PLANT-MEDIATED SYNTHESIS OF AG-CU-ZN NANOPARTICLES USING ANTIGONON LEPTOPUS FLOWER EXTRACT AND ITS BIOEFFICACY.

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Abstract

Introduction: The scientific discipline of nanotechnology encompasses a wide range of topics, including surface science, organic chemistry, molecular biology, semiconductor physics, and microfabrication.

Objective: The goal of the current work was to use *Antgonon leptopus* flower extract to swiftly synthesize Ag, Cu, and ZnNPs. The resulting nanoparticles were examined using a range of characterisation techniques, including as X-ray diffraction, UV-visible spectrum analysis, and scanning electron microscopy, and they were also examined in vitro against a few human pathogens.

Results: Spectral analysis verified that the AgNPs' UV-visible absorption spectra had a distinctive peak at 260 nm. ZnNPs' absorption spectra, which were taken from the reaction media, showed an absorbance peak with the distinctive absorption peak seen at 320 nm, while CuNPs' absorption spectra showed a distinctive absorption peak that was noticed at 290 nm. Silver nanoparticles with an XRD pattern displayed 2θ values of 7.6425, 20.1473, 23.2987, 27.4604, 31.8438, 33.4873, and 35.489. The XRD pattern of zinc nanoparticles was supported by the presence of peaks at 2θ values of 9.5770, 14.4925, 16.4033, 19.0455, 21.4664, 23.7485, and 24.7928, respectively, and copper nanoparticles peaks at 2θ values of 9.1995, 11.0902, 15.2634, 18.9091, 22.5422, 24.7211, and 27.9172. AgNPs are cube-shaped, as seen by the SEM analysis micrograph, and their average particle size was determined to be between 2 and 200 µm. Furthermore, substantial results were obtained from the biological activities of certain nanoparticles against antifungal (*Candida albicans*) and antibacterial (*E. Coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylocouus aureus*).

Conclusion: Throughout the world, *Antigonon leptopus* is utilized in ethnomedicine to treat a wide range of conditions, including pain, diabetes, coughing, stomachaches, and dermatological

issues. The current work demonstrates that the plant is the best resource for synthesizing AgNPs for future medicinal research.

Keywords: Green Synthesis, *Antigonon leptopus*, Ag-Cu-Zn, Characterization, Human Pathogens.

Introduction

Using plants to synthesize metal nanoparticles is a fast, convenient, and widely accepted method. In recent times, scientists have discovered that different parts of plants, such as bark, leaves, fruits, stems, and seeds, can be effectively utilized to create metal nanoparticles (Mittal *et al.,* 2013). Silver (Ag) nanoparticles have gained significant attention for their remarkable anti-bacterial, anti-fungal, and anti-proliferative properties. They have been extensively studied and utilized in various applications (Mohanta *et al.,* 2016a,b; Kim *et al.,* 2007; Nayak *et al.,* 2015). With their remarkable antimicrobial properties, silver (Ag) nanoparticles have found wide application in various fields such as food packaging, food and seed preservation, biofertilizers, cosmetics, and medicines (Marambio-Jones and Hoek, 2010; Dipankar and Murugan, 2012). In addition to these applications, silver nanoparticles have been extensively used in high sensitive bio-molecular detection, diagnostics, catalysis, and micro-electronics (Mohanta and Behera, 2014). Conventional methods for synthesizing nanoparticles often involve the use of chemicals that serve as both stabilizing and protecting agents. However, these chemicals can be toxic in nature. In addition, these traditional techniques allow for the production of nanomaterials in large quantities and in a shorter period of time. These methods involve the use of chemicals that can be harmful to both humans and aquatic organisms, posing potential life-threatening risks. To address the drawbacks of the traditional approach, green synthesis can serve as a viable alternative method (Xi-Feng Z *et al.,* 2016). It is an area of great importance in the field of bio-nanotechnology, offering significant benefits to the economy and the environment. It is notable for its ability to avoid producing any by-products or pollution. This method primarily favors the use of safe and non-toxic agents that are environmentally friendly and biologically safe. It also utilizes natural capping and stabilizing agents (Zhi G *et al.,* 2018). Green resources enable the synthesis of nanoparticles with excellent biomedical activity, thanks to the functional group found on their surface. One of the key benefits of utilizing plant resources is their widespread availability, affordability, and safety in handling. They offer a diverse array of components including alkaloids, flavonoids, terpenoids, tannins, and more (Zeqing B *et al.,* 2019).The characterization of the green synthesis of zinc oxide nanoparticles (ZnONPs) using Borassus flabellifer fruit extract involved various techniques such as UV-visible spectroscopy, FT-IR, XRD, TEM, Zeta potential, and EDS analysis. There is an absorption peak at 368nm in the UV-visible spectrum, which indicates the presence of surface Plasmon resonance (SPR) ZnONPs. The TEM photograph revealed that the ZnONPs synthesized were characterized by a porous nature and a rod-like structure, with an average size of 55nm. The surface charge of green synthesized ZnONPs was revealed by a Zeta potential value of -21.5mV. Our study focused on analyzing the cytotoxicity of the synthesized DOX-ZnONPs against MCF-7 and HT-29 cells, observing a dose-dependent response. The IC50 value for MCF-7 and HT-29 cells was determined to be 0.125μgmL−1. Evidence of apoptosis was observed through the nuclear stain Hoechst 33258. Through in vivo toxicity assessment, it was

