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Nnovative Applications of Nanoparticles in Biomedical Diagnostics, Drug Delivery, And Medical Treatments

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Abstract

The green synthesis of metal oxide nanoparticles has emerged as a sustainable alternative to conventional chemical and physical synthesis methods because it reduces the use of toxic reagents, minimizes waste generation, and utilizes renewable biological resources. Copper(I) oxide (Cu_2O) nanoparticles have attracted considerable interest owing to their distinctive optical, catalytic, and antimicrobial properties. The present study focuses on the environmentally benign synthesis of Cu_2O nanoparticles using plant- and agricultural-waste-derived extracts as natural reducing and capping agents. Waste materials, including *Daucus carota* peel, *Artocarpus heterophyllus* seed, and *Cocos nucifera* husk, were selected as potential bioresources for nanoparticle synthesis. The phytochemicals present in these materials, including phenolics, flavonoids, alkaloids, tannins, saponins, and other bioactive compounds, facilitate the reduction of Cu^{2+} ions and contribute to the stabilization of the synthesized nanoparticles. The proposed approach provides an economical and environmentally compatible route for producing Cu_2O nanoparticles while simultaneously promoting the valorization of agricultural waste. The synthesized nanoparticles can be investigated for their physicochemical characteristics and antimicrobial performance against representative Gram-positive and Gram-negative bacterial strains. Their antibacterial activity is associated with interactions between nanoparticles and bacterial cell envelopes, potentially resulting in membrane disruption, oxidative stress, and inhibition of essential cellular processes. The study highlights the potential of waste-derived Cu_2O nanoparticles as sustainable antimicrobial materials for applications in biomedical, environmental, and healthcare-related fields. Overall, the proposed green synthesis strategy combines waste utilization, nanotechnology, and antimicrobial research to develop potentially effective and environmentally responsible nanomaterials.

Keywords: Green synthesis, Copper(I) oxide nanoparticles, Cu_2O, Agricultural waste, Plant extracts, Phytochemicals, Nanotechnology, Antimicrobial activity, Gram-positive bacteria, Gram-negative bacteria, Sustainable nanomaterials

Introduction

A more environmentally benign process for creating metal oxide nanoparticles is made possible via green synthesis. By lowering macro/micro materials to the nanoscale and altering their shape and surface area, nanotechnology enhances their antibacterial efficacy in comparison to its bulkier version. The green production of metal oxides uses natural materials like plant component extracts and microbiological organisms like fungi and bacteria [1]. This process produces a nanoparticle with a certain shape and structure by using the waste by-products as a reducing and capping agent. (2014) Chen *et al*. In recent decades, metallic and metal oxide nanoparticles have garnered a lot of attention due to their exceptional properties. These unique qualities, which are controlled by their physical properties, have made it possible for these materials to make substantial contributions to a range of nanotechnological fields (Kerour *et al*., 2018) [2]. Out of all these metallic oxide nanoparticles, copper oxide nanoparticles have received the most interest because to their non-toxicity, affordability, accessibility, and notable optical absorptions in the UV-Vis ranges. Cupric oxide (CuO) crystalline monoclinic structure with a bandgap energy of 1.2 eV and cubic Cu_2O structure with an estimated absorption edge of 2.1 eV are the two forms of copper oxide nanoparticles, a p-type material. Cu_2O Nps' tendency to aggregate is a drawback that hinders the nanomaterial's capacity to carry out a variety of tasks (Liu, Li, Zhang, and Li, 2012) [3]. Chemical synthesis methods have been the main focus of study to solve this issue (Bhosale *et al*. (2013); Bhosale and Bhanage (2016); Lee *et al*. (2011); Wang *et al*. (2009)). Beyond the synthesis of metallic nanoparticles in general and copper nanoparticles in particular, the fundamental idea is to supply three important components: a precursor that can supply copper ions, a reducing agent that provides the electrons needed to reduce the copper ions into copper atoms, and a surfactant that controls the size of the copper nanoparticles at the appropriate pH and temperature [4]. When the reducing agent, or source of electrons, is a chemical substance, the method is chemical; when it is a physical source, such an electric current, it is physical; and when it is a creature, it is biological (Mohamed, 2020). Standard and green chemical techniques are the two categories of chemical processes. Conventional chemical methods employ hazardous synthetic compounds such sodium borohydride, hypophosphite, hydrazine, and others as reducing agents. Green chemical techniques, on the other hand, make use of organic substances like ascorbic acid and citric acid. Because they are often nontoxic, environmentally benign, and economical, green chemical processes are preferred over conventional chemical approaches. Many current techniques rely on energy-intensive, time-consuming processes or hazardous reducing agents. Because of this, several researchers have been inspired to create less hazardous, if not non-toxic, methods (Liu, Zhou, Yamamoto, Ichino, and Okido, 2012) [5].

