

GREEN SYNTHESIS OF COPPER NANOPARTICLE BY USING FRESH LEAVES OF MORINDA CITRIFOLIA AND EVALUATION OF ITS IN-VITRO ANTIBACTERIAL ACTIVITY

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Abstract

The present study informed on the phytochemical composition, antibacterial activities of the leaves juice of Morinda citrifolia. Thus, the major groups of secondary metabolites observed on that juice are polyphenols, alkaloids and glycosides. These results are consistent with those of several authors who have worked on the fruits and leaves of this plant. Morinda citrifolia is a plant with broad nutraceutical and therapeutic effects and used in the traditional treatment of several ailments. The objective of this work is to investigate the phytochemistry of the leaves of M. citrifolia on one hand and on other hand to evaluate antibacterial activity. The phytochemical investigation was carried out by using ethanolic extract leaves of M. citrifolia. Concerning the antibacterial activity, it was tested on the in vitro growth of Escherichia coli.

Keywords: Morinda Citrifolia, Polyphenols, alkaloids, glycosides

I. INTRODUCTION

A. Nanoscience and Nanotechnology: An Emerging Frontier

Nanoscience and nanotechnology have seen explosive growth in recent years, emerging as key interdisciplinary fields with vast applications worldwide. At the core of this field are nanomaterials, which play a critical role in shaping modern technologies. In research and development,

nanomaterials are increasingly utilized in a variety of sectors, including energy, environment, water purification, and pharmaceuticals. Their applications range from energy storage devices like fuel cells to enhancing security in defense systems, advancing drug delivery methods, and purifying water. The industrial revolution has significantly improved the quality of life, and without the contributions of nanoscience and nanotechnology, innovations such as high-speed personal computers and mobile communication would not be possible.

B. Defining Nanomaterials

The European Union's Commission defines nanomaterials as substances containing particles that are either natural, incidental, or manufactured. These particles are unbound or exist as aggregates or agglomerates, and at least 50% of the particles, based on the number size distribution, must have one or more external dimensions in the range of 1 to 100 nanometers. For context, a nanometer is about one millionth of a millimeter, approximately 100,000 times smaller than the width of a human hair. In essence, nanomaterials refer to materials where one or more dimensions of a single unit are between 1 and 100 nanometers, commonly referred to as the

nanoscale. While some nanomaterials occur naturally, engineered nanomaterials (ENMs) are of particular interest due to their widespread use in commercial products and processes. These materials can be found in everyday items such as sunscreens, cosmetics, sporting goods, stain-resistant fabrics, electronics, and tires. Furthermore, they hold significant promise in medicine, where they are employed for diagnostic purposes, imaging, and targeted drug delivery.

C. The Expansion of Nanophase Engineering

Nanophase engineering has rapidly expanded across a broad range of materials, both inorganic and organic. This field allows for the manipulation of properties such as mechanical, catalytic, electrical, magnetic, optical, and electronic functions. Nanomaterials are often created by assembling small clusters that are then fused into bulk-like materials or embedded into solid or liquid matrices. For instance, nanophase silicon, which differs from conventional silicon in both physical and electronic properties, holds potential for advanced semiconductor applications. Similarly, when glass is doped with quantum semiconductor "colloids," it can become a high-performance optical medium with applications in optical computing.

In 2016, the Nobel Prize in Chemistry was awarded to Jean-Pierre Sauvage, Sir J. Fraser Stoddart, and Bernard L. Feringa for their groundbreaking work in developing nano-molecular machines. These molecular-scale machines, which are approximately a thousandth of the width of a human hair, have the potential to revolutionize industries such as

manufacturing, materials science, medicine, and even human biology.

II. Unique Properties of Nanomaterials

Nanomaterials stand out due to their unique properties that emerge as their size approaches the nanoscale. Unlike bulk materials, where physical properties remain constant regardless of size, the properties of nanomaterials change significantly due to their small size and high surface area-to-volume ratio. At the nanoscale, surface atoms make up a larger proportion of the material's total atom count, which influences chemical reactivity and other material characteristics.

The key reasons behind the distinct properties of nanomaterials are their increased relative surface area and the emergence of quantum effects. As nanomaterials exhibit a much higher surface-to-volume ratio than their larger counterparts, they are often more chemically reactive and stronger. Moreover, quantum effects become more pronounced at the nanoscale, leading to new optical, electrical, and magnetic behaviors, further distinguishing these materials from their bulk counterparts.

