SYNTHESIS OF COPPER NANOPARTICLES(CU-NPS) AND IT'S APPLICATION

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Abstract

Chemicals make up the majority of microbiological growth inhibitors which are toxic and chemical solvents are bad for human health. Copper, silver, and gold metallic nanoparticles have several uses in biotechnology and medicinal research. Potent antifungal, antibacterial, anti-inflammatory, and anti-proliferative drugs are copper nanoparticles (CuNPs). In this study, nutmeg, a seed from the *Myristica fragrans* plant, was used to biogenically manufacture copper nanoparticles. The production of *Myristica fragrans* CuNPs involved dissolving copper sulphate in the extract. FTIR, EDX, and SEM were used to describe them. These CuNPs are between 20 µm and 200 nm in size, according to SEM tests. Copper and oxygen were detected, and the metal's oxidation state was validated by EDAX analysis. The nanoparticles' distinctive functional groups were discovered using FTIR spectroscopy. The anti-fungal activity of *Myristica fragrans* seed extract was assessed. The extract from the seeds of *Myristica fragrans* and caffeic acid ,a bioactive component exhibited the strongest antifungal activity. According to these findings, antifungal CuNPs Phyto-formulated with nutmeg extracts may one day be employed to treat microbial diseases.

Keywords: *Myristica fragrans, Caffeic acid, Copper nanoparticles, Scanning Electron Microscopy*

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1.Introduction

Due to their safety and absence of adverse effects, the use of medicinal and aromatic herbs to make antimicrobial medications has lately gained favour [1,2]. Herbal treatments have historically been used by many cultures to treat a variety of illnesses. India's food depends heavily on spices. Additionally, they contain a lot of potent anti-fungal chemicals [2]. Because they differ from bulk materials in terms of size, structure, dispersion, and morphology, nanoparticles hold the attention of many researchers [3,4,5]. Metallic nanoparticles are used in a variety of scientific and medical disciplines. Scientists continue to experiment with new scopes because of their small size. These metal nanoparticles exhibit outstanding chemical and optical qualities, great electrical conductivities, tiny diameters, and huge surface areas [6]. Copper is one of the many metallic nanoparticles that has attracted a lot of attention. Scientists are fascinated by plant-based molecules because of their variety in shapes, sizes, and biological functions [7]. Copper nanoparticles are used in materials such as multilayer metal ceramics and batteries. Additionally, they offer antibiotics, anti-oxidants, and medications [8]. From a medical and Phyto medical standpoint, turning an antibiotic into a pharmaceutical sounds like a wonderful idea. The anti-oxidant, anti-inflammatory, anti-hepatotoxic, anti-tumor, and anti-microbial properties of spices are well documented. Nutmeg contains antimicrobial compounds that have biological effects. Spices include active plant components that are necessary for these effects. Nutmeg's antibacterial properties are related to secondary metabolites, which are typically safe for food or have negative effects [9]. The copper nanoparticle surface-to-volume ratio is high. They eliminate fungus and bacteria. Antifungal action is produced by metal ions released into solutions and intimate contact with bacteria. If they make touch with the lipid membrane, they might kill. As a result of membrane lipid degradation and membrane collapse caused by free radicals, intracellular molecules can pass through. Necrobiosis results from this [10]. Recently, green synthesis has been employed to create nanoparticles using Phyto-derived bioactive chemicals [11]. Biogenic nano synthesis benefits the environment by using natural reducers and stabilisers [12]. Spices are plant-based bioactive seasonings [13, 14]. CuNPs were created in this study using nutmeg extract, and their anti-fungal effectiveness was tested.

2. Materials and Methods :

2.1.Plant material Collection:

Myristica fragrans (nutmeg) dry seeds were obtained from a nearby local source. The filth and other foreign matter was removed, and the area was carefully cleansed with twice-distilled water. The plant's active ingredients have to be extracted by using a mortar and pestle to pound the dried nutmeg seed into a uniform size [15].

2.2.Chemicals :

This experiment makes use of Copper sulphate, sodium carbonate, Folin-Ciocalteu reagent, methanol, and distilled water, among other materials.

2.3 Copper Nanoparticle Biosynthesis:

Only chemical reagents of analytical grade were used in this experiment. Nutmeg, or *Myristica fragrans,* was purchased at a shop in Visakhapatnam, India. In the dark, at 35°C, dried spice seeds were ground into tiny pieces and put in a jar before being sealed. Normally, 50 mL of water was used to dissolve 5 g of dried nutmeg spice. An 80°C heating mantle was used to heat the aqueous solution containing the seed material for 20 minutes before it was filtered through Whatmann No. 1 paper. Fresh 0.5 M copper sulphate solution was prepared, mixed 1:1 with dried seed extract, and then left at 37°C in the dark for 24 hours. Visual inspection revealed that the solution's colour had changed from blue green to brownish red (Fig.1A&B). *Myristica fragrans* seed extract was used to successfully create CuNPs [16,17].