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observed that DOX-ZnONPs exhibit minimal systemic toxicity in a murine model system. The results demonstrate the low toxicity and high therapy efficacy of DOX-ZnONPs, providing strong evidence for the potential of green biosynthesized ZnO as a promising drug delivery system (Soundarapandian Kannan *et al.*, 2014).

In this study, we have successfully synthesized Ag, Cu, and Zn nanoparticles using flowers from *Antigonon leptopus*, a member of the Polygonaceae family. This family is well-known among Angiosperms, which are flowering plants. This study utilizes a straightforward and budget-friendly green synthesis approach. The nanoparticles will undergo comprehensive characterization using a range of analytical techniques, such as UV-visible spectroscopy, Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), and Antimicrobial efficacy assessments.

Materials and Methods

Plant Material Collection:

The flowers selected for the current study were those depicted in Figure 1. The plant material was collected from the Gangaikondan village in the region of Tirunelveli district, Tamil Nadu, India. It belongs to the Polygonaceae family. The plant was identified and authenticated by Dr.R.Lakshmanan, Assistant Professor of Botany, G.Venkataswamy Naidu College, Kovilpatti, Tuticorin District, Tamilnadu and 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:

With the precision of a scientist, the flowers of *Antigonon leptopus* were gathered and thoroughly cleansed using tap water and distilled water to ensure their freshness and purity. Afterward, 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. The obtained extract was used to synthesize a variety of nanoparticles.

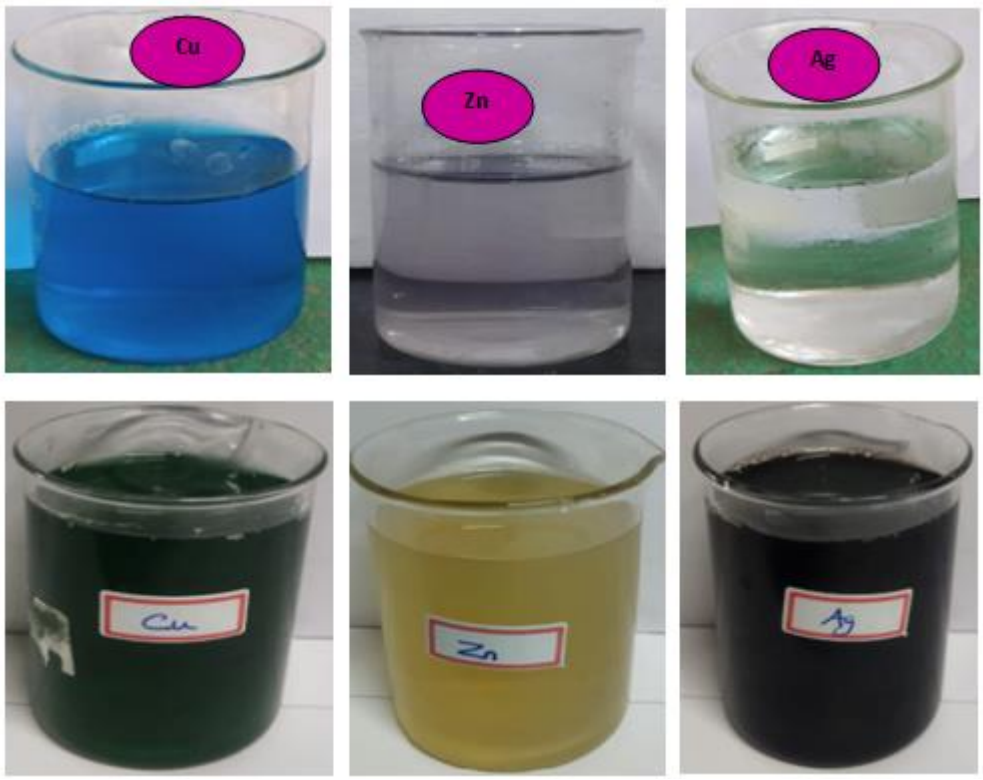

Figure.1: Green Synthesis of Ag, Cu and ZnNPs using the aqueous flower extract of *Antigonon leptopus*

Isolation of Nanoparticles:

Fresh and healthy flowers of *Antigonon leptopus* were carefully gathered and meticulously cleansed with tap water followed by distilled water to eliminate any dust or visible impurities. Afterward, a sample of fresh flowers was boiled in double-distilled water and the resulting extract was filtered through Whatman no.1 filter paper, collecting the filtrate in a conical flask. The obtained extract was used to synthesize a variety of nanoparticles.

