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COPPER OXIDE NANOPARTICLES SYNTHESIS USING *AERVA JAVANICA* AND THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT

In the field of nanomedicine, copper oxide nanoparticles (CuO NPs) are remarkable and foremost transition metal oxides having engrossing features. Their green synthesis is getting popularity as future antimicrobials due to cost effective, eco-friendly and simplicity. In this study, CuO NPs were synthesized from *Aerva javanica* (kapok bush or desert cotton) leaf extract which is well known for its medicinal properties. Antimicrobial potential of *A. javanica* synthesized CuO NPs was assessed against multi drug resistant bacterial and fungal strains. CuO NPs synthesised in this study were characterized using Uv-Visible, X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDXS). These fabricated CuO NPs were studied for their antimicrobial activity using disc diffusion method against multi drug resistant bacterial and fungal strains. Uv-Vis with absorbance band of 255nm confirmed the CuO NPs. XRD pattern distinctive structural peaks that confirmed the typical monoclinic CuO NPs structure. The average measured diameter of CuO NPs by XRD was 5.5 nm. FT-IR spectrum 1378 cm^{-1}-1524 cm^{-1} displayed CuO vibrations. SEM studies revealed the spherical and agglomerated synthesized CuO nanoparticles. EDXS showed strong peak of copper and oxygen and low peak of carbon elements due to capping of biomolecules. CuO NPs exhibited significant ($p<0.005$) antimicrobial activity against resistant bacterial strains. However, the significant inhibitory effect was reported in gram negative as compared to gram positive bacterial strain. CuO NPs showed significant ($p<0.005$) antifungal activity. However, *Aspergillus* exhibited higher sensitivity as compared to *Candida.* Based upon our results, it can be anticipated that biologically synthesized CuO NPs can play role as promising therapeutic agents in nanomedicine field.

Keywords: Cuo NPs, Uv-Vis, XRD, FT-IR, *Candida albicans, Aspergillus niger*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,*

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INTRODUCTION

Globally, the use of nanomaterial has gained popularity in biomedical sciences due to their distinguished property at cell and molecular level, increased specificity and augmented efficacy in treatment and prevention of diseases (Alharbi and Al-Sheikh, 2014). Nanomaterials features like small size, flexible designing and synthesis, and increased surface to volume ratio lead to their intensive use (Karlsson *et al*., 2009). Metal oxide nanoparticles (NPs) have utilization in the fabrication of both personal and commercial products. Zinc, copper, nickel, silver, antimony oxides nanoparticles are employed in wide array of industries (Baek and An, 2011).

Among different metal oxides NPs, CuO NPs have gained tremendous popularity due to simplest member of copper compounds and displays physical features like superconductivity at high temperature, spin dynamics and electron correlation (El-Trass., 2012). CuO NPs have potential applications in superconductors, gas sensors, ceramic pigments and gas sensors (Zhu *et al*., 2004). CuO NPs antimicrobial activity has made them potential contender as therapeutic agent (Nations *et al*., 2015). Currently, foremost challenge faced by researchers in health sector is with drug resistance.

Everyday different better synthesis approaches are evolving that encompass chemical, biological and physical procedures (Soomro *et al*., 2014). Microorganisms, due to their diversifying living nature in soil, air and water pose diverse microbial infections. Due to microbial infections and multi drug resistance, there is immense interest in developing the alternate antimicrobial agents like metal NPs, ketonic polymers and anti-microbial peptides (Kumar *et al*., 2010). Copper encompassing compounds like Cu $(OH)_2$ and $CuSO_4$ are applied as conventional inorganic antibacterial sources (Raffi *et al*., 2010). Liquid based copper solutions encompassing copper polymers and composite copper types are used as antifungal agents (Raffi *et al*., 2010). Copper ions exhibited antimicrobial action against broad diversity of microorganism like *Escherichia coli, Salmonella enteric* and *Listeria monocytogenes* (Gyawali *et al*., 2011). Recently, copper is the only metal with antimicrobial features registered first by Environmental Protection Agency (EPA) (Prado *et al*., 2012). CuO NPs demonstrated the higher antibacterial activity as compared to silver NPs against Bacillus subtilis and *E. coli* (Yoon *et al*., 2007). Beside the aforementioned uses, CuO NPs exhibited anti-cancer and antioxidant effectuality which extend them as a propitious tool for biomedical approaches (Maqbool *et al*., 2017).

