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Research Article
Biosynthesis of Copper Oxide Nanomaterials Using the Seeds of Date Fruits (*Phoenix dactylifera* L.) and Antibacterial Activity Evaluation

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Abstract

Background and Objective: Green chemistry approach is a simple, eco-friendly, less toxic, cost-effective and biological method. *Phoenix dactylifera* seed extract has been used as a reducing and capping agent for the green synthesis of copper oxide nanomaterials. This approach had forewarned the interest in researching natural waste products to increase the usage of alternative therapies for infectious diseases. The present investigation determined the production of biogenic copper oxide nanomaterials using the seeds of date fruits (*Phoenix dactylifera* L.) by green approaches and an eco-friendly process. **Materials and Methods:** Extract of seeds of date fruits acted as potential and effective bio capping and reductant agents for bio-synthesis of copper oxide nanoparticles. The properties of biogenic copper oxide nanomaterials were assessed and characterized by the FT-IR, SEM, EDX, XRD and TGA analysis. **Results:** All the characterization results were confirming that produced copper oxide nanomaterials are spherical in shape with a size of 30±6 nm. Synthesized copper oxide nanomaterials are highly pure forms and resistant to high temperatures. Further, the antibacterial activity of green synthesized copper oxide nanomaterials against human bacterial pathogens was evaluated by the agar well diffusion method. The maximum zone of inhibition was obtained in *E. coli* as compared to the positive control (tetracycline). **Conclusion:** The results of the antibacterial assay indicate that biogenic copper oxide nanomaterials should be considered as an antibacterial agent for the treatment and prevention of infectious diseases.

Key words: Biosynthesis, copper oxide nanoparticles, *Phoenix dactylifera* L., antibacterial activity, FTIR, XDR

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In current years, the green synthesis of metal nanoparticles is an attractive area of nanotechnology and nanoscience. Bio-fabrication of metal oxide nanomaterials using organisms is a vital role in green chemistry. The green synthesis route is a simple, one-step, eco-safe, low-cost, efficient, non-toxic, relatively reproducible and alternative method for the production of nanomaterials with the desired properties[1]. The nanotechnology field is offering various chemical and physical processes namely, photochemical reduction techniques, ultraviolet irradiation, laser ablation, lithography, aerosol technologies and laser ablation for the synthesis of different inorganic metal and metal oxide nanomaterials[2]. However, among these methods, the biological method seems to be the suitable candidate for the production of nanomaterials[3]. Green synthesized nanomaterials are more stable as compare to chemical and physical methods mediated nanomaterials. Hazardous chemicals or high energy requirements are not needed in biological methods. The process of purification/removal of chemical contamination in chemical and physical methods of nanomaterials is a very difficult method[4]. The extracts of naturally available plants and microbes have phytochemicals and polymers and are used as capping and reductant agents in green synthesis. Microbes can be employed for the synthesis of nanomaterials but the rate of production is slow as compare to phyto-mediated synthesis[5].

Metal oxide nanomaterials are broadly used in various fields like medicals, agricultural and environmental applications. These are showing the anti-cancer, anti-bacterial, anti-fungal, anti-inflammatory, anti-angiogenesis activities in a medical field[6]. Besides, nano metal oxide is utilized in drug delivery systems and drug formulations. Moreover, metal oxide nanomaterials are employed in wastewater treatment, adsorbents, dye degradation, photo-catalyst, electro-catalysts and gas sensor, etc[7]. Various metal oxide nanomaterials such as CuO, MgO, ZnO, AgO, CeO_2, TiO_2, SnO_2, Al_2O_3, etc are synthesized by green chemistry approach, physical and chemical techniques[8].

Copper and copper nanomaterials have attracted considerable attention because it is one of the most used in recent technologies and is readily available. Different methods have been proposed for the synthesis of nanomaterials. Copper is the king of organic materials. It is widely used in various fields due to its unique physical, chemical and biological properties[9]. Copper is used in various applications like gas sensors, photoconductive applications, superconductors and so on. Nowadays, copper oxide nanomaterials are used as antibacterial and antifungal agents against different types of bacterial and fungal pathogens[10]. Recently, so many researchers proposed new methods and novel ideas for the production of copper oxide nanomaterials using seed extract, plant extract and microbial extracts. The rice-shaped copper oxide nanomaterials were produced using the using *Caesalpinia bonducella* seed extract[11]. The wheat seed extract has been employed for the synthesis of copper oxide nanomaterials and assessed its catalytic potential properties[12]. *Persea americana* seeds were utilized for the bio-fabrication of copper oxide nanomaterials. Besides, its antimicrobial and antioxidant properties were analysed[13].

Phoenix dactylifera L., is usually identified as the date palm. It is belonging to the Arecaceae family. It is mostly cultivated in the desert oasis of the Arab countries for its edible fruits. The fruits have dietary fibres, vitamins, minerals, lipids and proteins[14, 15].