III. Physical Properties of Nanoparticles

Nanoparticles exhibit distinct characteristics due to their large surface area, which often dominates the physical contributions from the material's bulk. For example, zinc oxide nanoparticles display superior ultraviolet (UV) blocking properties compared to their bulk counterpart, which is why they are commonly used in sunscreen formulations. Similarly,

nanoparticles have unique optical properties; gold nanoparticles, for instance, display a deep red to black color in solution, which is not seen in bulk gold. Gold nanoparticles also have a much lower melting point (around 300°C for nanoparticles as small as 2.5 nm) compared to bulk gold (which melts at 1064°C). Additionally, when used in photovoltaic cells, nanoparticles show a significantly higher absorption of solar radiation compared to thin films of bulk materials, due to their smaller size and increased surface area.

One of the important physical properties of nanoparticles is their ability to form suspensions. This is due to the strong interaction between the particle surface and the solvent, overcoming the usual density differences observed in bulk materials that either sink or float. Furthermore, nanoparticles exhibit quantum effects, such as quantum confinement in semiconductor particles, surface plasmon resonance in metal particles, and super paramagnetism in magnetic nanoparticles. These unique phenomena make them valuable in various applications, including magnetic materials and memory storage.

Nanoparticles also possess diffusion properties, particularly at elevated temperatures, allowing sintering to occur at lower temperatures and shorter times compared to larger particles. Although this can affect the final product's density, it also increases the likelihood of particle agglomeration. On the other hand, nanoparticles made from materials like clay can reinforce polymers, resulting in stronger plastics when incorporated into the matrix. These

nanoparticles are also used in textiles to create smart, functional clothing.

In the realm of medical applications, soft or semi-solid nanoparticles, such as liposomes, are manufactured for drug delivery systems. These are widely used in the clinical delivery of anticancer drugs, antibiotics, antifungal drugs, and vaccines.

IV. **Classification of Nanomaterials**

Nanomaterials can be categorized in several ways, with the most common being by their dimensionality, origin, and structural configuration. The following classification is based on the dimensionality of the nanomaterial.

A. Zero-Dimensional(0-D)Nanomaterials

These nanomaterials have nanoscale dimensions in all three directions. Examples include metallic nanoparticles, such as gold and silver nanoparticles, and semiconductor quantum dots. Typically spherical in shape, their size ranges from 1-50 nm. They can be amorphous or crystalline, single or polycrystalline, and can consist of one or multiple chemical elements. These nanoparticles can exist individually or as part of a matrix, and may be metallic, ceramic, or polymeric in nature.

B. One-Dimensional (1-D) Nanomaterials

One dimension of these materials is outside the nanoscale range. Examples of 1-D nanostructures include nanowires, nanorods, and nanotubes. These materials are often long (several micrometers in

length) with a diameter of just a few nanometers. Nanowires and nanotubes made from metals, oxides, and other materials fall into this category. These nanoparticles may be crystalline or amorphous, pure or impure, and may exist independently or embedded within a medium. These materials can also be metallic, ceramic, or polymeric.

C. Two-Dimensional (2-D) Nanomaterials

In these nanomaterials, two dimensions exceed the nanoscale range, while the third remains within it. Examples include thin films, nano-coatings, and multilayer structures. The surface area of 2-D nanomaterials can be relatively large (several square micrometers), while the thickness is always in the nanometer range. Like 0-D and 1-D materials, 2-D nanomaterials can be crystalline or amorphous, consist of various chemical compositions, and may be deposited on substrates or integrated into matrices. They may be metallic, ceramic, or polymeric.

D. Three-Dimensional (3-D) Nanomaterials

Three-dimensional nanomaterials are bulk materials composed of individual components that are in the nanometer scale, typically ranging from 1 to 100 nm. These materials may contain dispersions of nanoparticles, bundles of nanowires, nanotubes, or multiple nanolayers. They represent more complex structures and offer enhanced properties compared to traditional bulk materials.

V. Applications and Commercialization of Nanomaterials

Nanomaterials have sparked significant interest in recent years due to their unusual mechanical, electrical, optical, and magnetic properties. Today, a wide range of commercial nanomaterials are available, including stain-resistant and wrinkle-free textiles, cosmetics, sunscreens, electronics, paints, and varnishes. Additionally, nano-coatings and nanocomposites are increasingly used in consumer products such as windows, sports equipment, bicycles, and automobiles. Novel UV-blocking coatings on glass bottles are protecting beverages from sunlight damage, and long-lasting tennis balls are made using butyl rubber/nano-clay composites. Furthermore, titanium dioxide at the nanoscale is used in cosmetics, sun-block creams, and self-cleaning windows, while nanoscale silica serves as a filler in products like cosmetics and dental fillings.

