

FACILE GREEN SYNTHESIS AND CHARACTERIZATION OF AG-CU-ZN NANOPARTICLES USING *CORDIA SEBESTENA* L. FLOWER EXTRACT AND THEIR ANTIMICROBIAL EFFICACY.

**R.Lakshmanan^{*1}, S. Mariselvi², A. Srinivasalu³, S. Jayapriya⁴, S.N.Suresh⁵,
B.Mohanapriya⁶, and S. Violet Beaulah⁷**

1. Assistant Professor, Department of Botany, G.Venkataswamy Naidu College (Autonomous), Kovilpatti – 628502, Affiliated to Manonmaniam Sundaranar University, Tamil Nadu, India.
2. Assistant Professor of Zoology, Nallamuthu Gounder Mahalingam College, Pollachi, Tamil Nadu, India.
3. Lecturer in Botany, Visvodaya Government Degree College, Venkatagiri, Andhra Pradesh, India.
4. Associate Professor, Department of Costume Design and Fashion, Nehru Arts and Science College, Coimbatore, Tamil Nadu, India.
5. Associate Professor, Department of Biotechnology, Rathinam College of Arts and Science Coimbatore-641021, Tamil Nadu, India. Affiliated to Bharathiar University, Coimbatore, Tamil Nadu, India.
6. Assistant professor, Department of Biotechnology, Rathinam College of Arts and Science Coimbatore-641021, Tamil Nadu, India. Affiliated to Bharathiar University, Coimbatore, Tamil Nadu, India.
7. Assistant Professor, Department of Microbiology, Rathinam College of Arts and Science Coimbatore-641021, Tamil Nadu, India. Affiliated to Bharathiar University, Coimbatore, Tamil Nadu, India.

Corresponding Author: Dr.R.Lakshmanan, Email: rlaxman84phd@gmail.com

Abstract

This investigation reports on the green synthesis of selected nanoparticles (CuSO₄, ZnSO₄, AgNO₃) using the flowers of *Cordia sebestena* and were subjected to comprehensive characterization through various techniques. In the UV-visible spectrum analysis, the silver nanoparticles exhibited maximum absorption at 240 nm followed by copper nanoparticles exhibited their absorbance peak at 280 nm and zinc nanoparticles displayed their peak at 310 nm. XRD analysis indicated that the silver particles generated in our experiments existed in the form of nanocrystals, as evidenced by peaks at 2θ values of 7.7440, 15.1928, 19.1919, 21.1040, 23.5136, 26.731, and 31.4932, corresponding to heights of 13.71, 30.89, 232.79, 368.01, 216.01, 66.91, and 197.92 counts, respectively. Copper particles exhibited peaks at 2θ values of 5.4670, 9.4437, 11.6355, 15.6593, 18.8709, 20.7264, and 23.0335 with heights of 15.88, 41.72, 109.27, 37.58, 102.33, 197.10 and 53.14 counts, respectively. Zinc particles displayed peaks at 2θ values of 9.1935, 13.1814, 14.2828, 16.3701, 19.3166, 21.2627, and 22.4356 with heights of 115.48, 72.72, 97.51, 160.30, 577.17, 1059.99 and 446.48 counts, respectively. SEM spectral analysis revealed the slices of sticks, with cube shape structure distribution of silver nanoparticles among the surface within a size range spanning from 2 μ m to 200 nm. The antimicrobial efficacy of

metallic nanoparticles derived from *Cordia sebestena* was evaluated using the disc diffusion method against both gram-positive and gram-negative pathogens, including *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, as well as the fungi *Aspergillus flavus* and *Candida albicans*. Remarkably, metallic Ag, Cu, and Zn nanoparticles exhibited substantial zones of inhibition against the selected pathogens, with the highest inhibition zone observed in copper and silver nanoparticles.

Keywords: Synthesis, *Cordia sebestena*, Ag-Cu-ZnNPs, Characterization, Antimicrobial Efficacy, Human Pathogens.

Introduction

Green synthesis of AgNPs has gained importance and can be considered a better alternative method over conventional methods due to their distinctive properties such as cost-effectiveness, ease of availability, and less harm to the environment (Phanjomet et. al.,2012). The AgNPs are reported to have the capability to absorb/adsorb and minimize pollutants in the environment, which shows the effective remediation of contaminated water by the nano phytoremediation process (G Palaniet et. al., 2021). The acronym nano-phytoremediation refers to the approach for eliminating contaminants from the environment by leveraging both nanotechnology and phytotechnology (Srivastavet et. al., 2018). Nano-remediation and phytoremediation are compatible, while nano-remediation is a quick and efficient method but involves a high cost whereas phytoremediation is a low-cost and sustainable technique that requires appropriate plant selection (Bhati and Rai 2018).

Bimetallic nanoparticles are considered valuable for their suppleness in catalytic, optical, and electronic properties. Hashemian Rahaghi et. al (2015) and Wang HK et.al (2012) reported that bimetallic nanoparticles do not only differ in size and effect but also in their pure components composition¹. Several studies have been reported in synthesizing various bimetallic nanoparticles Pd-Pt, Pt- Co, Ni-Mo, Ag-Cu, Au-Ag, etc. Oxidation of styrene was done using AgCu catalyst as this provides superior catalytic activity in different oxidation reactions². Bimetallic NPs provide hundreds of combinations of existing metal elements and could bring about a revolution in medicinal research.

