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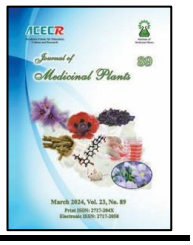

**Research Article**

# Evaluation of antioxidant and antimicrobial action of copper nanoparticles synthesized from *Moringa oleifera* pods

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ABSTRACT

**Background:** *Moringa oleifera* is a traditional South Indian medicinal plant and an excellent source of antioxidants. **Objective**: To evaluate the antimicrobial potential of copper nanoparticles (CuONPs) derived from *Moringa oleifera* pods against urinary tract-infecting microbes. **Methods**: Phyto constituents of the *Moringa oleifera* pod were screened. CuONPs were synthesized from *Moringa oleifera* pod, and it was characterized using UV-visible spectroscopy, FTIR, and SEM. Antioxidant activity of CuONPs was also determined. CuONPs discs at 50, 75, and 100 µg/ml were prepared and tested for antimicrobial action against *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Streptococcus aureus*, and *Candida albicans*. The integrity of the *E.coli* was assayed by determining the lactate dehydrogenase activity. **Results:** GCMS analysis revealed the presence of Di-n-Octyl Phthalate and 2, 2-diethoxy Propane presence in the *Moringa oleifera* pod. Antioxidant assay revealed the free radical scavenging property of CuONPs. UV- VIS confirmed the synthesis of CuONPs by showing the λ Vmax at 220nm. SEM analysis revealed the CuONPs size in 40 to 160nm. The FTIR analysis exhibited the presence of OH and C = C groups in the CuONPs. The *klebsiella pneumoniae* was the most susceptible strain for CuONPs, followed by *Escherichia coli* and *Candida albicans*. *E. coli* showed a significant LDH activity at high concentration of CuONPs. **Conclusion**: The CuONPs synthesized from the *Moringa oleifera* pod exhibited significant antimicrobial activity against the microbes.

## 1. Introduction

Nanotechnology provided a scientifically sound method for generating nanostructures with improved functionality [1]. Any particle with a 1–100 nm range in size is called a nanoparticle (NPs) [2]. Being a Nano-size range can afford a wide surface for the discovering of antimicrobial agent [3]. These Manipulative functionalities and wide applications sparked the scientific community to develop eco-friendly nanoparticles [4]. Eco-friendly NPs mean the green synthesis of NPs using bacteria,

*Abbreviations:* CuONPs, Copper nanoparticles; FTIR, Fourier Transform Infrared Spectroscopy; SEM, Scanning electron microscopy; LDH, Lactate dehydrogenase; GCMS, Gas Chromatography-Mass Spectrometry; NICM, National Collection of Industrial Microorganisms; NADH, nicotinamide adenine dinucleotide (NAD) + hydrogen; DPPH, 2,2-diphenyl-1-picrylhydrazyl; NBT, Nitro blue tetrazolium, KBr, Potassium bromide

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fungi, and plant extract rather than the deployment of conventional physical and chemical methods [5]. Numerous studies have been reported the use of plant extracts to synthesize metal-ion-based NPs with significant antimicrobial activity [6]. Basically, phytochemical constituents act as the reduced agent to synthesize green NPs [7]. *Moringa olifera* Lam, commonly called drumstick predominantly found in South Asia. The plant is highly valued because of its micronutrients used to treat various illnesses. Especially leaves have been reported as anticancer, cardioprotective, wound healing, hepatoprotective, and antimicrobial activities [8]. Moreover, *Moringa olifera is* considered the best resource for the rare combination of phytochemical constituents such as beta-sitosterol, caffeoylquinic acid, zeatin, quercetin, and kaempferol [9]. Virk *et al* 2023 recorded the rapid formation of NPs from *Moringa olifera* leaves [10]. Kalaiyan *et al* 2021 reported the significant antimicrobial action of the NPs from *Moringa olifera* leaves [11]. Recent studies have also shown the antimicrobial activity of silver nanoparticles synthesized from *Moringa olifera* [12]. Hence, the present study was focused on synthesizing the CuONPs using *Moringa oleifera* pod and evaluating the antimicrobial activity against the urinary tract infecting microbes.

## 2. Materials and methods

### *2.1. Chemicals*

The chemicals and reagents used in the study were procured from the HI Media Laboratories.

### *2.2. Preparation of aqueous extract*.

*Moringa oleifera* Pods were collected and washed with the distilled water. The cleaned pods were dried and grounded using distilled water. After grinding, the extract was filtered in the Whatman filter paper. The filtrated extract was used to synthesize the copper nanoparticle.

### *2.3. Moringa oleifera pod GCMS Analysis*

Phytochemical constituents present in *Moringa oleifera* pods were screened using a GCMS instrument (Shimadzu GC/MS-QP2010 Plus) equipped with a ZB-1 MS embedded silica column (30 m × 0.25 mm ID, 0.25 µm film thickness). The analysis was conducted in Electron Ionization mode (70 eV) with helium used as the mobile phase at a flow rate of 1 mL/min. The injection temperature was set at 250 °C. The column temperature started at 50 °C, held for 3 minutes, and then increased by 10 °C per minute up to 300 °C. The final temperature of 300 °C was maintained for 10 minutes. A 1.0 µL sample was injected in split mode. The relative percentages of each compound were recorded, and spectral configurations were obtained from the mass spectral database.

### *2.4. Synthesis of copper nanoparticle*

Copper nanoparticles (CuONPs) were synthesized as per Pagar et al. (2020) [13]. Copper sulphate pentahydrate at 1mM, 2mM, 2.5mM, 3mM, and 3.5mM/mL was added to a clean 50 mL beaker containing 10 mL of an aqueous extract of *Moringa oleifera* pods. The mixture was stirred constantly on a magnetic stirrer at 500 rpm for 25 minutes. After a short while, the colourless solution turned dark brown. The resulting solution was centrifuged at 4000 rpm for 15 minutes at room temperature. The residue at the bottom of the centrifuge tube was collected and subjected to combustion in a muffle furnace at 400 °C for 1 hour. After combustion, a fine dark-coloured powder was obtained, stored, and used for various assays.