In this present work, the aim was to produce the biogenic copper oxide nanomaterials using the *Phoenix dactylifera* seeds through a simple, eco-friendly, inexpensive method. Resource utilization and waste utilization approach has been involved in this method. In addition, the anti-bacterial properties of *Phoenix dactylifera* seeds mediated copper oxide nanomaterials were evaluated.

MATERIALS AND METHODS

Study area: The research was performed in Riyadh, Kingdom of Saudi Arabia during the 7 months from September, 2020 to March, 2021.

Material collection: *Phoenix dactylifera* (Date palm) fruits were obtained from the supermarket, Saudi Arabia and seeds were removed and collected for preparation of extract. High-grade chemicals and reagents were purchased for the synthesis of nanomaterials. The glasswares were rinsed by the acids and distilled water. The de-ionized water was employed for nanomaterial synthesis.

***Phoenix dactylifera* seed extract preparation:** The cleaned *Phoenix dactylifera* seeds were milled using the grinder and the powder was collected. Then, the fine powder was obtained after the segregation process. The 2 g of seed powder was mixed with 100 mL of deionized H_2O and boiled at 80°C on a water bath for 2 hrs. Next, the mixture was filtered by Whatman filter papers. The aqueous extract of *Phoenix dactylifera* seed extract was saved and stored in a cold room for more analysis.

Copper oxide nanomaterials synthesis: Copper oxide nanomaterials were synthesized by the green chemistry route and co-precipitation method. The 100 mL of 2% seed extract was mixed to the 0.1 M of copper sulphate at 80°C under constant stirring. The pH was maintained at 9. At the end process, the brownish-black colour pellet was received. The pellet was dried using the hot air oven. Finally, the black colour powder was collected and stored in cleaned and sterile bottles for characterization analysis[16].

Copper oxide nanomaterials characterization: The characterization of copper oxide nanomaterials was done to know the size, shape and purity of the synthesized nanomaterials. As-synthesized copper oxide nanoparticles shape was determined by Scanning Electron Microscope. The chemical moiety on green synthesized nanomaterials was assessed with help of Fourier Transform Infrared Spectroscopy. The crystalline nature and size of fabricated nanomaterials were characterized and estimated through X-ray Diffraction analysis. The Energy Dispersive X-Ray analysis was performed to find out the purity of the bio-synthesized nanomaterials. Thermogravimetric analysis was assessed by Mettle-Toledo TGA.

Copper oxide nanomaterials antibacterial activity: The antibacterial potential was investigated for green synthesized copper oxide nanomaterials by the agar well diffusion method. The gram-negative bacteria (*E. coil* and *P. aeruginosa)* were used in the assay. Various concentrations of copper oxide nanomaterials were prepared for assessing their antibacterial potential. The Muller Hinton agar plates were prepared and swab the sample culture on it. After that, wells were made and different concentrations of nanomaterials were added to each well. Then, the plates were incubated at 37°C for 24 hrs. The tetracycline was employed as a positive control for this assay. After 24 hrs, the zone of inhibition (in diameter) was calculated[17].

RESULTS AND DISCUSSION

Characterization: XRD analysis reveals the crystalline nature of nanomaterials, the size, structure of nanomaterials and the purity and phase of nanomaterials. Figure 1 showed the XRD pattern of the green synthesized copper oxide nanomaterials. The peaks at the 2θ region were corresponding to the (110), (111), (022), (200), (202), (112) and (222) planes based on the crystal of nanoparticles. Debye Scherrer's formula was used to calculate the size of particles and was found to be around

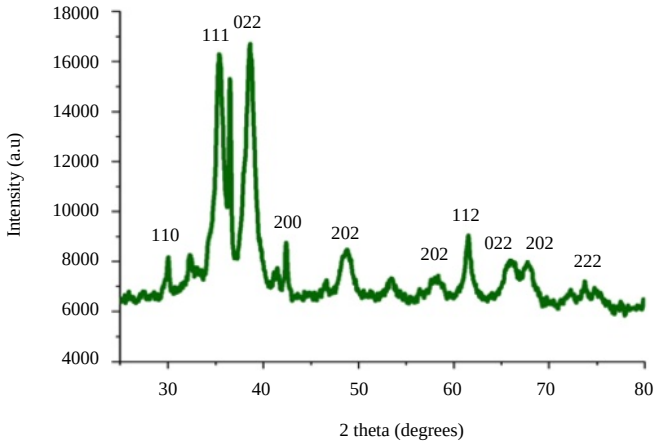

Fig. 1: XRD pattern of *Phoenix dactylifera* seed-mediated copper oxide nanoparticles

30±6 nm. Ghidan *et al.*[18] reported the green chemistry route for the synthesis of copper oxide nanoparticles with help of the *Punica granatum* peels extract. Current results are consistent with previous investigations on the biosynthesis of copper oxide nanoparticles[16-18].

