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SYNTHESIS OF COPPER NANOPARTICLES USING BIO METHOD IN *CASSIA AURICULATA* LEAVES EXTRACT

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ABSTRACT: Knowing the morphology of Copper nanoparticles was confirmed by Field Emission Scanning Electron microscopy (FE-SEM). Herein, we are reporting a novel biological approach for the formation of copper nanoparticles using leave. Copper sulfate was made to reduce with an aqueous solution of *Cassia auriculata* leave extracts. X-Ray diffraction (XRD) pattern reveals the formation of Copper nanoparticles, which shows crystallinity. Field emission scanning electron microscopy (FE-SEM) suggested particles size and spherical shape in the range of 38.1-43.5 nm. UV absorption studies of copper nanoparticles showed that have absorbance in the range 488.5 to 514.3 nm and FT-IR studies of copper nanoparticles were showed that the stretching frequency of fingerprint region at 509.21 cm^{-1} for Cu-O bond.

Keywords: *Cassia auriculata* leaves, Copper nanoparticles, UV-Vis, FT-IR, XRD, FE-SEM

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INTRODUCTION: Avaram (*Cassia auriculata* Linn.), family Caesalpiniaceae, is also known as Avaram tree, The leaves are alternate, stipulate, paripinnate compound, very numerous, closely placed, rachis 8.8-12.5 cm long, narrowly furrowed, slender, pubescent with an erect, linear gland between the leaflets of each pair. Leaflets are very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping, oval-oblong, obtuse at both ends, mucronate, glabrous or minutely downy, dull green, paler beneath, stipules very large, reniform-round, produced at the base on the side of next petiole into a filiform point and persistent. Plants have great potential uses, especially as traditional medicine and pharmacopoeial drugs.

A large proportion of the world population depends on traditional medicine because of the scarcity and high costs of orthodox medicine¹. Medicinal plants have provided modern medicine with numerous plant-derived therapeutic agents². Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases³.

It is one of the principle constituents of 'Avaarai panchaga chooranam'- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level⁴. The plant has been reported to possess anti-pyretic⁵, hepatoprotective⁶, anti-diabetic, anti-peroxidative and anti-hyperglycaemic⁷, microbicidal⁸ and antihyperlipidaemic activities⁹. The flowers are used to treat urinary discharges, nocturnal emissions, diabetes, and throat irritation¹⁰.

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They are one of the constituents of polyherbal formulation 'Diasulin' in the concentration range of 40 mg/dl, which is proven to have antidiabetic activity¹¹⁻¹².

MATERIALS AND METHODS:

Materials: *Cassia auriculata* leaves (collected from the Jameen Nathampatti village, Rajapalayam, virudhunagar district) and double distilled water used for the preparation of the aqueous extract.

Methods:

Preparation of *Cassia Auriculata* Leaves Extract in Aqueous Medium: *Cassia auriculata* leaves were cleaned thoroughly and then sun-dried for 5 – 7 days. Dried leaves were ground to a fine powder. The aqueous extract was prepared by refluxing 30 gm of leaf powder and 300 ml of distilled water by using Soxhlet apparatus at 100 °C for 5 h. Then, the extracts were collected in an airtight bottle and were kept in a deep freezer for further use.

Synthesis of Copper Nanoparticles from Leaf Extract: The 10 ml of *Cassia auriculata* leaves extract was added to 40 ml aqueous solution of 1 mM copper sulfate in a conical flask for reduction into Cu⁺ ions and kept for incubation (darkroom) at room temperature. Here the filtrate acts as reducing and stabilizing agent for 1mM of CuSO₄. Suitable controls (40ml distilled water + 10 ml Plant extract) in another test tube were maintained throughout the experiments. Reduction of copper sulfate to copper ions was identified by the color change from the light yellow color of the extract to dark brown color as given in **Fig. 1a**, and **b**.

