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Green Synthesis of Copper Nanoparticles by Using Aloe Vera Flower Extract and Its Antimicrobial Activity

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ABSTRACT:

This research paper is about to investigates the phytosynthesis of copper nanoparticles using Aloe Vera flower extract. Exposing aqueous copper ions to floral extract reduced them from Cu2C to Cu0, resulting in nanoparticle formation. The generated nanoparticles. The samples were analyzed using UV-Vis spectrophotometer, FESEM, and FTIR. The existence of an absorption peak at 578 nm using a spectrophotometer verifies the production of Cu nanoparticles. The form and morphology of copper nanoparticles were investigated using FESEM analysis. FTIR analysis suggested the presence of reductive groups on nanoparticle surfaces.

Keywords: Copper, Biosynthesis, Nanoparticles, Flower Extract, Aloe Vera

Introduction:

Nanotechnology is a promising area of contemporary study and the primary driving factor behind the present industrial revolution. Nanotechnology deals with nanoparticles, which are typically modest in size and range (1nm-100nm). They have a high surface area-to-volume ratio, which makes them even more remarkable. Over the last few years, the synthesis of metal nanoparticles (MNPs) has been a major focus of study in transitory materials science in order to maximize societal benefit. We focus our attention to the copper nanoparticles (CuNPs) since they demonstrate several significant qualities including thermal, electrical, and optical, as well as being cheap cost and widely available in the market. Nanotechnology is a very recent research approach. This method is widely employed in a range of areas. Nanomaterials have smaller dimensions, ranging from1 to 100 nanometers.

Nanotechnology research seeks to develop nanoparticles of varying sizes, shapes, and chemical compositions for potential human uses. Nanoparticles have been manufactured using a variety of physical and chemical approaches. The chemical process for producing nanoparticles is exceedingly expensive, with extra environmental and biological implications. Biogenic synthesis, which uses plants to produce nanoparticles, is an inexpensive and environmentally beneficial method. Metallic nanoparticles are versatile and hence useful in a variety of applications, including environmental, biological, and antibacterial research, solar power production, and catalysis. Plant extracts are used to manufacture copper and its oxide nanoparticles. Bio nanotechnology is a fast expanding branch of nanotechnology in which bio-organisms are widely employed to synthesis nanomaterials, which are then used to improve the organisms' quality of life. Biological synthesis employs the biological principle of oxidation and reduction via microbial enzymes or plant phytochemicals.

Numerous publications and research have shown that this green synthesis approach has already produced a great number of metal/metal oxide nanoparticles such as silver (Ag), gold (Au), selenium (Se), platinum (Pt), zinc oxide (ZnO), iron oxide (Fe2 O 3), graphene oxide, and so on . Furthermore, those research found that numerous metal nanoparticles have a wide range of biological and chemical functions, although CuNPs have lately received attention. In humans, copper serves as a cofactor for several enzymes involved in neuropeptide production, cell signalling pathway modulation, antioxidant defense, and immune cell function. Copper is required by plants for a number of metabolic and physicochemical activities.

One of the most important trace elements for plant development. It is found in modest amounts in both people and plants and regulates metabolic and biological activity by acting as a cofactor for many enzymes. It serves as a cofactor for several enzymes, including amino oxidase, cytochrome c oxidase, and plastocyanin, ensuring proper action. Copper oxide, on the other hand, exhibits antimicrobial, antibacterial, antifungal, anti-microbial, antifungal, magnetic phase change, gas sensing, biocidal, superconductive, catalytic, and optical properties.

Chemical A.R grade copper(II) acetate monohydrate $(CH_3COO)_2$ $Cu{\cdot}H_2O$ served as the chemical precursor, with a molecular weight of 199.65 g/mol Sodium hydroxide and hydrochloric acid (12N) were utilized in the synthesis.

CuNPs have potential uses in water treatment, gas sensing, catalysis, agriculture, high-temperature superconductor manufacture, medicinal research, and so on. Overall, this review focuses on recent advances in the biosynthesis and characterisation of CuNPs, as well as prospective medicinal applications (mostly antibacterial, antifungal, and anticancer properties). Bio-capped CuNPs allow future sustainable development and successful bio-medical uses in combating multi-drug resistance, bio-film generating pathogenic bacteria, and numerous health-related applications.