FT-IR spectroscopy analysis is used to examine the surface functional groups in biosynthesized nanoparticles. Moreover, it helps to find out the possible bio-molecules that act as reducing/stabilizing/capping agents for the production of copper oxide nanomaterials. Figure 2 refers to the spectra of bio-synthesized copper oxide nanomaterials and displayed the major strong peaks at 3677, 3302, 3080, 1709, 1454, 1149, 974, 877, 644 and 421 cm^{-1}. The occurrence of the C-H stretch of an alkenyl group on the surface of nanomaterials was confirmed by the presence of a peak at 3677 cm^{-1}. The bands at 1709, 1454 and 1149 cm^{-1} are referring to the C–O stretching vibrations, N–H bending vibrations, C=C stretching and –C=O stretching vibrations respectively. The peaks are 877, 644 and 421 cm^{-1} present in spectra due to metal oxide groups of copper oxide nanomaterials. The phyto-molecules from seed extract act as reducing/capping/stabilizing agents during the development of phyto-mediated CuO NPs[19] and present on the surface of nanomaterials.

Figure 3 depicts the SEM micrograph of biosynthesized copper oxide nanomaterials. It determines that as-synthesized nanomaterials are spherical. EDX spectra represent the purity of elements by reflects the two major groups of peaks (copper and oxygen elements). EDX study outcome the constitution of metals present in the sample of the present study synthesized nanoparticles contain cu as the major constituent element with intensity count 400 at 20 Kev in Fig. 4. The high

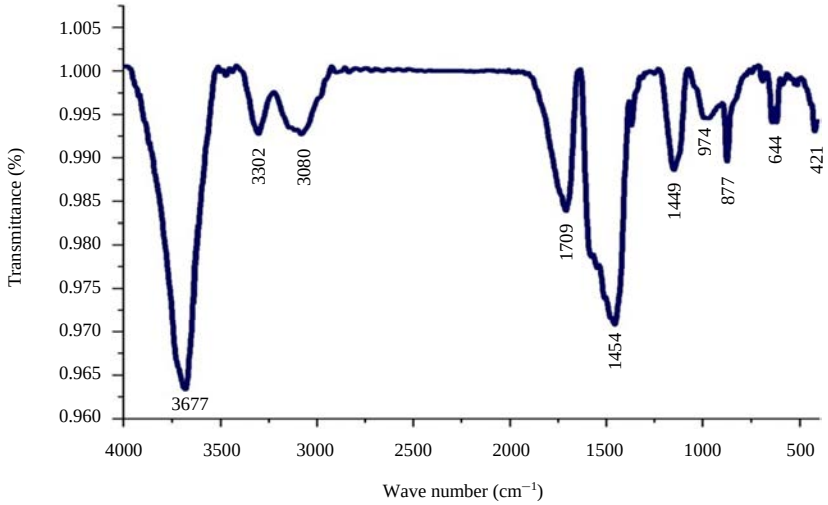

Fig. 2: FTIR spectra of *Phoenix dactylifera* seed-mediated copper oxide nanoparticles

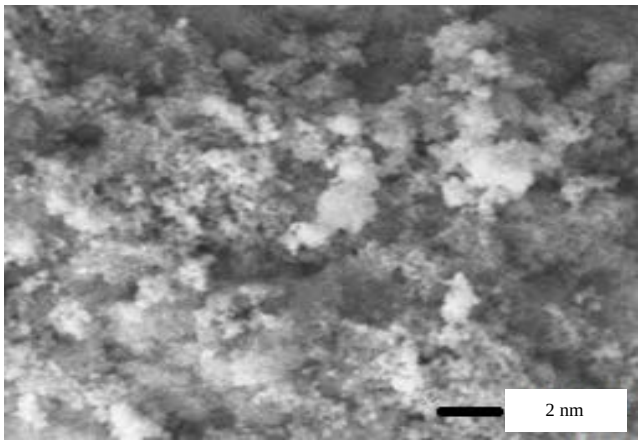

Fig. 3: SEM micrograph of *Phoenix dactylifera* seed-mediated copper oxide nanoparticles

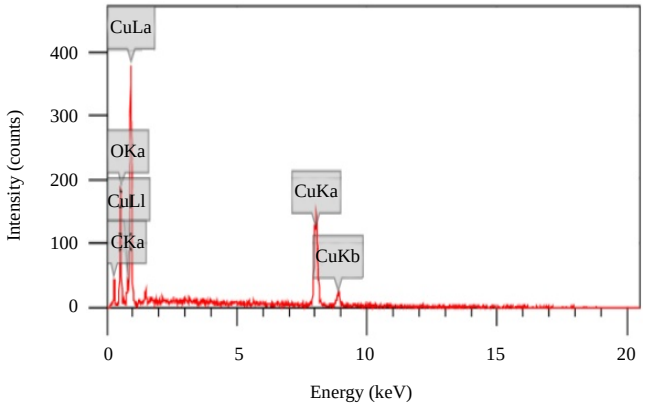

Fig. 4: EDX spectra of *Phoenix dactylifera* seed-mediated copper oxide nanoparticles