Synthesis of Silver Nanoparticles:

Similarly, 90 milliliters of Silver Nitrate solution was placed in a conical flask, to which 10 milliliters of the flower extract were added. The Silver Nitarte solution underwent a color shift from brown to dark brown. The conical flask was subjected to light exposure for a 72-hours incubation period.

Synthesis of Copper Nanoparticles:

Using a conical flask, a mixture was created by combining 90 milliliters of Copper Sulphate solution with 10 milliliters of the flower extract. The transformation of the extract's light green to

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dark green indicated the reduction of copper sulfate to copper ions. Afterward, the conical flask was placed under light for a 72-hour incubation period.

Synthesis of Zinc Nanoparticles:

In a conical flask, 90 milliliters of Zinc Sulphate solution was carefully combined with 10 milliliters of the flower extract. The solution of Zinc Sulphate experienced a change in color, transitioning from green to yellow. During a 72-hour incubation period, the conical flask was exposed to light.

Characterization

UV- Spectrophotometer Analysis:

To conduct UV-spectrophotometer analysis, around 1 milliliter of the sample suspension was placed into a quartz tube. To enable the monitoring of nanoparticle formation, the sample was subsequently diluted with 2 milliliters of distilled water. The UV-Visible spectra scans were performed using a UV-visible spectrophotometer (Shimadzu UV 1800, Germany) throughout a wavelength range of 200-900 nanometers.

XRD Analysis:

In order to analyze the pure produced nanoparticles, freeze-dried powder samples were used for XRD analysis. The examination was carried out at 40 kV/20 mA utilizing continuous scanning in 2 delta mode (Absar, 2003). The nanoparticle solution underwent first purification by subjecting it to multiple rounds of centrifugation at a speed of 5000 revolutions per minute for a duration of 20 minutes. Subsequently, the resulting nanoparticle pellet was dispersed again in 10 milliliters of deionized water.

SEM Analysis of Silver Nanoparticles:

A ZEISS machine was utilized to do SEM examination. The sample was applied onto a carbon-coated copper grid to create thin films. The surplus solution was eliminated using blotting paper, and the films on the SEM grid were dried by exposing them to a mercury light for 5 minutes.

Antimicrobial Activity of Nanoparticles:

The antimicrobial activity of flower extracts from *Antigonon leptopus* against specific human infections was assessed using the Agar well diffusion method in a clinical laboratory. The Kirby-Bauer method, as described by Bauer et al. (1996), was used to evaluate the antibacterial efficacy of the separated plant extraction pellets. The bacteria and fungi were injected into Nutrient agar, Sabouraud's Dextrose Agar (SDA), and Potato Dextrose Agar (PDA) plates after being cultured overnight. Wells were established in all plates, including those specifically intended for control purposes. Flower extracts containing Silver nitrate, Copper sulphate, and Zinc sulphate were made with a concentration of 50 mg/mL. Controls consisting of antibiotics (Amikacin and Nystatin) were used at equal concentrations. The bacterial plates containing *E.coli*, *Pseudomonas aeruginosa*, Bacillus subtilis, and *Staphylococcus aureus* were subjected to incubation at a temperature of 37°C for a duration of 24 hours. Similarly, the fungal plates containing *Aspergillus flavus* and *Candida albicans* were incubated at a temperature of 35°C for a duration of 48 hours. The measurement of the inhibitory zone's diameter was conducted in millimeters.

Results and Discussion

Synthesis and Characterization of Nanoparticles:

The flowers of *Antigonon leptopus* displayed discernible color variations when subjected to various metal compositions (Silver Nitrate, Copper Sulphate, and Zinc Sulphate), indicating the creation of three separate categories of nanoparticles. The presence of these nanoparticles was then verified using spectral investigations utilizing UV-Visible Spectroscopy, X-Ray Diffraction, and SEM analysis. Furthermore, these nanoparticles were utilized in a range of biological activities. Ensuring the reproducible quality of herbal products requires thorough control over the starting components. As a result, there has been an increased focus in recent years on standardizing medicinal plants that have the potential for therapeutic use. Although current methodologies are accessible, the identification and evaluation of plant-based medications through pharmacognostical research remain a more dependable, precise, and cost-efficient strategy. The World Health Organization (WHO, 2000) states that the first stage in determining the authenticity and purity of a medicinal plant is to provide a detailed description of its physical characteristics, both visible to the naked eye and under a microscope. This description should be done before any subsequent testing processes (Anonymous, 2002).

