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Green synthesis of *paspalum scrobiculatum* mediated copper oxide nanoparticles and its anti-bactericidal activities

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Abstract

This research mainly aimed to explore the sustainable green biosynthesis of copper oxide nanoparticles (CuONPs) derived from ethanolic extract of *Paspalum scrobiculatum*. *P. scrobiculatum* mediated copper oxide nanoparticles (PS-CuONPs) were synthesized by a simple, low-cost, and effective precipitation method. Qualitative screening tests on ethanolic extract of *P. scrobiculatum* reveal the flavonoids, phenols, terpenoids, proteins, and carbohydrates present in it. The green synthesized CuONPs were characterized by various techniques such as UV, Fourier Transform-Infrared (FT-IR), X-Ray Diffraction (XRD) analysis, Scanning Electron Microscope (SEM), and Energy Dispersive X-Ray Analysis (EDAX) to confirm their existence. The size and morphology of the synthesized PS-CuONPs were observed by XRD and SEM analysis respectively which found that the average size is to be 43.96 nm and has a rectangular shape. The EDAX of PS-CuONPs showed the existence of copper (Cu) and oxygen elements (O) in 53.46% and 46.44% respectively. The anti-bactericidal activity of synthesized PS-CuONPs against five bacterial strains was studied by the disc diffusion method. PS-CuONPs showed noticeable inhibitory action against the pathogen *Pseudomonas aeruginosa* (5.0±0.30 mm) and on *Proteus vulgaris* it profound no inhibitory effect. Thus, the synthesized PS-CuONPs played a vital role in bactericidal activity which can be an alternative antibacterial agent against infection-causing above pathogens.

Keywords: *P. scrobiculatum*, PS-CuONPs synthesis, Characterisation, Anti-bactericidal activity

1. Introduction

Nanochemistry is a new sub-discipline of the chemical and materials sciences that focuses on the development of innovative nanoscale material fabrication processes. These materials have been studied for applications in electronics, textiles, composite materials, nano-devices and systems, medicine, and biotechnology, among others [1,2]. Bacteria were mostly found in all habitats on Earth which can be living in (or) on other organisms such as plants, animals, and in humans. Some of these bacteria can cause food spoilage and crop damage and some can help in food digestion which kills the cells that cause sickness and gives vitamins to the human body. Rather few selective bacteria are known as parasites or pathogens that can cause the disease in humans, animals, and plants. Such types of pathogens will cause infectious diseases in humans by overwhelming the immune system [3]. The most common pathogenic/bacterial infection disease are cystitis, laryngitis, pharyngitis, tonsillitis, cellulitis, erysipelas, intestines, osteomyelitis, septic arthritis, pyoderma, necrotizing, neonatal meningitis, pneumonia, toxemia, and inflammation in the stomach [4,5].

Copper is an essential micro-mineral for human health which is considered a low-toxic metal. Copper nanoparticles demonstrate extraordinary performance as antimicrobial agents on bacteria and fungus. In metal nanoparticles, silver possessed greater microbial activity, but the main drawback is its cost-effectiveness however copper/copper oxide nanomaterials have a similar principle of action as that of silver. Copper was chosen as nanoparticles for the encapsulation mostly due to its cost-effectiveness [6,7].

Paspalum scrobiculatum Linn. belongs to the *Poaceae* family, commonly which is known as 'Kodo millet' in English. It is a tufted perennial grass and is widely distributed in Chhattisgarh, Madhya Pradesh, and Karnataka in India. Traditionally *P. scrobiculatum* grain is used to manage diabetes mellitus also it is used to treat general debility, inflammation, and hemorrhages [6-8]. The present investigation aimed to investigate the anti-bactericidal activity of copper oxide nanoparticles (CuONPs) derived from ethanolic extract of *P. scrobiculatum* [8,9].

2. Materials and methods

Ethanol solvent used for the extraction, also for synthesise and microbial activity chemicals like copper sulfate pentahydrate, KBr, amoxicillin, nutrient agar, and double-distilled water was used which all are analytical and lab grades. Analytical instruments Scanning Electron Microscope (SEM) (CAREL ZEISS, EVO 18), Fourier Transform-Infrared (FT-IR) (Perkin Elmer, Spectrum Two), UV-Visible (Perkin Elmer, Lambda 35), X-Ray Diffraction (XRD) (D8 ADVANCE, Bruker), and Energy Dispersive X-Ray Analysis (EDAX) (FEI Tecnai G2 F20) are performed on the samples.