Nanophase ceramics are another area of interest because they are more ductile at elevated temperatures compared to coarse-grained ceramics. This makes them suitable for use in a variety of high-performance applications. Nanoscale metal clusters and colloids, particularly those with mono- or polymetallic compositions, have proven valuable in catalytic applications. They serve as precursors for new heterogeneous catalysts and have shown significant advantages in chemical transformations and electrocatalysis, such as in fuel cells. Enantioselective catalysis has also been achieved using chiral modifiers on the surface of nanoscale metal particles. Moreover, polymer-based composites containing high levels of inorganic particles have a

high dielectric constant, making them ideal for photonic band gap structures.

A. Phytochemical and Antimicrobial Properties of Morinda citrifolia Leaves

A qualitative phytochemical analysis of *Morinda citrifolia* (*M. citrifolia*) leaves has revealed the presence of various secondary metabolites, such as polyphenols, reducing compounds, mucilage, and terpenoids. Antimicrobial assays further demonstrated that the leaves of *M. citrifolia* effectively inhibit the growth of multiple bacterial strains, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *S. epidermidis*, *Proteus vulgaris*, *Streptococcus oralis*, *Enterococcus faecalis*, and *Escherichia coli*. These inhibitory effects showed statistically significant differences for each bacterial strain ($p < 0.001$), confirming the antimicrobial potential of the plant.

These findings support the traditional medicinal use of *M. citrifolia* and offer a promising basis for developing new therapies to address conditions related to oxidative stress and infectious diseases. In many developing nations, where socio-economic challenges complicate health management, infectious diseases remain a leading cause of death. Approximately 17 million deaths occur annually due to infections, with more than half of these deaths taking place in Africa (Traoré et al., 2012). As a result, medicinal plants play an essential role in disease management, as they are commonly used to treat ailments (Bessong et al., 2006).

Medicinal plants, with their vast array of bioactive compounds, serve as valuable resources for the development of potential new drugs (Walton and

Brown, 1999; Ghasemzadeh et al., 2010). These plants are not only affordable alternatives for populations in developing countries but also provide effective treatments where synthetic drugs may fail (Conlon et al., 2003). Plants such as *M. citrifolia* offer a diverse range of therapeutic benefits, including antimicrobial, antifungal, anti-inflammatory, anticancer, and analgesic properties (Calzuola et al., 2006; Wang et al., 2002; Jasril et al., 2003; Pawlus et al., 2005; Potterat and Hamburger, 2007; Ruksilp et al., 2011).

Morinda citrifolia, widely known as Noni, has been used in Asia and Australia for over 2000 years to treat various diseases (Whistler, 1985). The plant, native to tropical and subtropical regions, is now used to treat conditions like cough, skin infections, urinary tract infections, and tuberculosis. The fruit juice, often marketed as a dietary supplement, is sold in the USA under the name “Noni” (Phakhodee, 2012). However, there remains a lack of scientific evidence supporting many of these therapeutic claims. Therefore, this research aims to provide a more comprehensive understanding by conducting phytochemical screenings and evaluating the antioxidant and antibacterial properties of *M. citrifolia* fruits.

B. Nanoparticles in Medicine

The integration of nanoparticles into disease diagnosis and treatment offers a promising avenue for medical advancements. Nanoparticles are typically classified into organic and inorganic categories. Organic nanoparticles include substances such as albumin, lipid nanoparticles, and chitosan, whereas inorganic nanoparticles are often made from metals like copper, gold, and silver, as well as magnetic

materials. The importance of nanoparticles in medical applications lies in their comparable size to cellular components, which allows them to interact at the cellular level, similar to how nature constructs biological systems at the nanoscale. This provides a foundation for their use in various treatments, such as gene correction, viral genome inhibition, cancer cell destruction, and cellular metabolism restoration.