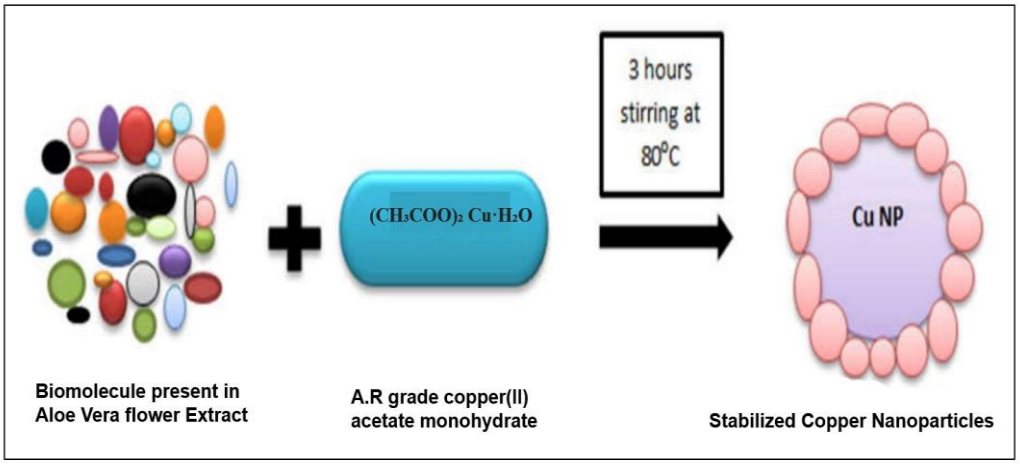


Figure 1. Schematic diagram of bio reduction of aqueous copper acetate solution by biomolecules present in aloe Vera extract form stabilized copper nanoparticles

Methodology:

Preparation of plant flower extract

First, running tap water was used to wash 50 grams of fresh aloe vera flowers, followed by distilled water. The flowers were smashed with a pestle and mortar. Following that, 200 mL of double distilled water and crushed fresh flower were combined in a 250 mL round-bottom flask, which was then placed in a water bath at 80° C. After that, it was removed using regular filter paper and Whatmann No. 1 filter paper. For later usage, the resultant flower extract was kept in the refrigerator.

Cu nanoparticles were produced using a slightly modified procedure from (Egziabher and Michael (2012).
To synthesize copper nanoparticles, combine 30 mL of fresh flower extract with 270 mL of 0.01 M $CH_3COO)_2$ $Cu·H_2O$

The aqueous solution was stirred at 80°C using a magnetic stirrer for approximately 3 hours. The suspension was centrifuged at 3000 rpm for 30 minutes. The supernatant liquid was decanted and the residue was rinsed with 10 mL of distilled water. Impurities were removed using three cycles of centrifugation, decantation, and washing. The precipitate was dried in an oven at 70oC for 30 minutes. The produced copper nanoparticles were further characterized. A bio-reduction technique is demonstrated.

Phytochemical screening of aloe Vera flower extract

Flavonoids (Sodium Hydroxide test)
Alkaloids (Wagner's test)
Test for phenols
Test for tannins
Test for reducing sugars
Test for saponin

Mechanism and Screening of Phytochemicals

Phytochemical screening detects bioactive substances such as tannins, saponins, flavonoids, alkaloids, phenols and reducing agent in flower extracts. Phytochemicals are natural bioactive molecules found in flower that help reduce and stabilize the biosynthesis process . They also play an important function in controlling the morphological structures of CuNPs.

The color shift in the reaction media indicates the reduction of copper salts (precursors) to CuNPs. Screening for biologically active phytochemicals is necessary for their uses in human healthcare, including heart disease, gastrointestinal infections, inflammation, and cancer treatment.

Alkaloids, flavonoids, tannins, and saponins have important roles in several health and clinical disorders (see Table 3 [58-62]). Numerous studies have emphasized the value of therapeutic plants based on phytochemical screening, highlighting the necessity for innovative research to address fundamental health requirements Caroling et al.

