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A NEW PARADIGM SHIFT TO PREPARE COPPER NANOPARTICLES USING BIOLOGICAL SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY

Wisam J. Aziz and Haneen A. Jassim*

Department of Physics, College of Science, Mustansiriyah-University, Baghdad, Iraq.

Abstract

Inthe present study, a simple way, fast and environmentally friendly "hydrothermal method" for preparation Cu nanoparticles. Cu NPs were prepared from *Syzygium aromaticum* (clove) bud extract. The synthesized nanoparticles were subjected to structural, optical, morphological, antimicrobial and antifungal studies. The nanoparticles structural properties and surface morphology formation were studied by X-Ray diffraction, Scanning Electron Microscopy and average grain size also have been calculated. Ultraviolet-visible (UV-Vis) absorption spectrum showed the absorption peak of CuNPs at 305 nm. The high crystalline nature of CuNPs with an (FCC) phase isobvious from the X-ray diffraction (XRD) pattern. The greatest intensity peak located at (111) orientation. Crystallite size was found to be ~12 nm. The obtained formation was homogeneous and relatively spherical and cluster nanoparticles. The antimicrobial studies of CuNPs effect against *Bacillus subtilis*, *Escherichia coli* and pronounced fungicidal activity against *Candida albicans* of microorganisms. The diameters of the inhibition zones of CuNPs were of *Bacillus subtilis* (42mm) and *Escherichia coli* (33 mm) at 200 µg/ml concentration. The diameter of the inhibition zones of CuNPs against the fungus strains was of *Candida albicans* (50 mm) at 200 µg/ml concentration. The current study clearly indicates that the *Syzygium aromaticum* bud extract mediated CuNPs exhibited the excellent antimicrobial activity.

Key words : Biological synthesis, hydrothermal, *Syzygium aromaticum* (Clove), copper nanoparticles, antibacterial and antifungal activities.

Introduction

During the last two decades, nanostructured materials were extensively studied because of their unique properties (Kalita, 2012; Ajitha, 2016). In particular, metal nanoparticles have received a great attention because of their, electronic and magnetic properties (Jain, 2008; Nasrollahzadeh, 2014). It has been reported that metal nanoparticles (Cu and Ag) show a broad spectrum of antimicrobial activity, including the (gram negative) and (gram positive) bacteria as well as fungi (Chatterjee, 2014; Ajitha, 2016; Sneha, 2011). Among all metallic nanoparticles (CuNPs) has drawn a much attending owing to their versatile applications, for instance, of electrical, optical, antibacterial and antifungal purposes (Prasad, 2016; Cerda, 2017). In addition, the availability and cost of Cu made it as a better choice comparing to the Ag and Au.

The usefulness of Cu as an antimicrobial activity, it is an effective agent with less toxicity, which is especially important in the biomedical field. Many synthesis methods were employed for the synthesis of Cu nanoparticles such as chemical, solvothermal, sonochemical and biosynthesis (Mandal, 2006; Ahmad, 2003). Nanotechnology need for to expand substitute methods for biochemical synthesis, which is safe, non-toxic, clean and eco-friendly. One such method is a greener synthesis, were decreasing and capping agents such as bacteria, fungi and plants are used (Salvadori, 2013; Abdul Hameed, 2012; Thakkar, 2010; Krumov, 2009; Stoytcheva, 2011). The green syntheses of nanoparticles through natural seed extracts have a significant potential to boost the nanoparticles product without the use of toxic and expensive chemicals. Due to an effective nature of low toxicity, Cu metal is an especially important in the typical antibacterial treatment. Many reports confirm the antimicrobial antiviral properties of spice plants (Shan, 20050. Clove is one of most

***Author for correspondence :** E-mail: hanenali940@yahoo.com

valuable spices and has attracted much attention due to antimicrobial activities (Cortes-Rojas, 2005). In this article, the cost-efficient and completely biogenic method for synthesizing Cu NPs by (cloves) bud extract and could be subjected to the structural, morphological, optical, antimicrobial and antifungal studies.

Experimental Work

Materials

In this study, Copper nitrate Cu $(NO_3)_2$ (Reagent World, USA, purity 99.99%), Sodium hydroxide and *Syzygium aromaticum* buds were collected from the local market. DW used as a solvent.

Bud extract

Syzygium aromaticum bud (20 g) was washed with (DW) and dried at room temperature for 24 hrs. The buds were crushed by grinderin 50 ml of sterile DW and heated for 30 min at 90°C. The extract was then filtered using Whatman's No.1 filter paper. The filtered extract wascollected and saved for more usage in CuNPs synthesis as shown in fig. 1.