Thermal synthesis approaches involving microwave, colloidal, hydro, sono and solo gel have been documented for the CuO NPs fabrication (Jeronsia *et al*., 2019). These approaches are based upon costly perilous chemicals, labour intensive, time consuming, and energy. Therefore, there is dire need of developing biocompatible methods for the nanomaterials synthesis that can overcome the cited limitations (Awwad *et al*., 2015). Studies displayed the remarkable increase in trend from physicochemical approaches of synthesizing metal oxides NPs to biological method called as green synthesis or biosynthesis of NPs (Pugazhendhi *et al*., 2018; Vasantharaj *et al*., 2019). The biological approach of NPs synthesis emphasis synthesis of reducing agents from different sources like fungi, yeast, bacteria, algae and plant extracts which brace biocompatibility and mega scale synthesis (Nasrollahzadeh *et al*., 2019a; Nasrollahzadeh *et al*., 2019b). Biological synthesis has attained more popularity currently due to its simplicity, cost effective and sustainability. Besides the environmental friendly benefit of synthesizing NPs from microorganisms there are constrains; bacterial toxicity, trouble in isolation and incubation steps (Mali. *et al*., 2019). Thus, the plants remain best suited and promising for NPs oxides synthesis, this credibility is related to quick reaction with decreased energy, cost effective, synthesis of several biomolecules, sound stability, no use of hazardous chemicals, safe and easy operation method (Duman *et al*., 2016). Plant extracts contain the both stabilizing and reducing agents during the CuO NPs preparation and other NPs (Ocsoy *et al*., 2013; Rupak *et al*., 2017). Biomolecules like terpenoids, tannins, tannins, flavonoids have been documented as potential stabilizing and reducing agents for CuO NPs fabrication (Rezaie *et al*., 2017).

Recent studies demonstrated the gold nanoparticles biosynthesis displaying high biocompatibility in cancer cell lines from *A. javanica* (Mu *et al*., 2021). Another recent report suggested that CuO-NPs synthesized from *A. javanica* showed potential antimicrobial activity against different bacterial as well as fungal pathogens (Amin *et al*., 2021). Cobalt oxide nanoparticles synthesized from *A. javanica* methanolic extract showed better performance against gram positive bacteria and *fusarium oxysporum* species (Mubraiz *et al*., 2021). All these recent studies suggest the antimicrobial potential of *A. javanica* as potential therapeutic plant.

Aerva javanica is known for its medicinal properties and used to cure skin diseases, rheumatism and cancers (Al-Shehri and Moustafa, 2019, Afzal *et al*., 2022). Based upon previous documented information, we hypothesized that *A. javanica* based nanoparticles might show better antimicrobial activity against multi drugs resistant strains. In this study, an assessment has been carried out to expound an environmental friendly, cheap green synthesis approach for fabrication of CuO NPs using *A. javanica* plant leaf extract. According to our knowledge to the best, this is the first study to assess the activity of CuO NPs against microorganism from *A. javanica* extract. Very few studies documented and evaluated the microbial CuO NPs in bacteria (Hu *et al*., 2009) and the yeast (Kasemets *et al*., 2009).

MATERIALS AND METHODS

Place of work and Materials: Nanoparticle synthesis was performed during June-September 2020 at Laboratory of Nano Science and Technology Division (NS & TD), National Centre for Physics (NCP), Islamabad, Pakistan. Antimicrobial activity was performed during September-November 2020 in Microbiology Laboratory, Department of Microbiology, Quaid-e-Azam University, Islamabad and The Islamia University of Bahawalpur (IUB), Pakistan. In this work, analytical grades chemicals were used. Copper sulphate pentahydrate ($CuSO_4.5H_2O$) (Sigma Aldrich) was used. *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger* used in this study were provided by Microbiology Department, IUB.

Methods: Fresh leaves of *A. javanica* were collected from Cholistan area, Bahawalpur, Punjab. After thoroughly washing leaves several times, they were kept at room temperature in shadow until drying completely. The dried leaves were later pulverized to fine powder. Aqueous extract was prepared in ratio of 1:10 w/v of

mixture and heated at 60 ºC for 50 min. Centrifugation for filtered extract was done out at 4000 rpm for 15 min. Filtered centrifuged extract was later stored at 4 ºC.