About 500g of *P. scrobiculatum* millets were dried in sunshade for 2 to 3 days and ground into a fine powder using the electrical grinder. Then transferred the powder millet into the 500 mL beaker, to that around 200 mL of ethanol solvent was added then mixed well. The solvent level should be maintained at 2 cm above the plant material and kept for 3 days to get the complete extraction of phytochemical constituents. After that, the ethanol extract was double filtered using the normal Whatmann No.1 filter paper. The obtained filtrate was stored in a refrigerator for further qualitative screening tests and nanoparticle synthesis.

In the present study, the precursor was found to be copper sulfate pentahydrate ($CuSO_4.5H_2O$). 0.1M of copper sulfate was taken in a 250 mL beaker then placed on the electrical magnetic stirrer. After getting a clear solution slowly added the 10 mL of prepared extract and maintained the constant stirring. For every 30 min, time interval added another 10 mL of extract. After the completion of 50 mL of the extract addition, kept it overnight at room temperature so that *P. scrobiculatum* mediated copper oxide nanoparticles (PS-CuONPs) were settled down completely at the bottom of the beaker. The pure nanoparticles were collected by centrifuging the reaction mixture at a speed of 6,000 rpm. The obtained PS-CuONPs were washed with double distilled water and ethanol solvent several times for getting maximum purity. The synthesized PS-CuONps were dried in an oven then UV-Visible spectroscopy, FT-IR spectral analysis, X-Ray Diffraction, SEM analysis, and EDAX analysis were performed on PS-CuONps to confirm the existence of CuO nanoparticles [10-12].

The antibacterial activity was performed in the precursor, extract, and synthesized PS-CuONps by a disc diffusion method against five strains [*Escherichia coli* (MTCC 25922), *Enterobacter aerogenes* (MTCC 2822). *Pseudomonas aeruginosa* (MTCC 27853), *Staphylococcus aureus* (MTCC 25923) and *Proteus vulgaris* (MTCC 7299)]. The strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37 °C and maintained on nutrient agar (Difco, USA) slant at 4 °C. The nutrient agar plates were scrubbed using the cotton swabs with the adjusted broth culture of the bacterial strains. The Petri dishes were prepared by inoculation with the test organisms with a diameter of 60 mm. At room temperature, the discs were allowed to dry at equidistance placement in each of the agar plates for 2 h using sterile forceps. Positive control was prepared by taking 10 μL of Amoxicillin used as an antibiotic standard disc. The Petri dishes were incubated at 37 °C for 24 h duration, the zone of inhibition was noted in millimeters [13,14].

3. Results and discussion

The phytochemical screening tests were performed in the ethanolic extract of *P. scrobiculatum* using standard procedure. Results indicated the presence of flavonoids, phenolic compounds, terpenoids, amino acids, carbohydrates, proteins, lignins, and lactones in the extract. These phyto-compounds are present in the extract which reduces the copper sulfate into copper oxide nanoparticles [15]. UV-Visible Spectroscopy is commonly performed to confirm the formation of any nanoparticles. UV-visible spectra of ethanolic extract of *P. scrobiculatum* showed the maximum absorbance at 211 nm, 286 nm, and 318 nm which are due to unsaturated groups or heteroatoms of primary and secondary metabolites (C=C, S, N, O) in the extract. This is because of metabolites such that flavonoids, phenolics, and alkaloids. In PS-CuONPs, the UV absorbance is found at 501 nm and 550 nm which confirms the formation of CuONPs in the synthesized material (due to surface plasmon resonance effect) which is displayed in Figure 1[16].

