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Green Synthesis of Copper Nanoparticles Using Leaves and Flowers Extract of Cassia auriculata L. and Study of It's Antimicrobial Activity.

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Abstract: ***Evolution of biologically inspired experimental processes for the synthesis of nanoparticles is developed into an important branch of nanotechnology. The leaf and flower extract of Cassia auriculata L. (Caesalpiniacece) in the synthesis of copper nanoparticles was investigated employing UV/Ultra visible spectrophotometer, SEM/scanning electron microscopy and P-XRD/Powder X-ray diffraction. Uv shows the sharp band of copper Nanoparticles is around 375nm of Cassia auriculata (fresh and dry leaf and flower) , SEM image has shown the morphological shape of the copper nanoparticles C.auriculata fresh leaf copper nanoparticles average size is 12.43nm, dry leaf is 40nm and fresh flower copper nanoparticles size is 57.87nm, dry flower is 7.14nm, X-ray diffraction patterns of synthesized fresh leaves mean size of copper nanoparticles calculated using the Debye-Scherrer's equation was found to be 12.43nm,mean size of dry leaves is 40nm,mean size of fresh flower is 57.87nm and mean size of dry flower is 7.14nm respectively.The results of antimicrobial activity of the C. auriculata green synthesized CuNPs against the Gram- negative bacterial strains (E. coli and S.typhi) and the Gram-positive bacterial strains (S.aureus and B.subtilis), Fungi (Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, Aspergillus flavus) under the well diffusion method had a considerable antimicrobial activities increased with higher concentration. The fresh leaves CuNPs shows more effective against S.aureus, dry leaves CuNPs against E. coli, fresh flowers CuNPs against S. aureus and dry flowers CuNPs shows more effective against B. subtilis. In case of fungi both fresh and dry leaves and flowers CuNPs shows more effective against F.oxysporum. C. auriculata is a medicinal shrub or tree which is native to the India, Myanmar and Sri Lanka. This plant reported for its uses in the Ayurvedic and siddha system of medicine for treating various diseases mainly both the diabetes types. The antimicrobial potential of the leaf and flower extract was tested. The anti-bacterial and anti-fungal property were found to be better for the aqueous leaf and flower extract and the extract containing the metal particles.***

Keywords: ***Green synthesis of copper nanopaeticals, Cassia auriculata, UV-visible spectrophotometer, Scanning electron microscopy, Power X-ray diffraction, Antimicrobial activity.***

I. INTRODUCTION

Nanotechnology is the most promising technology that deals with understanding and control of matter at the nanoscale, and at dimensions between approximately 1 nm to 100nm. Quantum effects rule the behaviour and properties of particles at nanoscale range. Nanoparticles are considered as building blocks of the next generation of technology with applications in many industrial sectors[1]. Green synthesis method was found to be the best method when compared to the other conventional methods such as chemical reduction, photochemical reduction, electrochemical reduction, heat evaporation etc [2].[6].metallic NPs were found to have 7-50 times less toxic effect to mammalian cells than their corresponding ionic forms and prolonged effect as a source of elements in an organism , copper and its complexes have been widely utilized as cheap and effective materials for sterilizing liquids, textiles and also human tissues for centuries[3].copper nanoparticles distribute in organs and tissues of animals and cause specific structural changes[4].

Currently there has been a significant increase in the area of nanotechnology in terms of research due to the applications of nanoparticles. They act as the whole unit in terms of the properties and activities. The properties of many materials change when formed as nanoparticles. This is typically because these particles will have greater surface area per weight which makes them more reactive than other molecules[5]. A wide variety of physical, chemical and biological processes results in the synthesis of nanoparticles, some of these are novel and others are quite common. Nature has devised various processes for the synthesis of nano - and micro-length scaled inorganic materials which have contributed to the development of relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials[7].

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For the past few decades, there has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules[8]. Metallic nanoparticles are multifunctional in nature and hence finds huge number of applications in various sectors for environmental, biomedical and antimicrobial, solar power generation and catalytic causes. Application of plant extracts to synthesize copper and its oxide nanoparticles is a green chemistry methodology which establishes strong relationship between natural plant material and nanosynthesis[9].Copper nanoparticles have been used as disinfectants in water treatment plants, food processing, wound healing ointments, bandages, and so on because of their antibacterial as well as antiviral properties[11].

Noble metal nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in there searchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains. These unique properties mainly depend on the size, shape and surface area of nanoparticles[12].[13].A few studies have suggested that the primary cause of the antibacterial function might be from the disruption of cell membrane activity . Another possibility could be the induction of intercellular reactive oxygen species, including hydrogen peroxide (H2O2), a strong oxidizing agent harmful to bacterial cells[15].

Objectives:

1) Synthesis and characterization of copper nanoparticles from **Cassia auriculata L.** leaves extract (fresh and dry leaves extract) .
2) Synthesis and characterization of copper nanoparticles from **Cassia auriculata L.** flowers extract(fresh and dry flowers extract).
3) Study of antimicrobial activity of Copper nanoparticles from **Cassia auriculata L.** leaves extract(fresh and dry leaves extract) .
4) Study of antimicrobial activity of Copper nanoparticles from **Cassia auriculata L**.flowers extract(fresh and dry flowers extract

II. MATERIALS AND METHOD

A. *Collection of Sample*



Figure :1 Cassia auriculata L.

Fresh leaves and flowers of Cassia auriculata was collected from the village Devanuru, Nanjangud taluk, Mysuru district.

B. *Chemicals*

The chemical used in this, is analytical grade copper sulphate pentahydrate($CuSO_4.5H_2O$) .

C. *Preparation of Fresh Leaves Extract*

20gm of fresh leaves were plucked from the plant, the leaves were washed cleanly under tap water for few minutes, again washed under double distilled water for few minutes. Left for drying for few minutes. Leaves were finely chopped as small pieces, then transferred into 500ml borosil Beaker. Added 100ml of double distilled water to 20gm of fresh chopped leaves and boiled it for 15 minutes. Then filtered it through filter paper (Whatman no: 1 filter paper, pore size 25µm) and used immediately for the biosynthesis of Copper Nanoparticles.

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D. Preparation of Dry Leaves Extract

Fresh leaves were plucked from the plant, the leaves were washed cleanly under tap water for few minutes again washed under double distilled water for few minutes. Left for shade dry for a week. Then leaves were finely powered then transferred into 500ml borosil Beaker. Added 100ml of double distilled water to 20gm of leaves powder and boiled it for 15 minutes. Then filtered it through filter paper (Whatman no: 1 filter paper, pore size 25µm) and used immediately for the biosynthesis of Copper Nanoparticles.

E. Preparation of Fresh Flower Extract

Fresh flowers were plucked from the plant, washed cleanly under tap water for few minutes again washed under double distilled water for few minutes. Left for shade dry for a week. Then flowers were finely chopped as small pieces then transferred into 500ml borosil Beaker. Added 100ml of double distilled water to 20gm of fresh chopped flowers and boiled it for 15 minutes. then filtered it through filter paper (Whatman no: 1 filter paper, pore size 25µm) and used immediately for the biosynthesis of Copper Nanoparticles.

F. Preparation of Dry Flower Extract

Fresh flowers were plucked from the plant, washed cleanly under tap water for few minutes again washed under double distilled water for few minutes. Left for shade dry for a week. Then flowers were finely chopped as small pieces then transferred into 500ml borosil Beaker. Added 100ml of double distilled water to 20gm of chopped flowers and boiled it for 15 minutes. Then filtered it through filter paper (Whatman no: 1 filter paper, pore size 25µm) and used immediately for the biosynthesis of Copper Nanoparticles.

G. Green Synthesis of Copper Nanopaeticals

For the synthesis of copper sulphate 1g of copper sulphate was taken in 100ml of double distilled water it makes 1% of $CuSO_4$. Adding of 5ml of extract to 25ml copper sulphate in 250ml of borosil conical flask at room temperature and kept it inside the incubator at 37°C for 48hours.