Green synthesis of CuO NPs: For CuO NPs, synthesis, Mary *et al.,* (2019) method was followed with minor changes. 0.1M of $CuSO_4.5H_2O$ was used and poured to extract of *A. javanica* in 1:3 ratio. pH was adjusted to 11 by NaOH pellets. Later, solution was heated to 60 ºC for 2 hrs under constant stirring until the color changes which show the synthesis of CuO NPs. Solution having the prepared CuO NPs was washed 3-4 times to remove any impurities, and then centrifugation was done at 4000 rpm for 10 min. Finally, the pellet was oven dried at 120 ºC for 6 hrs. Oven dried pellet was further pulverized to fine grinded powder and placed at 4 ºC.

Characterization of CuO NPs: For the confirmation of CuO NPs, physical characterization approaches were performed.

UV-Vis spectroscopy: Biosynthesized CuO NPs were studied for optical absorption characteristics by peaks visualization. UV-Vis Spectral scan was performed in range of 200 nm- 800 nm using spectrophotometer (UV-1900; Shimadzu Europa GmbH).

X-Ray Diffraction (XRD): To analyze the crystalline size and structure of synthesizes nanoparticles, X-ray diffractometer (D8 Advance, Bruker, Germany) with Cu Kα radiation (λ=1.54060 Å) was used. XRD analysis was performed using copper anode with range of 2θ, 20º – 80º.

Fourier Transform Infrared Spectroscopy (FTIR): The infrared spectroscopy (FTIR model Bruker Equinox 55, Germany) was utilized to ascertain the molecular analysis (bonds types and chemical structure). Biosynthesized CuO NPs were combined with KBr pellet and scanned in the range of 500-4000 cm^{-1} (Das *et al*., 2016).

Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy (SEM-EDXS): For morphological study (the shape lattice) of synthesized CuO NPs, scanning electron microscope was used (SEM; Model QUANTAX EDS for TEM, Bruker, Germany). EDXS was utilized to investigate the elemental constituent of NPs with imaging analyzed from 1000X – 30,000X with resolution of 0.5 to 1µm.

Biological Screening of CuO NPs

***In Vitro* Antibacterial Assay of CuO NPs:** Antibacterial activity of synthesized CuO NPs was studied for multi drug resistant gram positive and gram negative bacterial strains. These pathogenic strains were obtained from Microbiology Lab, IUB. The activity of CuO NPs against bacterial strains was ascertained through disc diffusion assay after following the instructions of Clinical Laboratory Standard Institute (CLSI) (Bauer *et al*., 1996; CLSI, 2006). Cultivated bacteria on separate plate were further incubated on nutrient agar media for 24 hours at 37 ºC. bacterial culture was later inoculated in nutrient broth medium at 37 ºC for overnight. 1mL of these overnight grown bacterial culture was shifted to nutrient agar. Different concentrations (1, 2, 4 µg / ml) of CuO NPs loaded discs over nutrient agar plates were kept for 24 hrs at 37 ^{0}C. After incubation, zone of inhibition was calculated for each disk through scale. Whole experiment at this was performed twice with each sample in triplicate. Double distilled deionized water was used as negative control while 50 uL aqueous extract of *A. javanica* as positive control, 1 mg ciprofloxacin disk for both gram positive and gram-negative bacteria strain were used as control. After incubation, inhibition zones were estimated to assess antibacterial activity.

***In Vitro* Antifungal Assay of CuO NPs:** CuO NPs fabricated were tested against antifungal strains; *C. albicans* and *A. niger* using diffusion method. Sabouraud dextrose agar (SDA) was media plates were prepared and inoculated with *C. albicans* and *A. niger* for 72 hrs at 30 ºC. Fungal suspension of 1.5×10^6 CFU/ml along with 50 µl of CuO NPs was placed at incubator shaker at 30 ºC for overnight. Using dilution series for CFU, fungal suspension were diluted 10 times. The aggregated mixtures were cultured later in SDA medium and placed at 30 ºC for 72 hrs. After incubation period, colonies were counted in each plate. Their average value was calculated. Experiment was performed twice with each sample in triplicate. 1 mg fluconazole disk for fungal strain were used as control.