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*2.5. Determination of Antioxidant activity*

*2.5.1. DPPH scavenging activity assay*

The antioxidant activity of CuONPs was assessed as per Ansar et al. (2020) [14]. CuONPs at concentrations of 50, 75, 100, and 125 µg/mL were mixed with 0.1 mM DPPH and incubated at room temperature for 15 minutes. Absorbance was then measured at 517 nm, with methanol as a blank. The decrease in DPPH content indicates the scavenging activity of CuONPs, which was calculated as a percentage inhibition compared to vitamin C used as the standard.

DPPH scavenging activity = standard absorbance (standard absorbance −blank absorbance) / standard absorbance × 10

*2.5.2. Determination of Nitric oxide scavenging activity*

The nitric oxide scavenging activity of CuONPs was determined following the method described by Patel Rajesh et al. (2011) [15]. Different concentrations of CuONPs were mixed with 2 mL of Fe-NPs and incubated at room temperature for 2 hours. Subsequently, 0.5 mL of the incubated mixture was combined with 1 mL of sulfanilic acid and left at room temperature for 5 minutes. To this reaction mixture, 0.1 % naphthyl ethylenediamine dihydrochloride was added and incubated again at room temperature for 30 minutes. The absorbance was then recorded at 546 nm. The nitric oxide scavenging activity of CuONPs was calculated and expressed as a percentage inhibition.

% scavenging activity = (T0 (control) − T (test)) = T0 × 100

*2.5.3. Determination of Superoxide anion scavenging activity*

The superoxide anion scavenging activity of CuONPs was determined as per Nishikimi et al 1972 [16]. In brief, reaction mixture was prepared by adding varying concentrations of CuONPs with 1 ml of 100 mM phosphate buffer (pH 7.4), 468 µM NADH, 156 µM NBT and 60 µM PMS. Then the reaction mixture was mixed well and incubated at room temperature for 5 mts. The amount of formazan complex is directly proportional to the scavenging activity, and it was detectable at 560nm.

*2.5.4. Determination of Hydroxyl radical-scavenging activity of CuONPs*

Hydroxyl radical-scavenging activity of the CuONPs was determined based on Smirnof et al. 1989 [17]. A reaction mixture was prepared by adding 1ml each of 9 mM ferrous sulphate, of 9 mM salicylic acid, and hydrogen peroxide with varying concentrations of 1ml of CuONPs and incubated in a boiling water bath for 1 hr at 37 °C. After the incubation reaction, the mixture was cooled and read at 510nm. The Hydroxyl radical-scavenging activity of the CuONPs was calculated and expressed in percentage.

*2.6. Characterization of copper nanoparticles synthesized from the Moringa oleifera*

*2.6.1. UV-Visible spectroscopic analysis*

The CuONPs synthesized using the *Moringa oleifera* were subjected to the UV Visible spectrophotometer (Shimandzu1700, Double beam, Japan). The reduction of copper nanoparticles was continuously monitored in the UV- spectrophotometer at 220-700nm [18].

*2.6.2. FTIR analysis for the nanoparticles*

The FTIR spectrum of the CuONPs was recorded based on the KBr Disc method. The dried CuONPs were ground in the presence of anhydrous KBr and compressed and formulated as a pellet. The pellet was scanned in the FTIR spectra in 4000-400 cm-1 [18].



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*2.6.3. Scanning electron microscopic (SEM) analysis*

The surface morphology dimension and the size of the CuONPs were visualized in the SEM (ZEISS Supra 40, Germany) under the standard atmospheric condition. The samples were placed on the glass slide and spread evenly and magnified [18].

*2.7. Microorganisms*

The following strains, *Escherichia coli* (NICM 2065), *Enterococcus faecalis* (NICM 5021), *Klebsiella pneumonia, Streptococcus aureus* (NICM 5021), and *Candida albicans* were procured from the national chemical laboratory.

*2.7.1. Antibacterial sensitivity assay*

The antibacterial activity of CuONPs was determined according to Kim et al. (1995) [19]. Mueller-Hinton agar plates were swabbed with an overnight culture. Filter paper disks were prepared by soaking them in CuONPs solutions of three different concentrations: 50 µg/mL, 75 µg/mL, and 100 µg/mL, until saturated. The soaked paper disks were then placed on the inoculated Mueller-Hinton agar plates. A standard ampicillin 20 µg disk was used as a positive control. All plates were incubated at 37 ± 2 °C for 24 hours. After incubation, the diameter of the inhibition zone was measured in millimeters using a vernier caliper.

*2.7.2. Antifungal assay*

The antifungal assay was conducted according to Bauer et al. (1996) [20]. The fungal strain inoculum was spread over a potato dextrose agar medium. Paper disks containing different concentrations of CuONPs (50 µg/mL, 75 µg/mL, and 100 µg/mL) were placed on the surface of the medium. The plates were then incubated at 37 ± 2 °C for 24 hours in triplicates. The antifungal activity of CuONPs was determined by measuring the diameter of the zone of inhibition in millimeters.

*2.8. Assay of Lactate dehydrogenase activity*

The protein leakage from *E. coli* was detected by assaying LDH activity. The LDH assay was performed according to Kalińska et al. (2019) [21], with slight modifications. Bacterial cells (5 x $10^3$ cells/well) were incubated in 96-well plates containing the medium and 50, 75, 100, and 125 µg/mL of CuONPs for 24 hours. For the control, *E. coli* was incubated in plates without CuONPs. After incubation, the plates were centrifuged, and 50 µL of the supernatant was transferred to new 96-well plates. Then, LDH working solutions were added to each well, and the absorbance was measured at 490 nm. LDH activity was expressed as nmole/min/mL of pyruvate liberated.

*2.9. Statistical analysis*

The inhibition diameters (mm) zone is represented in mean ± SD, where N = 3. The mean ± SD inhibition zone diameters of the CuONPs and the standard control (ampicillin) were analyzed in the one-way ANOVA variance and followed by Turkey –Kramer multiple comparisons were carried out using Graph pad prism. The P values $\leq$ 0.05 are considered significant when comparing one strain with another.

## 3. Results

*3.1. GCMS analysis of Moringa oleifera pod.*

Figure 1 depicts the characteristic absorption spectra of *Moringa oleifera* pod on GCMS analysis. A total of 51 phytochemicals were present in the *Moringa oleifera* pod. Among Di-n-Octyl Phthalate, 2,2-diethoxy Propane, and Piperidine, 1-5-1,3-benzodioxol occupied the major proportion. The area occupied by the respective phytochemicals and their retention times are shown in Tables 1 and 2.