H. Extraction of Nanoparticale Samples

Incubated samples were centrifuged at 5000 rpm for 25 minutes, the supernatant was pale yellow in colour and the pellet is brown colour, discard the supernatant and add a little distilled water to pellet and poured it in sterilized Petri plate, dried in hot air oven to observe nanoparticles powder.

I. Characterization Techniques

In order to track the decrease of Cu ions, the synthesized nanoparticles were examined using a UV-visible spectrophotometer (Beckman Coulter-DU739). The wavelength range for scanning the CuNps was 200 nm–550 nm, and the absorption maxima were noted. The foundation of UV-Visible Spectroscopy is the idea that different spectra are produced when chemical compounds absorb ultraviolet or visible light.

Scanning electron microscopy is a high-resolution microscopy technique that uses a beam of high energetic electrons to probe the objects on a very fine scale. SEM is a surface imaging technique that gives information about particle sizes, size distributions, nanomaterial shapes and the surface morphology of the synthesized particles at the micro and nanoscales [18]. The SEM images in the study were obtained using Hitachi S-3400N equipment.

X-ray Diffraction (XRD) is an analytical technique that is used for the analysis of both molecular and crystal structures, qualitative identification of various compounds, measuring the degree of crystallinity, particle sizes etc. The powdered form of extracted silver nanoparticles was analyzed using Powder XRD Smart Lab X-ray diffractometer. Each material gives out a unique diffraction beam which can be defined and identified by comparing the beams with the reference database in the Joint Committee in Powder Diffraction Standards (JCPDS) library [18]. The mean size of silver nanoparticles can be calculated using the Debye-Scherer's equation, which is a formula that relates the size of sub-micrometer crystallites in a solid.

$$D = K2/(\beta \cos \theta)$$

Where, D- Average size of the synthesised nanoparticles, K- Scherrer constant (0.94). -X-ray wavelength in (0.1540nm) , β- Full width at half maximum (FWHM) in radiance and θ- Diffraction angle in degrees[18].

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J. Antibacterial Assay

The selected bacteria are Escherichia coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus.The pure culture was provided by the department of Microbiology, manasagangothri campus, University of Mysore, Mysore. Nutrient agar media was prepared as per the requirement according to the number of plates and the pH was adjusted to 7. In this method, sterilized nutrient agar plates are inoculated with each bacterium by swab method with the help of sterile swab of cotton. Then these plates were permitted to dry. After this 5 wells were bored by sterile cork borer in each agar plate, subsequently, 25µl,50µl,75µl, of CuNPs solution was introduced to the well and also positive control and negative control. The antibiotic ampicillin was taken as positive control and distilled water as negative control. All these procedures was done inside the laminar air flow chamber to maintain the aseptic condition. Then the plates were kept for complete diffusion followed by incubation at 37 °C for 24 hrs. and measured the diameter of inhibitory zones in mm.

K. Antifungal Assay

The selected fungi are Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, Aspergillus flavus,. Which were sub cultured from the pure culture for further study. The pure culture was provided by the department of Biotechnology manasagangothri campus, University of Mysore, Mysore. PDA media was prepared as per the requirement according to the number of plates and the pH was adjusted to 7.The antifungal activity of the CuNPs was determined by well diffusion method. The fungal inoculums prepared were used to test the antifungal potential. The PDA media was poured into sterile Petri plates in aseptic condition, then plates were allowed to solidify in laminar air flow chamber. The 4 fungal pathogens were then coated over a media containing plates with the help of sterile swab of cotton. Then these plates were permitted to dry. After this 5 wells were bored by sterile cork borer in each agar plate. Subsequently, 25µl,50µl,75µl, of CNPs solution was introduced to the wells and also positive control and negative control. The antibiotic Bavistin was taken as positive control and distilled water as negative control. Then the plates were sealed and incubated at room temperature for 5-6 days, and finally antifungal activity was calculated by measuring the diameter of inhibitory zones in mm.

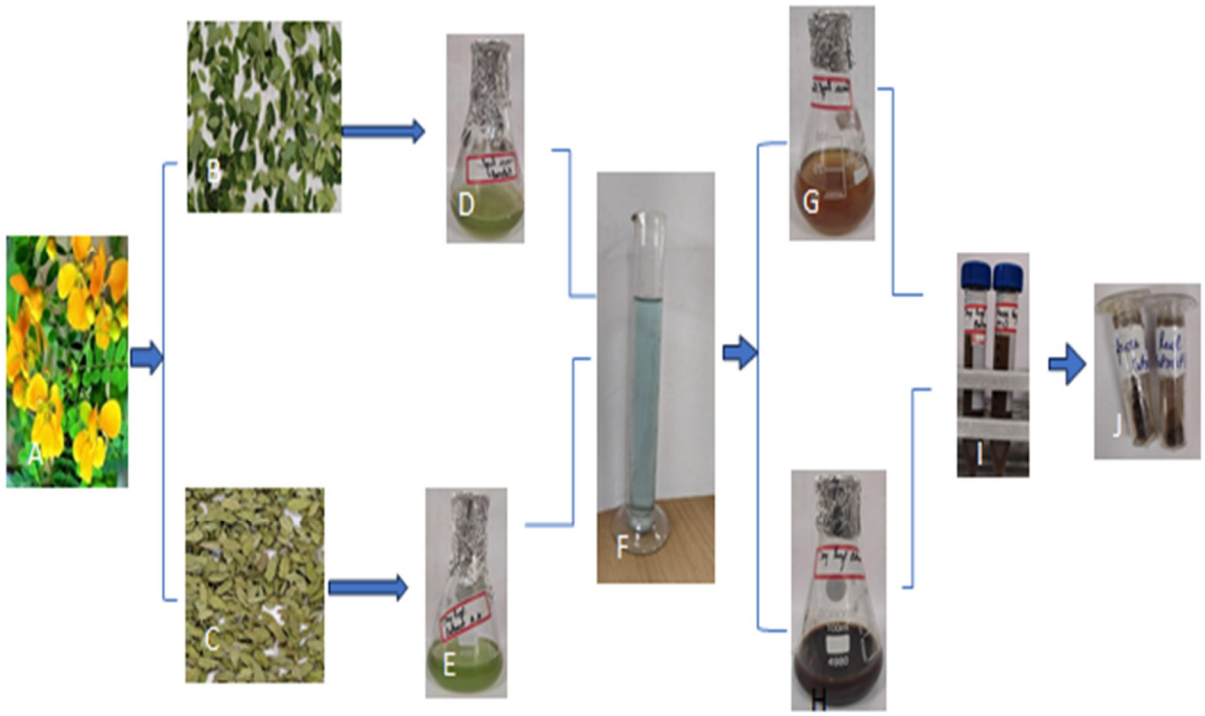


Fig 2 : Various steps involved in the preparation of NPs sample(A)C.auriculata plant(B) fresh leaves (C) dry leaves (D)fresh leaves extract (E)dry leaves extract (F) 1%CuSO4 (G) formation of CuONPs of fresh leaves (H) formation of CuONPs of Dry leaves (I) synthasised extract centrifuged at 5000r45pm (J) finely crushed CuONPs of fresh and dry leaves.