Statistical Analysis: We performed antimicrobial activity experiment with three different treatment each in triplicate. Calculated mean values of each treatment with standard error were compared with positive and negative control. ANOVA was performed to check the statistical significance (Steel *et al*., 1997). LSD test was employed to compare the statistically significant difference among means (Salkind, 2010). The values p <0.005 were counted statistically significant. The data was statistically analyzed using SPSS (Ver 25.0) software.

RESULTS

CuO NPs synthesized in this study were studied by UV-Vis spectrum for their optical features. Figure 1 shows UV-visible spectrum of green synthesized CuO NPs. Biologically synthesized CuO NPs display only one absorption sharp peak at 255 nm showing the formation of CuO NPs (Figure 1).

UV-Vis spectrum was done in 200-800 nm range. XRD was performed to study the crystalline

structure of CuO NPs. XRD diffractogram for CuO NPs is shown in figure 2 reveals very clear peaks attributed to 2θ values of 110, 111, 202, 020, 202,113, 020, 220, 312, 222 for the corresponding intensity of 32.51, 35.51, 38.72, 46.56. 48.78, 53.71, 58.32, 61.15, 65. 28, 68.31 respectively indicating the CuO NPs formation. Besides these, other peaks also displayed (Figure 2). The average crystalline size was 5.5 nm. The average crystal size was calculated using Debye Schrerrer equation as following.

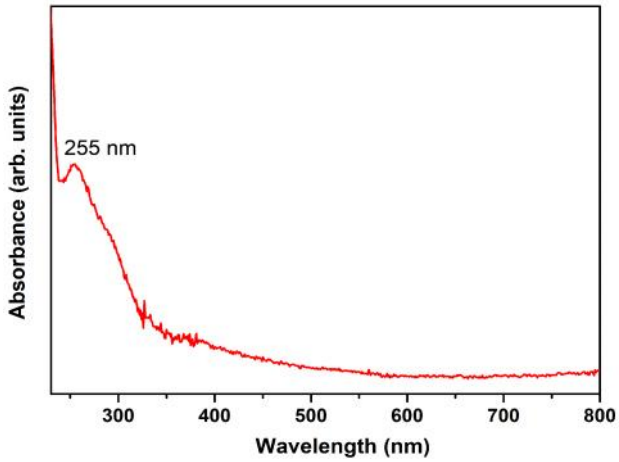


Fig.1 UV-Visible spectrum for CuO NPs. CuO NPs shows absorption peak at 255 nm due to inter band transition of core electrons of copper metal.

$D = 0.94\lambda / (\beta\cos\theta)$

D mentions average particle size, λ is wavelength of x-ray diffraction, θ is diffraction angle and β is full width at half maximum.

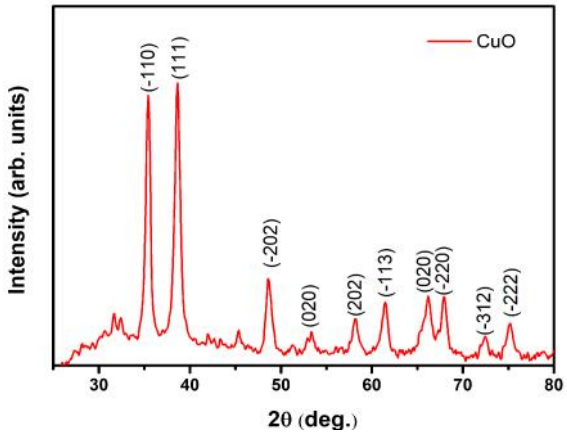


Fig 2. XRD spectrum of CuO NPs. Clear and strong peaks corresponding to 2Ɵ values of 110, 111, 202, 020, 202,113, 020, 220, 312, 222 for the respective marked intensity of 32.51, 35.51, 38.72, 46.56. 48.78, 53.71, 58.32, 61.15, 65. 28, 68.31 respectively indicating the CuO NPs formation

FTIR spectrum pattern displayed vibrational bands at 500 cm^{-1}, 1022 cm^{-1}, 1378 cm^{-1}, 1524 cm^{-1}, 2912 cm^{-1}, and 3336 cm^{-1} (Figure 3). The FTIR spectrum was performed in the range of 500- 4000 cm^{-1}. FTIR authenticated the presence of different chemical bonds responsible for the CuO NPs synthesis. Scanning electron microscopic analysis showed spherical particle. Figure 4 shows the SEM images of CuO NPs. SEM analysis revealed the clumped and clustered particles. EDS was performed to determine the purity and synthesized NPs chemical composition. Figure 5 confirms the Cu, O and C presence. EDS showed strong prominent peaks Cu, O and C that confirmed the CuO NPs synthesis. EDS analysis revealed the 13.99 %, 31.50 % and 54.51 % of Cu, O and C respectively (Figure 5).

