Research Article

Synthesis, characterization, and antimicrobial activity of fermented fruit juice-mediated cu powder: A traditional approach

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ABSTRACT

Aim: Traditional metallic medicines have innate discernable structures; systematic dissection and investigation of their synthesis, may lead to invaluable human benefits and proven applications. Transmutation of even toxic metals and minerals for internal administration has been a very interesting process; it has gathered much research attention these days. Such synthesized metals involved as drugs, for healing diversified human diseases are mercury, gold, silver, copper, zinc, lead and tin etc. The present work aims to synthesize copper in powdered form following traditional lines of thought that has biomedical application. **Materials and Methods:** The synthesized Cu powder is characterized using PXRD, FTIR, UV, SEM and TEM studies. Its anti microbial activity was also investigated against E.Coli., and S.Aureus bacteria. **Results and Discussion:** UV and XRD patterns confirmed the presence of Cu; XRD revealed the copper structure to be face centered cubic in nature. SEM and TEM revealed surface morphological properties; FTIR revealed vibration bands corresponding to C-H bending, C-O stretching and overlapping of OH with amine N-H stretching bonds. The powder exhibited sound anti microbial activity against the two selected species. **Conclusion:** An interesting green and biogenic synthesis involving pre and pro biotic beverages is presented here. This is a novel, cost cutting, eco friendly and excludes the usage of hazardous chemical.

KEY WORDS: Antimicrobial activity, Electron microscopy, Nano copper, X-ray diffraction

INTRODUCTION

The unique physical and chemical properties of materials when they are engineered to nanosizes (100 nm or less) have many applications in the fields of electronics, information technology, cosmetics food industry, and biomedical fields.[1] The striking feature of them in comparison to bulk materials is that, their particles have small size and large surface area providing totally different physical, chemical, electrical, magnetic, and optical properties.[2] Nanoparticles are increasingly becoming vital factor in strengthening the fight against pathogenic microorganisms which also results in a change in approach toward combating infectious diseases.[3] Nanoparticles are undergoing a revolution in treating critical diseases such as cancer and rheumatoid arthritis.[4] It is well known that their extreme small sizes enable them to be utilized for potential target-oriented drug delivery to organs such as brain which are usually protected by blood-brain barriers. For the past 5 years, there has been a rapid rise of interfacing between nanotechnology and biology incorporating diverse properties of these minute materials.[5]

Metal nanoparticles and nanomaterials have many applications in the field of biology and pharmacy due to their catalytic, optical, electrical, and magnetic properties.[6] The intensified biological activity of these particles against microorganisms needs profound investigation before their commercialization. There have been quite encouraging results depicting the bioactivity of different drugs and antimicrobial formulations incorporating nanoparticles. For centuries solids, disinfect fluids, and tissues were embodied with metal ions, either alone or in complexes. During the time of Hippocrates (400BC), ancient Greeks were the pioneers in unfolding the sanitizing power of copper. Copper was also prescribed for pulmonary diseases and for purifying drinking water.[7]

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By the 18th century, copper had come into wide clinical usage in the western world in the treatment of mental disorders and affrications of the lungs. Copper is one of the relatively small groups of metallic elements which are essential to human health. These elements along with amino and fatty acids and vitamins are required for normal metabolic processes. The adult body contains between 1.4 and 2.1 mg of copper per kg of body weight. It is estimated that a human eats and drinks about 1 mg of copper every day. After nutritional requirements are met, excess copper is released into bile and excreted in the feces.[8]

Extensive and prolonged usage of copper in intrauterine devices is one of the copper significant applications. In contrast, to the low sensitivity of human tissue (skin or other) to copper, microorganisms are extremely susceptible to copper.[9] It has been demonstrated that copper used in paints in rendering self-disinfecting surfaces is effectively bacteriostatic.[10] This steered the usage of copper in paints which reduced fouling and microbial biofilm formation in ships.[11] To kill bacteria in dental tubules, addition of Actifed copper remarkably enhanced the antibacterial properties of calcium hydroxide. Woven and non-woven fabrics produced from copper bound textile fibers have been developed as a groundbreaking technology.[12]

In agriculture, copper compounds are most extensively employed and it was discovered that copper sulfate solution dripped with seed grains interdicted seed burn fungi. In addition to copper sulfate, around 23 copper compounds were screened by international copper research association regarding their potency in killing snails of water of high and low alkalinity including and excluding high level of suspended solids.