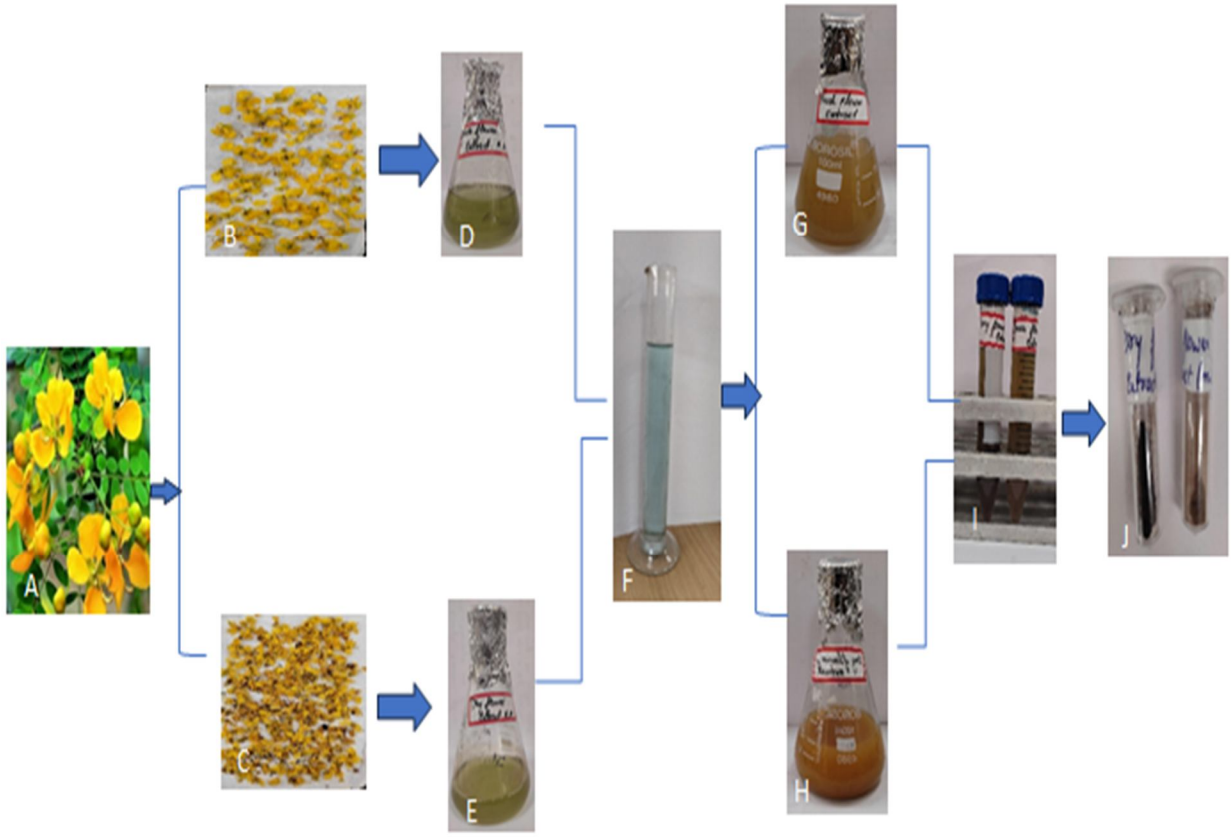


Fig 3: various steps involved in the preparation of NPs sample(A)C.auriculata plant(B) fresh flowers (C) dry flowers (D)fresh flowers extract (E)dry flowers extract (F) 1%CuSO4 (G) formation of CuONPs of fresh flowers (H) formation of CuONPs of Dry flowers (I) synthasised extract centrifuged at 5000rpm (J) finely crushed CuONPs of fresh and dry flowers.

III. RESULT AND DISCUSSION

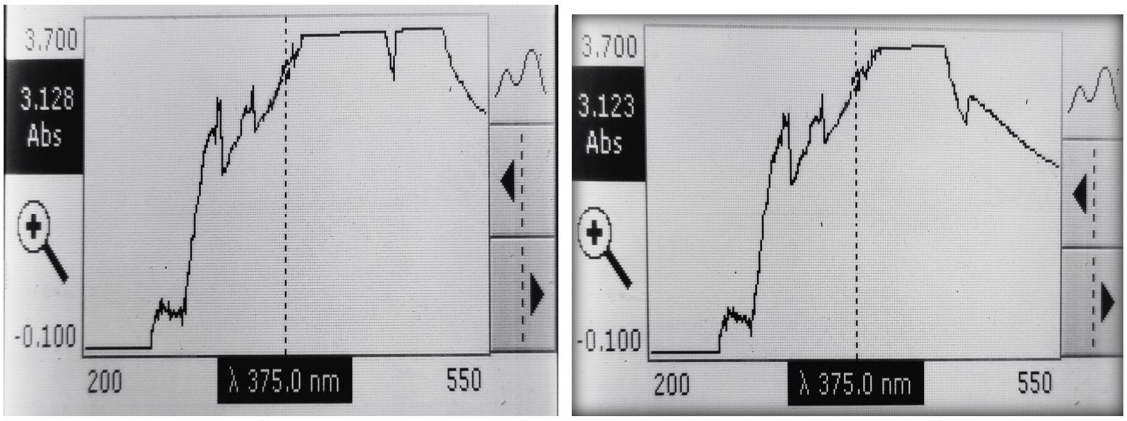
A. Green Synthesis of CuSO4 Nanoparticles

25ml of CuSo4 is mixed with 5ml of each plant extract the reaction was carried out slowly the colour changes from green to dark brown indicates the formation of copper nanoparticles. The incubated sample(around 5ml)is used for Uv-vis spectra test and the remaining incubated samples were centrifuged at 5000 rpm for 25 minutes, the supernatant was pale yellow in colour and the pellet is brown colour. Discarded the supernatant and added a little distilled water to the pellet and poured it in sterilized Petri plate, dried in hot air oven and scooped out the CuNps powder is used for SEM and P-XRD analysis.

B. UV-visible Spectroscopy Analysis

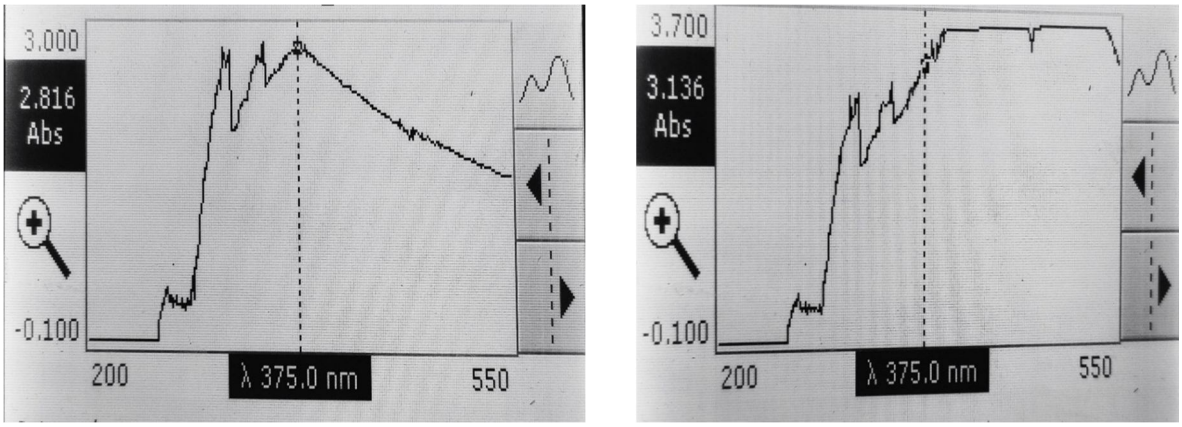
UV–vis is a commonly used technique to characterize nanoparticles. This technique allows to confirm the nanoparticles formation by measuring the Surface Plasmon Resonance (SPR). This procedure can provide information about the size, stability, and aggregation of the NPs. Wavelengths in the range of 200–700 nm are generally used to characterize the metal and metal oxide nanoparticles.

The metal nanoparticles have free electron, which gives the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp band of copper Nanoparticles is around 375nm of Cassia auriculata (fresh and dry leaf and flower). From different literature it was found that the copper Nanoparticles show SPR peak at around within 200-550nm. So we confirmed that Cassia auriculata leaf and flower extract has potential to reduce copper ions to copper nanoparticles.



A B

Fig. 4 : UV-Visible analysis of (A) fresh leaves extract, (B) dry leaves extract of Cassia auriculata copper Nanoparticles.



C D

Fig. 5 : UV-Visible analysis of (A) fresh flower extract, (B) dry flower extract of Cassia auriculata copper Nanoparticles.