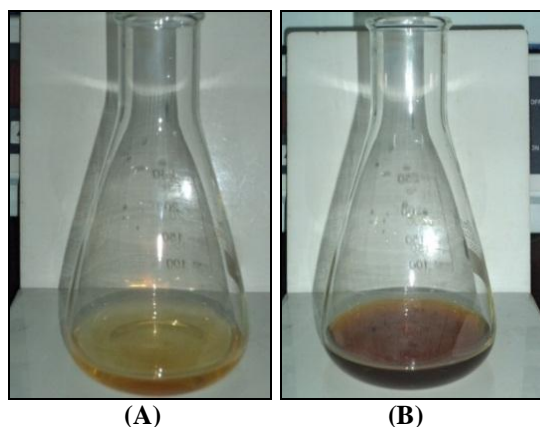


FIG. 1(A) AND 2(B): PHOTOGRAPHS SHOWING A) *CASSIA AURICULATA* LEAVES EXTRACT B) COLOR CHANGES AFTER ADDING LEAF EXTRACT TO CuSO_4 SOLUTION

A control setup was also maintained without adding copper sulfate to the plant extract. The formation of Copper nanoparticles was further confirmed by spectral analysis.

Characterization of Copper Nanoparticles:

Ultraviolet-Visible Spectrophotometer (UV-DRS): The UV-DRS spectrum was recorded in Shimadzu UV-2400PC series. Absorption spectra of copper nanoparticles formed in the reaction media have an absorbance peak in the range 488.5 to 514.3 nm **Fig. 2**.

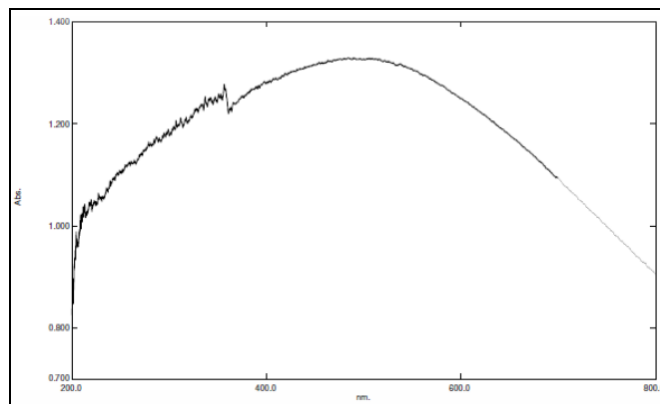


FIG. 2: UV-VISIBLE SPECTRUM OF COPPER NANOPARTICLES

Fourier Transform - Infrared Spectroscopy (FT-IR):

The FT-IR spectrum was recorded in Shimadzu FT-IR spectrometers 7600, Measurement of the samples were performed in transmission mode. To identify the binding groups of the plant extract with copper, FT-IR spectra of *Cassia auriculata* and copper nanoparticles were recorded. On comparing the IR stretching frequency of the functional groups present in *Cassia auriculata* **Fig. 3** and copper nanoparticles **Fig. 4**, it was revealed that copper binds to oxygen moiety indicated by M-O stretching at 509.21 cm^{-1} .

X-Ray Diffraction (XRD):

For the XRD (BRUKER ECO D8 ADVANCE) spectral analysis, prepared particles in solution was purified by centrifugation at 5000 rpm for 20 minutes. An XRD spectrum was recorded at Kalasalingam University, and the spectra, and their values were given in **Fig. 5**. The dried mixture of copper nanoparticles collected for the formation of Cu nanoparticles by X-ray diffractometer operated at a voltage of 40 kV and a current of 20 mA with Cu K α radiation in θ - 2θ configuration. Morphology of the interplanar distance spacing was

calculated using Bragg's equation. $n\lambda = 2d \sin \theta$. In the XRD curves of copper nanoparticles, intense

and sharp peaks were observed at 24° , 31.9° , 42° , 52.4° , 58° and 63.2° .