The antimicrobial mechanism of nanoparticles can be understood by studying their specific binding to the surfaces of microorganisms and the consequent metabolism of such materials inside microorganisms. However, the development of effective antibacterial materials with tailored properties that are capable of delivering a controlled release of copper ions is still an open research area.[13] Recent findings also report easy entry of these particles through respiratory, gastrointestinal, and dermal passages.[14,15] It is also found that nanoparticles may produce adverse effects in human cells on contact.

It can be said that the choice of copper in the present work is triggered by the above-mentioned factors. In addition, a vast body of growing literature reveals that copper in nano dimensions is reported to have antimicrobial activity against a number of species of bacteria and fungi.[3]

A number of synthesizing techniques were being reported to prepare copper nanoparticles including thermal reduction, a capping agent method, sonochemical reduction, laser irradiation, and induced radiation.[16] In all the above techniques, copper at nanosize can be extracted at high temperatures and also using highly concentrated chemicals which cause environmental destruction. Sastry *et al*. have synthesized copper nanoparticles using aluminum vessel and different types of surfactants mixed in aqueous solution of copper sulfate for a reaction period of 15 min. Sudhirshende *et al*. have synthesized copper nanoparticles by citrus medica using aluminum vessel by heating it with the mixture containing juice and copper sulfate solution. The temperature considered was 60–100°C.[17,18]

Ancient traditional approaches of medicine like Indian alchemy or herbal medicines such as Ayurveda transmute metals, minerals, and other materials into acceptable form for internal administration by following various pharmaceuticals processing methods.[19] The philosophy of Indian Alchemy believes matter as a combination of elements which are volatile and fixed in general.[20] Matter is laid on a scale of three philosophical principles, one is molten fluid with vitreous matter, and the other two are combustible oily matter and volatile fluid matter. It is to be considered here that these were not chemical substances rather these were philosophical principle ingredients in every matter. The theory also says that one of the volatile principle substances (known as essence of a material) can be extracted from minerals, metals, and metallic salts. This can be attributed to a typical pyrometallurgical process. All the procedures generally follow a three-step process in drug preparation; they are pre-treatment of material, calcinations or metal/essence extraction, and post-treatment.[20,21]

Pre-treatment of raw materials in any further usage of experimentation/processing is considered to be most vital aspect in Indian alchemical system.[22] It is generally termed as purification/pre-treatment, and it was believed by the ancient Indians that this process transforms the inherent attributes of a substance which leads in addition of new properties. This belief can also be seen in other alchemical systems, especially of Geber. Various modes of purification procedures are mentioned in Indian alchemical system such as boiling, churning, grinding, and impregnation.[23] Here, copper sulfate has gone the process of pre-treatment as mentioned in the alchemical texts.[21]

A growing body of literature has examined that nanoparticles may produce potential functional and toxicity effects on human neural cells. This was due to the ability of these particles to pass through biological membranes.[24] Fermented liquids are traditional ayurvedic alchemical beverages used as probiotics and purifying agents in cleansing metals. Many texts mention such recipe incorporating fruits/herbs/rice/cereals.[20,21,25]

The present study is carried out to synthesize the nano copper using the above-mentioned ancient ferment. Morphological and physiochemical properties of extracted copper were evaluated. We also investigated the antimicrobial properties of synthesized metallic copper powder.

MATERIALS AND METHODS

Fruits of pomegranate were selected from local market of Kurnool for fermenting process.[25] Commercially available $CuSO_4.5H_2O$ (Merk, (GR) 98% purity) was used for synthesis of the copper particles.

The copper (II) sulfate pentahydrate salt $CuSO_4.5H_2O$ (Merk, (GR) 98% purity) was pre-treated following alchemical procedure.[26] Where it is noteworthy that few researchers have attempted to investigate the effect of such pre-treatment/purification procedures increased the antimicrobial and antifungal properties of copper sulfate and they found that more than six such species were significantly executed. An emerging body of literature showed significant increase in percentage of copper and iron followed by decrease in sulphur after pre-treatment.[26-29] After drying, the powder was mixed with fermented pomegranate juice[25] in an iron vessel and left for a reaction period of 7 days. The iron vessel was subjected to fumigation using some pungent dried herbal powders such as piper nigam, shalom album, powder of zingiher, officinate, piper longum, and ferula asafoetida, respectively. On the past day, the mixture was removed and post-treated by further cleansing mechanisms usually followed in traditional copper purification methods.[30] This was intended as special purification. The dried powder was utilized for further studies.