C. Scanning Electron Microscopy (SEM)

Copper nanoparticles were further confirmed by knowing the morphology and size of synthesized copper nanoparticles. SEM image has shown the individual copper nanoparticles as well as number of aggregates. The morphological shape of the copper nanoparticles was spherical and showed in micrograph (fig. 6-9). The size of nanoparticles which should be between 1-100nm.C.auriculata fresh leaf copper nanoparticles average size is 12.43nm, dry leaf is 40nm and fresh flower copper nanoparticles size is 57.87nm, dry flower is 7.14nm. This size are within the 100nm it proves that C.auriculata strongly supports the synthesis of copper nanoparticles naturally.

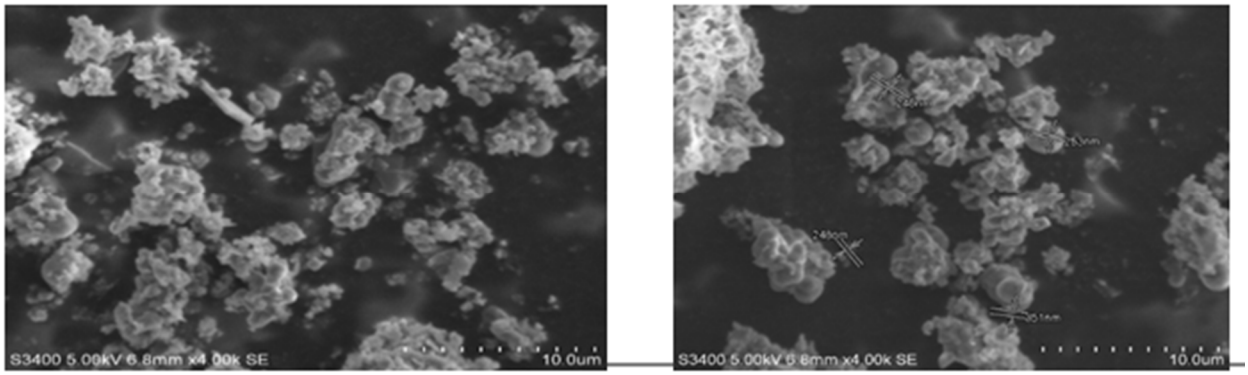


Fig. 6 : SEM image of fresh leaves Cassia auriculata copper nanoparticles

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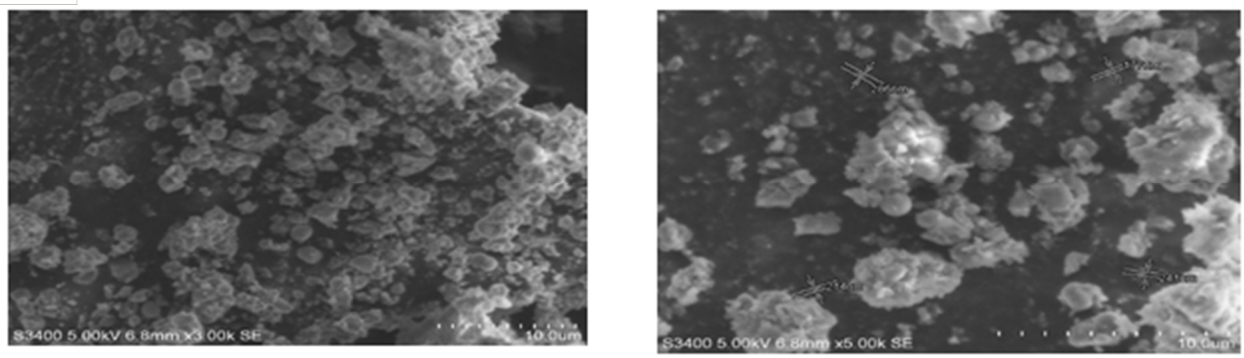


Fig. 7 : SEM image of dry leaves Cassia auriculata copper nanoparticles

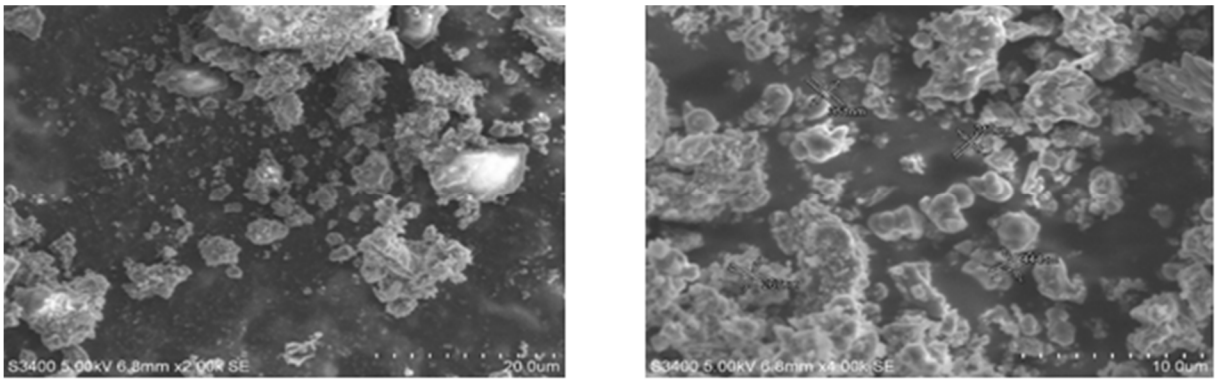


Fig. 8 : SEM image of fresh flowers Cassia auriculata copper nanoparticles

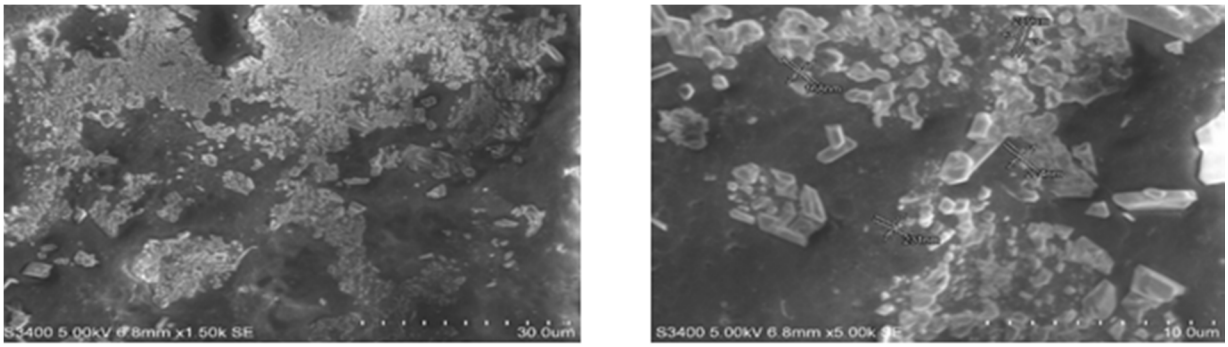


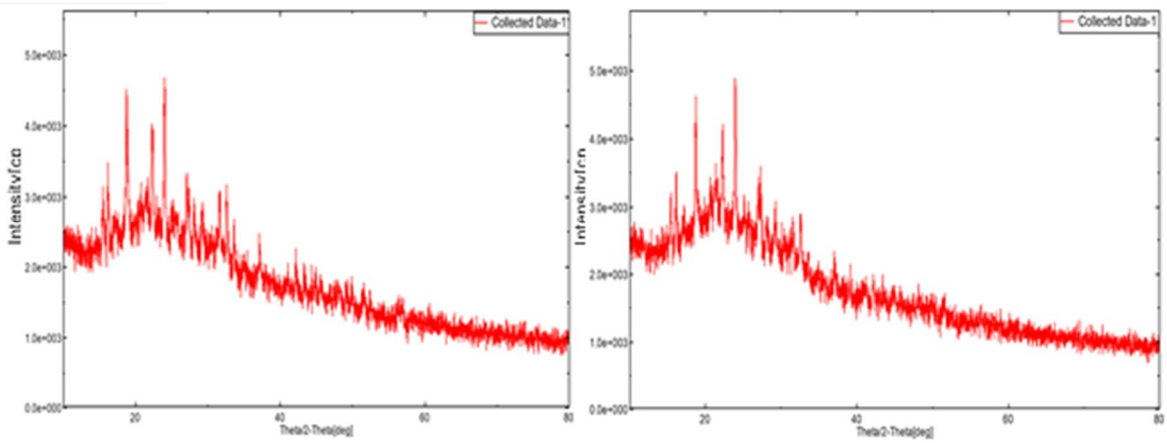
Fig. 9 : SEM image of dry flowers Cassia auriculata copper nanoparticles