In this study, we present the synthesis of Ag, Cu, and Zn nanoparticles (AgNPs) using flowers from *Cordia sebestena* a member of the Boraginaceae family and a prominent dicot family within Angiosperms. This study employs a simple and cost-effective green synthesis approach. The resulting nanoparticles will be thoroughly characterized through various analytical techniques, including UV-visible spectroscopy, Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), and Antimicrobial efficacy assessments.

Materials and Methods

Plant Material Collection:

Flowers from *Cordia sebestena* were chosen for the current study. The plant material, belonging to the Boraginaceae family, was sourced from the Ayyaneri village in the Kovilpatti region of the Tuticorin district, Tamil Nadu. Taxonomic attributes were verified using references

such as the 'Flora of Presidency of Madras' (Gamble, 1928) and the 'Flora of the Tamil Nadu Carnatic' (Mathew, 1981).

Isolation of Nanoparticles:

Fresh and healthy flowers of *Cordia sebestena* were carefully gathered and meticulously cleansed with tap water followed by distilled water to eliminate any dust or visible impurities. Subsequently, 10 grams of fresh flowers were boiled in 100 milliliters of double-distilled water, and the resulting extract was filtered through Whatman no.1 filter paper, collecting the filtrate in a conical flask. This obtained extract was utilized for the synthesis of various nanoparticles.

Synthesis of Copper Nanoparticles:

In a conical flask, 90 milliliters of Copper Sulphate solution was combined with 10 milliliters of the flower extract. The reduction of copper sulfate to copper ions was identified by the transformation of the extract's light yellow color to a dark brown hue. The conical flask was then exposed to light for a 72-hour incubation period.

Synthesis of Zinc Nanoparticles:

Similarly, 90 milliliters of Zinc Sulphate solution was placed in a conical flask, to which 10 milliliters of the flower extract were added. The Zinc Sulphate solution underwent a color shift from pale green to brownish green. The conical flask was subjected to light exposure for a 72-hour incubation period.

Synthesis of Silver Nanoparticles:

Similarly, 90 milliliters of Silver Nitrate solution was placed in a conical flask, to which 10 milliliters of the leaf extract were added. The Silver Nitrate solution underwent a color shift from light green to brownish green. The conical flask was subjected to light exposure for a 72-hour incubation period.

Characterization

UV- Spectrophotometer Analysis:

For UV-spectrophotometer analysis, approximately 1 milliliter of the sample suspension was transferred into a quartz tube. The sample was then diluted with 2 milliliters of distilled water to facilitate monitoring of nanoparticle synthesis. UV-visible spectra scans were conducted in the wavelength range of 200-900 nanometers using a UV-visible spectrophotometer (Shimadzu UV 1800, Germany).

XRD Analysis:

To characterize the purified synthesized nanoparticles, freeze-dried powder samples were employed for XRD analysis, which was conducted at 40 kV/20 mA using continuous scanning in 2 theta mode (Absar, 2003). The nanoparticle solution was first purified through repeated

centrifugation at 5000 rpm for 20 minutes, followed by redispersion of the nanoparticle pellet into 10 milliliters of deionized water.

SEM Analysis of Silver Nanoparticles:

SEM analysis was carried out using a ZEISS machine. Thin films of the sample were prepared on a carbon-coated copper grid by dispensing a small amount of the sample onto the grid. Any excess solution was removed using blotting paper, and the films on the SEM grid were allowed to dry by exposure to a mercury lamp for 5 minutes.

Antimicrobial Activity of Nanoparticles:

The effectiveness of flower extracts from *Cordia sebestena* against the growth of various human pathogens was evaluated using the Agar well diffusion method in a clinical laboratory (Scudder Diagnostic Laboratory Institute, Nagercoil). The Kirby-Bauer method (Bauer et al., 1996) was employed to assess the antibacterial activity of isolated plant extraction pellets. Overnight-grown cultures of the respective bacteria and fungi were inoculated onto Nutrient agar, Sabouraud's Dextrose Agar (SDA), and Potato Dextrose Agar (PDA) plates. Wells were created in all plates, including those designated for controls. Flowers extracts (Silver nitrate, Copper sulfate, and Zinc sulfate) were prepared at a concentration of 50 mg/mL. Antibiotics (Amikacin and Nystatin) at the same concentration were used as controls. Bacterial plates (*E.coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*) were incubated at 37°C for 24 hours, while fungal plates (*Aspergillus flavus* and *Candida albicans*) were incubated at 35°C for 48 hours. The diameter of the inhibition zone was measured in millimeters.

Results and Discussion

Synthesis and Characterization of Nanoparticles:

The flowers of *Cordia sebestena* when exposed to different metal compositions (Silver Nitrate, Copper Sulphate, and Zinc Sulphate) in aqueous solutions, exhibited distinct color changes, signifying the formation of three distinct types of nanoparticles. These nanoparticles were subsequently confirmed through spectral studies involving UV-visible spectroscopy, X-ray diffraction, and SEM analysis, and they were employed in various biological activities. The meticulous control of starting materials is imperative for ensuring the reproducible quality of herbal products. Consequently, recent years have seen a heightened emphasis on the standardization of medicinal plants with therapeutic potential. Despite the availability of modern techniques, the identification and evaluation of plant-based drugs through pharmacognostic studies remain a more reliable, accurate, and cost-effective approach. According to the World Health Organization (WHO, 2000), the macroscopic and microscopic description of a medicinal plant constitutes the initial step toward establishing its identity and purity, and this should precede any other testing procedures (Anonymous, 2002).