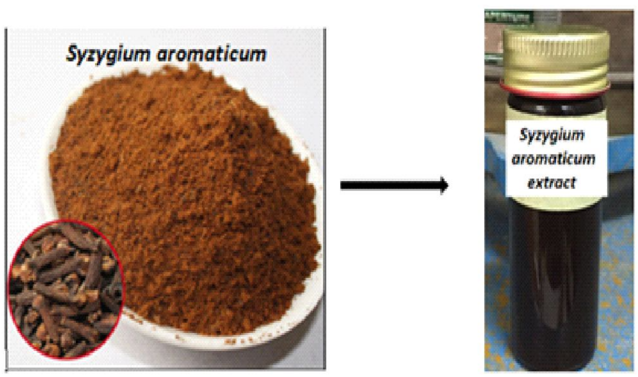

Fig. 1 : The conversion of *Syzygium aromaticum* into a *Syzygium aromaticum* extract.

Biosynthesis of Cu NPs

For the synthesis of CuNPs, 20 ml of 0.1 M of the copper nitrate sol prepared in a reaction vessel of 80 ml volume at (60-70°C) of temperature. Later, 25 ml of aqueous extract of *Syzygium aromaticum* bud was added and mixed homogeneously on the magnetic stirrer for 30 min. After that, add 5 ml of sodium hydroxide of 14 M to change the pH status in pH=14, the pH change was examined by pH test paper. To ensure the formation of CuNPs, Within 15-20 min, the blue color solution changes to pale bluish-green color due to formation of CuNPs. The mixture was added to seal Teflon-lined vessels of 100 ml capacity (autoclave) and heated in the oven at 185°C for 3 hours. NPs were purified and washed with accetone and distilled water 5 times by centrifuging at 4000 rpm for 20 min. Finally, dried in air at 30°C for 48 hours.

Properties of the Cu NPs

Synthesized of CuNPs were analyzed to different characterization techniques at room temp. The structural characteristics of the synthesized NPs were analyzed by X-ray system (Shimadzu - XRD6000, Shimadzu Company, Japan). The X-ray source was Cu-Kα radiation with 0.15406 nm wavelength. The system operates at 40 KV and 30 mA emission current. The sample is scanned from (20 - 90 degree). UV-Visible absorption study was assessed using a UV-visible spectrophotometer (CARY, 100 CONC plus UV-Vis-NIR, Split- beam Optics, Dual detectors) spectrophotometer equipped with a xenon lamp at a wavelength range at (300-900 nm). The morphology of Cu NPs was examined by SEM.

Results and Discussion

Structural analysis (XRD)

XRD was used to calculate the crystalline characteristics of the synthesized nanoparticles. All the peaks agree with the (FCC) structure and were in consistency with the JCPDS approved (No. 04-0836) data. It observed three diffraction peaks agree into (111), (200) and (220). The largest intensity peak implies the (111) orientation. The peaks placed at $2\theta^0 = 43.297^0$, 50.433^0, 74.130^0. The peak positions are in good agreement with literature values of metallic copper (Dowlatababdi, 2017; Soomro, 2014). The average size of the crystallites has been determined by the mostpre-eminent (111) orientation using the Debye-Scherrer's formula (Sampath, 2014):

$$D = K\lambda/\beta\cos\theta \quad (1)$$

Where, K denotes the Scherrer's constant (K = 0.94), D is the average crystallite size, β is the full width at half maximum (FWHM) of a Gaussian fit, λ is the wavelength of the Cu K_α radiation (λ = 0.1546 nm) and θ is the half diffraction angle. The crystallite size was determined to be ~12 nm. The lattice constant calculated from (111) plane was 4.078 Å for the CuNPs, which is in close agreement with the standard data (a = 4.0862 Å; JCPDS No: 04-0836) (Alagar, 2010). The XRD profile of CuNPs is given in fig. 2.

The Morphological study(SEM)

The SEM images of bio-synthesized Cu NPs were recorded at different magnifications as shown in fig. 3.

The formation of homogeneous and relatively spherical and cluster nanoparticles. Copper nanoparticles show nearly monodispersed distribution of particle sizes.

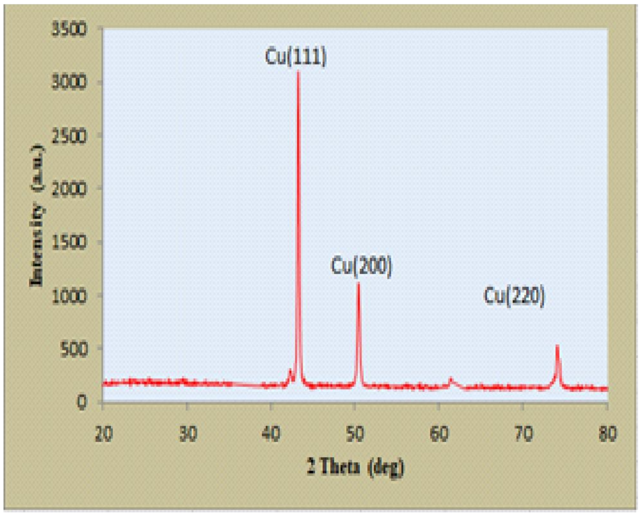

Fig. 2 : XRD profile of green synthesized CuNPs using *Syzygium aromaticum* bud extract.