D. X-Ray DiffractioN (XRD) Analysis

X-ray diffraction patterns of synthesized fresh leaves CuNps showed 8 distinct peaks with 2θvalues of 15.51, 16.31, 18.77, 22.35, 24.04, 25.26, 26.98 and 32.65.The following 2 values, 8.91, 8.91, 8.94, 10.85, 39.01, 0.64, 18.39 and 3.76 are assigned to the sets of planes (002),(002),(002),(101),(101), (110), (110) and (112) respectively. These sets of planes may be indexed to the Face Centred Cubic (FCC) lattice structure of the copper nanoparticles. The mean size of copper nanoparticles calculated using the Debye-Scherrer's equation was found to be 12.43nm.

X-ray diffraction patterns of synthesized dry leaves CuNps showed 11 distinct peaks with 2θvalues of 15.42, 16.16, 18.73, 21.36, 22.24, 23.93, 27.17, 29.49, 31.36, 32.64 and 36.97. The following 2 values, 48.74, 18.20, 45.95, 42.82, 43.08, 17.54, 39.55, 44.81, 53.02, 42.57 and 43.73 can be assigned to the sets of planes (002), (101), (101), (110) and (103) respectively. These sets of planes may be indexed to the Face Centred Cubic (FCC) lattice structure of the copper nanoparticles. The mean size of copper nanoparticles calculated using the Debye-Scherrer's equation was found to be 40nm.

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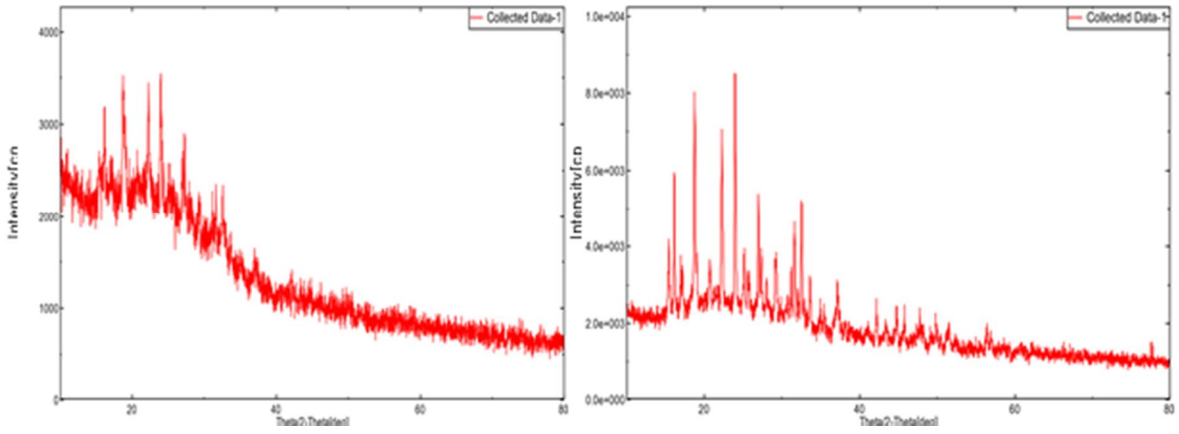


A B

Graph. 1: XRD of (A) fresh leaves, (B)dry leaves CuNPs.

X-ray diffraction patterns of synthesized fresh flowers CuNps showed 26 distinct peaks with 2θvalues of 15.44, 16.13, 16.97, 18.73, 20.69, 22.25, 23.96, 25.15, 26.95, 27.34, 27.99, 29.18, 31.18, 31.58, 32.05, 32.50, 33.60, 37.12, 42.17, 44.70, 45.76, 56.29 and 77.56. The following 2 values, 43.08, 63.77, 25.26, 50.61, 37.79, 43.47, 54.83, 47.93, 55.25, 57.44, 62.39, 33.27, 71.66, 50.26, 84.16, 62.93, 65.8, 46.39, 77.82, 60.31, 176.53, 27.83 and 206.8 are assigned to the sets of planes (002), (002), (002), (002), (101), (101), (101), (101), (110), (110), (110), (110), (112), (112), (112), (112), (112), (200), (022), (112), (211), (220) and (321) respectively. These sets of planes may be indexed to the Face Centred Cubic (FCC) lattice structure of the copper nanoparticles. The mean size of copper nanoparticles calculated using the Debye-Scherrer's equation was found to be 57.87nm.

X-ray diffraction patterns of synthesized dry flowers CuNps showed 6 distinct peaks with 2θvalues of 16.13, 18.70, 22.25, 23.89, 27.39 and 32.79. The following 2 values, 15.06, 11.56, 7.39, 3.22, 2.29 and 3.33 are assigned to the sets of planes (002), (002), (101), (101), (011) and (112) respectively. These sets of planes may be indexed to the Face Centred Cubic (FCC) lattice structure of the copper nanoparticles. The mean size of copper nanoparticles calculated using the Debye-Scherrer's equation was found to be 7.14nm.



C D

Graph. 2: XRD of (C) dry flowers, (D)fresh flowers CuNPs.

E. Antimicrobial Activity

Copper compounds have been shown to provide excellent antimicrobial activity against a number of microorganisms including bacteria, fungi etc, Copper ions have demonstrated antimicrobial activity against a wide range of microorganisms, such as Escherichia coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus and fungal species Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, Aspergillus flavus.

Copper has been exploited for health purposes since ancient times.

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The synthesized copper nanoparticles from fresh and dry leaves and flower extract of *Cassia auriculata* showed high degree of toxicity against bacteria and fungi. It shows clear diameter of the zone of inhibition around the well where in the suspension of CuNps is present. The obtained result is represented in tables for all four samples of CuNps.

Table. 1 : A table shows antimicrobial activity of CuNps from C.auriculata fresh leaves extract.

BACTERIAL CULTURE	Inhibition zones (mm)				
	+ve control	-ve_control	25µl	50µl	75µl
Escherichia coli	10	0	3	6	8
Salmonella typhi	0	0	0	0	0
Bacillus subtilis	2	0	2	5	8
Staphylococcus aureus	2	0	1	8	10

Table. 2 : A table shows antimicrobial activity of CuNps from C.auriculata fresh flowers extract.

BACTERIAL CULTURE	Inhibition zones (mm)				
	+ve control	-ve_control	25µl	50µl	75µl
Escherichia coli	25	0	10	13	17
Salmonella typhi	0	0	0	0	0
Bacillus subtilis	32	0	12	18	22
Staphylococcus aureus	18	0	3	11	15

Table. 3 : A table shows antimicrobial activity of CuNps from C.auriculata dry leaves extract.