A.Without Copper sulphate B.With Copper sulphate

Fig.1. Color change of *Myristica fragrans* seed extract from blue green to brownish red (A&B)

2.4. Characterization of Biogenic CuNPs :
Initially, biogenically produced CuNPs were evaluated by analytical UV-Vis spectroscopy. One millilitre of biologically produced test samples and one millilitre of a copper sulphate solution devoid of plant extract were used to assess copper ion reduction. Scanning electron microscopy was used to examine the copper particle's size and surface texture (SEM). Energy-dispersive X-ray (EDX) spectroscopy analysis was used to determine the presence of copper and the elemental compositions in synthesised particles. A detection graph was used to analyse the presence of copper. The percentage of total weight used to calculate elemental composition. A dried pellet of synthetic copper nanoparticles was studied using Fourier-Transform Infrared (FTIR) Spectroscopy while it was kept at 37°C in the dark for 24 hours [18]. Visual inspection revealed that the solution's colour had changed from blue green to brownish red. *Myristica fragrans* seed extract was used to successfully create CuNPs [17,18].

2.5. CuNPs Characterization :
SEM, elemental analysis, and Fourier transform infrared spectroscopy (FTIR) were used as methods for characterising the samples .Scanning electron microscopy was used to examine the copper nanoparticles' surface size and shape (SEM). SEM images of copper nanoparticles produced by *Myristica fragrans* seed extract obtained through the bioreduction process are shown in Fig.2A and B. The size of the copper nanoparticles, according to the results, ranged from 20 nm to 200 nm. [19,20]

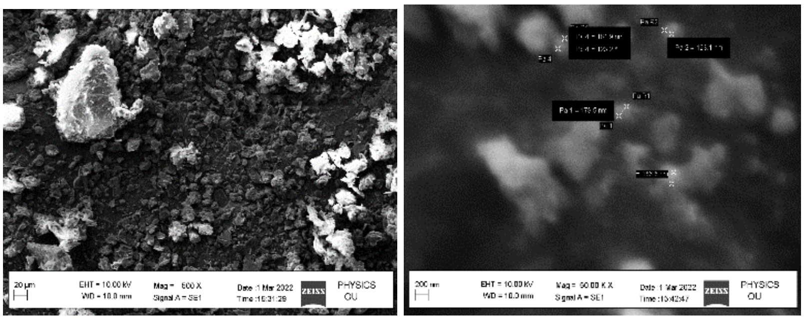

Fig.2. A&B: SEM micrographs of Copper nanoparticles with diameters of 20 µm to 200 nm.

2.6.Energy dispersive X-ray spectroscopy(EDX):
The SEM-EDX results obtained in Fig.3 and Table.1.confirm the presence of copper nanoparticles.

Element	Weight(%)	Atomic(%)
CK	63.22	73.20
OK	28.28	24.58
PK	1.58	0.71
Cu L	6.92	1.51
Total	100	

Table.1.Elemental composition of *Myristica fragrans* assisted Copper nanoparticles

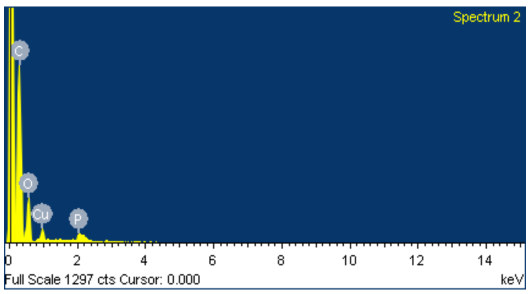

Fig.3.SEM- Energy Dispersive X- spectroscopy analysis of CuNPs

3. Results and Discussion:
The batch extraction process's physicochemical parameters were optimized[16]

3.1.Effect of various solvent percentages on Caffeic acid extraction*:*
It has been shown that mixes of methanol with different concentrations of water are more effective

at extracting phenolic compounds than a monocomponent solvent solution. An organic solvent usually becomes a more polar medium when a modest bit of water is added, making the extraction of polyphenols simpler. By extracting the dried *Myristica fragrans* (nutmeg) seed powder with different percentages of methanol (20%, 40%, 60%, 80%, and 100%), the maximum extraction yield of Caffeic acid was discovered. The results are shown in Fig.4.

