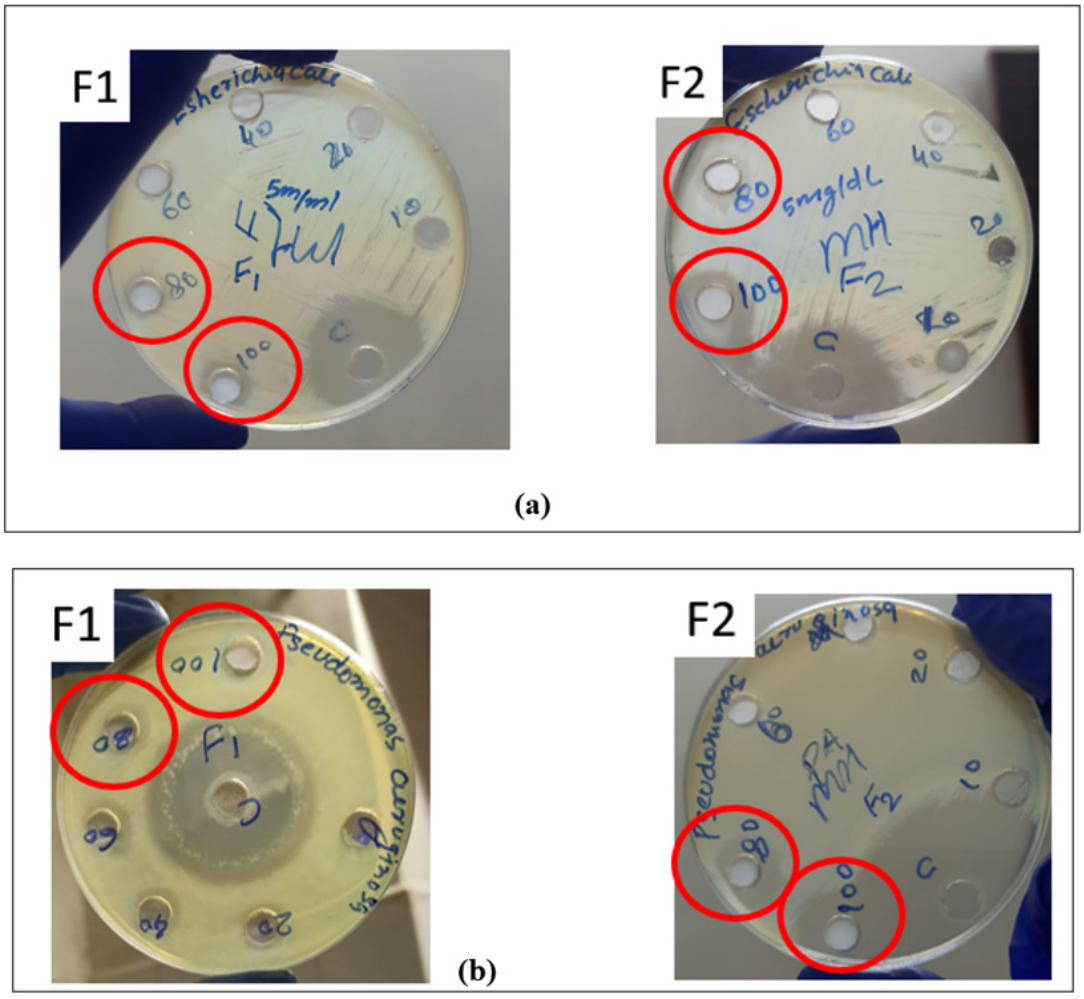

Figure 13: Antimicrobial analysis of antibacterial dusting powder. a. F1 (Formulation with plant powder), F2 (Formulation with copper complex) for *E. coli*. b. F1 (Formulation with plant powder), F2 (Formulation with copper complex) for *Pseudomonas aeruginosa*.

on our findings, the copper (II) complex effectively prevented heat-induced denaturation of albumin up to a certain temperature point.