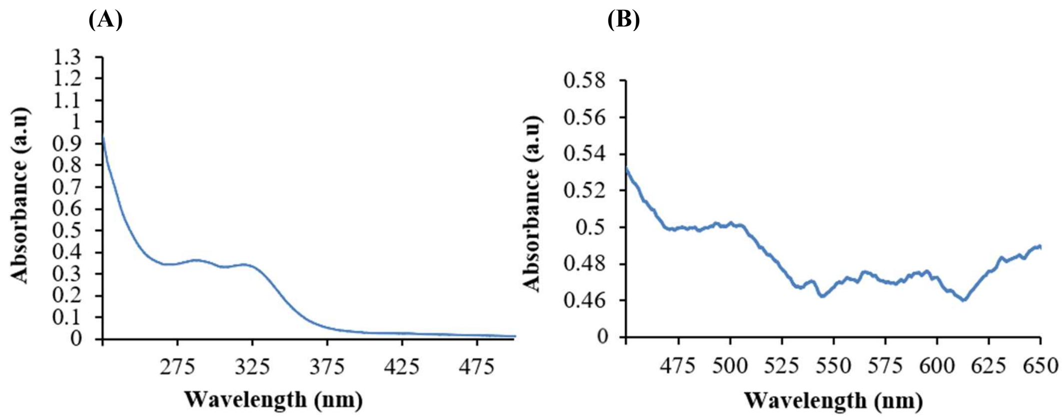

Figure 1 UV-Visible of (A) *P. scrobiculatum* extract and (B) Synthesised PS-CuONPs.

FT-IR spectrum of *P. scrobiculatum* extract and green synthesized PS-CuONPs was shown in Figure 2. It gives the functionalities in plant extract and synthesized CuONPs which explains that in extract the absorption peaks found at 3416 cm^{-1}, 2978 cm^{-1}, 1844 cm^{-1}, 1079 cm^{-1}, 1047 cm^{-1}, 879 cm^{-1}, and 678 cm^{-1} and are responsible for O-H stretching of alcohol (intermolecular bond), C-H stretching of alkane, C-H bending of an aromatic compound, C-O stretching of a primary alcohol, CO-O-CO stretching of anhydride, C=C bending of alkenes, and C-X stretching respectively. In the case of PS-CuONPs, the vibrational frequencies were found to be 3427 cm^{-1}, 1624 cm^{-1}, 1197 cm^{-1}, 1155 cm^{-1}, 900 cm^{-1}, 658 cm^{-1}, and 585 cm^{-1} which are due to O-H stretching of intramolecular bond, C=C stretching of α,βunsaturated ketone, C-O stretching of alcohols, C-O stretching of ether, C=C bending of alkenes, C-X stretching of halo compounds and M-O stretching of metal-oxygen bonds. The spectra reveal that after forming CuONPs the O-H frequency gets suppressed and M-O bond (below 600 cm^{-1}) peaks becomes elongated which confirms the Cu-O metal bonds in the synthesized materials [17].

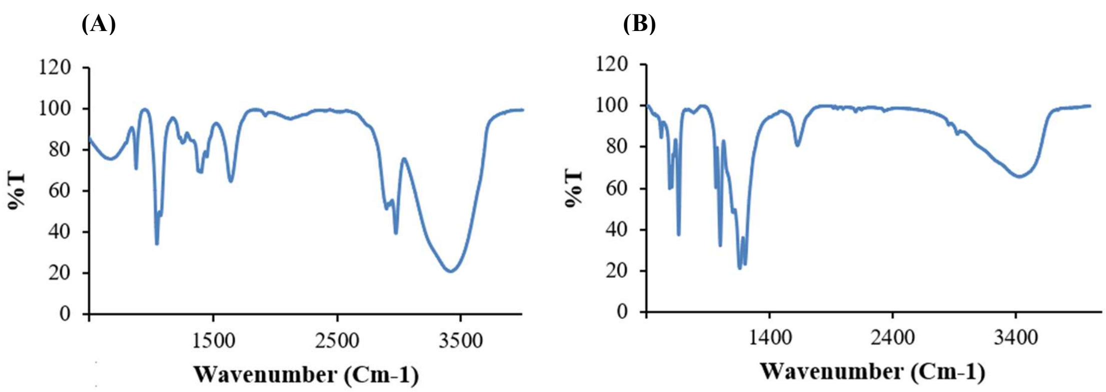

Figure 2 FT IR spectrum of (A) *P. scrobiculatum* extract and (B) Synthesised PS-CuONPs.

XRD spectral analysis on nanomaterials provides information on the crystallinity of synthesized PS-CuNPs. The average size of the nanoparticles PS-CuONPs was found by using the Debye-Scherer equation. XRD peaks of PS-CuONPs is obtained at the 2Θ values of 34.80^0, 35.67^0, 36.23^0, 38.02^0, 39.80^0, 47.30^0, 54.80^0, 57.80^0, 58.99^0 and 63.41^0 which has the plane of (110), (002), (200), (-112), (-202), (020), (202), (-113), (022) and (220). Along with the assigned peaks (JCPDS File No. 48-1548), a few unassigned peaks were found in the XRD which are due to phytoconstituents in the *P. scrobiculatum* extract and are shown in Figure 3. The average crystalline size of the PS-CuONPs was found to be 43.96 nm [18].