UV- Visible Spectrum Analysis:

The conversion of metal ions into metal nanoparticles, aided by the exposure to *Antigonon leptopus* flowers, was evident by alterations in color and then verified using UV-Vis spectroscopy. The change in color was caused by the stimulation of surface plasmon oscillations (Mulavney, 1996). UV-Vis spectroscopy is a commonly used method for studying nanoparticles of specific sizes and shapes in water-based solutions (Wiley *et al.*, 2006). The existence of nanoparticles was confirmed by acquiring spectra in the visible spectrum using a UV-visible spectrophotometer, with absorption wavelengths ranging from 200 to 900 nanometers (Fig. 2a, b and c)

Analysis of Ag, Cu and ZnNPs of *Antigonon leptopus* flowers

The absorption spectra of silver nanoparticles generated in the reaction medium showed a peak in absorbance between 200 and 400 nm. The typical peak of silver nanoparticles was found around 260 nm (Figure 2a). While examining the absorption spectra of copper nanoparticles, a noticeable absorption peak at 290 nm, which is a hallmark feature of copper nanoparticles, was clearly noticed (Figure 2b). The absorption spectra of zinc nanoparticles produced from the reaction medium showed a conspicuous absorbance peak at 320 nm, which is a hallmark feature of zinc nanoparticles (Figure 2c). A different investigation documented that the UV-Vis spectra obtained from the reaction solution of silver nitrate reduced by the leaf extract of *Barleria noctiflora* exhibited a peak absorbance at 480 nm (Lakshmanan, *et al.*, 2023).

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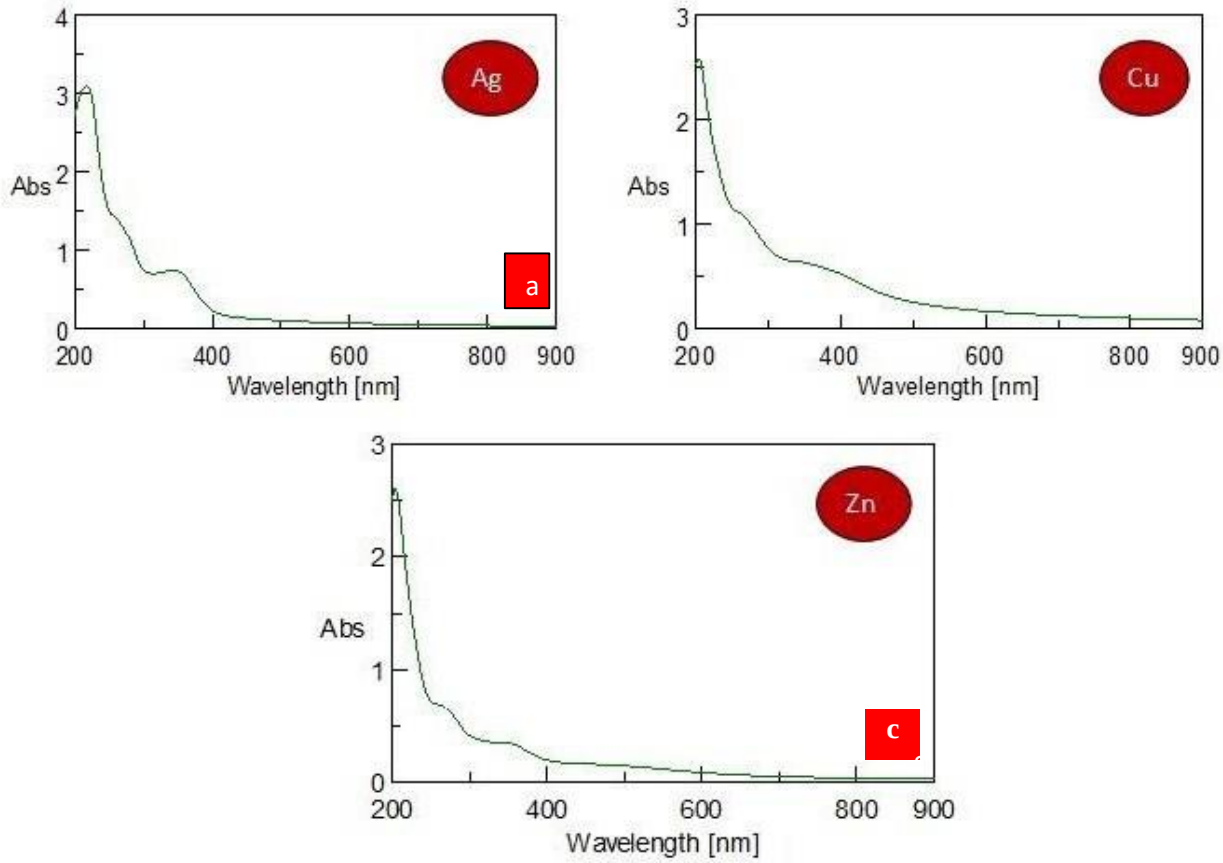