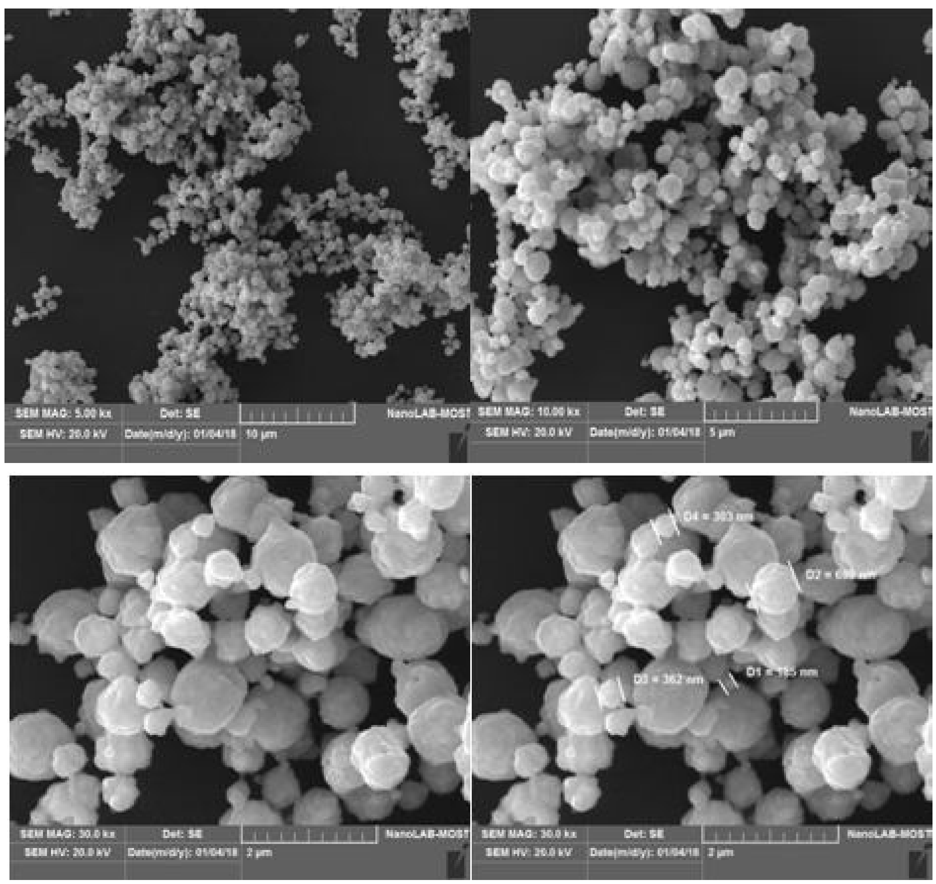

Fig. 3 : SEM images of biosynthesized CuNPs at different magnifications.

UV-Vis absorption studies

The CuNPs was also monitored through the UV-Vis spectroscopy. The reduction of copper nitrate tocopper was observed byreading UV-Vis spectrum of the reaction mixture after diluting a little aliquot of the sample with DW. The surface plasmon vibrations of copper nanoparticles produced a peak at ~ 302 nm (fig. 4). The peak is also intense, which shows that the particles are tiny in size and are easily dispersed in the solvent.

Antimicrobial activity

In general, fewer nanoparticles with a higher surface area to communicate more with bacteria compared to larger particlesthrough furnishing greater antibacterial activity. Cu worked as an antimicrobial agent for decades has shown a potent antimicrobial activity and was effective to reduce the microorganism concentration by 99.9%. Bio-synthesized CuNPs have shown prominent bactericidal activity on *E. coli* and *B. subtilis* test pathogens. The diameter of the inhibition zones of CuNPS on the bacterial strains, *B. subtilis* (42mm) and *Escherichia coli* (33 mm) at 200 μg/ml concentration. The control has no inhibition of both *E. coli* and *B. subtilis*. Antibacterial studies elucidate that the *B. subtilis* is a more effective bacterial activity than the *E. coli*. The bactericidal property from CuNPs is mainly due to the release of copper cations (Cu+) and these Cu+ ions have attached to the bacterial cell wall due to electrostatic attraction. Moreover, the metal ions are no just interacting by the surface of a membrane but can also penetrate inside the bacteria. Antifungal properties of bio-CuNPs