UV- Visible Spectrum Analysis:

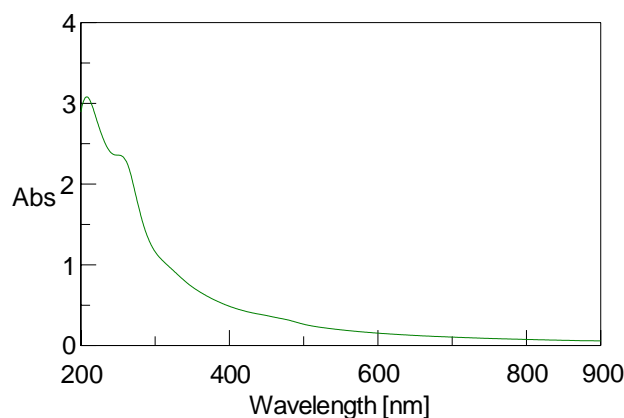
The reduction of metal ions to metal nanoparticles, facilitated by exposure to *G Cordia sebestena* flowers, was observable through color changes and subsequently confirmed via UV-Vis spectroscopy. This alteration in color occurred due to the excitation of surface plasmon vibrations (Mulavney, 1996). UV-Vis spectroscopy is a widely employed technique for investigating size and shape-controlled nanoparticles in aqueous suspensions (Wiley et al., 2006). The presence of nanoparticles was verified by obtaining spectra in the visible range using a UV-visible spectrophotometer, with absorption wavelengths spanning from 200 to 900 nanometers (Figures 1-3).

Upon introducing *Cordia sebestena* flower extract to a 1 mM Silver Nitrate solution, the solution transitioned from light green to dark green within 10 minutes, indicative of silver nanoparticle formation. Similarly, in a 1 mM Copper Sulphate solution, the color shifted from light yellow to dark brown after 20 minutes, signifying the formation of copper nanoparticles. In a 1 mM Zinc Sulphate solution, the color transformation ranged from pale green to brownish green, again occurring after 20 minutes, confirming the generation of zinc nanoparticles.

Analysis of AgNPs of *Cordia sebestena* flowers

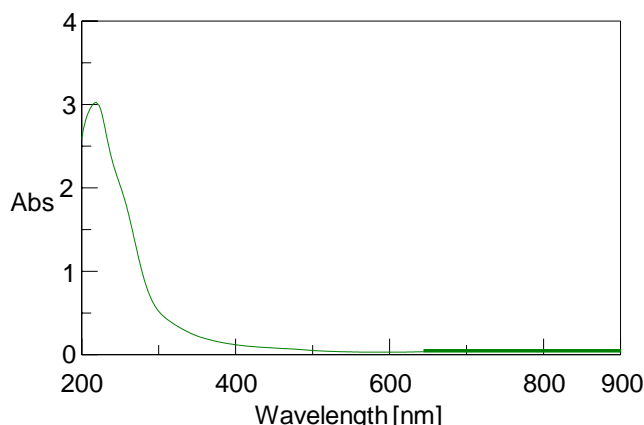
Absorption spectra of silver nanoparticles formed in the reaction medium exhibited an absorbance peak within the 200 to 400 nm range, with the characteristic peak of silver nanoparticles observed at 240 nm (Figure 1). Our findings align with the research of Ashok Kumar et al. (2015), who investigated the impact of varying leaf extract concentrations on the size of silver nanoparticles.

Figure No. 1: UV-Vis Spectrum of AgNPs in flowers of *Cordia sebestena*

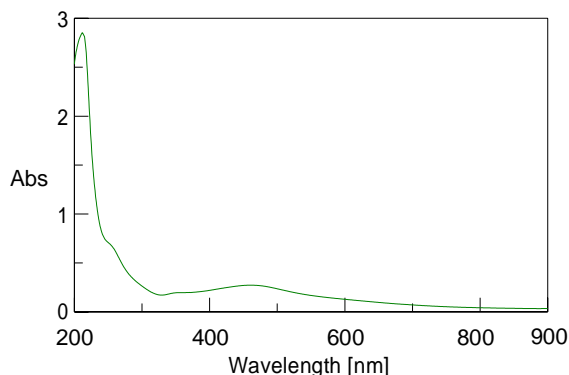


Analysis of CuNPs in flowers of *Cordia sebestena*

The formation of copper nanoparticles was effectively assessed through UV-visible spectroscopy. The absorption spectra of copper nanoparticles generated in the reaction medium displayed an absorbance peak within the 200 to 400 nm range, with the distinctive absorption peak observed at 280 nm, characteristic of copper nanoparticles (Figure 2). It is noteworthy that the sharpness of this absorption peak appeared to be contingent on the concentration ratio of the extract, as corroborated by Shen et al. in 2011.

Figure No. 2: UV-Vis Spectrum of CuNPs in flowers of *Cordia sebestena***Analysis of ZnNPs in flowers of *Cordia sebestena***

The absorption findings provided compelling evidence for the synthesis of zinc nanoparticles using the bio-reduction method in a liquid medium. Absorption spectra obtained from the reaction medium exhibited an absorbance peak within the 200 to 400 nm range, with the distinctive absorption peak evident at 310 nm, which is characteristic of zinc nanoparticles (Figure 3).