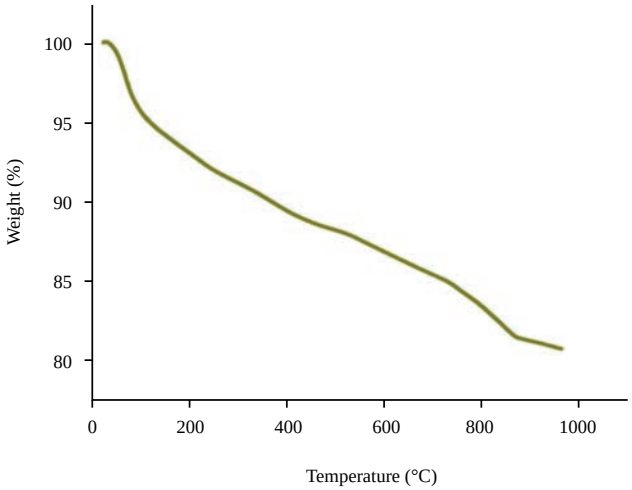

Fig. 5: TGA spectra of *Phoenix dactylifera* seed-mediated copper oxide nanoparticles

percentage of copper and oxide has confirmed the formation of copper oxide nanomaterials by green chemistry route[17]. Hence, the EDX spectra represent and confirm the formation of copper oxide nanomaterials by *Phoenix dactylifera* seed extract. The 19.5% of weight loss was occurred in green synthesized nanomaterials at 900°C in Fig. 5 due to the loss and decomposition of mass under the high temperature, time and atmosphere.

Antibacterial activity: The antibacterial activity was assessed using *Phoenix dactylifera* seed extract mediated copper oxide nanomaterials against human bacterial pathogens. The maximum zone of inhibition occurred in *E. coli* when compared to the positive control. The minimum zone of

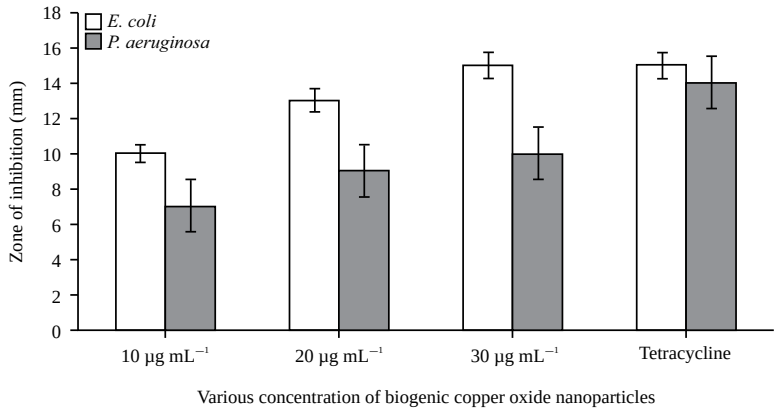

Fig. 6: Antibacterial activity of *Phoenix dactylifera* seed-mediated copper oxide nanoparticles

inhibition was obtained in *P. aeruginosa*. The tetracycline was used as a positive antibiotic to comparing the activity in Fig. 6. Sivaraj *et al.*[16] reported the synthesis of biogenic copper oxide nanomaterials using the *Tabernaemontana divaricata* leaf extract and tested its antibacterial activity against the urinary tract pathogen.

CONCLUSION

The copper oxide nanomaterials have been synthesized by the green chemistry approach. It is a simple, eco-friendly, less toxic, cost-effective and biological method. *Phoenix dactylifera* seed extract has been used as a reducing and capping agent for the green synthesis of copper oxide nanomaterials. These nanomaterials were investigated for the antibacterial activity against Gram-negative and human pathogens. Green synthesis copper oxide nanomaterials show the enhancement of antibacterial activity. The results determine that biogenic copper oxide nanomaterials are a new and novel antibacterial material.

SIGNIFICANCE STATEMENT

This study discovered the beneficial effects of copper oxide nanoparticles *Phoenix dactylifera* seeds against the antimicrobial activity. The study will help the future researcher to uncover the critical areas of eco-friendly, less toxic, cost-effective biogenic copper nanomaterials from *Phoenix dactylifera* seed that researchers were not able to explore to date. This may be a promising finding for the future will focus the formulating pharmaceutical drug using this biogenic copper nanomaterial and in the prevention of side effects.

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