As and when fermented pomegranate juice was mixed with powdered and pre-treated copper sulfate, the solution initially turned light pink in color. Displacement of a reactive metal with a lesser reactive metal from its compound is a well-known cementation process.[17] As the reaction proceeds, a thin wall of copper coating at various locations was observed on the 2nd day of the experiment. By now, the color of the liquid was darker in pink than earlier. The number of such bright copper layered zones started increasing during the succeeding days of the experiment. The vessel was left undisturbed for a week, and it was noticed from several trials that any sort of disturbance to the vessel in the form of severely mixing the mixture within these days drastically reduced the copper formation in a significant way.

Microorganisms

Two species of bacteria, one includes Gram-positive species, i.e., *Staphylococcus aureus*, the other Gram-negative species, i.e., *Escherichia coli* were obtained and maintained on Luria- Bertani agar. Before incubation with the nanoparticles, the microorganisms were cultured overnight in 5 ml of Luria-Bertani broth (Fluka) in a Certomat BS-T incubation shaker (Sartorius Stedim Biotech, Aubagne, France) at 37° and 150 rpm until the microbial culture reached on OD_{600} of 1.0, corresponding to 8×10^8 colony-forming units per ml, as determined used an Ultrospec ultraviolet-visible 3000 spectrophotometer. The antimicrobial activity of the synthesized microorganisms was investigated using zone of inhibition method.

Antimicrobial Susceptibility Test

The antibacterial properties of the synthesized nano copper particles were evaluated by the qualitative method against the mentioned microorganisms using the agar disk diffusion method as described previously as depicted in Figure 1a and b. Gram-positive and Gram-negative bacteria were cultivated on nutrient agar medium. Briefly, 2.8 g of agar powder is mixed in 100 ml of distilled water. Prepared agar medium was powered in sterilized Petri plates and allowed to solidify. The surfaces of the solidified agar plates were allowed to dry in the incubator before streaking of microorganisms onto the surface of the agar plates. The above-mentioned microorganisms were collected from the culture tube with a prior sterilized metal loop and streaked on solidified agar plates. Then, bores or cups were made in agar Petri plates with a sterilized metal borer, and the sample was placed in the cup. The Petri plates were placed in the incubator for 36 h.

RESULTS AND DISCUSSIONS

X-ray diffraction (XRD) is a technique used to identify the crystalline phases present in materials and to measure the structural properties. The XRD patterns for the nanoparticles are shown in Figure 2. The peaks at 43.0°, 50.1°, and 73.9° of the spectrum correspond to the (1 1 1), (2 0 0), and (2 2 0), respectively, represent face-centered cubic structure compared with the standard JCPDS of copper (JCPDS– 01-089-288). No other impurity peaks were observed, suggesting that the synthesized particles were of high purity. The average nanocrystalline size (d) was calculated using the Scherrer formula. The average crystallite size was calculated for Debye-Scherrer equation and was found to be the range of 6–5 nm. The synthesized

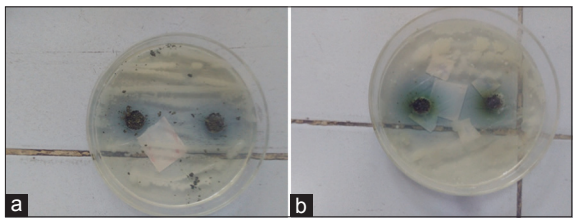

Figure 1: (a) Antibacterial activity of Gram-positive bacteria, (b) antibacterial activity of Gram-negative bacteria

nanoparticles show an absorption peak around 570 nm which is shown in Figure 3. Nanosized Cu particles generally exhibit an SPR at around 500–600 nm. Therefore, this peak can be ascribed to be the absorption of nanoparticles of copper.