Table 1. Health benefits of phytochemicals.

Phytochemicals	**Effects in Human Physiology**
Alkaloids	Antimicrobial properties, drugs discovery
Flavonoids	Antiallergic, antioxidant, therapeutic properties, antimicrobial, oestrogenic enzyme inhibition, vascular, anti-inflammatory, and cytotoxic antitumour activity
Saponins	Gastrointestinal infection along with having antitumor properties
Tannins	Antibacterial activity ,healing of wounds and in bleeding
Phenols	Antioxidant and anti-inflammatory
Reducing sugars	Improved weight management, blood sugar regulation, and cardiovascular health

Table 2. Phytochemical screening of Aloe Vera flower extracts.

S. No.	**Tests for Phytochemicals**	**Amount of Plant Extract**	**Chemical Compounds Added**	**Final Color Changes for Confirmation of Phytochemicals**
1	Tannins	2mL	2 mL of 5% ferric chloride	Dark blue or greenish black
2	Saponins	2mL	2mL of distilled water was added and shaken for 15min	1cm layer of foam on the surface
3	Flavonoids	2mL	1mL of 2N sodium hydroxide	Yellow
4	Alkaloids	2mL	2mL of concentrated hydrochloric acid and a few drops of mayer's reagent	Green or white precipitate
5	Phenols	2mL	2 mL of ferric chloride	Green
6	Reducing sugar	1mL	1 mL of Barfod's reagent heated for 2 min	Red ppt

Green Synthesis of Selected Copper Nanoparticles (F1)

Method 1: Steam Bath

It is used for heating or incubating samples, melting solids, preparing solutions, and maintaining specific temperatures for extended periods

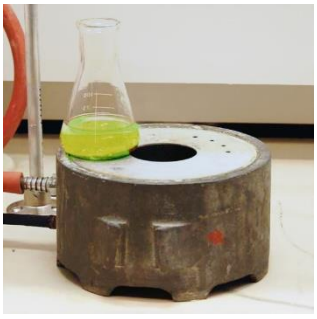


Fig 2. Steam Bath

Method 2: Magnetic Stirrer

A magnetic stirrer is a laboratory device used to mix liquids by creating a rotating magnetic field that spins a magnetic stir bar placed inside the liquid.

Fig 3. Magnetic Stirrer

Characterization Techniques

Characterization of Copper nanoparticles (F1)

1. UV Visible Spectroscopy
2.Fourier Transformed Infrared Spectroscopy
3.Scanning Electron Microscopy
4.Transmission Electron Microscopy
5.Antimicrobial Activity: Agar Well Diffusion Method

1. UV-Visible Spectroscopy Studies

Copper nanoparticles were examined using a UV single beam spectrophotometer. – (UV2510 TS) 300-700 nm with a resolution of 1 nm. Every hour, a tiny aliquot of the material was taken in a cuvette and diluted to the required level for measurements. Absorption peaks were adjusted using the water background spectrum as a reference.

2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy (Affinity) was used to record the Fourier Transform Infrared spectra.

The ATR mode has a wavelength range of 4000 to 750 cm-1 with an 8 cm-1 resolution.
FTIR (Fourier Transformed Infrared Spectroscopy) is used to analyze freshly synthesized nanoparticles.The produced copper nanoparticles were analyzed by Tra Jared Spectrum at 4000-550 c.

Measurements of Fourier transformed infrared radiation (FTIR) were done in order to locate the putative morcules in charge of the sapping of the copper nanoparticles generated by the Aloevera lewers extract and the reduction of Culons. After the process, the residual solution was centitfaged for 14000 rpн to remove any free biomass residue or compounds, such as loose biological contaminants and free proteins or enzymes that aren't the nanoparticles capping ligands. The resultant suspension was redispensed with 10 ml of sterile distilled water. During these times, the centrifuging and dispersion procedures were performed. Overnight, the suspension was dried in an oven at 60°C. A tiny piece of the purified suspemion (0.01 g) was crushed with KBr pellets and exposed to IR spectroscopy in diffase reflectance mode, with a resolution of 2 em in transmittance mode.