Since the biological approach to nanoparticle synthesis is an environmentally beneficial method that minimises waste creation and contamination, it is utilised to lessen the negative effects of chemical synthesis. One example of a biological method of making metal oxide nanoparticles is the use of plant extracts to help produce a range of metal oxide nanoparticles. Nps and natural products have been demonstrated to have a synergistic interaction that significantly increases both of their biological activity (Mali *et al*. (2019); Happy *et al*. (2019) [6]; Mata *et al*. (2015)). To date, morphology-dependent characteristics have been observed in the size and form of synthesised Cu_2O (Chang and Zeng (2004); Wang *et al*. (2003)). Precursors and reducing agents are frequently used in synthesis techniques under various reaction conditions, which lengthens the time it takes for products to develop (Zhao *et al*., 2008). With increased environmental concerns limiting the use of chemicals, researchers perform biosynthesis of different metal oxides using natural extracts. When analysing the infections caused by A. baumanni and E. coli pathogens, the biological reducing agents found in Callistemon viminalis leaves, which were utilized [7] to create Cu_2O nanoparticles, acted as an intrinsic antibacterial agent (Sharma *et al*., 2018). It has been demonstrated that biochar loaded into Cu_2O nanoparticles is a superior option in the antibacterial sector and serves as a carrier to increase the specific surface area (Yang *et al*., 2019). Cu_2O micro-nanoparticles have been synthesised using glucose and exhibit strong catalytic activity in both visible and dark conditions. (Xue and others, 2019). In order to create Cu_2O nanoparticles, this study has chosen three waste by-products that are typically regarded as trash: D. carota peel, A. heterophyllus seed, and C. nucifera husk. For the synthesis of Cu_2O Nps, these three plant/tree extracts are often regarded as waste. Alkaloids found in the carrot peel, the outer layer of the skin of the D [8]. carota plant, help reduce metallic salts to Nps (Nguyen and Le, 2018). It has been demonstrated that jack fruit has antioxidant, antifungal, and antibacterial qualities. Because of phytochemicals such polysaccharides, saponins, flavonoids, and coumarins, it exhibits increased activity (Baliga *et al*., 2011) [9]. A phytochemical investigation found that a coconut husk included tannins, phenolics, saponins, and glycosides. (Balogun and others, 2014). Cu2+ is decreased during the green synthesis process because of the phytochemicals in the extraction, which serve as reducing agents (Makarov *et al*., 2014). The phenolic functional group helps reduce Cu2+ to Cu+ ions. Numerous studies have discussed the use of different plant extracts, such Nerium oleander leaf aqueous extract (Gopinath *et al*., 2014) [10], to produce copper and copper oxide nanoparticles, some of which may be too costly or hard to get.

Green synthesis of cauliflower-like Cu_2O: antimicrobial activity

Materials and characterization

Purchased from SRL (Sisco Research Laboratories), cupric acetate (Cu (OAc)2.H2O) was utilised without purification [11]. A lyophilizer (Lark Innovative Fine Teknowledge) was used to lyophilise fresh, delicate coconut water that was bought from the neighbourhood market. Under Cu Kα (λ = 1.54A˚) radiation, phase identification was carried out using a Bruker D8 Advance X-ray diffractometer with a 2θ range of 10 to 80o and a step width of 0.02o.A Perkin Elmer Lambda 35 spectrophotometer was used to record the Diffuse Reflectance Spectrum (DRS) in the 200–800 nm wavelength range. Thermo Scientific's Nicolet Nexus 470 was used to record Fourier transform infrared spectroscopy at room temperature between 4000 and 500 cm−[12 .Morphological investigations have been carried out using transmission electron microscopy (FEI Tecnai G2 T20 S-Twin TEM) and scanning electron microscopy equipped with energy-dispersive X-ray spectroscopy (Carl Zeiss).

Nanoparticle synthesis

Ten millilitres of distilled water were mixed with 100 milligrams of lyophilised tender coconut water and diluted till the mixture turned translucent. A post-addition of 50 m of 0.1 M aqueous cupric acetate solution was made [13]. The solution was stirred for five minutes and then kept at 180 W in a microwave oven for ten minutes to continue the reaction. Centrifugation was used to get the brick-red precipitate when the reaction was brought to room temperature [14]. After being cleaned with pure ethanol, the residue was dried for 12 hours at 80°C in a vacuum oven (Figure 1).

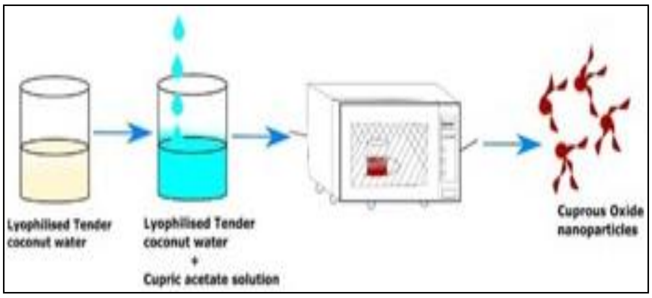

Fig 1: Representation of the synthesis of Cu_2O nanoparticles

Results and Discussion

Nanoparticles were obtained by lyophilising the coconut water. By freezing the coconut water throughout a low-temperature dehydration process, lyophilization preserves the essential sugars and other nutrients while dehydrating the coconut water [15]. Three distinct, distinct, and connected processes are freezing, primary drying (sublimation), and secondary drying (desorption). Some advantages of lyophilization include the following: Liquids are easy to process, which facilitates aseptic handling. The stability of a dry powder has been enhanced. Water is removed from the product without causing it to overheat [16]. The product is more stable when it is dried [17]. The reconstituted substance dissolves readily and swiftly. Among the drawbacks of lyophilization are the following: Processing and handling times have gone increased. The main disadvantage is the expense and complexity of the equipment.