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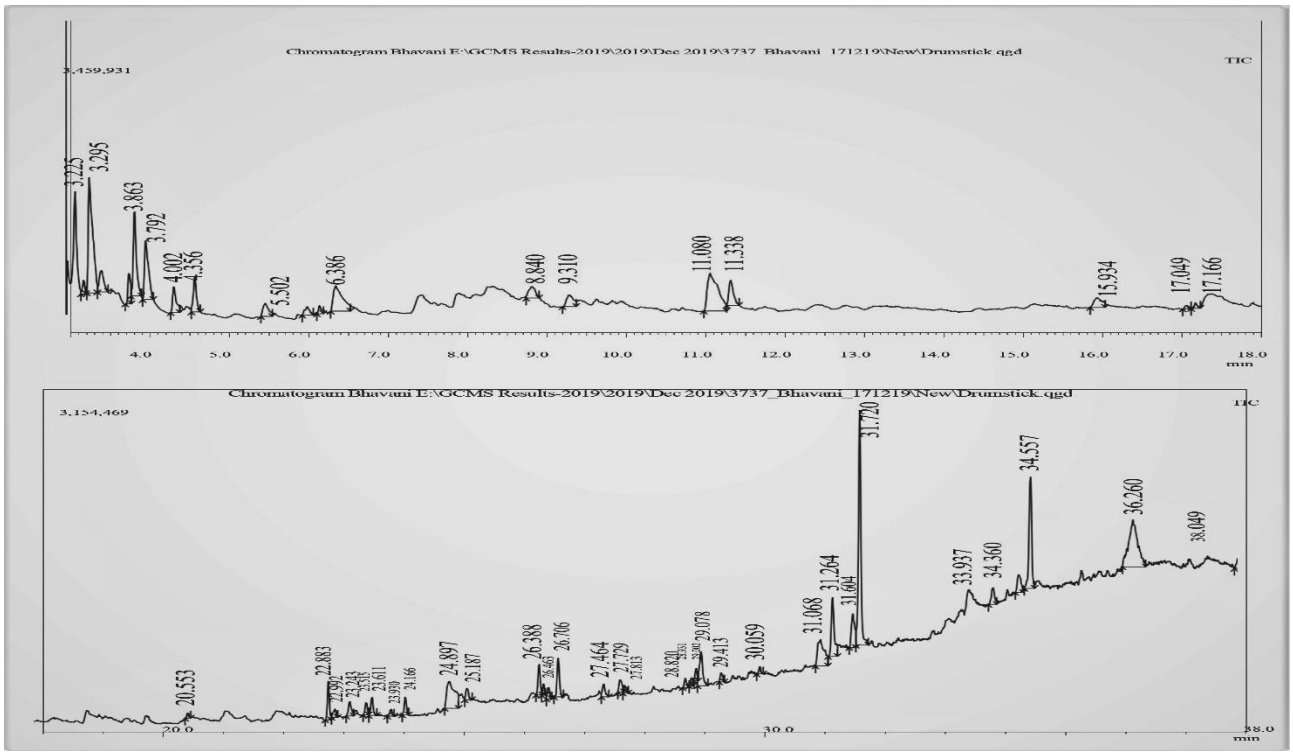


**Fig. 1.** The Chromatogram and the mass spectrometric profile of *Moringa oleifera* pod on GCMS analysis

**Table 1.** The phytochemical constituents in the *Moringa oleifera* pod on GCMS analysis

| Peaks | Retention Time (mts) | Area % | Name of the compound |
|---|---|---|---|
| 1 | 3.225 | 0.52 | 1.99 2,5-Furandione, 3,4-dimethyl |
| 2 | 3.295 | 8.44 | 3.79 2,2-diethoxy Propane |
| 3 | 3.448 | 1.65 | 3.99 2-Propyn-1-OL |
| 4 | 3.792 | 1.43 | 2.45 l-Alanine, N-methoxycarbonyl- methyl ester |
| 5 | 3.863 | 4.48 | 2.76 1-hexen-3-OL |
| 6 | 4.002 | 3.98 | 3.51 1-Propanol, 2,2-dimethyl-, acetate |
| 7 | 4.356 | 1.63 | 3.25 1-Butanamine, n-Butylidene |
| 8 | 4.617 | 1.85 | 2.72 2-pentanone, 4-hydroxy-4-methyl |
| 9 | 5.502 | 1.26 | 4.95 3,4-hexanedione, 2,2,5,5-tetrameth |
| 10 | 6.027 | 0.69 | 4.23 2H-Pyran, tetrahydro |
| 11 | 6.182 | 0.41 | 2.62 2-Butyl-(2-methylbutylidene)-AM |
| 12 | 6.386 | 3.98 | 8.11 2(3H)-Furanone, 5-Methyl |
| 13 | 8.840 | 1.26 | 5.66 6-Dodecanone |
| 14 | 9.310 | 1.36 | 6.04 2,5-Anhydro-1,6-Dideoxyhexo-3,4-D |
| 15 | 11.080 | 6.81 | 9.36 1,5-Anhydro-6-Deoxyhexo-2,3-dilouse |
| 16 | 11.338 | 1.94 | 4.03 Pentanedioic acid, Ethyl Methyl Ester |
| 17 | 15.934 | 1.35 | 7.02 Cyclohexane, 1,2,3-Trimethoxy-, S |
| 18 | 17.049 | 0.19 | 2.80 Tetradecane, 1-Chloro |
| 19 | 17.166 | 0.26 | 2.36 Cyclododecane |
| 20 | 20.553 | 0.23 | 2.77 E-11,13-Tetradecadien-1-ol |





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**Table 1.** The phytochemical constituents in the *Moringa oleifera* pod on GCMS analysis (Continued)

| Peaks | Retention Time (mts) | Area % | Name of the compound |
|---|---|---|---|
| 21 | 22.883 | 1.41 | 2.29 Neophytadiene |
| 22 | 22.992 | 0.44 | 3.27 2-Pentadecanone, 6,10,14-Trimethyl |
| 23 | 23.243 | 0.81 | 3.45 (8Z)-14-Methyl-8-Hexadecen-1-OL |
| 24 | 23.515 | 0.48 | 2.45 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol |
| 25 | 23.611 | 0.81 | 2.80 1-Hexadecanol |