3. Scanning Electron Microscopy (SEM)

The size, shape, and morphology of produced copper nanoparteles were studied using scanning electron microscopy (SEM JEOL/JSM6390LA, Jeol India Pvt. Lad).
A dried dispersion of copper nanoparticles generated by reduction. Copper ions and aloevera flower extract were employed for the analysis.

4. Transmission Electron Microscopy (TEM)

The size and morphological features of the produced Cu nanoparticles were measured using a TEM (JEM- 1400 Plus, electron microscope). After lacing and sonicating the CuNPs, carbon- coated TEM grids were drop- coated with Cu nanoparticles in medication for TEM trials. After allowing the film on the TEM grids to dry, the redundant material was removed with blotting paper.

5.Antimicrobial Activity: Agar Well Diffusion Method

CuNPs were produced and estimated for antibacterial efficacity against several mortal conditions using the agar well prolixity fashion (35). The strains studied comprised Staphylococcus aureus ATCC 25,923 as Gram-positive (G ve) bacteria, Pseudomonas aeruginosa ATCC 9027, Klebsiella pneumonia ATCC 13,883 and E. coli ATCC 10,231 as Gram-negative (G- ve) bacteria, and Candida albicans ATCC 10,231 as incentive. The pathogens were fitted into LB broth and dressed for 24 hours at 37 °C before being swabbed unevenly onto sterile Muller- Hinton Agar(MHA) plates with sterile cotton hearties. A sterilized stainless-steel cork borer was used to create agar wells with a diameter of 5mm. Under aseptic circumstances, 50 µl of copper nanoparticle concentrations (60, 80, and 100 µg/ml) were put into four wells using a micropipette. The plates were incubated at 37 °C for 24 hours, after which the zone of inhibition was measured using a centimeter ruler, and the result for each organism was recorded and represented in millimeters.

Result and Discussion:

1) UV Visible Spectroscopy -

The generation of copper ions in solution was measured using a UV- Visible spectrophotometer. The absorption peak conforms the formation of copper nanoparticles**.**

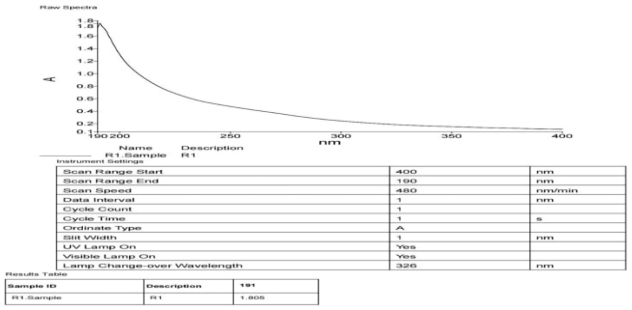


Fig 4. UV- UV-Visible spectra of green synthesized copper nanoparticles

2) Fourier Transformed Infrared Spectroscopy -

The FTIR analysis was used to determine the molecules and /or functional group that are probably present in synthesized copper nanoparticles

