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EVALUATION OF ANTIMICROBIAL PROPERTIES OF GARLIC-MEDIATED NANOPARTICLES

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Abstract - *Green synthesis of nanoparticles using plant extracts has emerged as a sustainable alternative to conventional chemical methods. This study focuses on the preparation and antimicrobial evaluation of nanoparticles synthesized using garlic (Allium sativum) extract as a natural reducing and stabilizing agent. Garlic contains bioactive compounds such as allicin, organo sulfur molecules, and flavonoids, which facilitate nanoparticles formation and contribute to their biological activity. The synthesized nanoparticles were characterized using UV–Visible spectroscopy, and FTIR to confirm particle formation, functional group interactions. Antimicrobial activity was assessed against selected Gram-positive and Gram-negative bacterial strains using agar well diffusion techniques. The garlic-mediated nanoparticles demonstrated significantly higher antimicrobial activity compared to crude garlic extract, suggesting that nanoscale size and enhanced surface reactivity improve their interaction with microbial cells*.

***Key Words*: Nanoparticles, Bioactive compound, UV-Visible, Diffusion, Microbial, Flavonoids, Synthesis**

1. INTRODUCTION

The evolution of nanotechnology has attracted young researchers towards eco-friendly biosynthesis of nanoparticles. Plant extracts containing biomolecules can be used to reduce metal ions to nanoparticles. The reduction of metal ions can be carried out readily at room temperature and pressure, and easily scaled up[1].These findings highlight the potential of garlic-mediated nanoparticles as efficient antimicrobial agents suitable for biomedical, pharmaceutical, and food-safety applications.[2] The green synthesis approach also offers advantages such as simplicity, cost-effectiveness, and environmental safety. Overall, this study supports the development of plant-based nanoparticle synthesis as a promising alternative to traditional antimicrobial strategies. Further research on toxicity assessment, stability, and application-specific optimization is recommended to expand their practical use.

2. EXPERIMENTAL

2.1. Materials

Copper sulphate, distilled water (all at high laboratory standard) were used in this study, Allium sativum extract was used as the reducing agent for the preparation of the copper nanoparticles which was also used as the stabilizer for the newly synthesized nano sized copper colloids, Muffle furnace, Hot air oven, Mortar and Pestle.

2.2. EXPERIMENTAL PROCEDURES:

2.2.1 Preparation of the extract of Allium sativum and the synthesis of copper nanoparticles:

Aqueous extract of Allium sativum was prepared using fresh bulbs of garlic (10 g) which were collected from local market. The surfaces of the bulbs were cleaned with running tap water followed by distilled water and subsequently boiled in 100 ml distilled water at 120° C for 10 min. The extract was filtered through Whatman No.1 filter paper and filtrate was collected.

2.2.2 Synthesis of the Copper Nanoparticles:

Copper sulphate solution was prepared using 100 ml of deionized water. To 50 ml of above solution, 50 ml of extract of Allium sativum was added and placed in the magnetic stirrer at 40°C turned for 3 hours. The colour of the solution turned from dark green to pale green which indicated the formation of nanoparticles. The formed copper nanoparticles were dried at 100° C for an hour. Dried green coloured copper nanoparticles were collected and stored in air tight container.

3. RESULTS AND DISCUSSION

3.1. ULTRAVIOLET VISIBLE SPECTROSCOPY

UV – visible absorption spectrum of copper nanoparticle is shown in Figure 1. The characteristic absorption peak at around 575 nm is due to the surface plasmon band of Cu colloids [3]. The strong surface plasmon absorption band observed at 575 nm may be due to the formation of non-oxidized Cu NPs. The broadness of the absorption band probably arises from the wide size distribution of copper nanoparticles.