Figure: 2. UV-Vis Spectrum of Ag, Cu and ZnNPs in flowers of *Antigonon leptopus*

XRD Analysis

The nanoparticles produced through the synthesis of *Antigonon leptopus* flower extracts were thoroughly examined and confirmed using X-ray diffraction (XRD) analysis (Figure 3). The purpose of the XRD investigation was to verify the crystalline properties of the nanoparticles. An analysis comparing our XRD spectrum to recognized standards confirmed that the nanoparticles produced in our studies were really present in the form of nanocrystals, as seen by the noticeable peaks at specified 2θ values.

XRD Analysis of Ag, Cu and ZnNPs of flowers of *Antigonon leptopus*

Regarding silver nanoparticles, the flowers of *Antigonon leptopus* is involved. The XRD study indicated seven different peaks in the XRD picture, spanning a range of 0 to 90 Å. The peaks observed in the data corresponded to specific 2θ values: 7.6425, 20.1473, 23.2987, 27.4604, 31.8438, 33.4873, and 35.489. These peaks had corresponding heights of 9.14, 158.66, 167.21, 531.90, 1095.48, 91.67, and 51.69 counts, respectively. The "d" spacing values for silver nanoparticles were determined to be 11.5680 Å, 4.40753 Å, 3.81800 Å, 3.24810 Å, 2.81029 Å, 2.67603 Å, and 2.52951 Å (Fig.3a). The peaks of copper nanoparticles occur at 2θ values of 9.1995, 11.0902, 15.2634, 18.9091, 22.5422, 24.7211, and 27.9172. These peaks correspond to heights of 188.33, 3.54, 118.29, 157.66, 340.42, 150.69, and 300.70 counts, respectively. The "d" spacing values for copper nanoparticles were determined to be 9.61338 Å, 7.97828 Å, 5.80506 Å,

4.69326 Å, 3.94439 Å, 3.60145 Å, and 3.91598 Å (Fig.3b) for the produced nanoparticles. The presence of peaks at specific 2θ values of 9.5770, 14.4925, 16.4033, 19.0455, 21.4664, 23.7485, and 24.7928 in the XRD pattern confirmed the existence of zinc nanoparticles. These peaks corresponded to heights of 56.98, 74.06, 11.21, 62.83, 671.06, 377.51, and 223.01 counts, as shown in Table 1, 2, and 3, and Figure 3a,b, and c. The "d" spacing values for zinc nanoparticles were determined to be 9.23526 Å, 6.1120 Å, 5.40413 Å, 4.65994 Å, 4.13958 Å, 3.74670 Å, and 3.59120 Å (Fig.3c) for the produced nanoparticles.

Table No. 1: XRD Pattern of AgNPs Synthesized by flowers of *Antigonon leptopus* extract with $AgNO_3$ Solution.

Pos.[°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
7.6425	9.14	0.4330	11.5680	0.83
20.1473	158.66	1.2121	4.40753	14.48
23.2987	167.21	0.3164	3.81800	15.26
27.4604	531.90	0.3164	3.24810	48.55
31.8438	1095.48	0.4330	2.81029	100.00
33.4873	91.67	0.3464	2.67603	8.37
35.489	51.69	0.4330	2.52951	4.72

Table 2: XRD Pattern of CuNPs Synthesized by flowers of *Antigonon leptopus* extract with $CuSO_4$ Solution.

Pos.[°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
9.1995	188.33	0.3464	9.61338	30.96
11.0902	3.54	1.0391	7.97828	0.58
15.2634	118.29	0.5196	5.80506	19.45
18.9091	157.66	0.6927	4.69326	25.92
22.5422	340.42	0.8659	3.94439	55.96
24.7211	150.69	0.6927	3.60145	24.77
27.9172	300.70	0.4330	3.91598	49.43

Table 3: XRD Pattern of ZnNPs Synthesized by flowers of *Antigonon leptopus* extract with $ZnSO_4$ Solution.

Pos.[°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	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

The X-ray diffraction pattern obtained for the Copper oxide nanoparticles synthesized using *Antigonon leptopus* leaf extract were subjected to XRD analysis, given a clear picture on the presence crystalline cubic phase of monoclinic Copper oxide (CuO) exhibiting 2θ values 32.28, 34.46, 35.98, 38.66, 47.12, 54.80 and 57.98. In addition, the peak observed at 2θ value of 28.31 might be due to the presence of trace amount of hollow CuO nanoparticles (M.Sravanthi *et al.*, 2016).