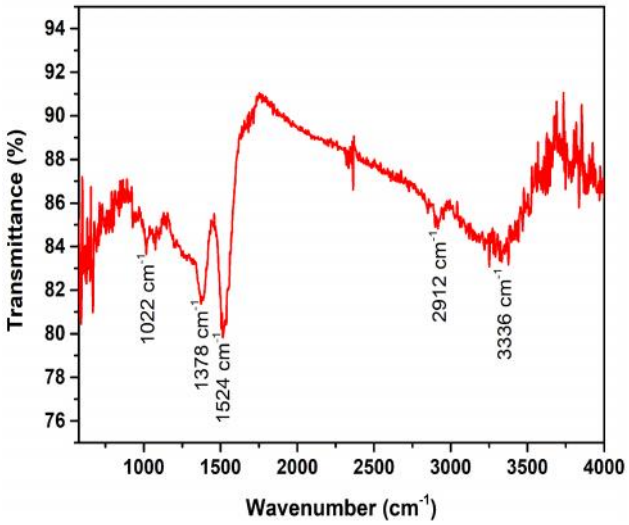
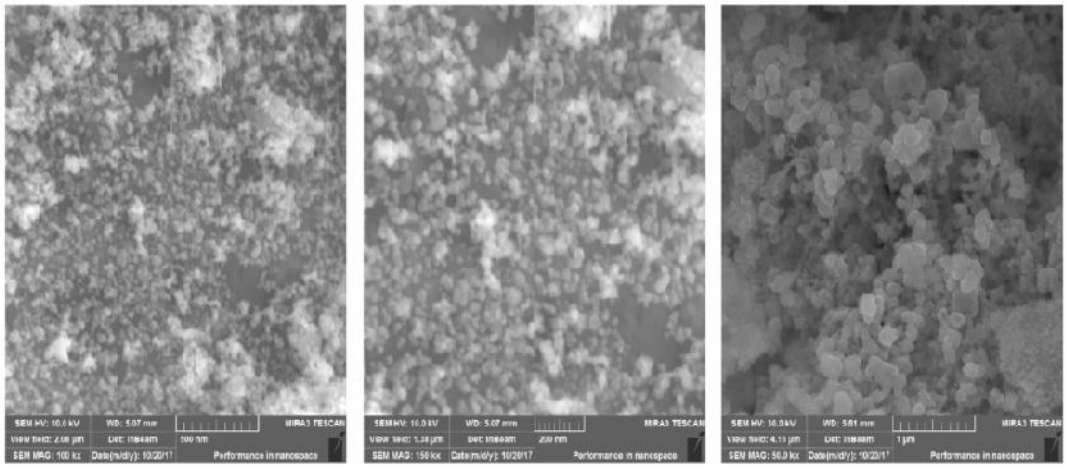


Fig. 3. FT-IR spectrum of CuO NPs

Table mentions the different concentrations of CuO NPs their antibacterial and antifungal activity. The antimicrobial efficacy of CuO NPs was found to be significant compared to that of positive control with $p<0.005$ in all bacterial and fungal strains. Results displayed significant variations in zone of inhibition. Among bacterial strains at highest concentration (4 µg/ml), higher inhibition zone was recorded followed by 2 µg/ml and 1 µg/ml concentration (Table). In our results, *P. aeruginosa* showed higher inhibition zone compared to *S. aureus*. Antifungal activity was found to be higher at higher concentration with no difference at minimum concentration. *A. niger* showed higher zone of inhibition as that of *C. albicans* at higher concentration while both *A niger* and *C. albicans* revealed no significant differences at dose of 2 mg/ 100 µl. (Table). The antifungal activities displayed *A. niger* to be more sensitive in comparison to *C. albicans.*