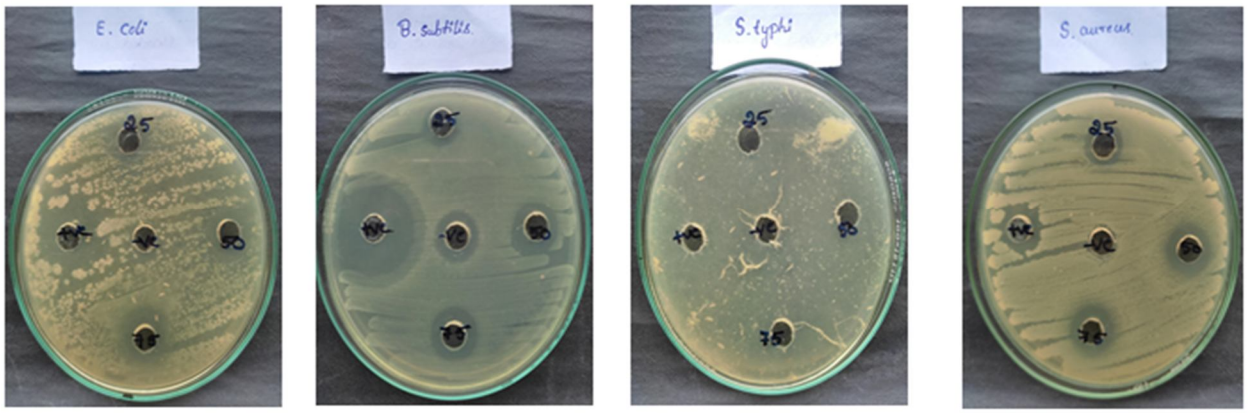
BACTERIAL CULTURE	Inhibition zones (mm)				
	+ve control	-ve_control	25µl	50µl	75µl
Escherichia coli	15	0	5	7	10
Salmonella typhi	0	0	0	0	0
Bacillus subtilis	25	0	4	13	12
Staphylococcus aureus	10	0	2	5	4

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Table. 4 : A table shows antimicrobial activity of CuNps from C.auriculata dry flowers extract.

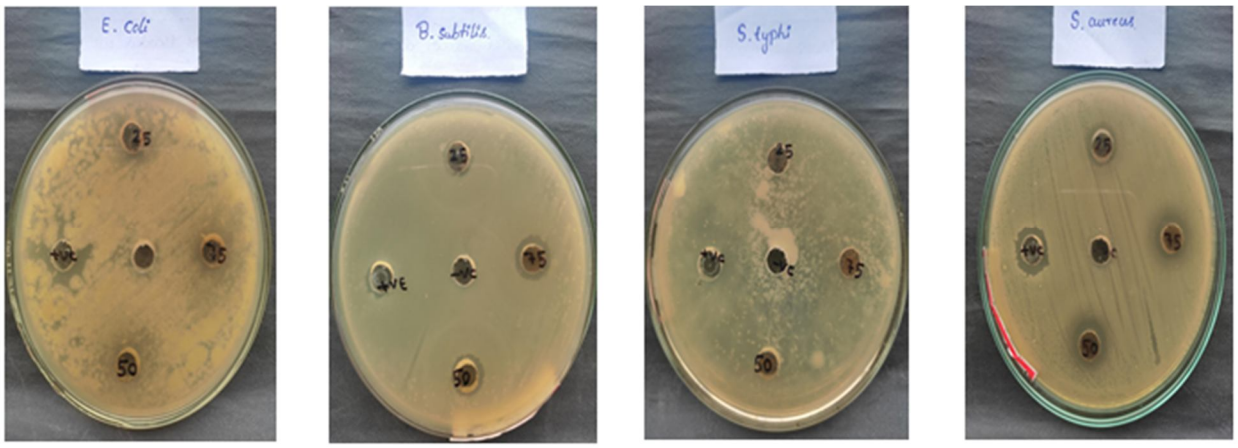
BACTERIAL CULTURE	Inhibition zones (mm)				
	+ve control	-ve_control	25µl	50µl	75µl
Escherichia coli	15	0	8	10	13
Salmonella typhi	0	0	0	0	0
Bacillus subtilis	3	0	1	3	4
Staphylococcus aureus	9	0	3	7	6

The results of antimicrobial activity of the C. auriculata green synthesized CuNPs against the Gram- negative bacterial strains (E. coli and S.typhi) and the Gram-positive bacterial strains (S.aureus and B.subtilis), under the well diffusion method had a considerable antimicrobial activities increased with higher concentration. The fresh leaves CuNPs shows more effective against S.aureus, dry leaves CuNPs against E. coli, fresh flowers CuNPs against S. aureus and dry flowers CuNPs shows more effective against B. subtilis.



A B C D

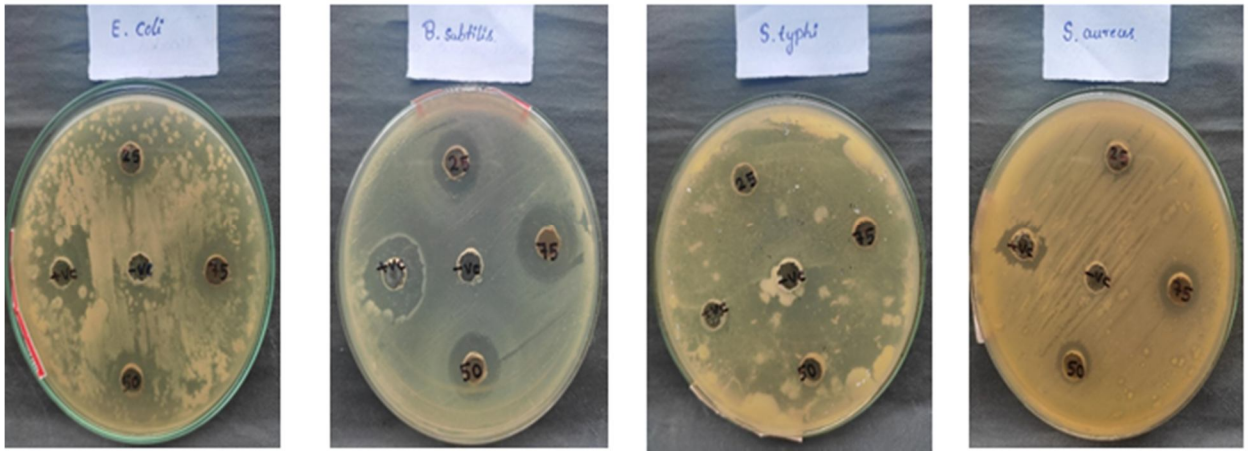
Fig 10: The antibacterial effect of CuONPs on pathogens fresh leaves extract (A) E. coli (B) B. subtilis(C) S. typhi(D) S. aureus



A B C D

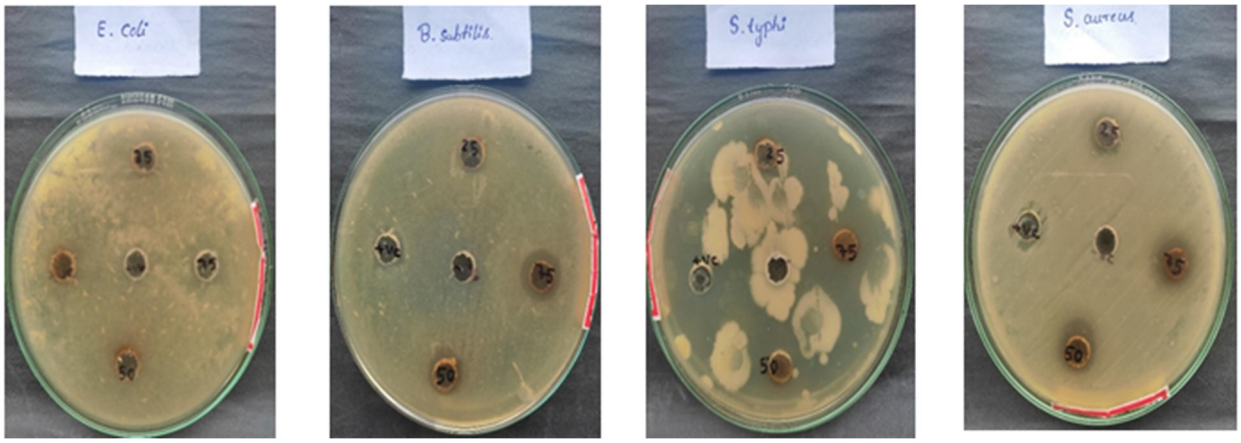
Fig 11: The antibacterial effect of CuONPs on pathogens dry leaves extract (A) E. coli (B) B. subtilis(C) S. typhi (D) S. aureus

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A B C D

Fig 12: The antibacterial effect of CuONPs on pathogens fresh flowers extract (A) E. coli (B) B. subtilis(C) S. typhi (D) S. aureus



A B C D

Fig 13: The antibacterial effect of CuONPs on pathogens dry flowers extract (A) E. coli (B) B. subtilis(C) S. typhi (D) S. aureus

Using the well diffusion method, the antifungal agent (NPs) was tested for its ability to inhibit fungal cells. The green synthesized nanoparticles suspension of varying concentrations was tested for its antifungal activity against four different fungal species: Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, and Aspergillus flavus.