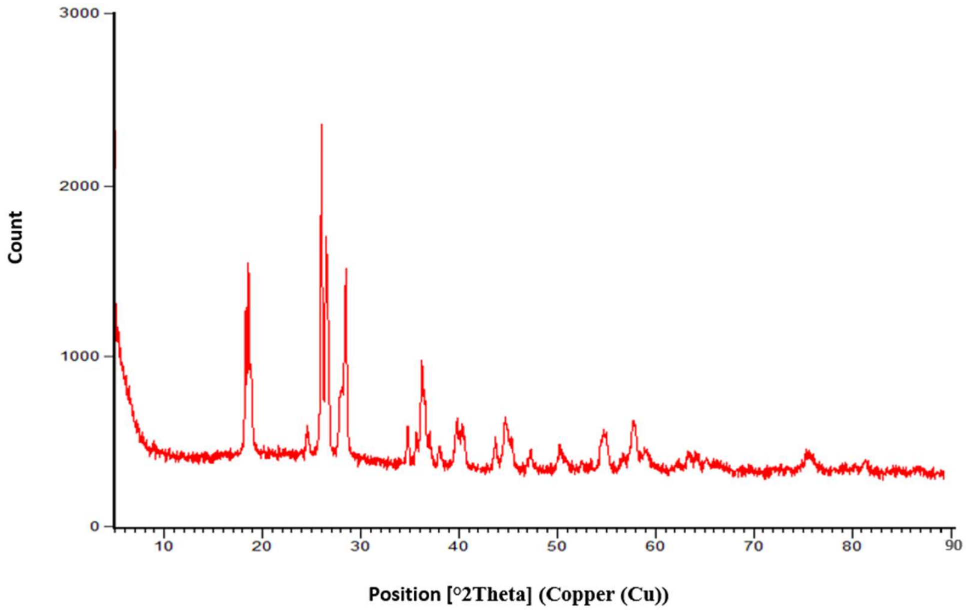

Figure 3 XRD pattern of synthesized PS-CuONPs.

The structural features of the synthesized nanoparticles PS-CuONPs were investigated using the Scanning Electron Microscope (SEM). The accelerating voltage used here is 15.00 kV. The images were represented in two different magnifications (Figure 4) which exhibit a mostly rectangular-shaped morphology with accumulations are found in them.

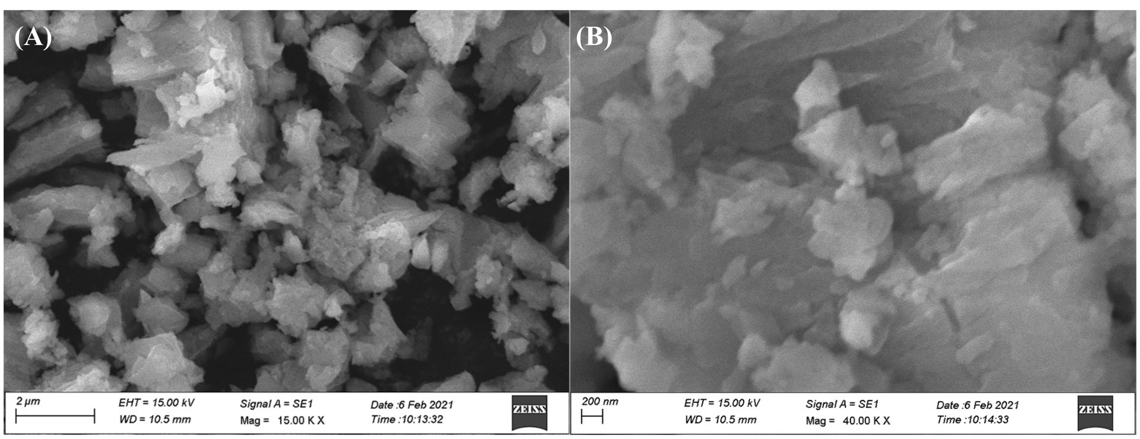

Figure 4 SEM images of synthesized PS-CuONPs; (A) Magnification at 15KX and (B) Magnification at 40KX.

The elemental composition of prepared PS-CuONPs nanoparticles was found out by the EDAX analysis. The EDAX image shows prominently large peaks of Copper at the weight percentage of 53.46% and Oxygen at 46.44% (Figure 5). The strong and narrow diffraction peaks of the synthesized CuO nanoparticles revealed that they are highly crystalline in nature. The existence of Copper and Oxygen peaks along with a few peaks were found in the EDAX which was due to the metabolites present in the extract (involved in the M-O complexation) [19].