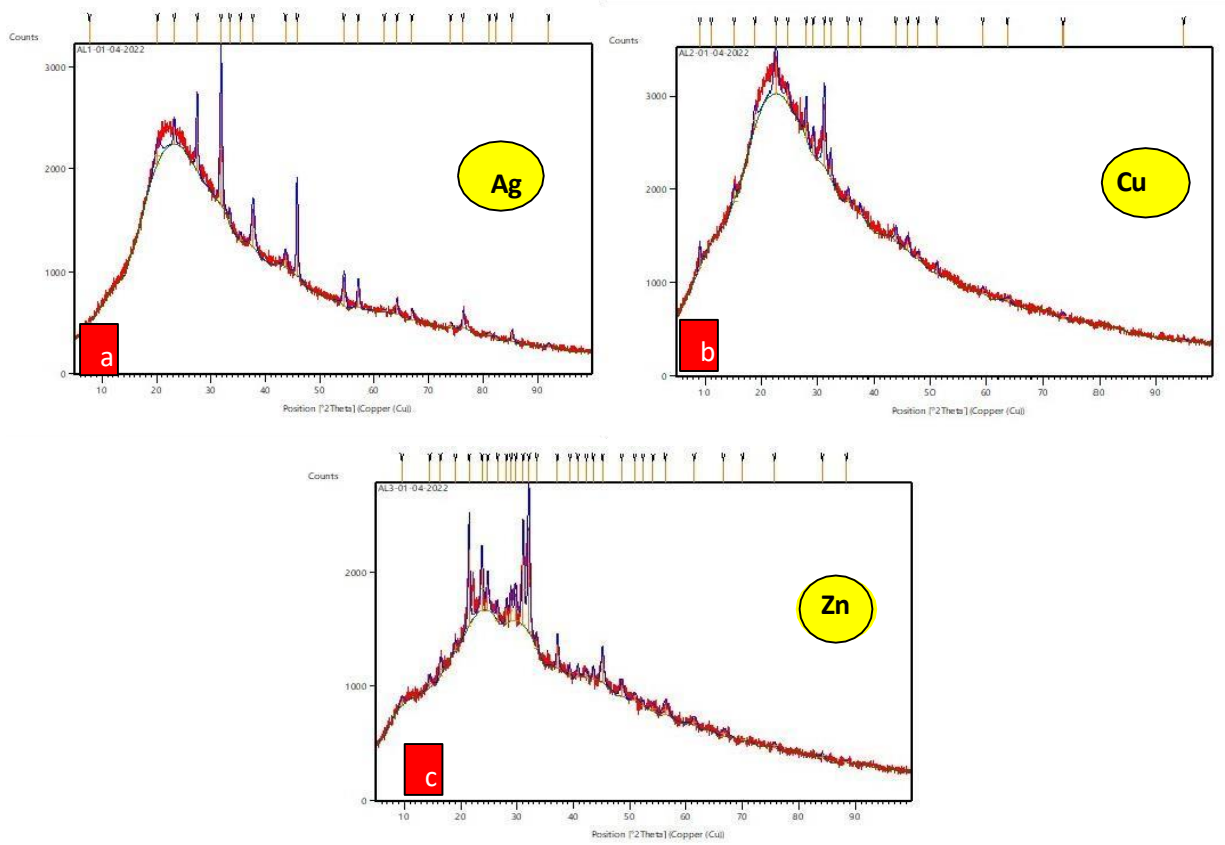

Figure: 3. XRD Spectrum of Ag, Cu and ZnNPs in flowers of *Antigonon leptopus*

SEM Analysis of Silver Nanoparticles:

The scanning electron microscope (SEM) was used to examine the surface morphology and particle size of the AgNPs manufactured by a green method. A typical scanning electron micrograph reveals the cubic structure of AgNPs. Figure 4 (a) & (b) demonstrate that the average particle size ranged from 2 nanometers to 200 micrometers. The plant extract of *Capparis zeylanica* was used to produce copper oxide nanoparticles. The nanoparticles were found to have a spherical shape and were generally consistent in size, measuring between 60-100nm (Saranyadevi *et al.*, 2014).