Figure No. 3: UV-Vis Spectrum of ZnNPs in flowers of *Cordia sebestena***XRD Analysis**

The biosynthesized nanoparticles, derived from *Cordia sebestena* flower extracts, were meticulously characterized and validated through X-ray diffraction (XRD) analysis (Figure 4-6). The XRD analysis aimed to confirm the crystalline nature of the nanoparticles. A comparative assessment of our XRD spectrum against established standards substantiated that the nanoparticles generated in our experiments indeed existed in the form of nanocrystals, as indicated by the discernible peaks at specific 2θ values.

AgNPs in flowers of *Cordia sebestena*

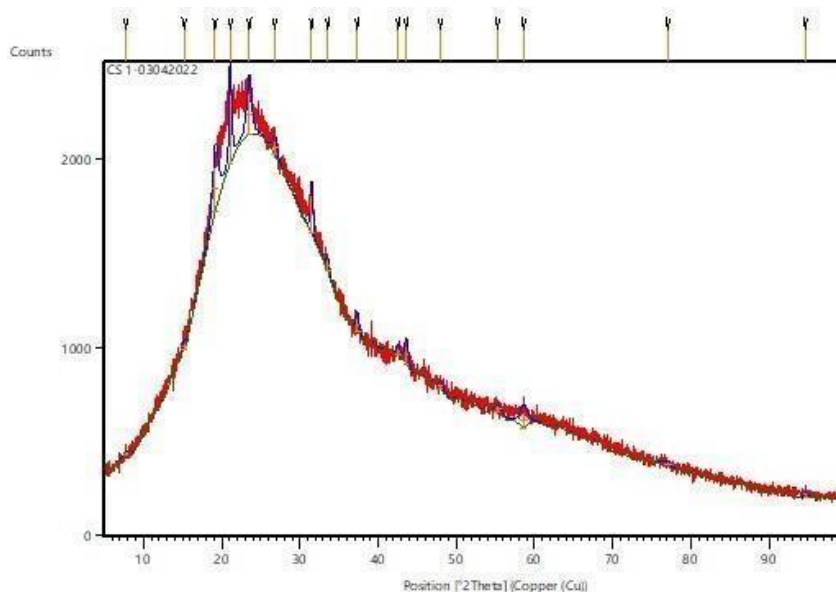
In the case of silver nanoparticles, the XRD analysis revealed seven distinctive peaks in the XRD image, ranging from 0 to 90° (Figure 4). These peaks corresponded to 2θ values of

7.7441, 15.1928, 19.1919, 21.1040, 23.5136, 26.7931 and 31.4932, with corresponding heights of 13.71, 30.89, 232.79, 368.01, 216.01, 66.91 and 197.92 counts, respectively. The corresponding "d" spacing values for silver nanoparticles were measured as 11.4165 Å, 5.8318 Å, 4.6247 Å, 4.20984 Å, 3.78359 Å, 3.32746 Å, and 2.84076 Å.

Table No. 1: XRD Pattern of AgNPs Synthesized by flowers of *Cordia sebestena* Extract with AgNO₃ Solution.

Pos.[°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
7.7440	13.71	0.3464	11.4165	3.73
15.1928	30.89	0.4330	5.8318	8.39
19.1919	232.79	0.8659	4.6247	63.26
21.1040	368.01	0.3464	4.20984	100.00
23.5136	216.26	0.6927	3.78359	58.77
26.7931	66.91	0.6927	3.32746	18.18
31.4932	197.92	0.3464	2.84076	53.78

Figure No. 4: XRD Spectrum of AgNPs in flowers of *Cordia sebestena*



CuNPs in flowers of *Cordia sebestena*.

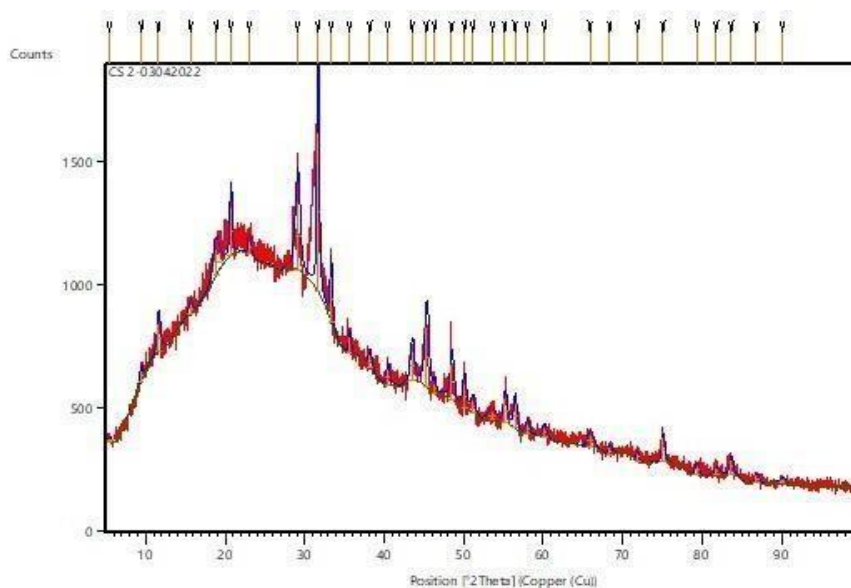
The presence of copper nanostructures was unequivocally confirmed through the distinctive peaks evident in the XRD pattern. Figure 5 illustrates the successful biosynthesis of copper nanostructures using *Cordia sebestena* flower extract, as validated by the emergence of seven characteristic peaks in the XRD image, spanning 2θ values from 0 to 50 Å. A meticulous