Table. 5 : A table shows antimicrobial activity of CuNps from C.auriculata fresh leaves extract.

FUNGAL CULTURE	Inhibition zones (mm)				
	+ve control	-ve_control	25µl	50µl	75µl
Aspergillus niger	19	0	5	8	8
Fusarium oxysporum	18	0	15	25	27
Penicillium atramentosum	0	0	0	0	0
Aspergillus flavus	25	0	8	10	12

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Table.6 : A table shows antimicrobial activity of CuNps from C.auriculata dry leaves extract.

FUNGAL CULTURE	Inhibition zones (mm)				
	+ve control	-ve_control	25µl	50µl	75µl
Aspergillus niger	20	0	3	8	10
Fusarium oxysporum	14	0	10	13	15
Penicillium atramentosum	0	0	0	0	0
Aspergillus flavus	27	0	9	12	13

Table.7 : A table shows antimicrobial activity of CuNps from C.auriculata fresh flower extract.

FUNGAL CULTURE	Inhibition zones (mm)				
	+ve control	-ve_control	25µl	50µl	75µl
Aspergillus niger	23	0	6	9	12
Fusarium oxysporum	15	0	12	20	18
Penicillium atramentosum	0	0	0	0	0
Aspergillus flavus	30	0	16	9	10

Table.8 : A table show antimicrobial activity of CuNps from C.auriculata fresh flower extract

FUNGAL CULTURE	Inhibition zones (mm)				
	+ve control	-ve_control	25µl	50µl	75µl
Aspergillus niger	25	0	5	0	0
Fusarium oxysporum	25	0	8	12	15
Penicillium atramentosum	0	0	0	0	0
Aspergillus flavus	22	0	5	8	10

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The results of antimicrobial activity of the C. auriculata green synthesized CuNPs against the fungal species: Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, and Aspergillus flavus. In case of fungi both fresh and dry leaves and flowers CuNPs shows more effective against F. Oxysporum, a considerable antimicrobial activities increased with higher concentration.

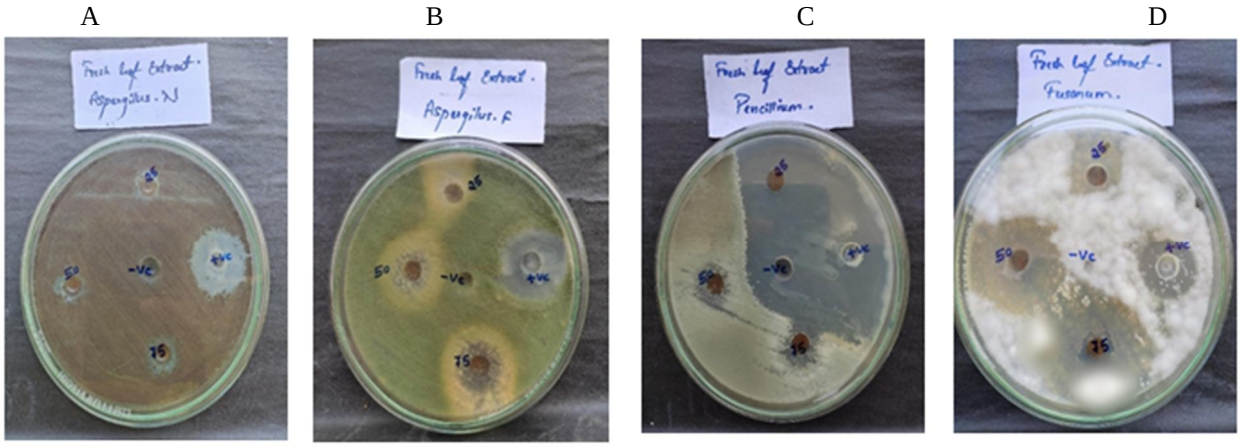


Fig 14: The antifungal effect of CuNPs on pathogens fresh leaves (A) A. niger (B)A.flavus (C) P.atramentosum(D)F.oxysporum.

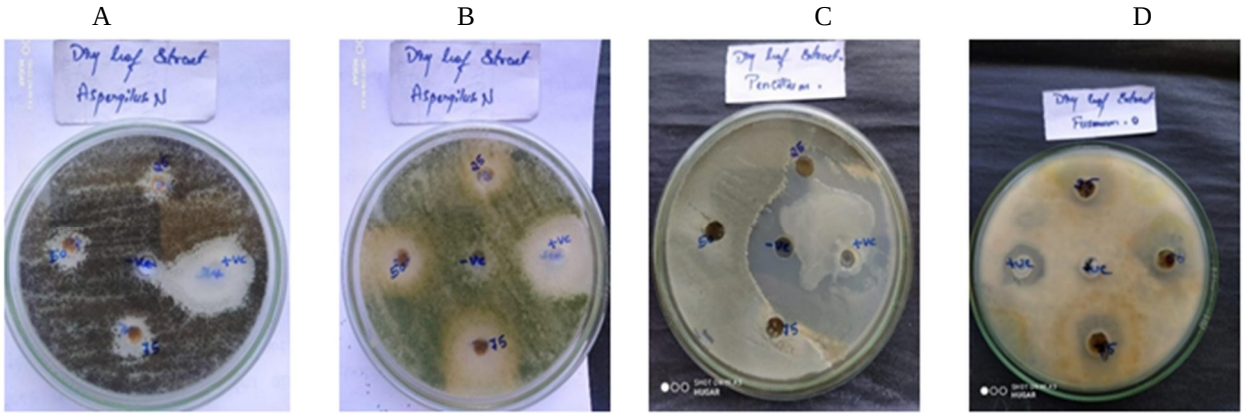


Fig 15 :The antifungal effect of CuNPs on pathogens dry leaves (A) A. niger (B)A.flavus (C) P.atramentosum(D)F.oxysporum.

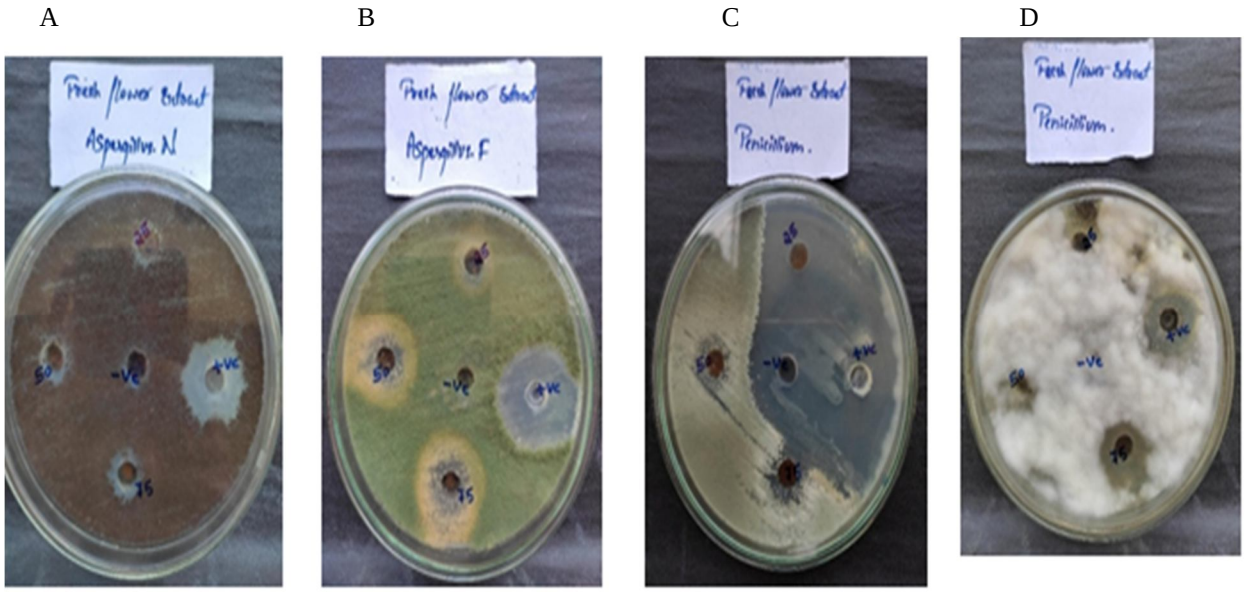


Fig 16: The antifungal effect of CuNPs on pathogens fresh flowers (A) A. niger (B)A.flavus (C) P.atramentosum(D)F.oxysporum.