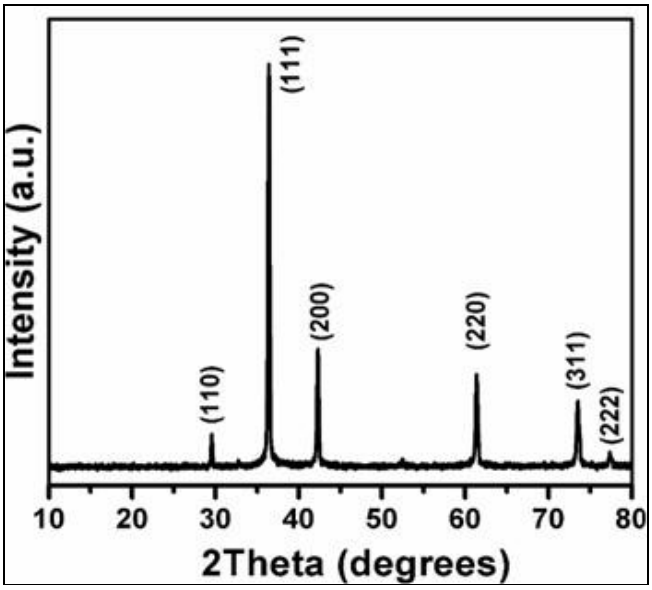

Fig 2: XRD pattern of Cu_2O nanoparticles

X-Ray diffraction studies

The XRD pattern of the material including the crystalline type of Cu_2O nanomaterial is acknowledged in Fig. 2. The careful crystal planes are comparable to the discrete diffraction peaks at the 2 theta degree [18]. Using the JCPDS card number 05-0667, a compelling lattice parameter of a = 4.27 A˚ was obtained. Cu_2O nanoparticle development is confirmed by the results, which support the structure as a cubic cuprite phase [19]. The purity of the produced nanoparticle was confirmed by the presence of sharp peaks with a sparse background. The lack of an additional Bragg position in the pattern-matched profile further supported the creation of a single-phase [20]. The development of a highly crystalline product is indicated by the strong diffraction peaks with an FWHM of 0.24o. However, we were unable to identify any unusual plane for CuO or Cu(0) nanoparticles in the matching diffraction pattern. The Scherrer equation yielded the following anticipated average particle size 0.9λ $D = \beta \cos\theta$, where D is the crystallite size, λ is the incoming radiation's wavelength (1.5406 A˚), β is the FWHM, and θ is the Bragg's angle. As a result, 39 nm is the average particle size found using the calculation above [21]. According to all of these findings, the produced nanoparticles are pure Cu_2O nanostructures.

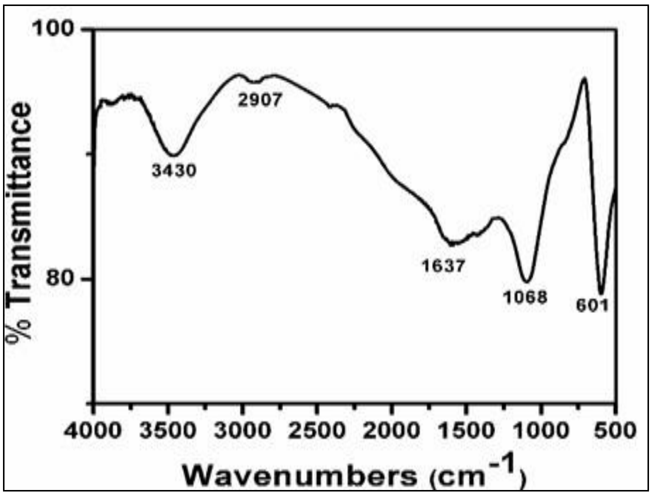

Fig 3: FTIR spectrum of the synthesized Cu_2O nanoparticles

Optical behavioral study

Cu(I) oxide is represented by the distinctive peak at 601 cm^{-1} in the FTIR spectrum seen in Fig. 3 (Andal and Buvaneswari, 2011). The alcoholic and phenolic hydroxyl groups (-OH stretching vibration) are clearly visible in the bands at 3430 cm^{-1} [22]. The symmetric stretching vibration of the -CH2 group is shown by the clear band at 2900 cm^{-1}, while the existence of the aromatic ring is indicated by the peak at 1600 cm^{-1} (Yong Cai Zhang, Jing Yuan Tang, 2006). This completes the list of accessible organic moieties. At 1074 cm^{-1}, a prominent band indicates the C-O stretching caused by the methoxy groups. Furthermore, the Cu-O vibrational mode in the Cu_2O phase was identified as the source of the distinctive peak at 601 cm^{-1} [23]. The presence of these groups suggests that the organic moieties absorbed the synthesised Cu_2O, which influenced the shape of the nanoparticles.

UV-Visible spectroscopy studies

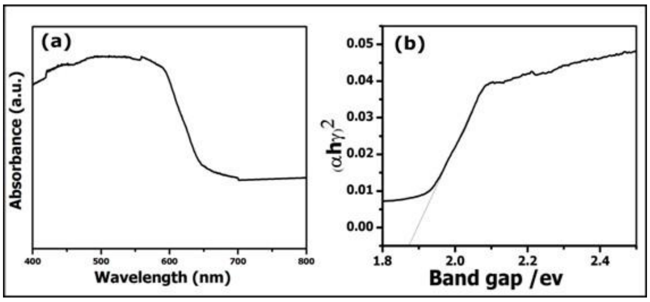

Fig 4: (a) UV-Visible spectra and (b) DRS spectra [24]

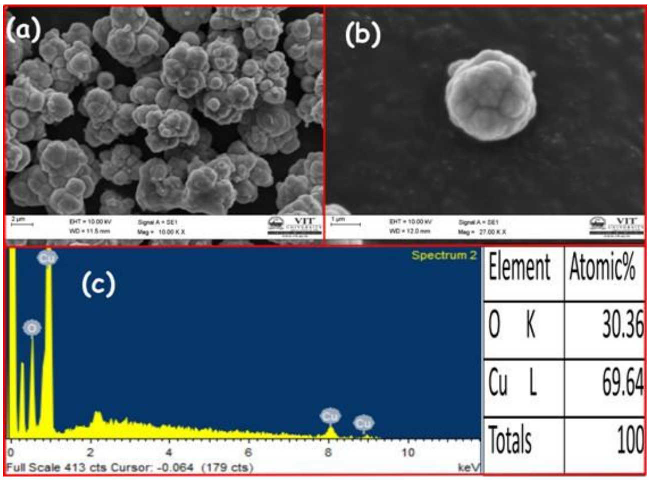

Fig 5: (a) and (b) SEM image c) EDAX spectrum and their corresponding elemental composition of the synthesized Cu₂O nanoparticles.

The surface morphology of the nanoparticle is examined using SEM [25]. In a SEM, the electron lenses are used to produce a tiny electron beam that is focused on the specimen's surface and scanned in a raster of lines and frames by the deflection coils. McMullan (1995) Figs. 5(a) and (b) show the SEM micrograph of the biogenic synthesised nanoparticles. The data displays the Cu_2O's cauliflower-like shape [26], with nanoclusters seen in a few rough-surfaced areas. Additionally, the pictures demonstrated that the Nps had almost spherical morphologies and were monodispersed and aggregated. The chemical makeup of the extract may support this variation in the size and form of the particles (Ganesan *et al*., 2020). The EDAX spectrum is seen in (Fig. 5c) [27]. This demonstrates the phase purity of the produced Cu_2O nanoparticles by confirming the existence of copper and oxygen without any residues of other elements. Additionally, the product's stoichiometry is confirmed by the 2:1 copper to oxygen ratio. The structure of the as-prepared nanoparticles has been described using TEM [28]. The archetypal model of nanoparticles is shown in Fig. 5, with a focus on the SAED pattern, particle size, and shape. In order to detect TEM pictures, the specimen was exposed to electron beams under high vacuum, which caused the transmitted beam to magnify the image from fifty to over a million times [29]. The TEM image that the particles are spherical with an average diameter of nm, despite the SEM image showing agglomerated structures. The crystallinity of the produced nanoparticles is demonstrated by the bright spots in the SAED pattern [30] Because of their direction toward the electron beam, the crystallinity may enhance the diffraction contrast. The Image J program yielded an average particle size between 10 and 20 nm.