comparison between our XRD spectrum and the established standard unequivocally affirmed that the copper nanoparticles produced in our experiments indeed assumed the form of nanocrystals. This was substantiated by the appearance of peaks at 2θ values of 5.4670, 9.4437, 11.6355, 15.6593, 18.8709, 20.7264, and 23.0335 corresponding to heights of 15.88, 41.72, 109.27, 37.58, 102.33, 197.10 and 53.14 counts, respectively, for flowers of *Cordia sebestena*. The corresponding "d" spacing values for copper nanoparticles were calculated as 16.16556 Å, 9.36528 Å, 7.60557 Å, 5.65914 Å, 4.70267 Å, 4.28567 Å and 3.86136 Å for the synthesized nanoparticles.

Table 2: XRD Pattern of CuNPs Synthesized by flowers of *Cordia sebestena* Extract with CuSO₄ Solution.

Pos.[°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
5.4670	15.88	0.6061	16.16556	2.45
9.4437	41.72	0.4330	9.36528	6.45
11.6355	109.27	0.5196	7.60557	16.89
15.6593	37.58	0.5196	5.65914	5.81
18.8709	102.33	1.0391	4.70267	15.82
20.7264	197.10	0.3464	4.28567	30.46
23.0335	53.14	0.3464	3.86136	8.21

Figure 5: XRD Spectrum of CuNPs in flowers of *Cordia sebestena*



ZnNPs in flowers of *Cordia sebestena*

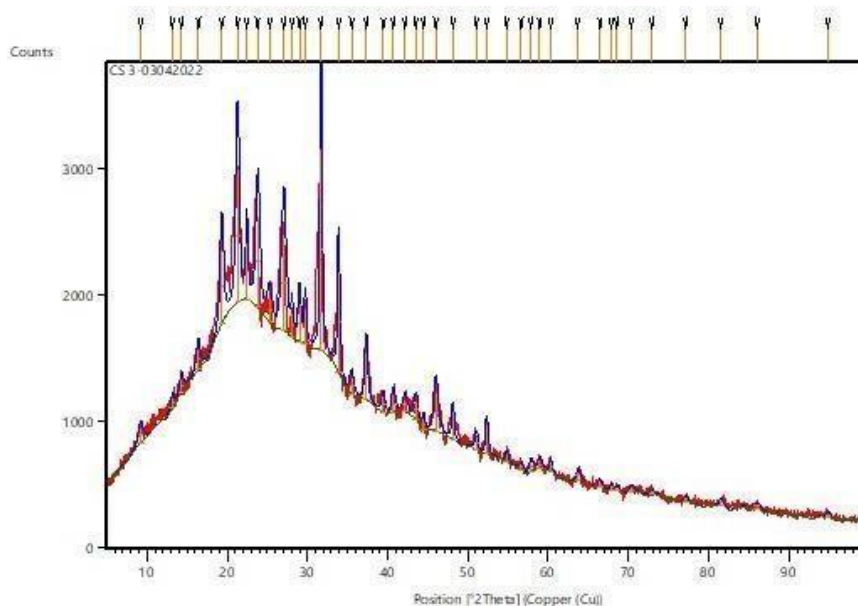
The successful biosynthesis of zinc nanostructures utilizing leaf extract from *Cordia sebestena* was unequivocally demonstrated and validated through the emergence of seven

characteristic peaks in the XRD image, covering 2θ values ranging from 0 to 60° . A thorough comparison of our XRD spectrum against the established standard irrefutably confirmed that the zinc particles generated in our experiments assumed the form of nanocrystals. This assertion was substantiated by the presence of peaks at 2θ values of 9.1935° , 13.1814° , 14.2828° , 16.3701° , 19.3166° , 21.2627° and 22.4356° , corresponding to heights of 115.48, 72.72, 97.51, 160.30, 577.17, 1059.99 and 446.48 counts (Figure 6) (Table 3) for flowers of *Cordia sebestena*. The corresponding "d" spacing values for zinc nanoparticles were calculated as 9.61957 \AA , 6.71686 \AA , 6.20129 \AA , 5.41500 \AA , 4.59514 \AA , 4.17876 \AA , and 3.96289 \AA for the synthesized nanoparticles.

Table 3: XRD Pattern of ZnNPs Synthesized by flowers of *Cordia sebestena* Extract with ZnSO_4 Solution.

Pos.[$^\circ 2\theta$.]	Height [cts]	FWHM Left [$^\circ 2\theta$.]	d-spacing [\AA]	Rel. Int. [%]
9.1935	115.48	0.6927	9.61957	7.27
13.1814	72.72	0.5196	6.71686	4.58
14.2828	97.51	0.5196	6.20129	6.14
16.3701	160.30	0.5196	5.41500	10.10
19.3166	577.17	0.5196	4.59514	36.35
21.2627	1059.99	0.5196	4.17876	66.76
22.4356	446.48	0.3464	3.96289	28.12