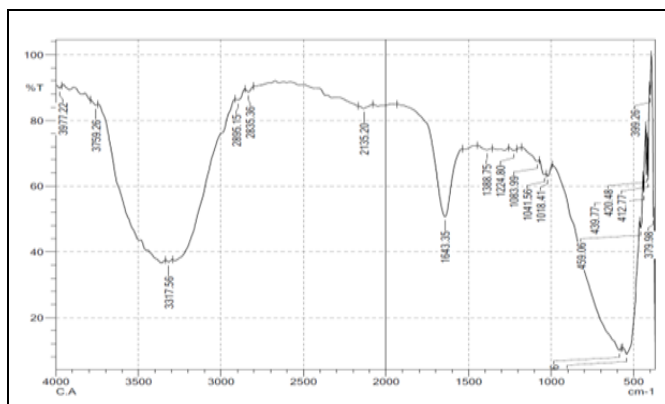


FIG. 3: FT-IR SPECTRUM OF *CASSIA AURICULATA*

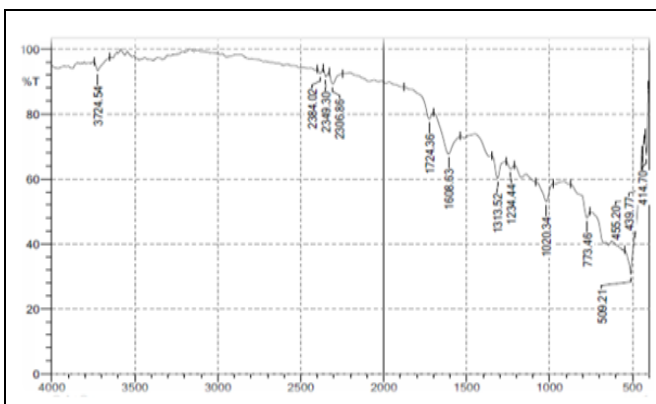


FIG. 4: FT-IR SPECTRUM OF COPPER NANOPARTICLES

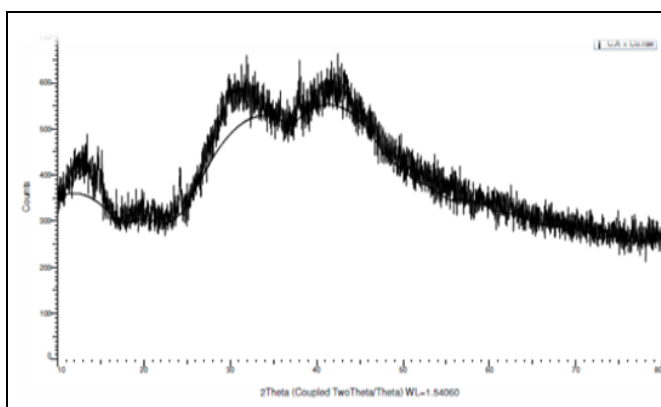


FIG. 5: XRD SPECTRUM OF COPPER NANOPARTICLES

Field Emission Scanning Electron Microscope (FE-SEM): The copper nanoparticles sizes were determined using Field Emission Scanning Electron Microscope. The FE-SEM images of copper nanoparticles at 50°C . The formation of copper nanoparticles as well as their morphological dimensions in the FE-SEM study demonstrated that the average size was from 38.1-43.5 nm with interparticle distance, whereas the shapes were uniformed spherical **Fig. 6**.

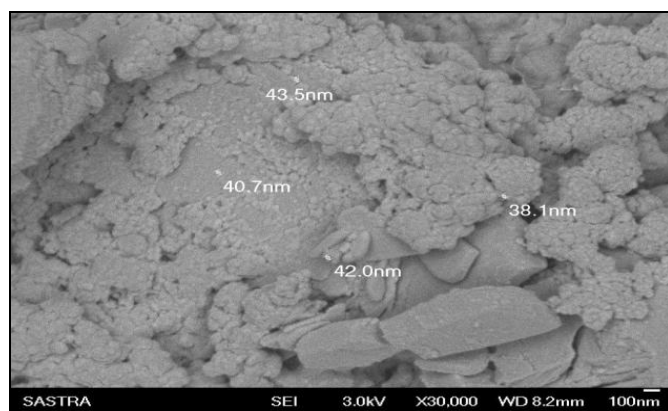


FIG. 6: FE-SEM IMAGES OF COPPER NANOPARTICLES

However, further observation with high magnification reveals that these Cu nanoclusters were assembled by smaller nanoparticles, which exhibit good uniformity, and the average diameter is about 38.1 nm.