**Table 2.** The phytochemical constituents in the *Moringa oleifera* pod on GCMS analysis

| Peaks | Retention Time (mts) | Area % | Name of the compound |
|---|---|---|---|
| 26 | 23.930 | 0.31 | 2.75 N,N-Bimethyl-3-Buten-1-Amine |
| 27 | 24.166 | 0.75 | 2.61 Pentadecanoic acid, 14-Methyl-, Methyl Ester |
| 28 | 24.897 | 3.98 | 9.24 9-Octadecenoic acid (Z) |
| 29 | 25.187 | 0.55 | 2.97 Hexadecanoic acid, 2-Oxo-, Methyl ester |
| 30 | 26.388 | 1.29 | 2.64 3-Eicosene |
| 31 | 26.463 | 0.39 | 2.11 9,12-Octadecadienoic acid, Methyl Ester, (E,E) |
| 32 | 26.541 | 0.50 | 2.72 9-Octadecenoic acid (Z)-, Methyl E |
| 33 | 26.706 | 1.73 | 2.78 2-Hexadecen-1-OL, 3,7,11,15-Tetrame |
| 34 | 27.464 | 0.67 | 3.26 2-Ethylhexyl (2E)-3- 4-Methoxyphenyl |
| 35 | 27.729 | 0.73 | 3.82 Dodecanamide |
| 36 | 27.813 | 0.36 | 3.16 Heptyltriacontyl ether |
| 37 | 28.820 | 0.44 | 2.86 Carbamic acid, 2-(Dimethylamino) Ethyl ester |
| 38 | 28.931 | 0.36 | 2.68 Dioctadecylphosphonate |
| 39 | 29.002 | 0.54 | 2.65 3-Methyl-2-Octanol |
| 40 | 29.078 | 2.18 | 4.03 12-O-acetylingol 8-tiglate |
| 41 | 29.413 | 0.42 | 2.81 2-Ethylhexyl (2e)-3-4-methoxyphenyl |
| 42 | 30.059 | 0.27 | 2.22 Hexanedioic acid, Dioctyl ester |
| 43 | 31.068 | 3.22 | 7.60 2-Methyl-Z,Z-3,13-Octadecadienol |
| 44 | 31.264 | 3.35 | 3.55 Trichloroacetic acid, Pentadecyl ester |
| 45 | 31.604 | 2.07 | 3.83 hexadecanoic acid 2-hydroxy-1- (hydroxymethyl)ethyl ester |
| 46 | 31.720 | 10.69 | 2.84 Di-n-Octyl Phthalate |
| 47 | 33.937 | 1.02 | 3.79 1,3-Benzenedicarboxylic acid, bis 2-ethylhexyl ester |
| 48 | 34.360 | 1.25 | 4.37 Piperidine, 1-5-1,3-benzodioxol |
| 49 | 34.557 | 5.22 | 2.93 Squalene |
| 50 | 36.260 | 7.95 | 10.53 Piperidine, 1-5-1,3-benzodioxol |
| 51 | 38.049 | 2.09 | 5.33 Ethyl Iso-Allocholate |

### *3.2. Copper nanoparticle synthesis*

The synthesis of copper nanoparticles was confirmed by visualizing the appearance of a reddish-brown colour from a colorless solution at 3 mM of copper sulphate. Whereas, at 1 mM, 2 mM, 2.5 mM, and 3.5 mM of copper sulphate there is no specific colour change inferred in the solution. Hence, the optimum concentration of copper sulphate is 3 mM for copper nanoparticle synthesis.

### *3.3. Antioxidant activity of copper nanoparticles*





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The free radical scavenging activity of copper nanoparticles was screened; the results obtained are depicted in figure 2. 83 % DPPH free radicals were scavenged at 125 µg/ml of copper nanoparticles. Whereas, in the presence of 50, 75, and 100 µg/ml copper nanoparticles scavenge 60 %, 64 %, and 70 % DPPH of free radicals. Copper nanoparticles at 125 µg/ml scavenge 83 % of superoxide anion, whereas at 50, 75, and 100 µg/ml scavenge 59 %, 64 %, and 78 %, respectively. Copper nanoparticles at 125 and 100 µg/ml scavenge 80 % of the nitric oxides. But at 50 and 75 µg/ml, copper nanoparticles scavenged 65 % and 70 % of nitic oxide radicals. The hydroxyl ions scavenging activity of copper nanoparticles was also analyzed. At 125 µg/ml, copper nanoparticles scavenged 83% of hydroxyl ions. But at 50, 75, and 100 µg/ml, copper nanoparticles scavenged 63 %. 69 % and 73 % of hydroxyl ions.