Figure 6: XRD Spectrum of ZnSO_4 Nanoparticles in flowers of *Cordia sebestena*

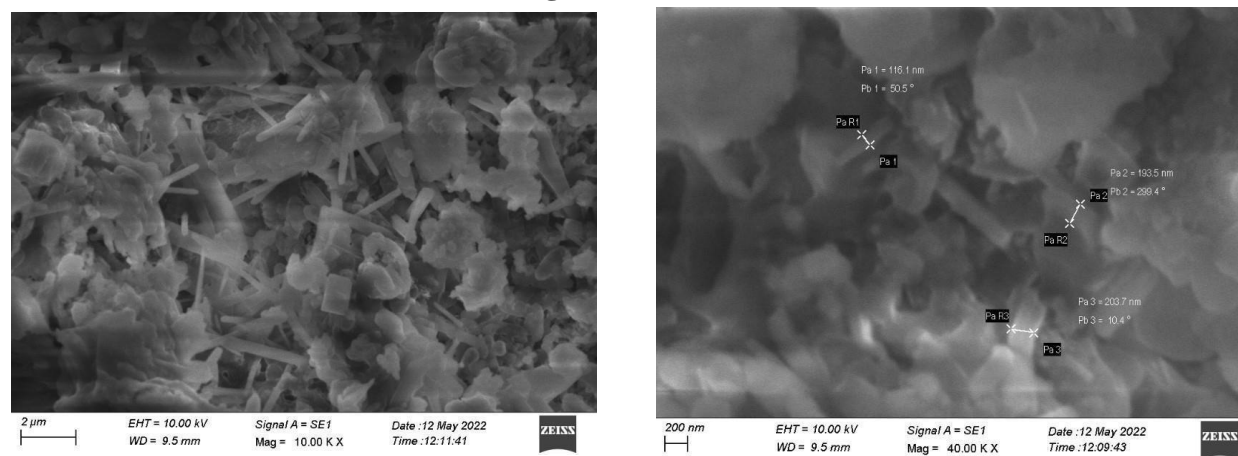


SEM Analysis of Silver Nanoparticles:

Examination of the Scanning Electron Microscopy (SEM) images provided insights into the formation and morphology of stable silver nanoparticles derived from *Cordia sebestena* flower

extract. The SEM analysis revealed the uniform distribution of silver nanoparticles appeared to be slices of sticks, with cube shapes on the cell surfaces with an average size ranging from 2 μm to 200 nm (Figure 7). The presence of phytochemicals found in the *Cordia sebestena* flowers such as tannins, phenols, flavonoids, etc act as reducing agents during the formation of AgNPs (Devaraj et al., 2013).

Figure No. 7. SEM image of SNPs synthesized by flowers of *Cordia sebestena* extract with AgNO_3 Solution.



Antimicrobial Activity Studies:

Infections caused by a range of bacterial agents, including pathogenic strains of *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*, are pervasive and on the rise. In recent years, the global emergence of drug resistance in human pathogenic bacteria has become a concerning trend, as documented by various researchers (Piddock and Wise, 1989; Singh et al., 1992; Mulligen et al., 1993). The relentless use of antibiotics has led to the development of resistance among microorganisms, posing substantial challenges in clinical settings for the treatment of infectious diseases (Davis, 1994).

The findings from this study have demonstrated the substantial antimicrobial activity exhibited by various metal nanoparticles against a diverse array of microorganisms. This trend is particularly pertinent in light of the gradual escalation in drug resistance among microorganisms (Amin et al., 2012). While synthetic drugs have been employed to combat a wide range of diseases caused by pathogenic microbes in humans, they often entail side effects, especially when overdosed. As a result, several medicinal plants have been identified and harnessed for their effectiveness in treating bacterial infections in humans. In the present investigation, the antimicrobial activity of *Cordia sebestena* flowers was assessed against six pathogens, and the results, including the zones of inhibition, are depicted in Plate 3 and tabulated for reference.

a) Antibacterial Activity Assessment

In this study, we assessed the antibacterial potential of various nanoparticle extracts derived from *Cordia sebestena* flowers. Our investigation revealed significant antibacterial activity against the following bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*.

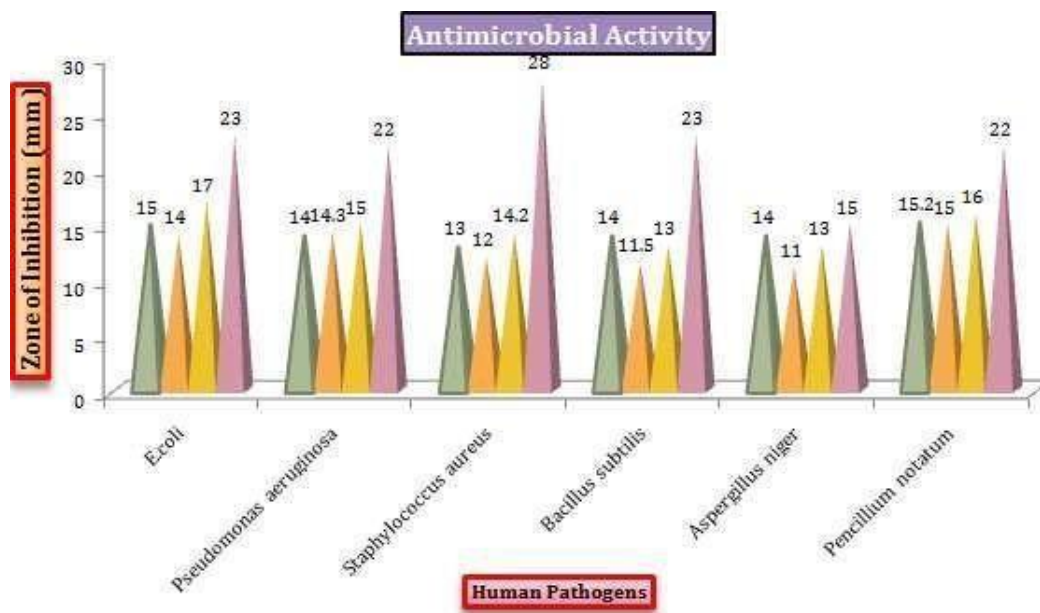
b) Antibacterial Studies on *Cordia sebestena* flowers