(a) (b) (c)

Fig 4. SEM images of CuO NPs (a) 500 nm, (b) 200 nm, (c) 1µm agglomerated spherical NPs

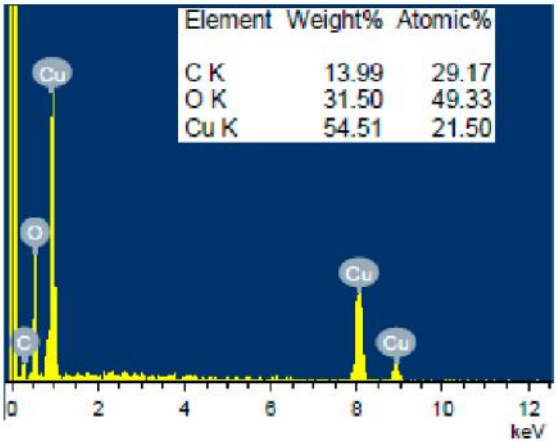


Fig 5. EDS analysis of CuO NPs

Table. *In vitro* antimicrobial assay readings against bacterial and fungal strains.

Microbial strain		Zone of inhibition (mm) results expressed as Mean ± SEM					
		CuO NPs (1 µg/ml)	CuO NPs (2 µg/ ml)	CuO NPs (4 µg/ ml)	Positive Control	Negative Control	*P* value
Gram positive bacteria	*Staphylococcus aureus*	1.00 ± 1.00	3.00 ± 1.52	5.33 ± 2.72	20.00 ± 0.57	1.56 ± 0.13	<0.005
Gram negative bacteria	*Pseudomonas aeruginosa*	1.16 ± 1.16	8.33 ± 0.88	20.66 ± 1.76	28.00 ± 0.57	1.56 ± 0.13	<0.005
Fungus Candida	*Candida albicans*	0.00 ± 0.00	3.00 ± 0.57	3.33 ± 1.76	12.00 ± 0.57	1.56 ± 0.13	<0.005
Fungus Aspergillus	*Aspergillus niger*	0.00 ± 0.00	3.66 ± 0.88	7.66 ± 1.20	10.00 ± 0.57	1.56 ± 0.13	<0.005

DISCUSSION

The current study encompasses use of Cholistani shrub *A. javanica* for the CuO NPs biological synthesis. *A javanica* is well known for its medicinal properties. Based upon this assumption and hypothesis, the mechanism of CuO NPs can be assumed and elaborated by using metabolic contents of leaf extract of *A. javanica* as they have been documented for metabolites (Ahmed-el

et al., 2010). The CuO NPs synthesized in our study were characterized using physical methods Uv-Vis, XRD, FTIR, SEM-EDXS. Uv-Vis spectrum exhibited only one absorption peak at 255 nm indicating CuO NPs synthesis. Our results are supported by previous studies documented (Nasrollahzadeh *et al*., 2016; Wang *et al*., 2016). Peak at 255 nm is considered best due to inter band shift of core electrons of copper (Fragoon *et al*., 2016). XRD peaks in our study reveal the different planes of specific monoclinic phase of CuO NPs and are in consensus with standard values documented by Joint committee on Powder Diffraction Standards (JCPDS) (card no. 80-0076). Similar results were reported previously (Manyasree *et al*., 2017; Imani *et al*., 2020). In XRD pattern in our studies, no peak showing impurities were detected which concludes the promising quality of copper oxide nano particles synthesized (Ahamed *et al*., 2014). The broaden pattern in diffraction peaks may be due to size effect and the crystal size.

FTIR spectral analysis showed broad range spectrum peaks. The peak observed at 3336 cm^{-1} resembles to N-H and O-H bonds stretching vibrations due to phenolics compounds in the solution (Qamar *et al*., 2020) and O-H group of surface water absorbed by (Imani *et al*., 2020). The diffraction peak at 2912 cm^{-1} corresponds to C-H stretching (Niaz *et al*., 2018; Imani *et al*., 2020). The sharp and prominent peak at 1524 cm^{-1} corresponds to C = O and C = N stretching (Qamar *et al*., 2020). Likewise, another sharp peak at 1378 cm^{-1} displays stretching of C-H band of alkane (CH_2 and CH_3) (Javadhesari *et al*., 2019). Furthermore, peak at 1022 cm^{-1} corresponds to primary and secondary alcohols C-O (Javadhesari *et al*., 2019). Unsaturated C-H stretching pattern that appeared below 1000 cm^{-1} that may be because of existence of phytochemicals like carbohydrates, proteins, alkaloids, steroids, terpenes and triterpenes. These bioactive compounds play role as capping and stabilizing agents through the synthesis of CuO NPs (Qamar *et al*., 2020).