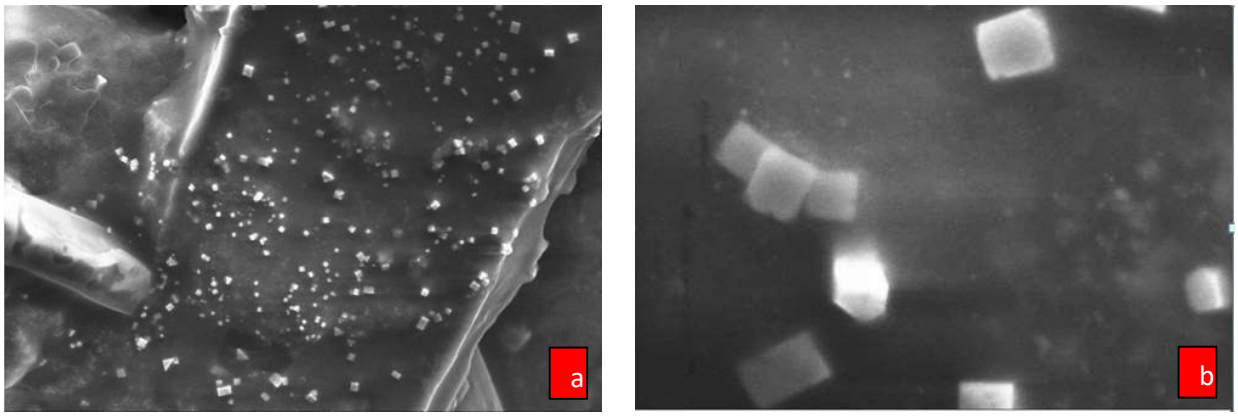

Figure: 4. SEM image of AgNPs synthesized by flowers of *Antigonon leptopus* extract with $AgNO_3$ Solution.

Antimicrobial Activity:

The antimicrobial efficacy of nanoparticles synthesized using green methods, flower extract, amikacin, and Nystatin were assessed against six human pathogens, namely Escherichia coli, *Pseudomonas aeruginosa*, Bacillus subtilis, *Staphylococcus aureus*, *Aspergillus flavus*, and *Candida albicans*. The outcomes, including the zones of inhibition, are illustrated in Figure 5 and 6. The quantification was done using the minimum inhibitory concentration (MIC) assay, where the zone of inhibition was determined by culturing the organisms on a Muller-Hinton agar plate and using the disc diffusion procedure. The antibacterial activity of Ag, Cu, and Zn nanoparticles derived from *Antigonon leptopus* extract, as well as amikacin and Nystatin, was found to be substantial against the tested pathogens. The activity was observed to be dependent on the dosage. The summary results may be found in Table 4. In this study, we found that the greatest inhibition of bacterial growth occurred when using the highest concentration of ZnNPs against *Pseudomonas aeruginosa* (18 ± 0.2). This was followed by CuNPs against *Staphylococcus aureus* (18 ± 0.4) and *E.coli* (16 ± 0.2) on AgNPs. Furthermore, the maximal inhibitory concentration of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) on *Candida albicans* was found to be 14.2 ± 0.2. The minimum inhibitory concentration of zone observed for ZnNPs was 10 ± 0.3. The fungus *Aspergillus flavus* showed the highest inhibitory concentration of CuNPs, with a value of 14 ± 0.3. On the other hand, the lowest inhibitory concentration was seen for ZnNPs, with a value of 8 ± 0.4.

In a study conducted by Rudhra (2024), it was shown that the ethanol extract exhibited the most potent antibacterial activity at a concentration of 200 µg/mL. The extract showed an inhibition zone of 20.4±0.13mm against *A.leptopus* and 20.3 ± 0.27 mm against *Staphylococcus aureus*. The methanol extract demonstrated a moderate level of antibacterial activity at a concentration of 200 µg/mL. It produced an inhibition zone of 16.1 ± 0.08 mm against *A.leptopus* and 13.2 ± 0.12 mm against *E.coli*.

.**Table 4: Antimicrobial Efficacy of Ag, Cu, and ZnNPs of flowers of *Antigonon leptopus***

	Pathogens	Antibacterial activity - Zone of Inhibition (mm)

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S. No		Silver Nanoparticles ($AgNO_3$)	Copper Nanoparticles ($CuSO_4$)	Zinc Nanoparticles ($ZnSO_4$)	Control (Amikacin)
1.	*Escherichia coli*	16 ± 0.2	13± 0.4	13± 0.3	23 ± 0.2
2.	*Pseudomonas aeruginosa*	15 ± 0.3	14± 0.2	18± 0.4	24 ± 0.3
3.	*Bacillus subtilis*	12 ± 0.4	12± 0.3	14± 0.2	28 ± 0.3
4.	*Staphylococcus aureus*	16 ± 0.2	18± 0.4	15± 0.3	25 ± 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.	*Candida albicans*	14 ± 0.2	14 ± 0.4	10 ± 0.3	15 ± 0.4
2.	*Aspergillus flavus*	11 ± 0.3	14 ± 0.3	8 ± 0.4	17 ± 0.3