Among the various nanoparticles, silver nanoparticles (AgNPs) are particularly recognized for their broad therapeutic applications, including antibacterial, antifungal, antiviral, and anticancer properties. AgNPs have been shown to damage DNA by disrupting mitochondrial respiratory chains in cancer cells, ultimately leading to cell death. This unique dual effect of AgNPs is attributed to their increased surface area and reactivity, which aligns with quantum chemistry and physics principles at the nanoscale. Green synthesis methods have been employed to produce AgNPs in an eco-friendly manner, utilizing non-toxic materials to ensure nanoparticle stability (Eshleman, 2011). For example, research has demonstrated that Ag⁺ can be produced from alfalfa biomass under specific pH conditions (Hera et al., 2011).

Additionally, studies have examined the cytotoxic effects of AgNPs synthesized from plant extracts, such as the leaves of *Abutilon indicum*, against human colon cancer cells (COLO 205) and normal MDCK cells. These studies have observed that AgNPs inhibit cancer cell growth in a concentration-dependent manner, causing morphological changes that lead to cell death (Mata et al., 2012).

C. Satureja hortensis and Green Synthesis of AgNPs

Satureja hortensis, belonging to the Lamiaceae family, is a medicinal plant that is known for its therapeutic properties, including antioxidant, antibacterial, antifungal, and anti-inflammatory effects. In recent years, *S. hortensis* has been studied for its potential in green synthesis of AgNPs, which are then used to investigate their antimicrobial and anticancer effects. Although no previous research has examined the synthesis of AgNPs from *S. hortensis* using both light and ultrasonic techniques, this study represents a novel approach in exploring the rapid and simple production of AgNPs using this plant extract.

The development of such innovative methods for synthesizing nanoparticles using plant-based reducing agents is a promising step forward in the field of nanomedicine. The use of traditional medicinal plants, such as *S. hortensis*, as natural resources for nanoparticle synthesis offers a sustainable and cost-effective alternative to synthetic chemical processes.

D. Nanotechnology and Antimicrobial Applications

The advent of nanotechnology has brought about significant advancements in medicine, particularly in the design and application of nanoparticles (NPs). Due to their nanoscale size (typically 10–9 m), NPs are able to penetrate microorganisms easily, making them ideal candidates for antimicrobial treatments. Nanostructured materials (NMs) have shown effectiveness in inhibiting the growth of a variety of microorganisms, making them crucial in developing novel therapeutic and diagnostic approaches.

NMs, such as metallic nanoparticles (Ag, Au, Cu, and Pt) and metal oxides (e.g., Ag₂O, TiO₂, SiO₂,

CuO, ZnO, CaO, MgO, and Fe₂O₃), have demonstrated significant antimicrobial properties. Among these, AgNPs are particularly effective in various biomedical applications. AgNPs can act by disrupting the biochemical pathways of microorganisms, including the formation of reactive oxygen species (ROS) and interrupting electron transfer reactions. Several reviews have highlighted the importance of the synthesis, characterization, and biomedical application of AgNPs, underscoring their potential in antimicrobial treatments.

In conclusion, the antimicrobial mechanisms of NPs, particularly AgNPs, are a promising avenue for future research, with the potential for significant impacts in treating bacterial, fungal, and viral infections. Additionally, the synthesis of nanoparticles from plant extracts offers a green and sustainable approach to developing new antimicrobial agents. **Copper (II)**

E. Nitrate and Its Applications

Copper (II) nitrate refers to a group of inorganic compounds with the general formula Cu(NO₃)₂(H₂O)_x. These compounds, when hydrated, appear as blue solids, while the anhydrous form of copper nitrate presents blue-green crystals. At elevated temperatures, specifically in a vacuum, anhydrous copper nitrate sublimates between 150°C and 200°C. Among its various forms, the hemi pentahydrate and trihydrate are the most common.

The primary use of copper (II) nitrate is in the production of copper (II) oxide, a compound used extensively as a catalyst in various organic chemical processes. Additionally, copper nitrate solutions have applications in textiles and as polishing agents for metals. Copper nitrate is also employed in the

pyrotechnics industry and is often used in educational settings, particularly in school laboratories, to demonstrate voltaic cell reactions. Furthermore, copper nitrate is incorporated in some ceramic glazes and is a key component in creating metal patinas.

F. Copper (Cu) and Copper-Based Nanoparticles in Catalysis

The applications of copper (Cu) and copper-based nanoparticles, derived from the abundant and cost-effective copper metal, have attracted significant attention in recent years. This is particularly true in the field of catalysis, where these nanoparticles have shown great potential. The growing interest in copper nanoparticles can be attributed to the ability to modify their chemical and physical properties through various synthetic techniques, as well as post-synthetic chemical treatments. These modifications allow for the fine-tuning of the nanoparticles for specific applications, thus broadening their utility in industrial processes.