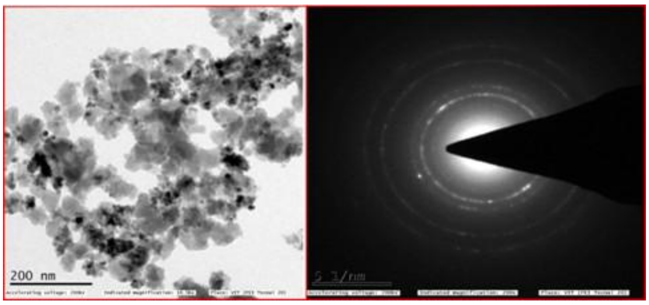

Fig 5: (a) TEM image and (b) SAED pattern of the synthesized Cu_2O nanoparticles.

TGA and DTA analysis

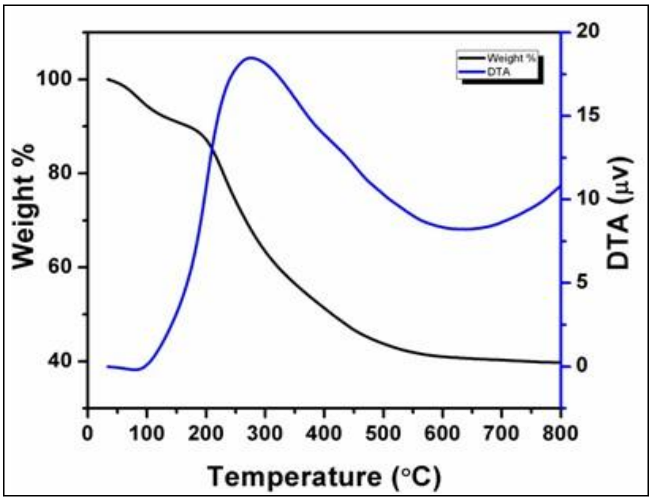

Fig 6: TGA and DTA plots of the prepared Cu_2O powder.

To validate the presence of organic moieties and determine the phase development, another TGA analysis (Fig. 6) was carried out. A two-step weight reduction is depicted in the TGA and DTA graphs At 100 °C, which corresponds to the loss of water molecules, the initial phase of weight loss begins [31]. Perhaps as a result of the extract's low molecular and biochemical composition, weight loss starts in the second stage at 200 ºC. In the 200–480 ºC range, a significant loss was seen as a result of the carbonisation of the reducing sugars and sugar alcohols attached to the Cu_2O nanoparticles. The breakdown and removal of water and other adsorbed molecules is shown by a large peak up to 300 ºC in the DTA plot. Additionally, the oxidation and crystallisation of nanoparticles are responsible for the significant exothermic peaks between 300 and 450 ºC. The provided literature is consistent with our approach (Ma *et al*., 2014).

Antimicrobial action

An agar well diffusion method was used to assess the antibacterial activity of the as-prepared Copper (I) oxide. Sterile Petri plates with a diameter of 90 mm were filled with sterile nutritional agar prior to the creation of wells with a diameter of 6 mm. The nutrientagar plates were covered with 0.1 mL of the bacterial species. The suspension was applied to the wells at several concentrations (50g, 100 µg, 250 µg, and 500 µg) [32]. To allow the particles to diffuse into the medium, the plates were kept at room temperature for half an hour. The antibacterial property was next assessed by measuring the inhibition zone that developed around the wells after these agar plates were incubated for 24 hours at 37 °C in the dark. When compared to the Gram-negative E [33]. coli bacterial strain, the results (Fig. 7 and Table 1) show that Cu_2O has greater action for the Gram-positive S. aureus strain with a zone of inhibition of 20 mm and for C. albicans with a zone of inhibition of 19 mm. Li *et al*. predict that the Cu_2O Nps will have low cytotoxicity values (Li, Lv, and Ai, 2016). Nonetheless, a concentration of 4g/L has been shown to have 100% antibacterial efficacy.

Similarly, after subjecting the nanoparticles to a 500 mg/L copper concentration for four hours, (Du *et al*., 2017) noted that the antibacterial activity reached over 100%. The possible method suggested by Hu *et al*. (Hu *et al*., 2005) can spread the NPs' antimicrobial activity [34]. Toxicity may be primarily caused by the Cu2+ ions scattered from the copper-based nanoparticles and the bacterial cell adsorption on the surface of the Cu2+ carriers (Applerot *et al*., 2012). Gu *et al*. investigated how Cu_2O NPs and montmorillonite affected the algae's degradation. The surface dissolving caused by the antialgal impact of Cu2+ ions released from Cu_2O was not strong enough to stop the development of algae [35]. Therefore, the crucial element causing this occurrence was the photocatalysis of the Cu_2O.