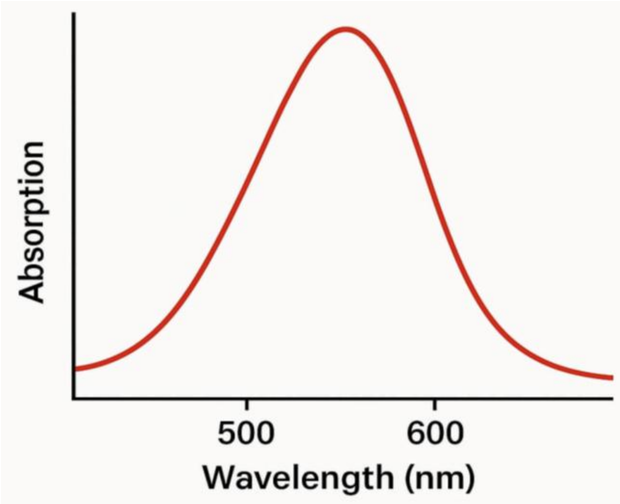

Fig-1: UV Spectrum of Cu- Allium Sativum NPs

3.2. FT-IR SPECTROSCOPY

FT-IR spectroscopy, a type of vibrational spectroscopy, is used to identify the stretching and bending frequencies of molecular functional groups attached to Cu NP surface. The FTIR spectrum of biosynthesized Cu NPs is presented in Figure.2. Which shows a number of absorption peaks at different IR frequencies. Broad bands at around 3200-3600 cm^{-1}, 1600-1800 cm^{-1} and 2400 cm^{-1} attribute for the presence of (OH) stretching of phenolic group, (>C=O) and (>NH) stretching. Hence, FTIR spectra of copper nanoparticles confirmed the presence of various biomolecules such as saponins, tannins, flavonoids, alkaloids and polyphenols etc. surrounding Copper nanoparticles which are responsible for reduction as well as in the stabilization of nanoparticles [4]

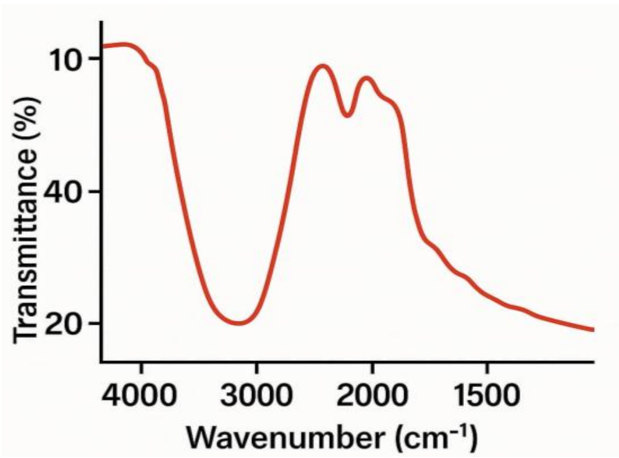

Fig-2: IR Spectrum of Cu- Allium Sativum NPs

3.3. MICROBIAL ACTIVITY

To assess the antimicrobial activity of the drug, 100mg of the synthesized nanoparticle was dissolved in 1ml of DMSO, mixing thoroughly until the drug was completely dissolved. This stock solution was then used to prepare test samples at concentrations of 10, 20, 40, and 60 micrograms (µg) for antimicrobial assays.The antimicrobial activity of the sample was evaluated using a well diffusion assay against three microorganisms: A) *Limosilactobacillus fermentum*, B) *Escherichia coli*, and C) *Candida albicans[5]*.Preparation of Culture Broth: Nutrient broth was prepared by dissolving 13g per litre in the required volume and sterilised using standard autoclaving methods. The target microorganisms were inoculated into the sterilized broth and incubated at 37°C for 24 hours.

3.4.WELL DIFFUSION ASSAY

The antimicrobial activity of the sample was assessed using a well diffusion assay. Mueller-Hinton agar medium was prepared at a concentration of 38g/L and supplemented with 1% agar agar. The medium was sterilised by standard autoclaving and then poured into petri dishes, allowing it to solidify undisturbed.

After solidification, the prepared culture broth was evenly swabbed onto the surface of the Mueller-Hinton agar. Wells of 6mm diameter were made in the agar, and test samples at varying concentrations (10, 20, 40, and 60µg), labelled II, III, IV, and V, were added to the respective wells. Ampicillin (10mcg) served as the standard reference and was labelled I. The plates were incubated at 37°C for 24 hours. After incubation, the antimicrobial effect was evaluated by measuring the zone of inhibition (in millimetres) around each well, with results recorded in table 1.