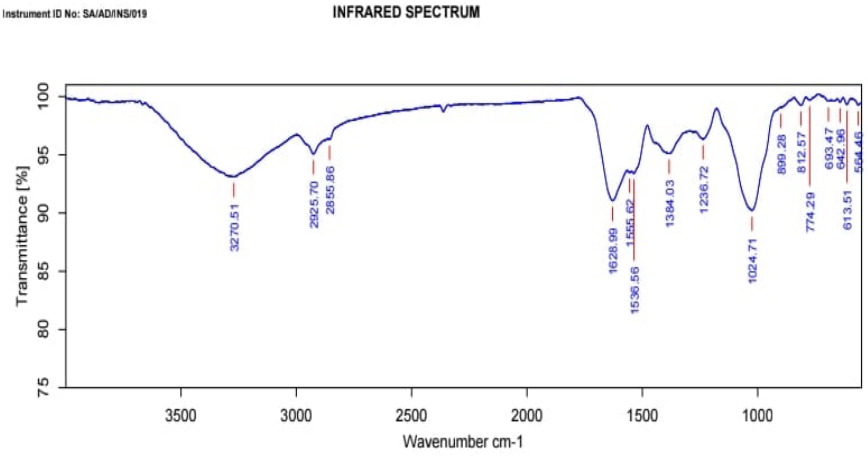
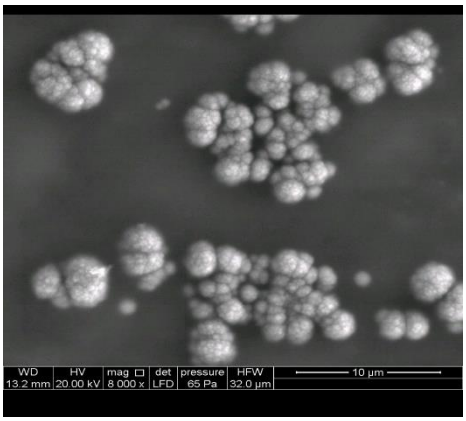
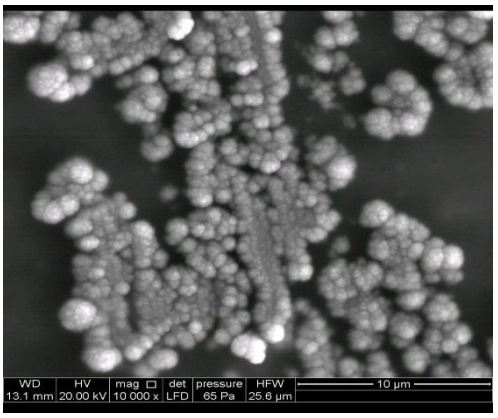


Fig 5. FTIR spectra of green synthesized copper nanoparticles

3) Fourier Transformed Infrared Spectroscopy -

The size of prepared nanoparticles was analysed in different magnification power and pressure.





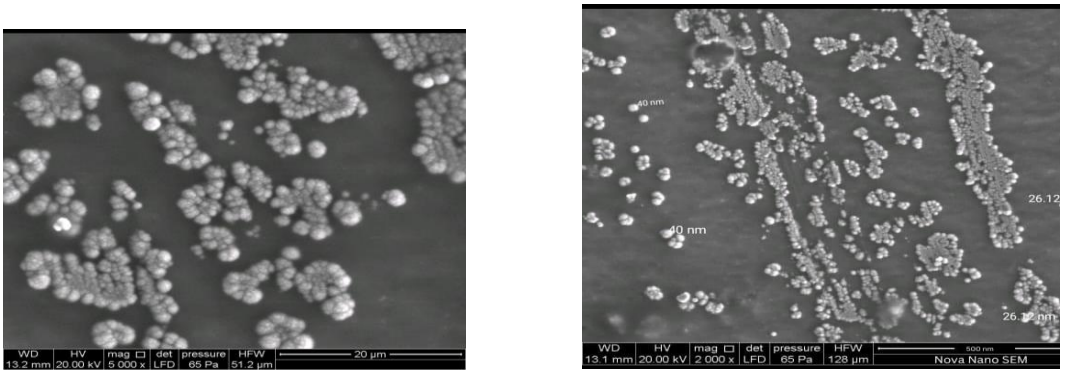


Fig 6. The Scanning Electron Microscope Image of Synthesized Copper Nanoparticles (F1)

4) Transmission Electron Microscopy (TEM) –

The size of prepared nanoparticles was analysed in different magnification power and pressure.

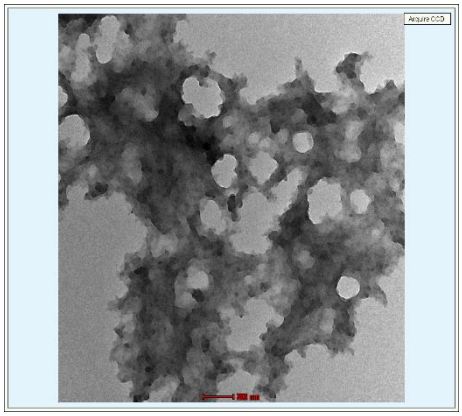


Fig 7: TEM image of synthesized Copper Nanoparticles (F1)