Cu nanoparticles' increased action against Gram-positive Staphylococcus aureus may be caused by the different amines and carboxyl group compounds found in the cell walls of Gram-positive bacteria. Cu nanoparticles' greater effectiveness against Gram-positive Staphylococcus aureus may be attributed to the different amines and carboxyl group compounds found in the cell walls of these bacteria. Carboxyl group compounds may therefore disintegrate in the presence of copper [36]. The greater antibacterial efficacy of copper nanoparticles against Gram-positive Staphylococcus aureus compared to Gram-negative strains may be explained by copper's catalytic reactivity toward amines and carboxyl groups found in the cell walls of Gram-positive bacteria [37]. There are several methods to comprehend how metallic nanoparticles interact with bacterial cells, even to the precise mechanism of copper nanoparticles' antibacterial activity is unknown. According to earlier studies, metal nanoparticles-such as copper and silver-can attach to biofilm walls by electrostatic attraction before penetrating them. Leakage occurs when "pits" are formed in the cell membrane, which reduces its selective permeability. Cytoplasmic fluid leakage occurs when "pits" are made in the cell membrane, which decreases the membrane's selective permeability. As a result, cellular transport is interfered with, and the cells eventually perish. Reactive oxygen species (ROS) are produced when metal ions produced by nanoparticles during interactions with bacterial cells inhibit respiratory enzymes, which may be another reason for cell death [38]. After then, the cells experience oxidative stress, which causes significant cellular damage. Additionally, phosphorus and sulphur, two soft bases in cellular DNA, may react with metal nanoparticles to prevent DNA replication and protein binding. Bacterial cells thus cease to function completely and ultimately perish (Roy *et al*., 2016).

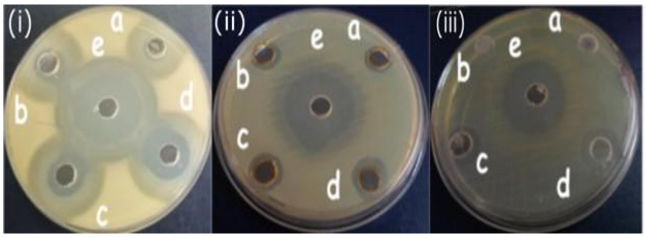

Fig 7: Antimicrobial activity of Cu_2O nanoparticles against i) *E. coli*, ii) *S. aureus*, and iii) *C. albicans*

Table 1: Antimicrobial activity of Cu_2O nanoparticles against i) *E. coli*, ii) *S. aureus*, and iii) *C. albicans*

S. No	Strains	50 μg	100 μg	250 μg	500 μg
1.	*E. coli*	10	13	15	20
2.	*S. aureus*	8	9	10	13
3.	*C. albicans*	-	-	13	17

We have described a simple synthetic technique for producing the Cu_2O nanoparticles. In the realm of nanotechnology, the green approach for nanoparticle synthesis has emerged as an intriguing substitute for conventional methods due to its affordability, simplicity, and environmental friendliness [39]. Lyophilised tender coconut water extract is used as an in-situ reducing agent in this process. The cauliflower-shaped Cu_2O nanoparticles are primarily formed by the reducing sugars in the tender coconut water extract. The characterisation techniques demonstrated the existence of intrinsic bioactive moieties involved in nanoparticle capping and surface stabilisation in the reacting solution, in addition to the crystallinity and size distribution of biogenic Cuprous Oxide nanoparticles. These green-synthesised nanoparticles shown strong antibacterial action against the pathogens S. aureus, E. coli, and Candida albicans [40]. The produced Cu_2O nanoparticles exhibit improved antibacterial activity against the fungal strain as well as Gram-positive and Gram-negative bacteria. A beaker with 100 mL of deionised water and about 10 grams of finely ground coconut flesh was heated in a microwave oven for 15 minutes. The extract was then filtered thru cellulose nitrate membrane filter paper (0.22 µm) and kept in the refrigerator until it was needed for additional testing.

Cuprous oxide nanoparticle synthesis

A beaker containing 2g of copper (II) acetate monohydrate dissolved in 50 mL of water was filled with an equivalent volume of freshly made coconut flesh extract. It was well mixed until the extract was completely dissolved. After that, the mixture was cooked in a microwave oven for ten minutes at 170 W while being stirred for fifteen minutes [41]. After filtering the mixture and washing it with ethanol, the brown precipitate was collected. After that, it was dried at 80 degrees Celsius in a vacuum oven to remove any remaining solvent and produce Cu_2O nanoparticles.

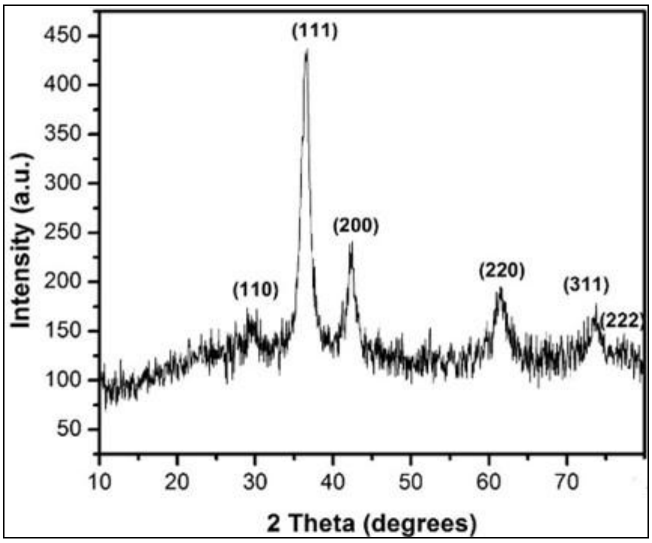

Fig 8: XRD pattern of Cu_2O nanoparticles

The powder XRD-diffraction pattern of the generated Cu_2O nanoparticles is displayed in Fig. 8. The planes (110), (111), (200), (220), (311), and (222) are represented by the diffraction peaks at 2θ = 29.43, 36.56, 42.36, 61.09, 73.68, and 77.63, respectively (JCPDS #01-077-0199). The strong diffraction peaks clearly show the presence of very pure crystalline Cu_2O NPs. We were surprised to see that Cu (0) nanoparticles did not seem to have a specific plane in our XRD pattern [42]. Following the treatments, there are no longer any impurity peaks or significant shifts in the peak locations in the XRD spectra, indicating that pure Cu_2O powders were produced under the current circumstances.

Elemental and morphological analysis

Scanning electron micrograph images of cuprous oxide nanoparticles obtained with a scanning electron microscope are shown in Fig. 9. Agglomerates of spherically shaped nanoparticles that are created by the aggregation of the nanoparticles make up cuprous oxide nanoparticles.