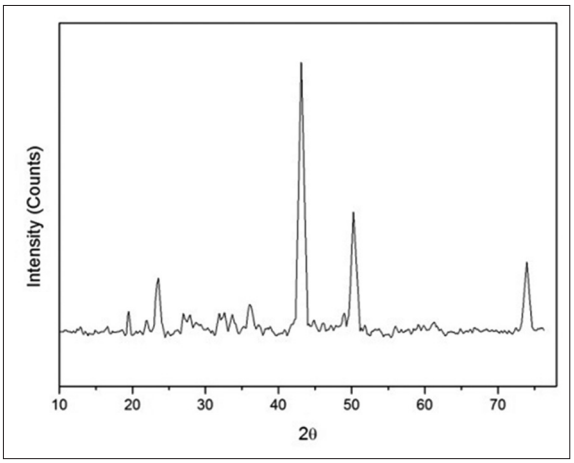

Figure 2: X-ray diffraction pattern of copper powder

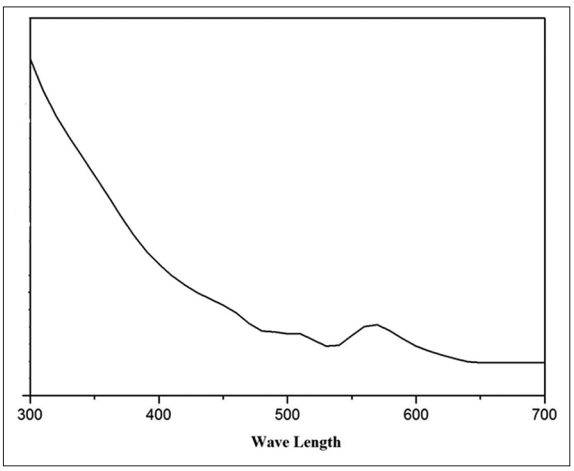

Figure 3: The ultraviolet-visible spectra of copper powder

An additional confirmatory test was performed using Fourier transform infrared (FTIR). The following Figure 4 indicates the spectra of synthesized nano copper using pomegranate juice. The FTIR spectra show vibration bands at 3410.72 cm^{-1} which may be due to the overlapping of OH and amine N-H stretching bonds, peaks at 1638.89 cm^{-1} and 1380.14 cm^{-1} indicates C-H bending and 1090.24 cm^{-1} indicates C-O stretching.[30] In addition, new moderate intensity peak representing copper nanoparticles were evident at 607.86 cm^{-1}.

Scanning electron microscope is one of the most widely used techniques for characterization of nanomaterials. The signals from electron sample interact and reveal the information of surface morphology, i.e., texture and chemical composition of the sample. The morphology of the copper nanoparticles was carried out and shown in Figure 5. It was shown that they are spherical in nature and uniform. The grain size of nanoparticles is in the range of 181–228 nm.

Transmission electron microscopy (TEM) is widely used to observe the size of small nanoparticles through imaging, revealing phases. TEM image was obtained with an accelerating voltage of 200kV. TEM is commonly used for imaging and analytical characterization of the nanoparticles to access the shape, size, and morphology. TEM image of the prepared spherical-shaped copper nanoparticles is represented in Figure 6. In addition to the individual particles, some aggregates are also present the particle sizes are estimated in the range of 79–120 nm.