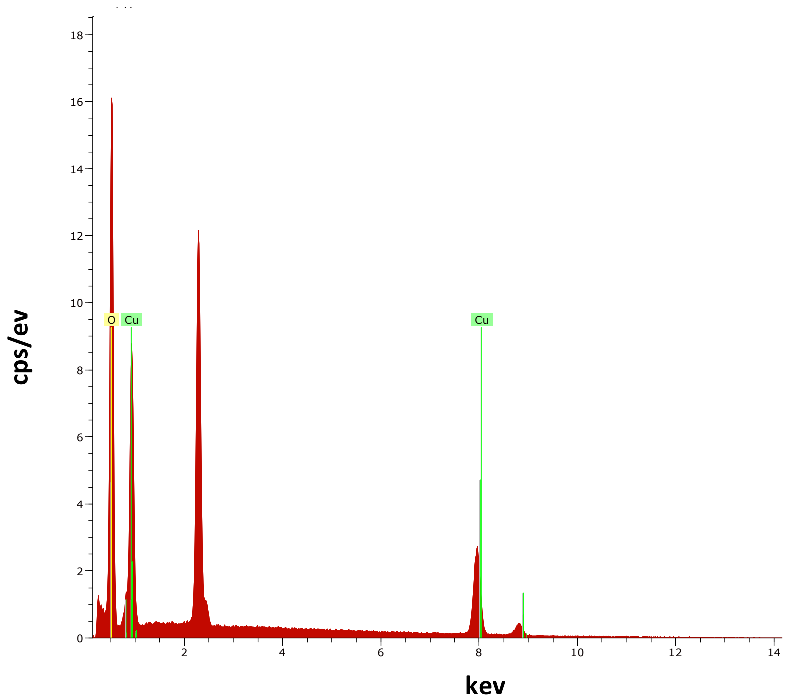

Figure 5 EDAX spectrum of synthesized PS-CuONPs.

The antibacterial activity was performed on the precursor copper sulfate, *P. scrobiculatum* extract, and synthesized PS-CuONPs against five different pathogenic bacteria was tested by disc diffusion method and their results were shown in Figure 6 and Table 1. Anti-bactericidal activities of synthesized copper oxide nanoparticles PS-CuONPs were investigated with the amoxicillin standard drug. The results show that, on *E. coli* and *E. aerogenes,* the precursor copper sulfate, *P. scrobiculatum* extract doesn't show any inhibitory effect but the synthesized nanomaterials PS-CuONPs showed a noticeable inhibitory effect of 13.0±0.15 and 14.0±0.21 mm. Likewise, on *Pseudomonas aeruginosa* and *Staphylococcus aureus* the nanomaterial PS-CuONPs showed a greater inhibition effect than the plant extract. On the contrary the copper nanomaterial PS-CuONPs doesn't reveal any inhibition effect against the pathogen *Proteus vulgaris*. CuONPs have effective antimicrobial potential due to their enormous surface region which enhances the interactions of nanoparticles with micro-organism surface (cell wall) that destroys microorganism cells. The exact mechanism of cell destruction in microbes is still now not known furthermore it has to be studied in detail. The literature reports that gram-negative bacteria are more inhibited by most of the anti-bacterial agents than gram-positive bacteria [20,21]. The synthesized copper nanoparticles PS-CuONPs exhibits noticeable inhibitory action on both the gram-negative and positive pathogenic bacteria. Similarly, reports were reported in other research that reveal the average inhibition zone of 15 to 25 nm [22-24].

Table 1 Zone of inhibition of chosen microorganism.

Microorganisms	Zone of Inhibition (mm/10 µL)			
	A (Amoxicillin)	B (Copper Sulphate)	C (Plant extract)	D PS-CuONPs
E. coli	18.0±0.73	0	0	13.0±0.15
E. aerogenes	18.0±0.81	0	0	14.0±0.21
P. aeruginosa	18.0±0.30	0	5.0±0.30	16.0±0.10
S. aureus	18.0±0.29	0	2.0±0.93	13.0±0.19
P. vulgaris	18.0±0.31	0	0	0