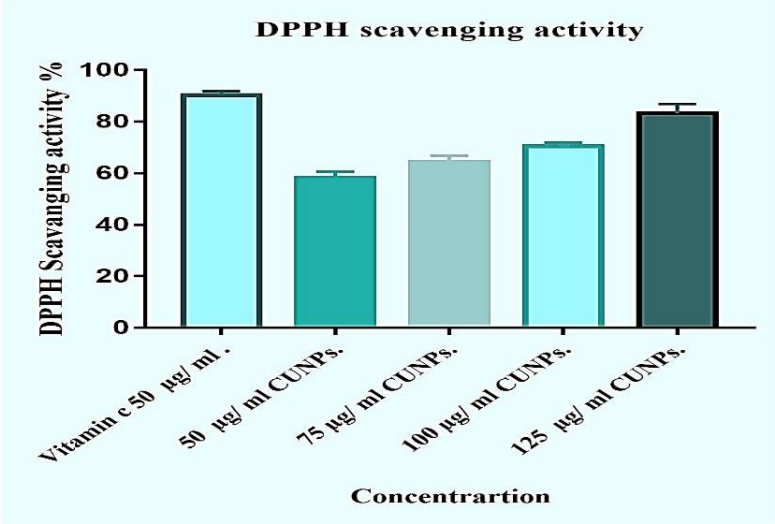


**Fig. 2.** (A) DPPH scavenging activity of copper nanoparticles

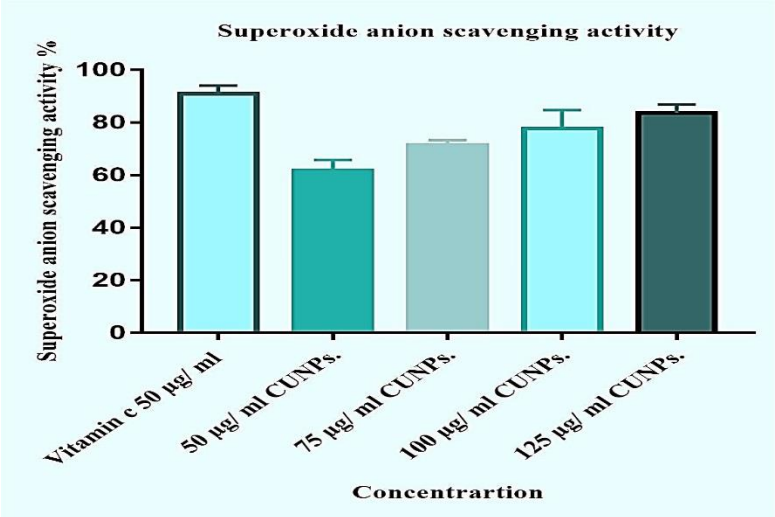


**Fig. 2.** (B) Superoxide anion scavenging activity of copper nanoparticles



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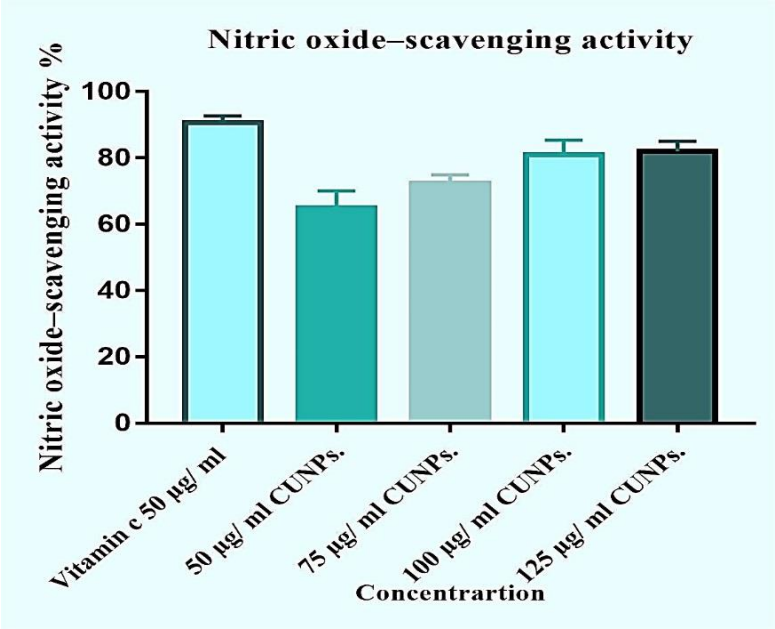


**Fig. 2.** (C) Nitric oxide scavenging activity of copper nanoparticles

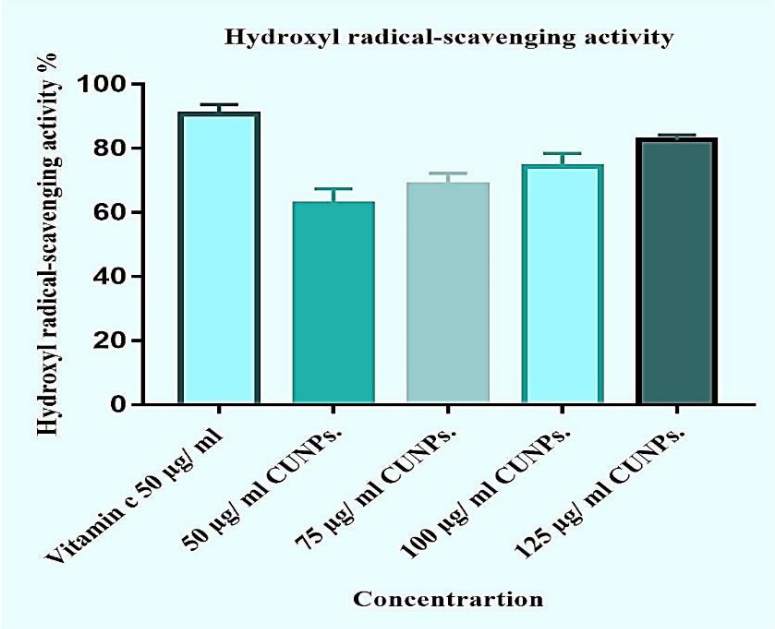


**Fig. 2.** D Hydroxyl radical scavenging activity of copper nanoparticles

*3.4. UV-Visible spectroscopic analysis of copper nanoparticles synthesized from the Moringa oleifera pod.*

Figure 3 depicts the λ Vmax of copper nanoparticles. At 220 nm, copper nanoparticles showed the maximum absorption. Hence, the absorption spectra of the copper nanoparticle are found to be 220 nm.

*3.5. SEM analysis of copper nanoparticles*

The size and surface morphology of the copper nanoparticles were studied using the SEM. Figure 4 depicts the surface morphology





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of copper nanocrystals analyzed by the SEM. Spherical and tubular-shaped copper nanocrystals were observed in aggregates, and each nanocrystal ranged in between 40.1 and 180.3 nm.

*3.6. FTIR spectra of copper nanoparticles*

The FTIR spectra of copper nanoparticles are shown in Figure 5. The broad peak at 3300 cm^-1 and the sharp peak at 1635.64 cm^-1 correspond to the OH and C=C stretch, respectively.