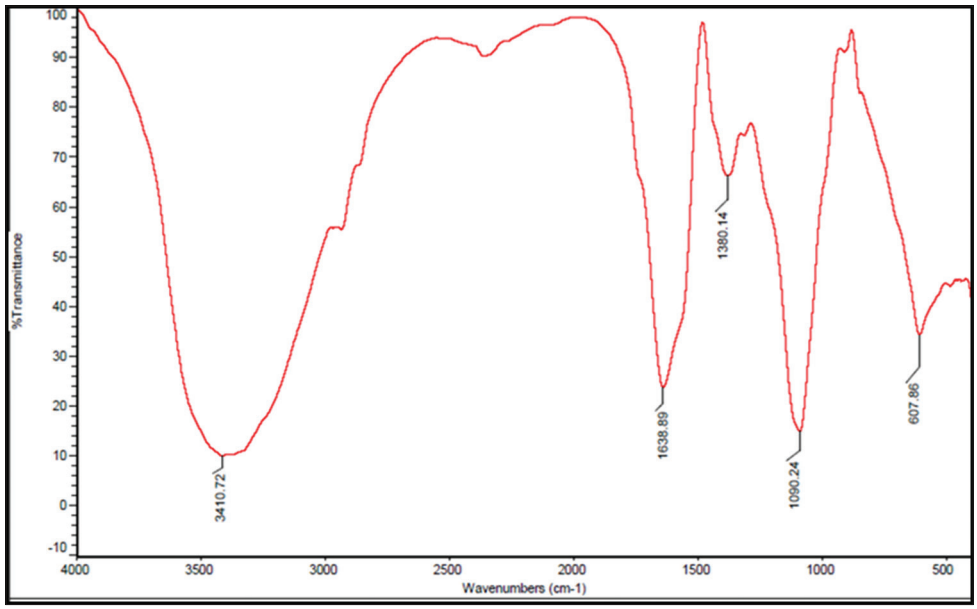

Figure 4: Depicts Fourier transform infrared analysis of copper powder

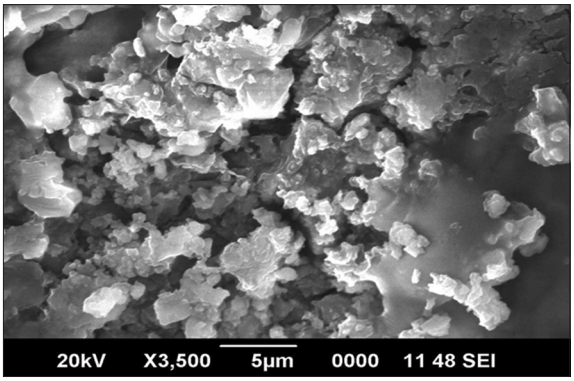

Figure 5: Scanning electron microscopy analysis of copper powder

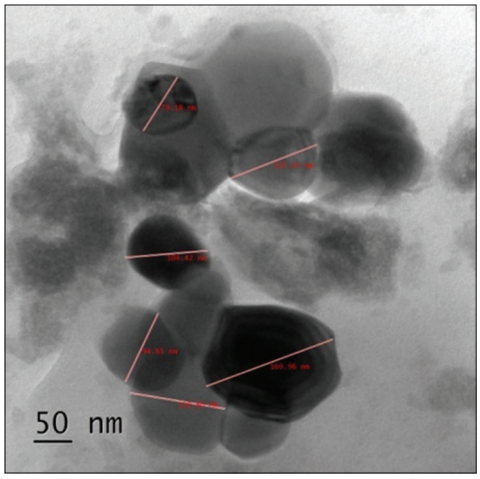

Figure 6: Represents transmission electron microscopy analysis of copper powder

Antimicrobial Activity of Cu-Np's

After placing the Petri plates in the incubator for 36 h, they were removed from incubator and examined zone of inhibition occurred around the cups, which indicates that the synthesized nano copper particles have antimicrobial activity.

It has been observed that high concentration of copper nanoparticles demonstrates complete cytotoxicity against *E. coli* and *S. aureus*. These nanoparticles adhere to the bacterial cell wall and break through the cell membrane and copper ions destroy the bacterial cell wall, which becomes thick and coarse, the cytoplasm is then degraded and disappears, leading finally to cell death.

The antibacterial mechanism is attributed chiefly to the strong adsorption of copper ions to the bacterial cells which imparts antibacterial efficacy in a concentration-dependent manner. Nanoparticles have a large surface to volume ratio, which enhances their bioactivity and makes them effective bacterial agents. It can be aspired that in future copper nanoparticles could replace some antibiotic medicines.

CONCLUSION

In this article, an interesting green synthesis method is incorporated following the ancient traditional routes and inspired by its fermented probiotic beverages that act as synthesizing agents. This approach is a novel, cost-cutting, eco-friendly, and energy efficient which excludes usage of chemicals. The as-synthesized metal powder was characterized with different modern approaches, and further, its antimicrobial activity was also studied using bacterial and fungal species. This will allow for the application and usage of these metal powder particles in the formulation of new types of antimicrobial materials such as topical gels for pharmaceutical and biomedical applications.

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