A concentration of 100 µg/mL inhibited albumin protein denaturation more effectively than the usual aspirin medication at the same concentration, with a maximum inhibition of 70.7% compared to the control. This was due to the suppression of the green produced copper (II) complex. Evaluation of Antibacterial efficacy of compounds

By adding different amounts of dusting powder based on plant extract and dusting powder based on green manufactured copper (II) complex, the compounds' *in vitro* antibacterial effectiveness was investigated (10-100 mg/mL). Placed on agar plates and then incubated with strains of bacteria that are relevant in therapeutic settings, such as *E. coli* and *Pseudomonas aeruginosa*. To compare the anti-bacterial activity of substances, Streptomycin was employed as a control at the same doses. The result showed that the growth of bacteria was significantly decreased in the case of green synthesized copper (II)" complex-based dusting powder due to the presence of copper ions which act as strong antibacterial agents. The higher antibacterial efficacy of green synthesized copper (II) Complex based dusting powder, maybe due to the orbital linkage property of Cu^{2+} ions, which tends to make the copper complex as powerful and potent antibacterial agent. In copper complex, positive charges are present on copper ions and the positive charges are shared with the bioactive agents which are present in plant extract. As a result of this, delocalization of π electrons takes over the copper complex. The enhanced antibacterial efficacy of copper complex may be explained on the basis of the chelation theory of metal complexes shows in Figure 13.

CONCLUSION

Our study has shown a new way to make anti-bacterial dusting powder with copper ions using an extract from *Catharanthus roseus* leaves. This method is quick, easy, and sustainable. The solution's color changed from blue to green when the phytochemicals in the leaf extract interacted with the copper salt, signifying the creation of a copper (II) complex. The results of the

UV-visible spectroscopy verified this as well. The development of the stable copper complex from plant extract was verified by the absorption peaks at 304 nm and 328 nm in the UV-visible spectral data. A number of structural and morphological investigations have shown that the concentration of the plant extract used as a precursor for the green synthesis of copper complex determines the size and form of the copper complex. Aqueous extract of *Catharanthus roseus* leaves is a key component in our study because of the significant role it plays in decreasing response time and minimizing the likelihood of negative effects. This environmentally friendly process shows great promise for the industrial-scale manufacture of plant-based antibacterial dusting powder. In the future, biomedical applications such as antibacterial, antioxidant, and anti-inflammatory medicines for humans may benefit from the biosynthesized copper (II) complex, according to all pharmacological properties seen in an aqueous environment.

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ABBREVIATIONS

XRD: X-ray Diffraction; **UV-vis:** Ultraviolet-visible Spectroscopy; **FT-IR:** Fourier Transform Infrared Spectroscopy; **DSC:** Differential Scanning Calorimetry; **FRSA:** Free Radical Scavenging Assay; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **DDW:** Double Distilled Water; **IC_{50}:** Half Maximal Inhibitory Concentration; **Cu (II):** Copper in +2 Oxidation State; **DMSO:** Dimethyl Sulfoxide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This research focuses on the green synthesis of copper (II) nanoparticles using the aqueous leaf extract of *Catharanthus roseus*, aiming to develop a novel, eco-friendly antibacterial dusting powder. The phytochemicals present in the plant extract, such as flavonoids, tannins, and alkaloids, served as natural reducing and stabilizing agents during the synthesis process. The resulting copper complex was characterized using UV-vis, FT-IR, XRD, and DSC techniques, confirming the formation of stable nanoparticles with an average size of 3.4 nm and demonstrating long-term stability. The synthesized complex was incorporated into a dusting powder formulation along with zinc oxide, starch, and talc. Pharmacological evaluations showed that the copper-enriched formulation exhibited significantly enhanced antibacterial activity against *E. coli* and *Pseudomonas aeruginosa*, superior antioxidant effects in DPPH assays, and notable anti-inflammatory activity through inhibition of protein denaturation. The study concludes that the green synthesized copper (II) complex has strong potential as a therapeutic agent in the treatment of wounds, burns, and skin infections, offering a sustainable alternative to conventional antimicrobial products.

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