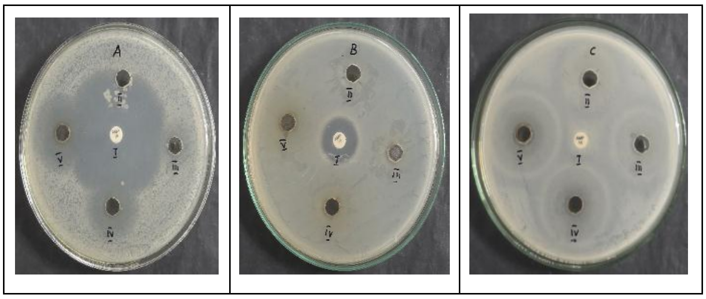

Fig-3: Effect of a) *Limosilactobacillus fermentum*, b) *Escherichia coli*, and c) *Candida albicans* on Cu-Allium Sativum nanoparticles

Table -1: Mean of zone of inhibition obtained by Cu-Allium Sativum nanoparticles against A) *Limosilactobacillus fermentum*, B) *Escherichia coli*, and C) *Candida albicans*

Organism	Zone of inhibition (mm)												
	I-Amp	II-10µg			III-20µg			IV-40µg			V-60µg		
	Zone	Zone	Reduced Microbial load	Diffusion	Zone	Reduced Microbial load	Diffusion	Zone	Reduced Microbial load	Diffusion	Zone	Reduced Microbial load	Diffusion
A	51	-	-	-	10	-	-	10	-	18	12	-	20
B	18	-	-	-	-	-	14	-	-	16	-	-	18
C	-	-	-	22	-	-	24	-	18	28	9	19	30

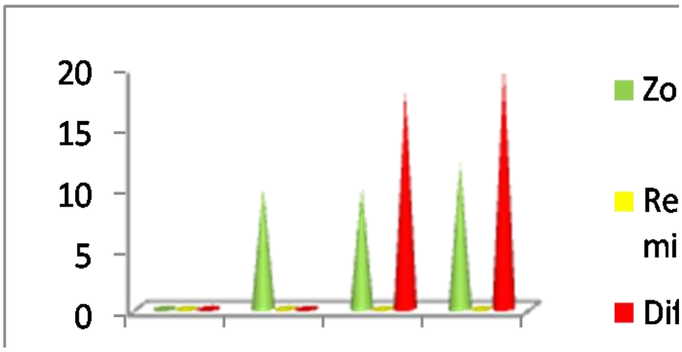

Fig- 4 : Antimicrobial activity Zone of inhibition obtained by Cu-Allium Sativum nanoparticles by well diffusion method

Many researchers have shown that Cu-garlic extracts have a great antibacterial activity against *Limosilactobacillus fermentum[6].* The phytochemicals present in cu -garlic nanoparticles are responsible for their antibacterial activity [7].The sample demonstrated greater antimicrobial activity against the Gram-positive organism at concentrations of 20, 40, and 60µg.Additionally, notable activity was observed against *Candida albicans* at 40 and 60µg, while no inhibition zones were detected for Gram-negative organisms

4. CONCLUSIONS

Bio-synthesis of copper nanoparticles using Allium sativum (garlic) extract was successfully carried out at room temperature. During the synthesis, biomolecules present in the garlic extract are mainly responsible for the reduction and stabilization of the nanoparticles. Optical properties, compositional analysis and functional group content of as-synthesized copper nanoparticles were studied using UV-Vis spectrophotometer and Fourier Transform Infrared Spectroscopy (FTIR) respectively. FTIR analysis illustrated the presence of chief functional groups; phenol, carbonyl and amide groups at the surface of CuNPs which are responsible for reducing and stabilizing the nanoparticles.

Cu Nps have been widely used for antimicrobial and biomedical products. Present study concluded that, the Cu Nps has strong antibacterial activity. The significance of over results is that CuNps solutions have a significant antibacterial activity against the Gram-positive organism. These nanoparticles may capable of entering into the bacterial cell through cell wall and cell membrane, where they might inhibit the enzymatic activity and finally stopped the growth and division of bacterial cell. Hence use of our nanoparticles may help in inhibiting the studied bacterial growth.

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