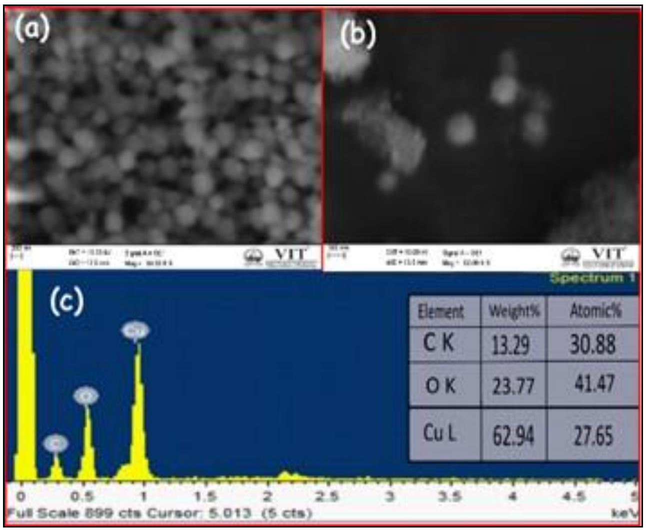

Fig 9: SEM image of Cu_2O NPs (a and b), EDAX Spectra (c) of NPs.

Energy-Dispersive X-ray Spectroscopy (EDS)

Energy-dispersive X-ray spectroscopy (EDS or EDX) is an elemental characterization technique commonly coupled with scanning electron microscopy (SEM) to determine the elemental composition of synthesized nanoparticles. When a high-energy electron beam interacts with a material, characteristic X-rays are generated as a result of electronic transitions between atomic energy levels [43]. The energies of these X-rays are characteristic of individual elements, allowing the qualitative identification and quantitative estimation of the elements present in the sample.

For copper oxide nanoparticles, EDS is particularly useful for confirming the presence of copper and oxygen and for evaluating the elemental purity of the synthesized material. The relative weight percentage and atomic percentage of copper and oxygen provide information about the elemental composition and can support the formation of Cu_2O. Minor carbon signals may originate from carbon-based sample-supporting materials or conductive coatings used during SEM-EDS analysis [44]. EDS therefore provides complementary evidence for the successful formation and elemental composition of copper oxide nanostructures.

Nuclear Magnetic Resonance (NMR) Analysis

Nuclear magnetic resonance (NMR) spectroscopy is an important analytical technique for identifying the molecular composition and chemical environment of organic compounds present in plant extracts. The technique is based on the interaction between nuclei possessing non-zero spin and an externally applied magnetic field. When exposed to radiofrequency radiation, these nuclei undergo transitions between different spin energy states, producing characteristic resonance signals [45]. Proton NMR (^1H-NMR) is particularly useful for investigating plant-derived extracts because it provides information about hydrogen-containing organic molecules, including sugars, sugar alcohols, phenolic compounds, and other phytochemicals. Chemical shifts are generally reported in parts per million (ppm) relative to an internal reference compound such as tetramethylsilane (TMS). The position, intensity, and integration of individual signals provide information regarding the chemical environment and relative abundance of molecular components. In green nanoparticle synthesis, NMR analysis can help identify reducing and stabilizing compounds present in plant extracts. Reducing sugars and sugar alcohols, for example, may participate in the reduction of metal ions during nanoparticle formation [46]. Therefore, NMR characterization provides valuable molecular-level evidence regarding the phytochemical constituents that may contribute to the reduction and stabilization of synthesized Cu_2O nanoparticles.

UV–Visible Spectroscopic Analysis

Ultraviolet–visible (UV–Vis) spectroscopy is widely used to investigate the optical properties and formation of metal and metal oxide nanoparticles. The technique measures the absorption of electromagnetic radiation in the ultraviolet and visible regions of the spectrum. Nanoparticle formation can result in characteristic changes in the absorption profile because of electronic transitions and collective interactions of charge carriers within the nanostructured material [47]. The optical response of Cu_2O nanoparticles is associated with their electronic structure, particle size, morphology, and surrounding chemical environment. Consequently, UV–

Vis spectroscopy can be used as a rapid and non-destructive method for monitoring nanoparticle formation and evaluating their optical characteristics. Changes in absorption intensity and spectral position may also provide information regarding variations in particle size, aggregation, and surface interactions.

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is an effective technique for identifying the functional groups and chemical bonds associated with synthesized nanoparticles and the biomolecules present on their surfaces. The technique is based on the absorption of infrared radiation by molecular bonds undergoing vibrational transitions. Different functional groups exhibit characteristic absorption bands, allowing the chemical constituents associated with a sample to be identified. In the green synthesis of Cu_2O nanoparticles [49], FTIR analysis is particularly important for determining the role of phytochemicals present in plant extracts. Functional groups such as hydroxyl, carbonyl, C–O, and other organic groups can participate in the reduction of copper ions and subsequently become associated with the nanoparticle surface. Such biomolecules may also act as capping or stabilizing agents, thereby limiting particle aggregation and improving nanoparticle stability [50]. The appearance of characteristic Cu–O vibrational bands provides additional evidence for the formation of copper oxide nanostructures. Thus, FTIR spectroscopy can provide information about both the inorganic Cu_2O framework and the organic molecules associated with its surface.

Antimicrobial Activity of Cu_2O Nanoparticles

The antimicrobial activity of nanoparticles refers to their ability to inhibit the growth or viability of microorganisms. Copper-based nanoparticles have attracted considerable attention because copper and its oxide forms can interact with microbial cells through multiple mechanisms. These mechanisms may include attachment to the cell surface, disruption of membrane integrity, generation of reactive oxygen species, release of copper ions, and interference with essential cellular and metabolic processes. The agar well diffusion method is a commonly employed qualitative approach for evaluating the antimicrobial activity of synthesized nanoparticles. In this method, microorganisms are cultured on an appropriate solid growth medium, and the test material is introduced into wells formed within the microbial culture. Following incubation, the inhibition zone surrounding the well is examined as an indication of antimicrobial activity. A larger inhibition zone generally indicates stronger growth inhibition under the specified experimental conditions. The antimicrobial response of Cu_2O nanoparticles can vary depending on nanoparticle concentration, particle size, morphology, surface chemistry, microbial species, and physiological characteristics of the microorganisms. Both Gram-positive and Gram-negative bacteria can exhibit different levels of susceptibility because of differences in their cell-envelope structures. Cu_2O nanoparticles may also demonstrate activity against selected fungal organisms, indicating their potential for broader antimicrobial applications.