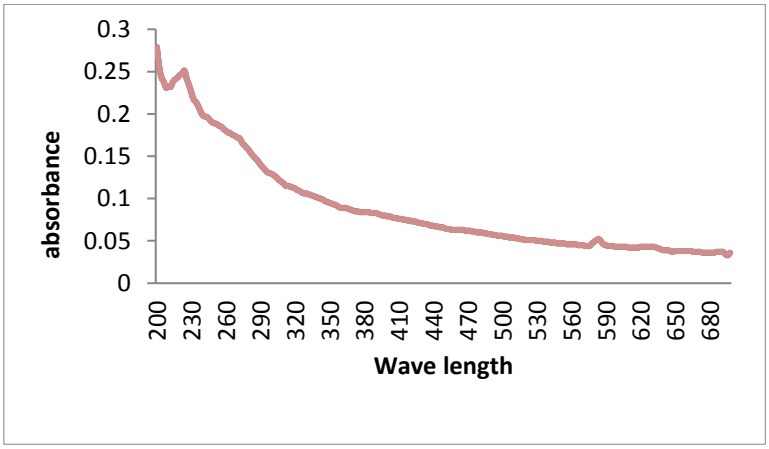


**Fig. 3.** The UV-Visible spectroscopy analysis of the copper nanoparticles

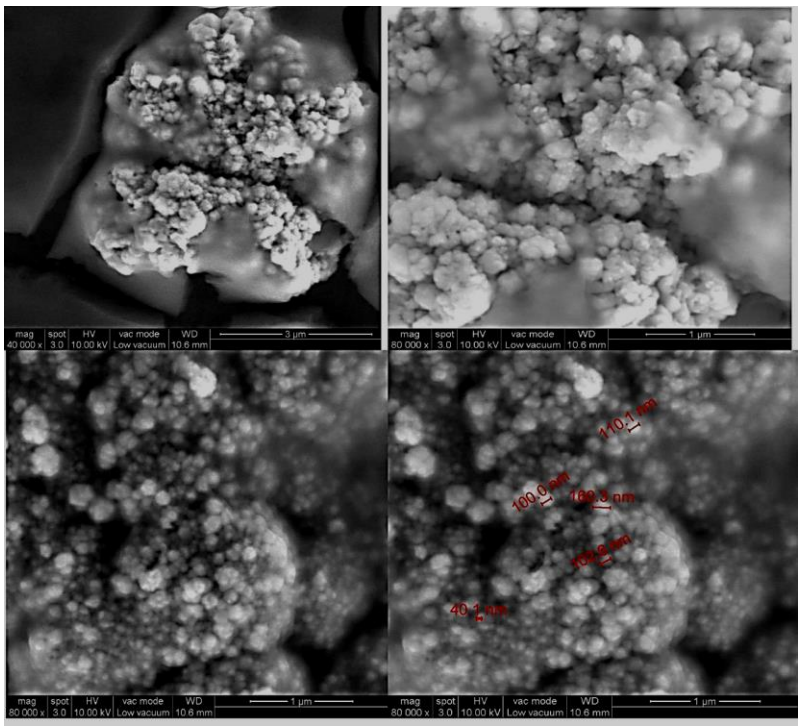


**Fig. 4.** SEM images of copper nanoparticles synthesized from the *Moringa oleifera* pod.
The Spherical and tubular copper nanoparticles in aggregates range from 40.1 to 110.3nm on SEM analysis.



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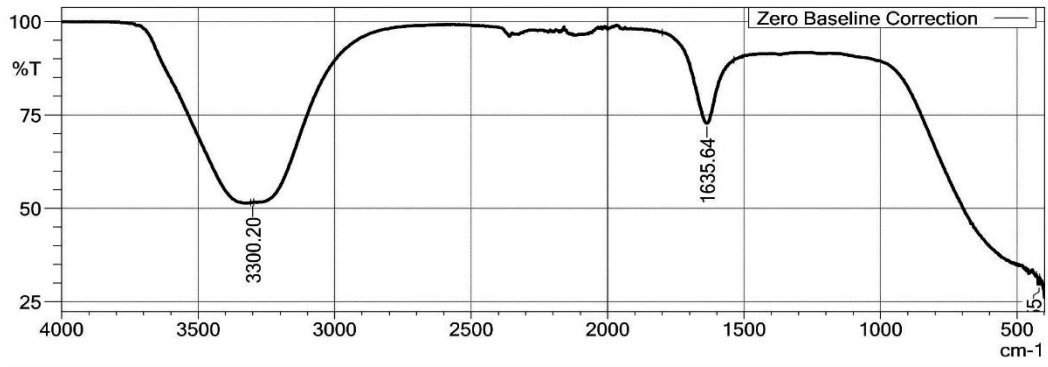


**Fig. 5.** The FTIR spectra of the copper nanoparticles
FTIR spectra for the CuONPs. The broadband at 3300 cm-1 and a sharp band at 1635.64 cm-1 correspond to OH and C = C vibration stretches.

*3.7. The Antimicrobial activity of copper nanoparticles*

The antimicrobial activity of copper nanoparticles was studied, and the results obtained are depicted in table 3 and figure 4. Figure 6 depicts the antimicrobial activity of copper nanoparticles. The *Klebsiella pneumoniae* was found to be most sensitive among given species to the copper nanoparticles. Followed by *E. coli*, C. albicans, Enterococcus faecalis, and Streptococcus aureus were considered to be least susceptible for the copper nanoparticles. However, all the species were susceptible at a high concentration (100 µg/mL) of copper nanoparticles. The *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Streptococcus aureus*, and *Candida albicans* scored the least susceptibility at 50 µg/mL and 75 µg/mL of copper nanoparticles.