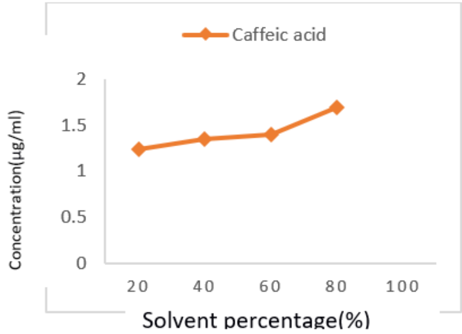

Fig.4. The concentration of Caffeic acid vs Solvent percentage(%)

The results showed the maximum concentration of Caffeic acid is 1.891 µg/ml was observed at 60% methanol.

3.2.Effect of soaking time on Caffeic acid extraction:

Soaking time is another important factor to consider when optimizing Caffeic acid extraction. The nutmeg seed powder was steeped in 60 percent methanol for 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 160 minutes, and the extraction result was observed as shown in Fig.5.

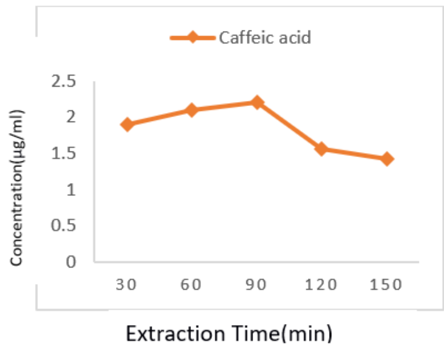

Fig.5. The concentration of Caffeic acid vs Extraction Time(min)

Caffeic acid content was lost when the extraction duration was extended beyond 120 minutes. The lower the level of polyphenol recovered, the longer the extraction time. This might be due to the oxidation of phenolic chemicals, which then polymerize into insoluble molecules. At 90 minutes of incubation of nutmeg seed powder in 60 percent methanol, greater concentrations of Caffeic acid of 2.202 µg/ml were detected.

3.3.The Effect of pH on Caffeic acid Extraction:

At varied pH levels of 5,6,7,8,9, and 10, the extraction yield of components was measured. Fig.6 depicts the results.

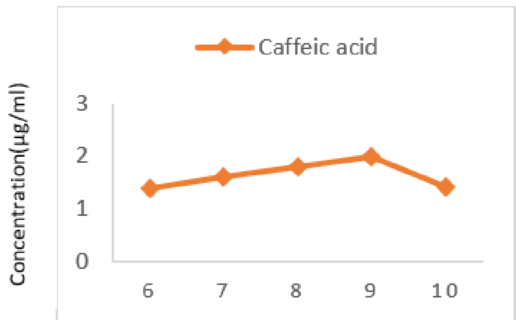

Fig.6. The concentration of Caffeic acid vs pH

The highest concentrations of Caffeic acid of 1.978 μg/ml were reached at pH-9 for 90 minutes using 60 percent methanol as a solvent.

3.4. The Effect of Temperature on Caffeic acid Extraction:

The success of the extraction process of phenolic compounds is highly governed by several parameters, the most important of which is the extraction temperature. Extraction at various temperatures, such as 32°C, 34°C, 36°C, and 38°C, was employed to obtain the highest yield of Caffeic acid .Fig.7 depicts the outcomes.

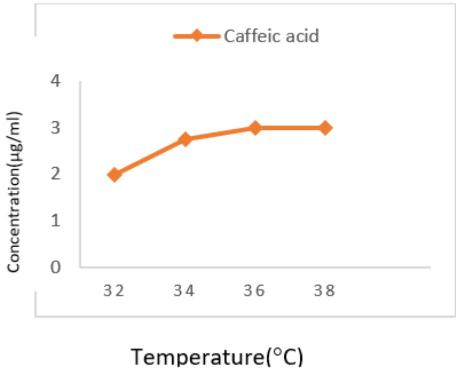

Fig.7. The concentration of Caffeic acid vs Extraction Temperature

The highest concentrations of Caffeic acid of 2.985 μg/ml were reached at pH-8 for 90 minutes using 60 percent methanol as a solvent at 38°C .

3.5.Fourier Transform Infrared Spectroscopy Analysis[FTIR]:

To discover possible interactions between protein and Copper nanoparticles, FTIR investigations of green-produced Copper nanoparticles were done. For the adsorbent properties of phytochemicals, FTIR analysis revealed possible biomolecules in plant extracts responsible for stabilising CuNPs. These bands are found in the FT-IR spectra of green-synthesized nutmeg nanoparticles (Fig.8). These functional groups were found in green-synthesised nutmeg nanoparticles, indicating the presence of esters, alcohols, and phenolic compounds. For example, alkaloids, steroids, tannins, flavonoids, phenolics, and glycosides are known secondary metabolites with antimicrobial properties[18,19] .Their antimicrobial properties are attributed to these functional groups.