Green Synthesis of Cu_2O Nanoparticles Using Agricultural Waste

Agricultural and plant-derived wastes contain numerous biologically active compounds that can participate in the synthesis of metal and metal oxide nanoparticles. Materials such as fruit peels, seeds, husks, leaves, and other plant residues may contain phenolics, flavonoids, alkaloids, carbohydrates, proteins, tannins, saponins, and other phytochemicals. These naturally occurring compounds can perform functions that are traditionally fulfilled by synthetic reducing, stabilizing, and capping agents. The use of agricultural waste for nanoparticle synthesis provides a sustainable strategy because it converts low-value biological residues into useful functional nanomaterials. In addition to reducing the dependence on hazardous chemicals, this approach can minimize waste generation and decrease the environmental impact associated with conventional nanoparticle synthesis. Plant extracts can facilitate the conversion of metal ions into nanoscale materials through the action of their constituent phytochemicals. During Cu_2O synthesis, reducing compounds present in the extract can participate in the reduction of copper ions, while other organic molecules can adsorb onto the developing nanoparticle surface and provide stabilization. The composition of the plant extract, extraction conditions, precursor concentration, reaction temperature, pH, and reaction time can influence the nucleation and growth of the resulting nanoparticles.

Mechanism of Cu_2O Nanoparticle Formation

The formation of Cu_2O nanoparticles through a green synthesis route generally involves several interconnected stages, including metal-ion reduction, nucleation, particle growth, and surface stabilization. Initially, copper ions interact with phytochemicals present in the plant extract. Electron-donating functional groups can facilitate the reduction of copper ions to a lower oxidation state. Subsequent nucleation produces small Cu_2O nuclei, which undergo controlled growth to form nanostructures. Phytochemicals adsorbed on the developing particle surfaces can act as capping agents. Surface capping reduces uncontrolled aggregation and influences the final morphology and particle-size distribution. The chemical composition of the biological extract therefore plays an important role in determining the physicochemical characteristics of the synthesized Cu_2O nanoparticles.

SEM can be combined with energy-dispersive X-ray analysis to obtain both morphological and elemental information from the same sample. The SEM image provides information about the physical appearance and distribution of nanoparticles, whereas the EDX spectrum identifies the constituent elements based on their characteristic X-ray emissions. For Cu_2O nanostructures, the detection of copper and oxygen signals provides elemental evidence supporting the formation of the copper oxide material. Comparison of elemental profiles obtained from nanoparticles synthesized using different plant or agricultural-waste extracts can also provide information about the compositional consistency of the products. The combined application of XRD, SEM, EDX, UV–Vis, FTIR, and NMR techniques provides complementary information about the structural, morphological, elemental, optical, and chemical characteristics of green-synthesized Cu_2O nanoparticles. Such multi-technique characterization is essential for establishing relationships between the synthesis route, nanoparticle properties, stability, and antimicrobial performance.

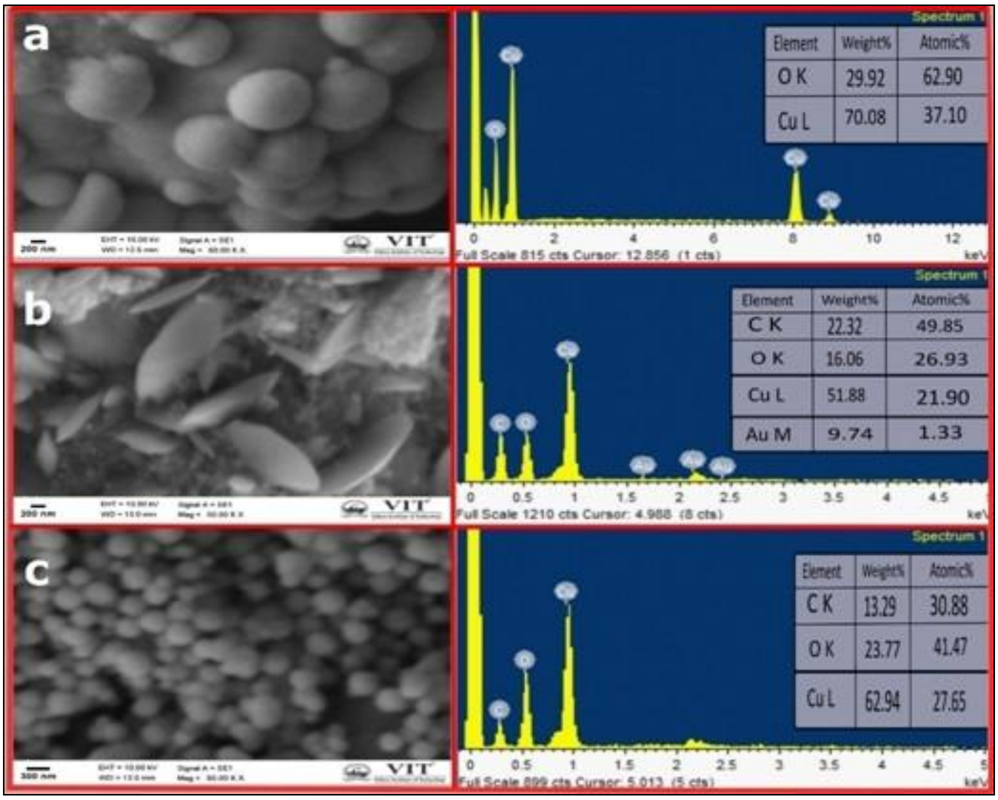

Fig 10: (a) Carrot peel, (b) Coconut husk, and (c) Jack fruit seed extract produced SEMimages and EDAX spectra of Cu_2O nanoparticles.