In addition, the design and development of novel supports, or even multimetallic systems such as alloys, have played an important role in advancing the applications of copper-based nanoparticles. This review covers the various synthetic methods employed to produce copper and copper-based nanoparticles, which include metallic copper, copper oxides, and hybrid copper nanostructures. It also highlights the use of copper nanoparticles immobilized or supported on materials like silica (SiO₂) and magnetic supports, as well as their diverse applications in catalysis.

The synthesis section of the review explores a wide range of preparative methods for the production of copper and copper-based nanoparticles. Meanwhile,

the application section delves into the use of these nanomaterials, particularly in catalytic processes, demonstrating their value in enhancing the efficiency and selectivity of chemical reactions.

VI. MATERIAL AND METHODS

A. Collection of Plant Materials

The plant material **Morinda Citrifolia** was sourced from local areas in the Ariyalur region. The entire freshly collected plant was utilized for the synthesis of copper nitrate nanoparticles.

B. Preparation of Plant Extract

The plant extract was prepared using the leaves of **Morinda Citrifolia**. Fresh leaves were thoroughly washed with deionized water and then cut into small pieces. These pieces were boiled at 100°C, followed by filtration using Whatman No.1 filter paper. The extract was then stored at 4°C for subsequent experiments.

C. Synthesis of Copper Nitrate Nanoparticles

For the synthesis of copper nitrate nanoparticles, **Cu(NO₃)₂·5H₂O** (0.1g) was dissolved in deionized water. This solution was combined with 10 mL of the **Morinda Citrifolia** plant extract and stirred vigorously using a magnetic stirrer at 1000 rpm at room temperature for 3 hours. To adjust the pH of the reaction mixture, 1 mL of 10% sodium hydroxide solution was added. The resulting precipitate was filtered and dried. The crude product was kept at 150°C for 12 hours in an oven. Afterward, it was calcined at 500°C for 3 hours, and

the resulting powder was ground into a fine powder using a pestle and mortar.

D. Qualitative Phytochemical Analysis

Test for Alkaloids (Dragendorff's Test):

1 mL of the extract was mixed with a few drops of Dragendorff's reagent, and the color change was observed. The appearance of a green color indicates the presence of alkaloids.

Test for Terpenoids:

1 mL of the extract was added to thionyl chloride in a test tube. A pink color formation indicates the presence of terpenoids.

Test for Steroids (Liebermann-Burchard Test):

The powder was dissolved in 2 mL of chloroform in a dry test tube. Then, 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid were added. The solution turned blue or bluish, indicating the presence of steroids.

Test for Coumarins:

1 mL of the extract was mixed with 1 mL of 10% sodium hydroxide. The presence of coumarins is indicated by a yellow color formation.

Test for Tannins:

A few milligrams of powder were treated with 10% alcoholic ferric chloride. The formation of a dark black or greenish-black color indicates the presence of tannins.

Test for Flavonoids:

A few milligrams of powder were mixed with magnesium and 2-3 drops of hydrochloric acid. The formation of a red or pink color suggests the presence of flavonoids.

Test for Phenols:

2 mL of the extract was mixed with 2 mL of distilled water and a few drops of 10% ferric chloride. The appearance of a blue or green color indicates the presence of phenols.

Test for Volatile Oils:

2 mL of the extract was mixed with 0.1 mL of dilute sodium hydroxide and a small amount of dilute hydrochloric acid. The formation of a white precipitate indicates the presence of volatile oils.

Test for Quinones:

2-3 drops of concentrated sulfuric acid were added to a methanolic extract. The formation of a red color indicates the presence of quinones.

Test for Sugars:

1 mL of the extract was combined with Fehling's solutions. The appearance of a red color suggests the presence of sugars.

VII. RESULTS AND DISCUSSION

The *Morinda citrifolia* plant was collected around Ariyalur and was identified using the flora of presidency of Madras and the fresh plant extract

plays a key role in copper nitrate nanoparticle synthesis.

A. Phytochemical Screening

The phytochemical investigation revealed mainly the presence of some large chemical groups both in the fermented and fresh fruit. As summarized in following table, the main chemical group detected are glycosides, polyphenols, and alkaloids. In addition, it was observed that the composition is not influenced by the fermentation stat of the juice. Thus, all the chemical compounds observed in the fresh juice were in the fermented one. To end, compounds such as anthocyanin, coumarines, anthraquinones, triterpenoids, steroids, cardenolides, cyanogenic derivatives, free anthracene, C-heterosides, mucilage.