The extracts obtained from *Cordia sebestena* flowers, containing a variety of nanoparticle extracts, exhibited in-vitro inhibitory effects on the growth of the tested microorganisms. The results, showcasing the zones of inhibition measured in millimeters (mm), are succinctly presented in Figure 8. Generally, we observed moderate inhibition of the growth of these test organisms, evident by cleared zones on the culture plates (Table 4 & Plate 1).

Table 4: Antimicrobial potential of Ag-Cu-Zn Nanoparticles of flowers of *Cordia sebestena*

S. No	Pathogens	Antibacterial activity - Zone of Inhibition (mm)			
		Silver Nanoparticles (AgNO ₃)	Copper Nanoparticles (CuSO ₄)	Zinc Nanoparticles (ZnSO ₄)	Control (Amikacin)
1.	<i>Escherichia coli</i>	17 ± 0.2	18 ± 0.4	14 ± 0.3	23 ± 0.2
2.	<i>Pseudomonas aeruginosa</i>	16 ± 0.3	14 ± 0.2	16 ± 0.4	22 ± 0.3
3.	<i>Bacillus subtilis</i>	17 ± 0.4	10 ± 0.3	16 ± 0.2	28 ± 0.3
4.	<i>Staphylococcus aureus</i>	15 ± 0.2	16 ± 0.4	14 ± 0.3	23 ± 0.4
S. No	Pathogens	Antifungal activity - Zone of Inhibition (mm)			
		Silver Nanoparticles (AgNO ₃)	Copper Nanoparticles (CuSO ₄)	Zinc Nanoparticles (ZnSO ₄)	Control (Nystatin)
1.	<i>Candida albicans</i>	8 ± 0.2	12 ± 0.4	11 ± 0.3	15 ± 0.4
2.	<i>Aspergillus flavus</i>	15 ± 0.3	16 ± 0.3	18 ± 0.4	22 ± 0.3

Figure 8: Antimicrobial Efficacy of Ag-Cu-Zn Nanoparticles from *Cordia sebestena* flowers



The various nanoparticle extracts obtained from *Cordia sebestena* flowers demonstrated substantial antimicrobial activity, as indicated by the zone of inhibition against four human pathogens, as depicted in Figure 8. The overall antimicrobial efficacy varied among the selected pathogens. Remarkably, *E. coli* exhibited the largest zone of inhibition, measuring (18 mm) in copper nanoparticles in connection to *Bacillus subtilis* (17 mm) in silver nanoparticles and *P. aeruginosa* (16 mm) in zinc nanoparticles.

Plate 1: Antimicrobial Efficacy of Ag-Cu-Zn Nanoparticles from *Cordia sebestena* flowers





The size of metallic nanoparticles plays a crucial role in maximizing the surface area available for interaction with bacterial cells, thereby enhancing the efficacy of bacterial eradication (Parameswari et al., 2010). This phenomenon can be attributed to the interaction between copper nanoparticles and the bacterial cell wall, which is particularly facilitated by the abundance of negative charges present in gram-negative bacteria (Ruparelia et al., 2008).

(c) Antifungal activity

The antifungal activity of various nanoparticles derived from flowers of *Cordia sebestena* was assessed against two strains, namely *Aspergillus flavus* and *Candida albicans*.

(d) Antifungal studies on flowers of *Cordia sebestena*

Selected nanoparticles from flowers of *Cordia sebestena* were tested against selected fungi. *Aspergillus flavus* exhibited the highest inhibition zone, measuring 18 mm with zinc and 15 mm in silver nanoparticles, while the minimum zone of inhibition, measuring 8 mm, was observed with silver nanoparticles. For *Candida albicans*, the most substantial inhibition zone was achieved with silver nanoparticles at 12 mm, followed by 11 mm with copper and zinc nanoparticles and 8 mm with silver nanoparticles.

In this study, the antimicrobial activity of flowers of *Cordia sebestena* was evaluated against six pathogens, and the corresponding inhibition zones were documented. The medicinal properties of silver have been recognized for over 2,000 years, and since the nineteenth century, silver-based compounds have found applications in various antimicrobial uses. Silver nanoparticles, known for their diverse physical, biological, and pharmaceutical applications, are being employed as effective antimicrobial agents in public spaces such as railway stations and elevators in China, where they have demonstrated notable antimicrobial properties.

Conclusion

In this study, we successfully employed a safe and straightforward biosynthesis method to synthesize various nanoparticles, including Cu, Zn, and AgNPs, using flowers of *Cordia sebestena*. These nanoparticles hold significant promise for diverse applications such as pharmaceuticals, cosmetics, food packaging, and water treatment, owing to their antimicrobial properties. They represent valuable tools for combatting drug-resistant pathogens and controlling the spread of infections. However, a comprehensive understanding of their mechanisms of action and thorough assessments of potential toxicity to human cells and the environment are essential for further research. Additionally, optimization of synthesis processes, characterization techniques, and scale-up production are pivotal for practical implementation. Overall, the synthesis of different nanoparticles utilizing flowers of *Cordia sebestena* opens up exciting possibilities for developing novel broad-spectrum antimicrobial agents. The combination of sustainable synthesis methods and potent antimicrobial properties positions these nanoparticles as a promising solution in addressing microbial infections and the growing challenge of antimicrobial resistance.