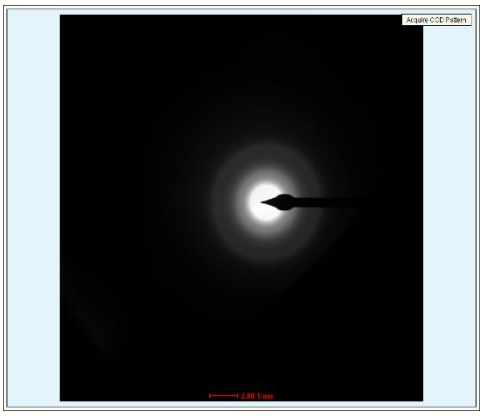


Fig 8. SAED Pattern image of Copper Nanoparticles

5) Antimicrobial Activity: Agar Well Diffusion Method –

CuNPs were produced and estimated for antibacterial efficacity against several mortal conditions using the agar well prolixity fashion (35)

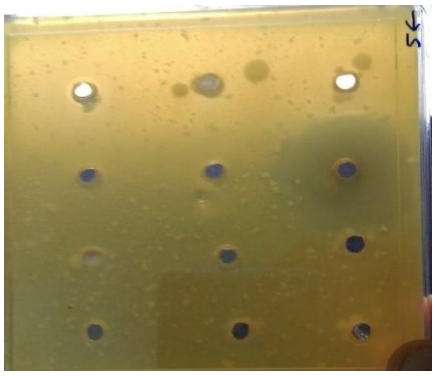


Fig 9. Zone of inhibition against the bacteria (Staphylococcus aureus)

Table 3: Zones of inhibition (Diameter mm)

Solvents	1:1	1:10	1:20
Dimethyl Sulfoxide	17 mm	--	--
Methanol	--	--	--
Ethanol	--		
Reference (Ampicillin)	32 mm	--	--

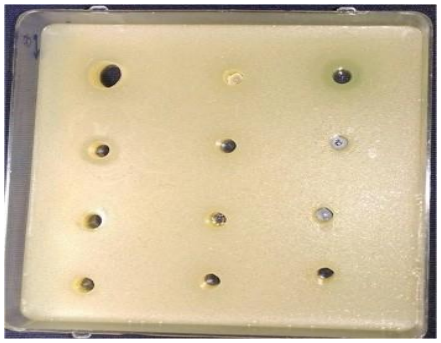


Table 4: Zones of inhibition (Diameter mm)

Solvents	1:1	1:10	1:20
Dimethyl Sulfoxide	15 mm	--	--
Methanol	11 mm	--	--
Ethanol	--		
Reference (Ampicillin)	17 mm	--	--

Fig 10. Zone of inhibition against the bacteria (E Coli)

Conclusion

Aloe Vera is botanically known as Aloe barbadensis belonging to family Liliaceae. It is a crucial component of traditional medicine system such as Ayurveda, Homoeopathy, Siddha and Unani.

This study presented the eco-friendly, cost effective green synthesis of copper nanoparticles by using Aloe Vera flower extract (Aloe barbadensis) as a reducing agent in the presence of copper acetate monohydrate. The phytochemical analysis of extract was carried out. The presence of phytochemicals such as alkaloids & flavonoids played important role in reducing and capping agent during formation of copper nanoparticles.

The maximum production of copper nanoparticles was noted at 50°C after 30 minutes of reaction was carried out on a steam bath.

The Uv visible spectroscopy confirmed its formation with absorption peak at 191nm. The Fourier Transformed Infrared Spectroscopy confirmed the presence of functional groups. The scanning electron microscope and Transmission electron microscope showed the average copper nanoparticles size 26.12 to 40 nm and with a spherical shape and highly crystalline. Antimicrobial activity of synthesized copper nanoparticles was performed using a agar well diffusion method.

Ampicillin (50µg .ml−1) was used as reference and Muller Hinton agar medium are used. DMSO, Methanol, Ethanol were used as a control for the preparation nanoparticles suspension. The synthesized copper nanoparticles showed significiant antimicrobial activity against staphylococcus aureus and Escherichia coli.

It can conclude that green synthesized copper nanoparticles by using aloe Vera flower extract was environment friendly with low cost and have significiant antimicrobial activity.

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