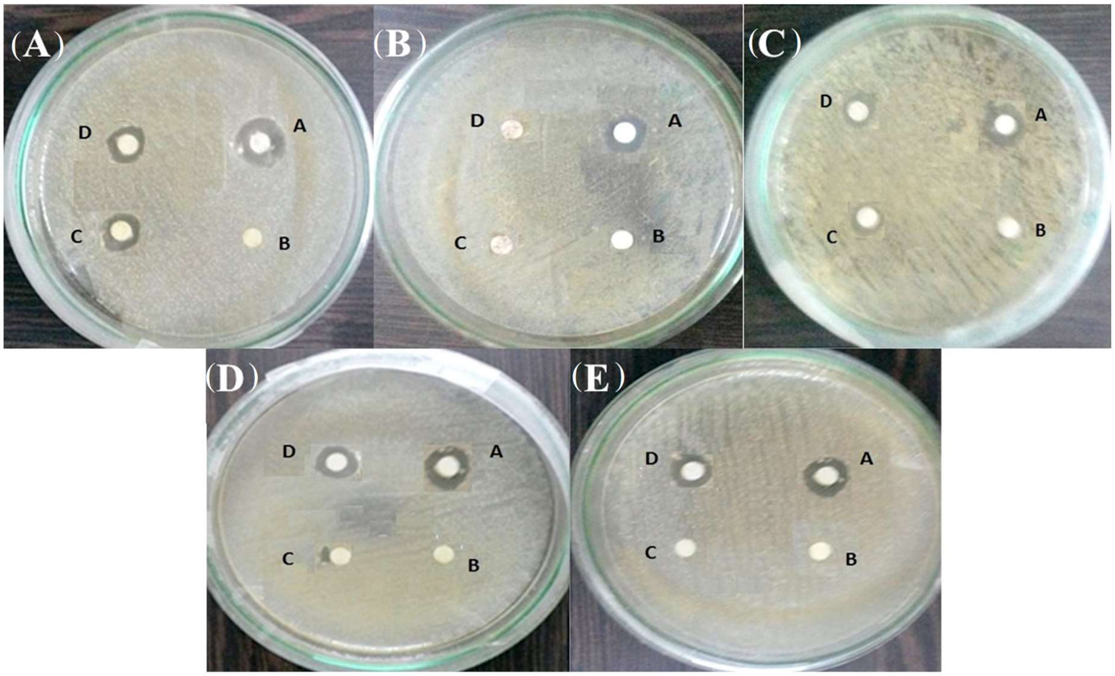

Figure 6 Antibacterial activity of *P. scrobiculatum* extract, Precursor, PS-CuONPs, and standard Amoxicillin on infection-causing pathogens; (A) *P. aeruginosa*, (B) *P. vulgaris*, (C) *S. aureus*, (D) *E. coli*, and (E) *E. aerogenes*.

Ahamed M, et al [25] studied the anti-bacterial activity of sodium hydroxide mediated CuONPs against seven-gram negative bacteria strains and the authors found that CuONPs have high sensitivity on *E. coli* and *E. faecalis* while least on *Klebsiella pneumonia*. Gultekin DD, et al [26] reported different peroxidase enzymes (fig extract) treated nanoparticles with their antibacterial potent on *Pediococcus acidilactici*. Bougainvillea extract-mediated CuONPs record noticeable microbial activity which is studied by [27-34] examine the anti-bactericidal activities of green synthesized CuONPs and their results evidenced the noticeable activities with the average inhibition zone between 15 to 25 mm which is also proved the concentration depended on effect.

4. Conclusion

A novel green method of synthesis of copper nanoparticles was used in the present research work which was found to be eco-friendly, non-toxic, and have less usage of chemicals than the physical and chemical methods. The phytochemicals present in the ethanolic leaf extract itself help in the metal oxide nanoparticle synthesis process (Cu-O). The secondary metabolites such as flavonoids and phenols were found to stimulate the formation of copper oxide nanoparticles. From the literature, it is understood that 70% ethanol extract possesses flavonoid compounds (major phyto-molecule) which is also confirmed by qualitative and UV-Visible. Furthermore, the XRD analysis proved the spherical crystalline nature of the CuONPs with an average size of 43.96 nm. The existence of a major elemental percentage of copper and oxygen without any additional peaks confirmed the elemental composition of the CuONPs. PS-CuONPs revealed the well inhibitory action on the gram-negative bacteria *P. aeruginosa* and it doesn't possess an inhibitory effect on *P.vulgaris*. Also, the antibacterial activity of PS-CuONPs has proved that they can act as an anti-bacterial agent against the chosen infection bacteria.

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