Table- 1, Phytochemical Screening of *Morinda citrifolia* leaves

S.No	Chemical compounds	Fermented juice
1	Test for Alkaloids	+++
2	Test for Terpenoids	-
3	Test for Steroids	-
4	Test for Coumarin	-
5	Test for Tannins	+++
6	Test for Flavanoids	+++
7	Test for Phenols	+++
8	Test for Volatile Oils	+++
9	Test for Quinones	-
10	Test for Sugar	-

B. Characterization of Cu NPs

The Cu NPs were characterized using UV-Visible, FT-IR, SEM techniques.

C. UV-Visible spectral Analysis

The UV-visible absorbance spectrum recorded for g-Cu NPs exhibited λ_{Max} of 403nm.

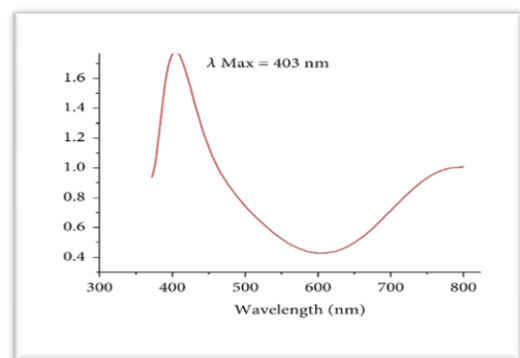


Figure 1 UV-Visible absorbance spectrum of g-Cu NPs.

D. FT-IR Spectral Analysis

The FTIR spectra of g-Cu NPs showed peaks at 3351, 3284, 2920, 2350, 1720, 1625, 1342, 1160, 1058, 740, and 590 cm^{-1} (Figure 3). From those indicated peaks, the broad absorption peaks of g-Cu NPs observed at 3351 cm^{-1} and 3254 cm^{-1} represent the presence of higher concentration of alcohols, with O-H stretches and N-H stretches of amines.

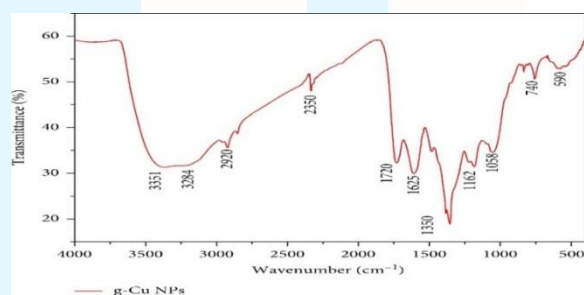


Figure 2 FT-IR Spectrum of g-Cu NPs

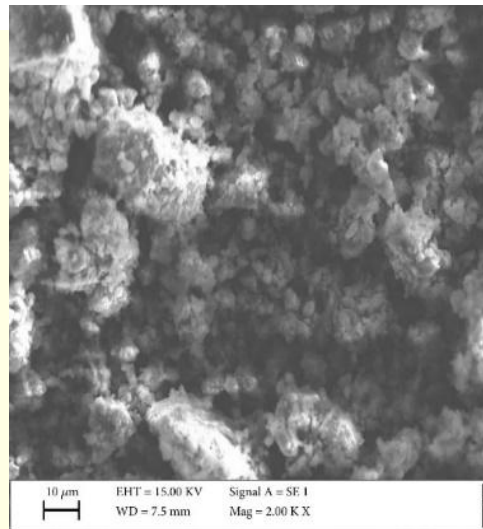
The intense peaks shown at 2929.0 cm^{-1} and 2890.6 cm^{-1} correspond to asymmetric C-H stretching of the $-\text{CH}_2$ and $-\text{CH}_3$ groups, respectively. The presence of atmospheric CO_2 resulted in a stretching vibration located at a

wave number of 2350 cm^{-1} . It is also believed that the amine and carboxylate group present in the leaf extract of *Morinda citrifolia* is responsible for the binding of proteins with the surface of Cu and thereby leading to the stabilization of the biosynthesized nanoparticles.