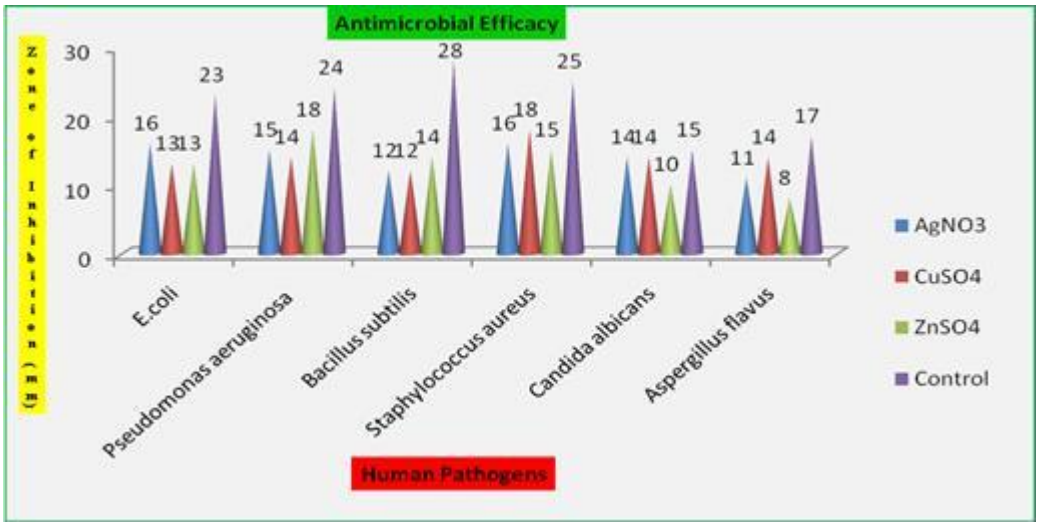

Figure 5: Antimicrobial Efficacy of Ag, Cu, ZnNPs from *Antigonon leptopus*

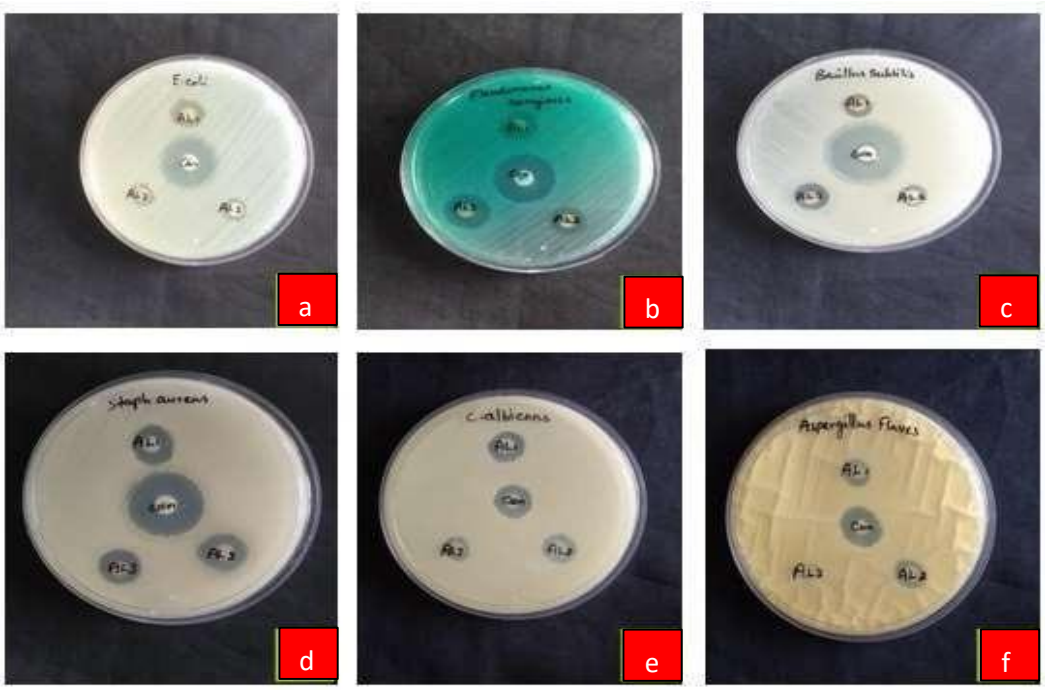

Figure: 6. Antimicrobial Efficacy of Ag, Cu and ZnNPs from *Antigonon leptopus* flowers

Conclusion

The present study utilized a non-hazardous and straightforward biosynthetic technique to successfully synthesize certain nanoparticles, including Ag, Cu, and Zn. The herb is utilized in ethnomedicine across different regions of the globe to address a range of health conditions, including pain, cough, diabetes, dermatological issues, flu, and stomachache. Various components of the plant have been studied for their pharmacological characteristics, and the plant has been found to exhibit a range of bioactivities including antibacterial, antioxidant, hepatoprotective, analgesic, anti-inflammatory, cytotoxic, and antidiabetic activities. The bioactivities of antibacterial, antioxidant, and cytotoxic activity were demonstrated in nanoparticles made utilizing *Antigonon leptopus*. The wide literature search reveals the potential ethnomedicinal uses of *Antigonon leptopus* and the plant's potential for developing pharmaceutical medicines.

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