References

1. Absar, A, Shankar, S and Murali, S. 2003. Geranium leaf biosynthesis of silver nanoparticles, *Biotechnology prog*, vol. 19, pp. 1627–31.
2. Amin M, Anwar, F, Janjua, MRSA, Iqbal, MA and Rashid, U. 2012. Green Synthesis of Silver Nanoparticles through Reduction with *Solanum xanthocarpum* L. Berry Extract: Characterization, Antimicrobial and Urease Inhibitory Activities against *Helicobacter pylori*, *Int. J. Mol. Sci.*, vol. 13, pp. 9923-9941.
3. Anonymous, 2002. The Drugs and Cosmetics Act and Rule, (The Drugs and Cosmetics Act 1940. The Drugs and Cosmetics Rule 1945), *Government of India, Ministry of Health and Family Welfare*, vol. 2, pp. 5.
4. Ashok kumar, S, Ravi, S, Kathiravan, V and Velmurugan, S. 2015. Synthesis of silver nanoparticles using *A. indicum* leaf extract and their antibacterial activity. *Spectrochim, Acta A, Mol.Biomol. Spectrosc.*, vol. 134, pp. 34-39.
5. Bauer, A.W, Kirby, W.M Sherris, J. C and Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* Apr; 45(4):493-6.
6. Bhati, M., & Rai, R. (2018). Nano-phytoremediation application for water contamination. *Phytoremediation: Management of Environmental Contaminants*, Volume 6, 441-452.
7. Davis, J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science*, vol. 264, pp. 375-382.
8. Devaraj P., Kumari P., Aarti C., & Renganathan A. (2013). Synthesis and characterization of silver nanoparticles using cannonball leaves and their cytotoxicity activity against MCF- 7 cell line. *Journal of Nanotechnology*, 2013.
9. Hashemian Rahaghi SH, Poursalehi R, Miresmaeili R. ScienceDirect Optical Properties of Ag-Cu Alloy Nanoparticles Synthesized by DC Arc Discharge in Liquid. *Procedia Mater Sci.* 2015;11:738-742. doi:10.1016/j.mspro.2015.11.062.
10. Mathew K..M 1981. The Flora of the Tamil Nadu Carnatic, 2; 1459-1460.

11. Mulvaney, P. 1996. Surface plasmon spectroscopy of nanosized metal particles. *Langmuir*. 12, 788.
12. Palani G., Arputhalatha A., Kannan K., Lakkaboyana S. K., Hanafiah M. M., Kumar V., & Marella R. K. (2021). Current trends in the application of nanomaterials for the removal of pollutants from industrial wastewater treatment-a review. *Molecules*, 26(9), 2799.
13. Parameswari, E., Udayasoorian, C., Sebastian, S.P., and Jayabalakrishnan, R.M. 2010. The Bactericidal Potential of Silver Nanoparticles. *International Research Journal of Biotechnology*, vol. 1, no. 3, pp. 044-049.
14. Phanjommet, P., Borthakur M., Das R., Dey S., & Bhuyan T. (2012). Green synthesis of silver nanoparticles using leaf extract of *Amaranthus viridis*. *Int J. Nanotechnol Appl*, 6, 53-59.
15. Ruparelia, J.P., Chatterjee, A.K., Duttagupta, S.P., and Mukherji, S. 2008. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomaterialia*, vol. 4, no. 3, pp. 707-716.
16. Sheny, D.S., Mathew, J., and Philip, D. 2011. *Photosynthesis* of Au, Ag and Au-Ag bimetallic nanoparticles using aqueous extract and dried leaf of *Anacardium occidentale*. *Spectrochimica Acta Part A Mol Biomol Spectrosc.*, vol. 79, no. 1, pp. 254–262.
17. Singh, A.P., Rawat, V.K., Behera, S.K., and Khare, P.B. 1992. Perspectives of pteridophytes biodiversity: a source of economy elevation. *National conference on biodiversity, development and poverty alleviation, Uttar Pradesh State Biodiversity Board*, pp. 46-49.
18. Srivastavet A., Yadav K.K., Yadav S., Gupta N., Singh J.K., Katiyar R., & Kumar V. (2018). Nano-phytoremediation of pollutants from contaminated soil environment: current scenario and future prospects. *Phytoremediation: Management of Environmental Contaminants*, 6, 383-40.
19. Wang HK, Yi CY, Tian L, et al. Ag-Cu bimetallic nanoparticles prepared by microemulsion method as catalyst for epoxidation of styrene. *J Nanomater*. 2012. doi:10.1155/2012/453915.
20. WHO. 2000. Methods and data sources for global burden of disease estimates 2000-2011. *Geneva: Department of Health Statistics and Information Systems*.
21. Wiley, B.J., Im, S.H, Li, Z, McLellan, J., Siekkinen, A., and Xia, Y. 2006. Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. *Journal of Physical Chemistry B*, vol. 110, no. 32, pp. 15666-15675.