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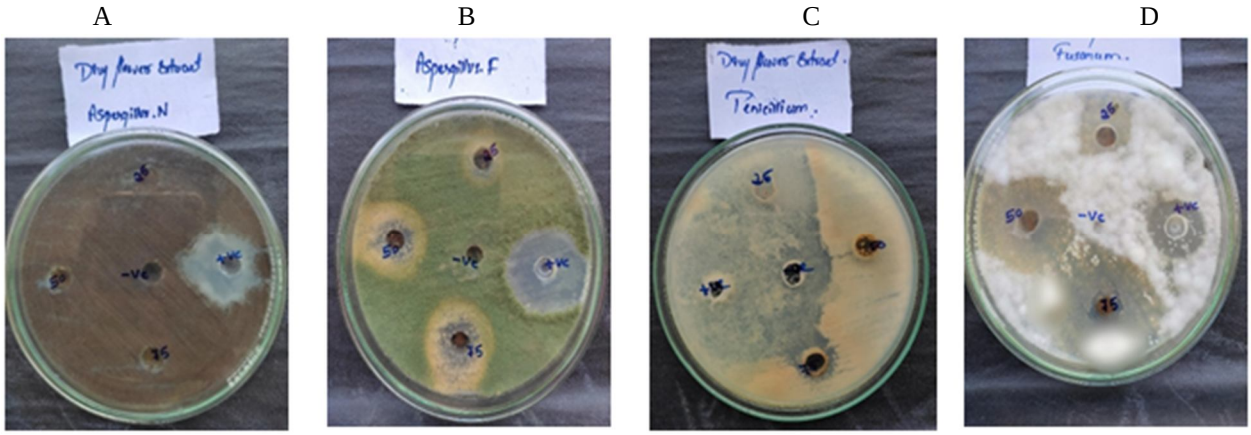


Fig 17: The antifungal effect of CuNPs on pathogens dry flowers (A) A. niger (B)A.flavus (C) P.atramentosum(D)F.oxysporum.

IV. CONCLUSION

In the present work copper nanoparticles were synthesized from *C.auriculata* leaves and flowers extract, which can act as reducing agents in the presence of aqueous copper sulphate solution and result in the synthesis of copper nanoparticles. The characterization studies performed using methods like XRD, UV-spectroscopy, SEM and also support the fact that the synthesized nanoparticles were large, spherical and polydispersed nonetheless were purely copper in composition. X-ray diffraction technique used for analyzing the crystal structure of material, UV-visible spectroscopy analysis was used to principally monitor the reduction of copper ions into copper oxide nanoparticles, the closer examination of SEM results indicates that there is a fair prospect of synthesizing smaller sized, monodispersed and non-aggregated copper nanoparticles. Nanoparticle characterization is of at most significance to establish the synthesis and biological properties of nanoparticles.

These nanoparticles were also tested for antimicrobial activity by using different bacteria and fungi. The synthesized nanoparticles exhibited potential antimicrobial activity against both bacteria and fungi. Hence this method facilitates best alternative for both chemical and other physical methods and can be employed in large- scale production for the purpose of biological applications.

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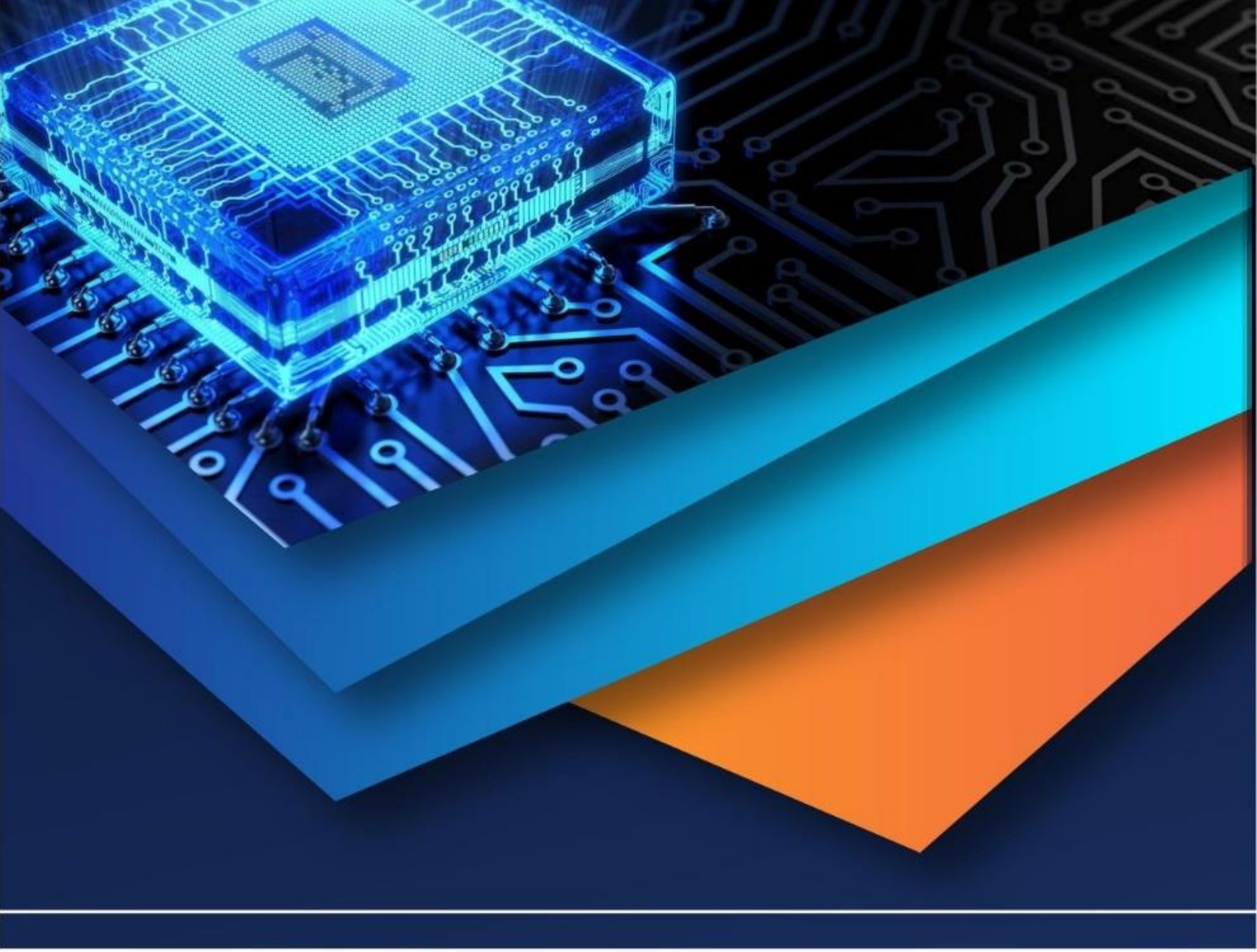
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