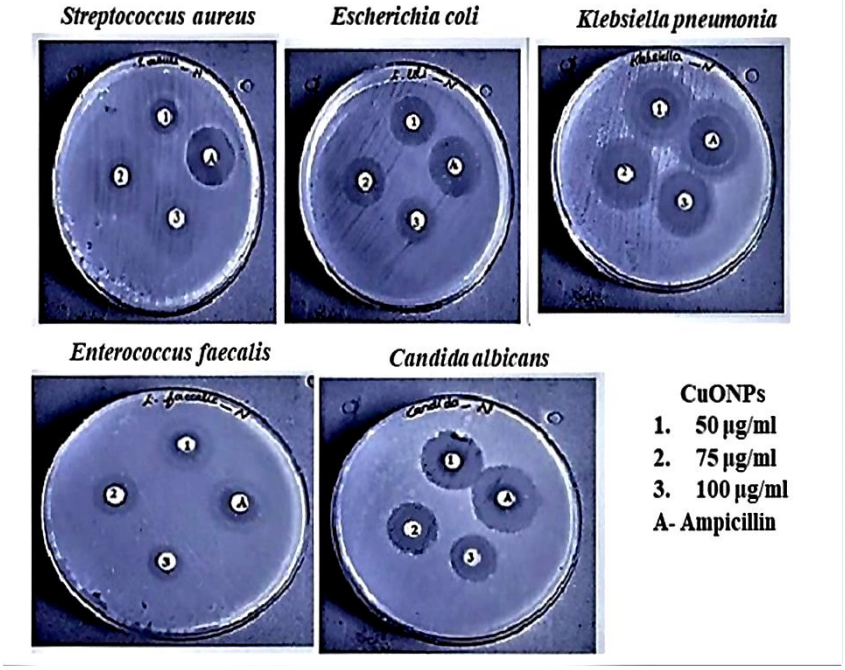


**Fig. 6.** The antimicrobial activity of the Copper nanoparticles





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**Table 3.** The antimicrobial action of copper nanoparticles

| Test Organisms | 50 µg/ml CuONPs | 75 µg/ml CuONPs | 100 µg/ml CuONPs | 20 µg/ml Amp |
|---|---|---|---|---|
| *E. coli* | $6.1 \pm 0.9^{bc}$ | $7.2 \pm 0.8^{c}$ | $9 \pm 1.0$ | $11.6 \pm 1^{c}$ |
| *Klebsilla pneumonia* | $9.4 \pm 0.7^{acd}$ | $10.4 \pm 1.2^{abd}$ | $11.2 \pm 0.7^{abc}$ | $12.8 \pm 0.8^{ac}$ |
| *Enterococcus faecalis* | $4.2 \pm 0.8$ | $7.2 \pm 1^{c}$ | $8.1 \pm 1.1$ | $10.2 \pm 1.1$ |
| *Staphylococcus aureus* | $4.3 \pm 0.7$ | $5.4 \pm 0.7$ | $8.0 \pm 1.3$ | $10.1 \pm 1$ |
| *Candida albicans* | $5.9 \pm 0.8^{bc}$ | $8.2 \pm 0.9^{abc}$ | $11.2 \pm 1^{abc}$ | $12.3 \pm 0.9^{c}$ |

Values are expressed as mean ± SD of zone of inhibition in diameter (mm). The superscript [a] is p≤0.05 significant different in between *E. coli* vs other strains. The superscript [b] is p≤0.05 significant different in between *E. faecalis* vs other strains. The superscript [c] is P ≤ 0.05 significant different in between *S. aureus* with other strains and superscript [d] significant different in between C. *albicans* with others.

### *3.8. E.coli integrity assay*

LDH activity in culture medium was assayed to ascertain the integrity of the *E. coli in* the presence of copper nanoparticles. Figure 7 represents the LDH activity of the *E. coli*. in the medium. At 100 and 125 µg/ml of copper nanoparticle treatment, *E. coli* displayed significant activity in the medium when compared to the control. At the same time, a non-significant LDH activity was inferred at 50 and 75 µg/ml of copper nanoparticle treatment.

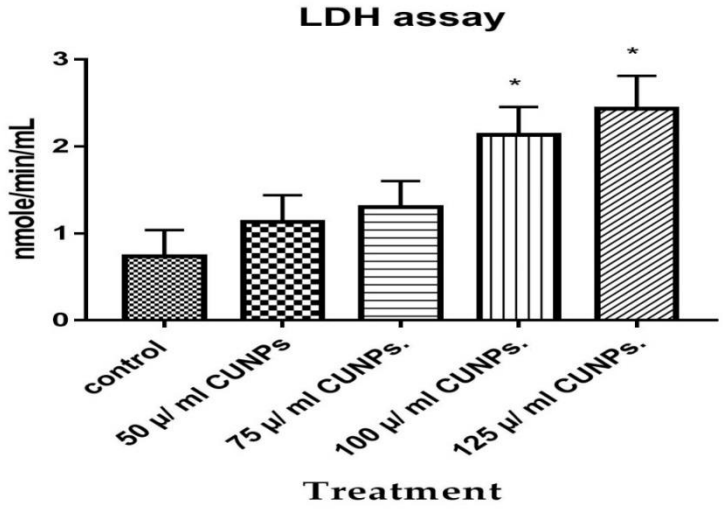


**Fig. 7.** Impact of copper nanoparticle on *E. coli* membrane integrity.
N = 3, valves are expressed as in the form of mean ± SD, P ≤ 0.05* considered significant compared to 50 µ/ ml CUNPs.

## 4. Discussion

Over the years, traditional antibiotics used to treat bacterial infections have been developed, but multidrug-resistant strains (MDR) have become a significant concern. Therefore, it is crucial to seek alternatives to combat these MDR strains and their associated complications [22]. In recent years, nano-based antimicrobial agents have garnered attention and are considered alternative approaches to controlling pathogenic strains with minimal risk. [23]. Therefore, the study aimed to synthesize CuONPs using *Moringa oleifera* pods and evaluate their antimicrobial potential against urinary tract infection-causing microbes. CuONPs were synthesized using copper





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sulphate as the precursor and *Moringa oleifera pods* as the capping agent. The colourless solution changed to reddish-brown, indicating successful synthesis of CuONPs, which was confirmed by UV-visible spectroscopy. A strong absorbance peak at 220 nm confirmed the presence of copper nanoparticles and marking the λ Vmax. The reddish-brown colour change and the characteristic absorbance at 220 nm are attributed to surface plasmon resonance and serving as signatures of copper nanoparticle synthesis [24]. FTIR is used to examine the functional groups in given particles [25]. In the current investigation, the FTIR spectrum of CuONPs shows two peaks: a broad peak at 3300 $cm^{-1}$ and a sharp peak at 1635.64 $cm^{-1}$. The broad peak at 3300 $cm^{-1}$ is related to OH stretching, while the sharp peak at 1635.64 $cm^{-1}$ corresponds to C = C stretching [26, 27]. The OH stretching is attributed to phenolic compounds such as flavonoids, alkaloids, and terpenoids present in *Moringa oleifera*, which may act as capping agents for the synthesis of CuONPs [28]. Previous studies have also indicated that C = C stretching is involved in the formation of CuONPs from *Moringa oleifera* [29].