The absorption peaks represented at 1720 cm^{-1} and 1625 cm^{-1} indicate the presence of C=O and C=C bending modes of vibrations. The peaks represented by 1350 cm^{-1} show C-N stretching of amide. The medium peak at 1160 cm^{-1} corresponds to C-O stretching of phenolic compounds. The C-O-C stretching appears at 1058 cm^{-1} . The last peak at 590 corresponds to bending modes of vibrations of the C-H bond. FTIR analysis results confirmed the presence of various phytochemicals of *Morinda citrifolia* leaf extract such as phenolic, tannins, and proteins involved in the synthesis of g-Cu NPs. In addition to this, the FTIR spectrum of Cu NPs shows peaks corresponding to the broad band centred at 740 cm^{-1} which represents a characteristic peak believed to be due to the interaction of Cu with biomolecules of extract.

E. Morphological and Compositional Analysis by SEM

The visual displays of size and shape of synthesized g-Cu NPs as depicted by SEM micrographs are shown in Figures 5(a) and 4(b). The SEM images also demonstrated the non-homogeneity of the particles in terms of their shape and size. All the possible spherical and irregular shapes such as truncated hexagonal, cylindrical, triangular, and prismatic shapes of Cu NPs with varying particle sizes were found in the micrographs.



The average grain size of Cu NPs was found to be in the range of 10–50 nm. It seems particles were found to agglomerate moderately due to a high surface area resulting in the formation of medium-sized particles. The chemical composition of the NPs was studied by SEM analysis. The peaks corresponding to elemental Cu, C, and O were clearly identified, and no additional peaks were present, which demonstrates the purity of the synthesized NPs, and this was consistent with the XRD studies.

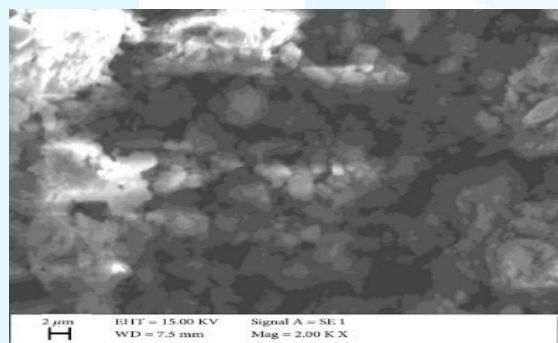


Figure 3 SEM micrographs of g- Cu NPs

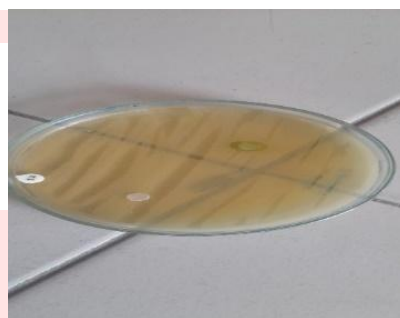
It is also possibly believed that the presence of C and O is basically from the capped bioactive compounds. In addition, it is also expected that very few copper atoms at the surface of the NPs might have been oxidized to yield small amounts of CuO

and Cu₂O. The reduction of copper ions to Cu NPs is facilitated by the biomolecules of plant extract containing surface hydroxyl groups.

F. Antibacterial activity of M.citrifolia juice

The mean inhibition diameters induced by our M. citrifolia leaves juices on the tested strains are shown in Fig. 1. Diameters vary significantly depending on strain and type of M. citrifolia juice ($p < 0.001$). Thus, at 20 mg/ml, both juices have no effect on *Micrococcus luteus* and *Salmonella typhi*.

However, the juices inhibited the growth of strains such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Streptococcus oralis*, *Enterococcus faecalis* and *Escherichia coli* with average inhibition diameters ranging from 10.0 cm to 14.5 cm for M. citrifolia fresh juice and from 9.5 cm to 12.0 cm for fermented juice. In addition, for each of these strains, the average inhibition diameter of fresh juice is greater than or equal to that of fermented juice. Thus, fresh juice is more active than three-month juice.



VIII. CONCLUSION

In conclusion the Copper Nitrate nanoparticle was synthesized using fresh leaf extract of *Morinda Citrifolia* album. The synthesized nano particle was

characterized and confirmed using UV-Visible, FT-IR, and SEM mapping analysis, the results showed that Cu (NO₃)₂ Nps were synthesized properly. The in-vitro antibacterial assay depicts the effective antibacterial activity of Cu (NO₃)₂ Nps. The in-vitro antiradical assay depicts the effective antiradical activity of Cu (NO₃)₂ Nps. This concludes that the further studies on Cu (NO₃)₂ Nps helps for the drug development.

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