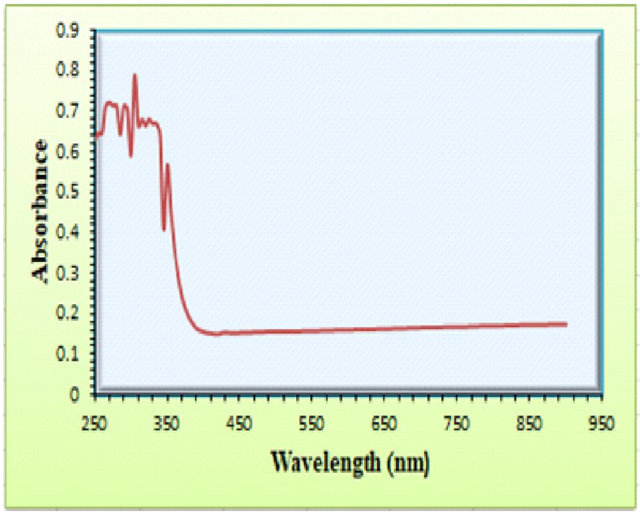

Fig. 4 : UV-Vis absorbance spectra of *Syzygium aromaticum* bud extract and bio-synthesized Cu NPs.

Table 1 :

S. no.	Test pathogens	Zone of inhibition(mm) at 200 µg/ml concentration	Control (Dmso)
1.	*Escherichia coli*	33	-
2.	*Bacillus subtilis*	42	-
3.	*Candida albicans*	55	-

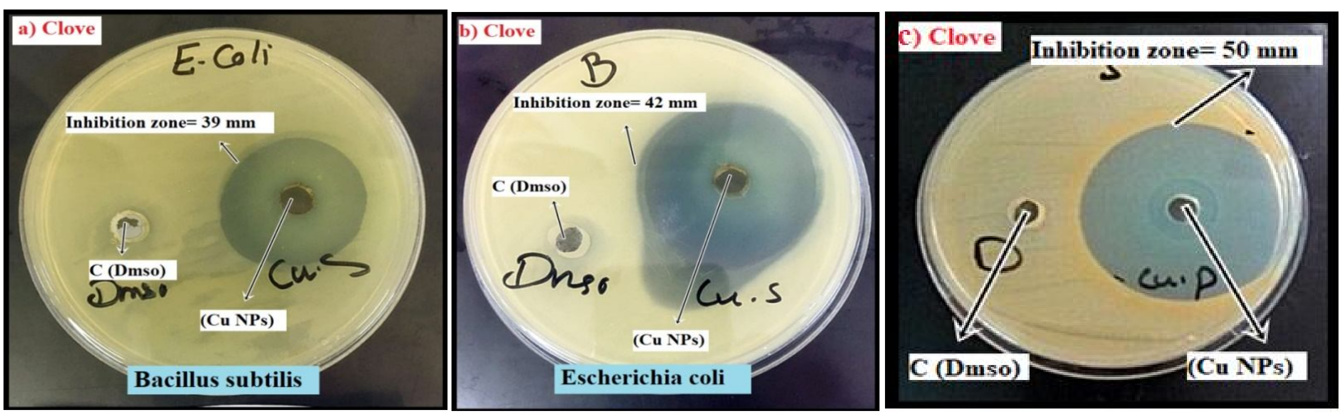

Fig. 5: Antimicrobial activity against the **a)** Bacillus subtilis, **b)** Escherichia coli, and **c)** Candida albicans.

were also studied against *Candida albicans* fungal test pathogens. The diameter of the inhibition zones of AgNPs against the fungus strains, *Candida albicans* (50 mm) at 200 µg/ml concentration as shown in table 1. In fig. 5, the inhibition zone is seen in *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*.

Finally, the current study clearly indicates that the *Syzygium aromaticum* bud extract mediated CuNPs exhibited the excellent antimicrobial activity.

Conclusion

Green synthesis of copper nanoparticles with the help of green plants is a highly cost-effective, safe, nontoxic, eco-friendly way of synthesis which can be made on abig scale. In conclusion, copper nanoparticles were synthesized using *Syzygium aromaticum* bud extract using eco-friendly hydrothermal method of temperature (185°C). The production of aqueous Cu+ ions of the bud extract has been demonstrated. The phytoconstituents present in the *Syzygium aromaticum* bud extract was effective in the reduction and stabilization of CuNPs. The pH was adjusted in 14 to get the optimum results.

The crystal size has been estimated from the most prominent (111) was found to be ~12 nm. The shape and size of the CuNPs were examined from the morphological studies (SEM). The formation of homogeneous and relatively spherical and cluster nanoparticles. The UV absorption peak at 302 nm clearly indicates the synthesis of CuNPs. The antimicrobial studies of CuNPs showed an excellent bactericidal effect on *B. subtilis*, *E. coli* and pronounced fungicidal activity against *Candida albicans* of microorganisms. As a result, the bio-synthesized of CuNPs can be done as a futuristic active antimicrobial agent in the biomedical field.

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