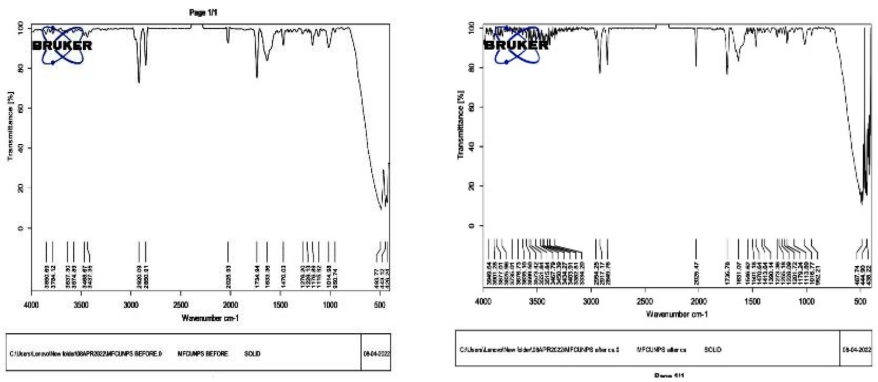

Fig.8. A&B: FT-IR measurement of green produced CuNPs before & after extraction.

Nutmeg nanoparticle FT-IR spectra (Fig.8.A&B) obtained revealed bands at 3334.20 cm^{-1} corresponding to –OH stretching, proving the presence of carboxylic acid –OH; 2954.25 cm^{-1} corresponding to C–H stretching of alkane C– H; 1736.78 cm^{-1}corresponding to C = O stretching of the ester group; 1470.64 cm^{-1} for Esters, phenolic compounds, and alcohols were found to be present in green-synthesised nutmeg nanoparticles, similar to findings from a previous study[16]. *Myristica fragrans* seeds were found to contain carbonyl groups, alkane groups, and ether groups as functional groups of biological compounds such as alkaloids, steroids, tannins, flavonoids, phenolics, and glycosides, all of which are known to have antimicrobial properties[21]. The antimicrobial properties of these functional groups have been determined to be a result of this conclusion.

3.6.Antifungal activity testing:

The effects of *Myristica fragrans* seed extract's antifungal properties on the *Saccharomyces cerevisiae* yeast strain. We assessed the antimicrobial effects of synthesised Copper nanoparticles against *Saccharomyces cerevisiae* using a well-diffusion process Fig.9. Gentamicin served as one of the experiment's positive controls[19]. 2.42± 0.049 cm ZOI was discovered. The plate was then incubated for 48 hours at 30°C. Zone of Inhibition (ZOIs) of 1.16± 0.078 cm was observed for CuNPs produced with *Myristica fragrans* (nutmeg) against yeast strain. The lowest extract concentration that, as compared to control, resulted in an 80% reduction in detectable growth—as well as when the mic value was 100 g/ml or less was identified as the Zone of Inhibition. The antifungal activities of the extracts increased with seed extract concentration (mg/ml). The test Fungi was shown to be resistant to the methanolic extract of *Myristica fragrans*. The results showed that *Saccharomyces cerevisiae* is susceptible to these strong, dose-dependent antifungal copper nanoparticles.

Fig.9. Zone of inhibition of *Myristica fragrans* CuNPs against *Saccharomyces cerevisiae.*

4. Conclusion: Microbiological growth can be inhibited by the use of chemical solvents. On the other hand, chemical solvents pose a health hazard to users and have only weak antibacterial properties. In biotechnology and biomedicine, nanoparticles made of metallic elements (such as copper, silver, and gold) have many applications. Copper nanoparticles (CuNPs) are effective because of their antimicrobial, anti-inflammatory, and anti-proliferative properties. The goal of this study was to produce copper nanoparticles using nutmeg, a seed from the *Myristica fragrans* plant, and to study their antibacterial properties. Seed extract was used to dissolve *Myristica fragrans* CuNPs in order to create *Myristica fragrans* CuNPs and was described using FTIR, EDX, and SEM analysis. The diameters of these CuNPs are between 20µm and 200 nm, according to SEM analysis. EDX analysis confirmed the presence of copper and oxygen, as well as the fact that copper had been oxidised. Nanoparticles produced by this method contained distinct functional groups, as demonstrated by FTIR spectroscopy. *Myristica fragrans* seed extract was tested for its antibacterial properties using growth zone inhibition. The seed extract of *Myristica fragrans* has the best antibacterial properties, according to the research. Antibacterial CuNPs phyto-formulated with nutmeg extracts, according to these findings, could be used to treat bacterial diseases.

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