RESULT AND DISCUSSION: Green synthesis of copper nanoparticles using *Cassia auriculata* leaves extract was first identified by color changes of the extract from light yellow to dark brown color. Reduction Cu^+ ion into copper nanoparticles during exposure to the leaves extract was observed as color changes (as given in **Fig. 1a** and **b**). Secondly, copper nanoparticles were confirmed by the spectral studies like UV-Visible Spectroscopy, FT-IR Spectroscopy, X-Ray Diffraction, and Field Emission Scanning Electron Microscopy.

Ultraviolet-Visible Spectrophotometer Analysis (UV-DRS): It is generally analyzed that UV-Visible Spectroscopy could be used to examine the formation of copper nanoparticles. Absorption spectra of copper nanoparticles formed in the

reaction media have shown the absorbance peak in the range 488.5 to 514.3 nm.

Fourier Transform - Infrared Spectroscopy Analysis (FT-IR): On comparing the FT-IR spectra of *Cassia auriculata* leaves extract **Fig. 3**, and the copper nanoparticles **Fig. 4** revealed the following observation. The FT-IR spectra of *Cassia auriculata* leave extract showed broadband at 3317 cm^{-1} indicating the presence of bonded - OH group. Stretching at 1643 cm^{-1} indicated the presence of $>\text{C}=\text{O}$ group. The FT-IR spectra of copper nanoparticles showed the following stretching frequencies (i) at 1608 cm^{-1} (due to carbonyl stretching) (ii) 509.21 cm^{-1} (due to the formation of Cu-O bond).

On comparing the IR spectra of both the extract and its assisted copper nanoparticles formation revealed the following observation. During the formation of copper nanoparticles assisted by *Cassia auriculata*, the O-H bonds present in the extract (observed by 3317 cm^{-1}) diminishes that showed that the O-H group oxygen might bind to copper in copper nanoparticles. $>\text{C}=\text{O}$ stretching frequency at 1643 cm^{-1} of the extract shifted to 1608 cm^{-1} in copper nanoparticles that showed that this might enter into the binding with copper in copper nanoparticles.

M-O stretching at 509.21 cm^{-1} also confirms the existence of Cu-O bond. Hence these observations indicated the formation of copper nanoparticles binding strongly to the oxygen atom present in the phytoconstituents of *Cassia auriculata* extract.

X-Ray Diffraction (XRD): Copper nanostructure was confirmed by the characteristic peaks observed in the XRD pattern. The analysis was carried out 2θ value ranging from 10° to 80° , with step size 0.020. All diffraction peaks correspond to the characteristic face-centered cubic observed at a 2θ angle at 24° , 31.9° , 42° , 52.4° , 58° and 63.2° respectively. Morphology of the interplanar distance spacing was calculated using Bragg's equation ($n\lambda = 2d\sin\theta$).

d) Field Emission Scanning Electron Microscope (FE-SEM): The formation of copper nanoparticles as well as their morphological dimensions in the FE-SEM observation showed that the average size was from 38.1-43.5 nm with interparticle distance,

whereas the shapes were uniformed spherical (as given in **Fig. 6**).

However, further observation with high magnification reveals that these Cu nanoclusters are assembled by smaller nanoparticles, which exhibit good uniformity, and the average diameter is about 38.1 nm.

CONCLUSION: Green synthesis of copper nanoparticles using *Cassia auriculata* leaves extract revealed the following observation. The formation of copper nanoparticles was confirmed by color changes from a light yellow color to dark brown color for *Cassia auriculata* leaves extract and the characterized UV-Vis, FT-IR spectral studies and their structure space and size by XRD and FE-SEM analysis. UV absorption studies of copper nanoparticles showed that have absorbance in the range 488.5 to 514.3 nm and FT-IR studies of copper nanoparticles were showed that the stretching frequency of fingerprint region at 509.21 cm^{-1} for Cu-O bond. Copper nanostructure was confirmed by the characteristic peaks observed in the XRD pattern.

All diffraction peaks correspond to the characteristic face-centered cubic observed at a 2θ angle at 24° , 31.9° , 42° , 52.4° , 58° and 63.2° respectively. Morphology of the interplanar distance spacing was calculated using Bragg's equation ($n\lambda = 2d\sin\theta$). FE-SEM observation with high magnification reveals that these Cu nanoclusters were assembled by smaller nanoparticles, which exhibit good uniformity and the average diameter was about 38.1 nm.

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CONFLICT OF INTEREST: Nil

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