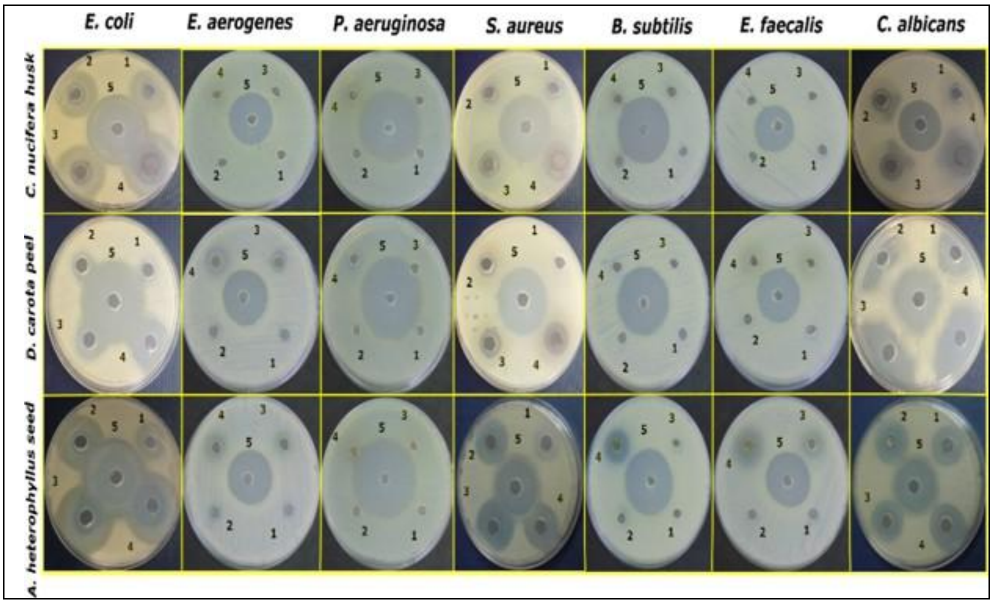

Fig 11: Anti-microbial activity of Cu_2O nanoparticles produced by the extract where 1- 50 μg 2-100 μg 3-250 μg and 4-500 μg of Cu_2O nanoparticle and 5- positive control.

A probable mechanism for antibacterial action has been proposed, even if the exact mechanism is still being debated. Cu_2O Nps generate ROS (reactive oxygen species), inhibiting DNA replication and protein synthesis in microorganisms (Kumari *et al*., 2020). This is also believed to be the case, and it is hypothesized that the Nps adhere to the walls of the bacteria, triggering membrane burst and cell death (Ghadiri *et al*., 2020). In one standard method of metal ion release from metal oxides, the metal ions are slowly released into the epithelial cells, which then pull the proteases and amino acids into the cell, resulting in a variety of impacts, including modifications to the physical structure of the cell and restrictions of enzymatic activity, to name a few (Yaqoob *et al*., 2020). When we looked at the fungal strain *C. albicans*, we found that nanoparticles were able to inhibit its growth and development. Candida albicans stay in virtually everyone for the rest of their lives as a harmless commensal organism. In rare cases, it may result in skin infections and more severe illnesses like those associated with an innate immune response. A diverse array of virulence characteristics and fitness features strengthen the *C. albicans*' capacity to infect its host habitats. This bacterium has several physiological characteristics that aid its pathogenicity. Yeast hyphae development, biofilm formation, and the ability to produce the hydrolase enzymes are all virulence factors. When the organism transitions from rounded "blastopores" to elongated cells that are connected end-to-end, it goes through a reversible morphological change known as "filamentation." The filamentous form of mycelium is divided into two distinct cellular morphologies, which are referred to as false-hyphal cells and real hyphal cells, in order to better visualise the individual cellular morphology. When *C. albicans* was cultured in the presence of the antifungal drug, programmed cell death occurred, which was demonstrated by the presence of snicking DNA, the accumulation of ROS, and an increase in fungal caspases (Laprade *et al*. (2016); Mayer *et al*. (2013)). It seems that the NPs use their antifungal properties bycreating a "rattle" within the cell membrane, which stops the fungal growth.

Procuring the statistical data was done using the Gram-negative bacteria (*E. coli*), the Gram-positive bacteria *(S. aureus)*, and the fungal strain (*C. albicans*). Table 1 offer statistical data that demonstrate the significant differences in the concentrations of the extracts on the development of bacteria and fungus. As far as the *S. aureus* and *E. coli* bacteria go, the jack fruit seed extract has been found to have no antibacterial action (Tramontin *et al*., 2019). The coconut husk inhibits *S. aureus*. However, it has no impact on *Cˆ. albicans*. (Esquenazi *et al*., 2002). According to reports, carrot peel has minimalaction against *C. albicans*, *E. coli*, and *S. aureus*, but it impacts *C. albicans* (El Zawawy, 2015). This research demonstrates that the antibacterial properties of Cu_2O NPs are attributable to Cu_2O NPs and the additional activity aided by the extract. However, when the Carrot peel extract was used to manufacture Cu_2O NPs, they tend to be more active. This Carrot peel-based Cu_2O Nps was effective against *S. aureus* and *C. albicans*, with improved antibacterial and antifungal activities. NPs exhibited greater efficacy against the *E. coli* bacteria due to adding husk and seed extracts of Carrot and Jack fruit. In this study, NPs synthesized using extracts from Carrot and Jack fruit seeds exhibit higher antibacterial action for Gram-positive bacteria. In contrast, NPs synthesized using Coconut husk extract showed superior antimicrobial activity forGram-negative bacteria.

Conclusion

Daucus carota (Carrot) peel, *Cocos nucifera* (Coconut) husk, and *Artocarpus heterophyllus* (Jackfruit) seed extracts were used to synthesize nanoparticles of cuprous oxide (Cu_2O). Through XRD analyses, the produced nanostructures exhibited a cubic cuprite structure. The SEM pictures of the spherical cluster morphology in the Carrot peel and Jack fruit seed extract display SEM photos of the globular cluster morphology, while Coconut husk extract reveals the rice spikelet shape. The several functional groups that helped produce the nanoparticles may be confirmed via FTIR spectra. Although the nanoparticles created using the extracts had similar antibacterial effects, the one synthesized using *D. carota* extract resulted in the most significant outcomes.

The mixture of produced Cu_2O NPs and extracts is most likely responsible for the anti-microbial action.

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