SEM analysis is widely employed to reveal the surface morphology and size of nanostructures [30]. In the present study, a tubular and spherical-shaped copper nano crystal was observed in aggregates and each nanocrystal ranges from 40 to 110 nm. Our findings are in correlation with the report of Andualem et al. (2020) [31]. The aggregation of copper nanocrystals is attributed to the presence of secondary metabolites in *Moringa oleifera* and is also influenced by the polarity and electrostatic attraction of copper nanocrystals with salt precursors [32].

The antimicrobial assay indicated that *K. pneumoniae* was the most susceptible to the copper nanoparticles, followed by *E. coli, S. aureus*, and *C. albicans*. Particularly, at high concentrations of CuONPs, all organisms displayed growth inhibition. Notably, gram-negative bacteria such as *K. pneumoniae* and *E. coli* exhibited the maximum susceptibility to CuONPs compared to the gram-positive strains such as *E. faecalis* and *S. aureus*. The difference in susceptibility between the gram-positive and gram-negative bacterial strains to CuONPs observed in our current finding is highly correlating with earlier reports [33]. The variation in the susceptibility of bacterial strains to CuONPs is attributed to opposite charges and electrostatic interactions. Additionally, since the peptidoglycan layer of bacteria is negatively charged, differences in the binding of $Cu^{2+}$ ions to the bacterial cell wall may cause variations in susceptibility. Another reason for the susceptibility differences is that Gram-negative bacterial strains allow more copper ions to reach the plasma membrane compared to Gram-positive bacteria [33]. In the current observation, fungicidal activity against *C. albicans* was also noted at certain concentrations of CuONPs. The fungicidal effect of the CuONPs might be due to disturbances in the replication and transpiration of the fungi [34].The LDH assay indicates the status of cellular integrity. In the current observation, higher LDH activity was detected in the medium of *E. coli* incubated with CuONPs. The increased LDH activity may be the result of leakage from bacterial cells due to oxidative stress resultant damage in the cell wall of bacteria [35].

## 5. Conclusion

The copper nanoparticles synthesized from the *Moringa oleifera* pod displayed significant antimicrobial activity against infecting urinary tract microbes.





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**Author contribution**

B. S. carried out the entire work under the supervision of M. S.

**Conflicts of interest**

There is no conflict of interest.

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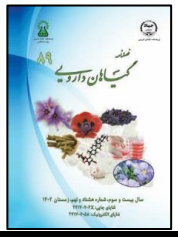

مقاله تحقیقاتی

# ارزیابی اثر آنتی‌اکسیدانی و ضد میکروبی نانوذرات مس سنتز شده از غلاف مورینگا اولیفرا

**باوانی سوندار، سانکار مدهاوان***

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**اطلاعات مقاله**

*گل‌واژگان:*
نانوذرات مس
غلاف مورینگا اولیفرا
فعالیت آنتی اکسیدانی
منطقه بازدارندگی
فعالیت ضد میکروبی

**چکیده**

**مقدمه:** *Moringa oleifera* یک گیاه دارویی سنتی جنوب هند و منبع عالی آنتی‌اکسیدان است. **هدف:** ارزیابی پتانسیل ضد میکروبی نانوذرات مس (CuONPs) مشتق شده از غلاف‌های *Moringa oleifera* در برابر میکروب‌های آلوده کننده دستگاه ادراری. **روش بررسی:** ترکیبات موجود در غلاف *Moringa oleifera* غربالگری شدند. نانوذرات مس از غلاف *Moringa oleifera* سنتز شدند و با استفاده از طیف‌سنج UV، FTIR و SEMمورد بررسی قرار گرفتند. فعالیت آنتی‌اکسیدانی نانوذرات مس نیز تعیین شد. دیسک‌های نانوذرات مس در غلظت‌های ۵۰، ۷۵ و ۱۰۰ میکروگرم بر میلی‌لیتر تهیه و برای اثر ضد میکروبی علیه اشریشیاکلی، انتروکوکوس فکالیس، پنومونی کلبسیلا، استرپتوکوک اورئوس و کاندیدا آلبیکنس مورد آزمایش قرار گرفتند. فعالیت *E. coli* با تعیین لاکتات دهیدروژناز مورد سنجش قرار گرفت. **نتایج:** تجزیه و تحلیل طیف GCMS حضور Di-n-Octyl Phthalate و 2, 2-diethoxy Propane را در غلاف *Moringa oleifera* نشان داد. سنجش آنتی‌اکسیدانی خاصیت مهار رادیکال‌های آزاد را توسط نانوذرات مس نشان داد. طیف سنج UV سنتز نانوذرات مس را با نشان دادن جذب در ۲۲۰ نانومتر تایید کرد. بررسی SEM اندازه نانوذرات مس را در ۴۰ تا ۱۶۰ نانومتر نشان داد. تجزیه و تحلیل FTIR حضور گروه‌های OH و C = C را در نانوذرات مس نشان داد. کلبسیلا پنومونیه حساس‌ترین سویه به نانوذرات مس بود و پس از آن اشریشیاکلی و کاندیدا آلبیکنس قرار داشتند. *E. coli* فعالیت لاکتات دهیدروژناز قابل توجهی را در دوز بالای نانوذرات مس نشان داد. **نتیجه‌گیری:** نانوذرات مس‌های سنتز شده از غلاف *Moringa oleifera* فعالیت ضد میکروبی قابل توجهی را در برابر میکروب‌ها نشان دادند.

*مخففها:* CuONPs، نانوذرات مس؛ FTIR، طیف‌سنجی مادون قرمز تبدیل فوریه؛ SEM، میکروسکوپ الکترونی روبشی؛ LDH، لاکتات دهیدروژناز؛ GCMS، گاز کروماتوگرافی – طیف سنجی جرمی؛ NICM، مجموعه ملی میکروارگانیسم‌های صنعتی؛ NADH، نیکوتین آمید آدنین دی نوکلئوتید + (NAD) هیدروژن؛ DPPH، 2،2-2 دی فنیل-۱-پیکریل هیدرازیل؛ NBT، نیترو آبی تترازولیوم؛ KBr، برمید پتاسیم

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