SEM analysis of our studies revealed the fabricated CuO NPs may be spherical in shape. However clear morphology and shape could not be evaluated due to cluster formation and agglomerations. SEM images of our studies coincide with previous reports (Qamar *et al*., 2020; Kumar *et al*., 2020). Purity and elemental composition of copper and oxide peak with no other element purity in our studies coincide with previous reports (Manyasree *et al*., 2017; Qamar *et al*., 2020). Presence of carbon peak confirmed the presence of carbon based stabilizers in synthesized CuO NPs (Khan *et al*., 2016).

CuO NPs efficacy was found significant as that of standard drug with $p<0.005$ in both bacterial strains. Among the bacterial strains, gram negative showed higher sensitivity against CuO NPs in comparison to gram positive as documented earlier (Manyasree *et al*., 2017). Increased zone of inhibition in both bacterial strains is related with higher concentration of CuO NPs (Manyasree *et al*., 2017). More sensitivity of gram-negative bacteria *P. aeruginosa* may can be best described as it allows increased Cu^{2+} ions to access membrane but is mainly approached with reduced susceptibility to antibacterial agents and antibiotics (Koch and Woeste, 1992). The difference in resistance or sensitivity may be due to cell wall structural differences, metabolism, physiology and magnitude of contact of microbes with nanoparticles (Gopalakrishnan *et al*., 2012).

Merah *et al.,* (2019) described similar results of CuO NPs which showed enhanced efficacy on gram negative as that of gram-positive bacteria that would be probably owing to size difference and due to Zn^{2+} and Cu^{2+} characteristics. Less efficacy of CuO NPs on gram positive bacteria may be due to augmented multi drug resistance. Reports highlighted that due to alterations in bacterial membrane structure, CuO NPs enter the bacterial cell that promisingly augments cell permeability and effect transit through cell membrane that ultimately causes cell death (Auffan *et al*., 2009). So far, antibacterial activity of CuO NPs have been elaborated by divergent mechanisms including damage of cell membrane, lipid peroxidation, reactive oxygen species and DNA damage in bacterial cells (Chatterjee *et al*., 2014). It can be concluded that, increased antibacterial resistance prevails to traditional drugs, to combat the development of multi resistant strains it has become mandatory to find new ways and approaches. Our recent published reports from *A. javanica* ZnO NPs showed promising antimicrobial activity in bacterial and fungal strains (Afzal *et al*., 2022).

In our study, less inhibition zone was observed in fungi as compared to bacteria. This is due to the more rigid fungal cell wall composed of chitin, chemically comprised of polysaccharides. Hence it does not permit to pass CuO NPs to inside cell (Qamar *et al*., 2020). Our study displayed the *Candida* strain considerable resistance as that of *Aspergillus*. Our studies are similar with earlier studies who also documented the CuO NPs as inhibitory particle against candida species (Weitz *et al*., 2015; Devipriya and Roopan. 2017; Imani *et al*., 2020). The antifungal approach of CuO NPs based upon cell wall distortion, triggering oxidative stresses and dissolution of CuO NPs (Dizaj *et al*., 2014; Ingle *et al*., 2014).

Increasing the CuO NPs concentration resulted into the increased inhibition in our studies, similar resulted were reported in past (Hou *et al*., 2017). This is due to higher dose of CuO NPs through cell membrane enter into cytoplasm and their permeability increases into cytoplasm which results into distortion of pathogens after several biochemical processes (Hou *et al*., 2017). *Candida* infections require dimorphic shift from yeast to

mycelial shape. In this study, *Candida* growth was inhibited with no significant difference at two consecutive higher doses of CuO NPs. CuO NPs antifungal activity is attributed to its effects on the mycelia (Sangeetha *et al*., 2012) It is noteworthy to disclose that factors like ionic strength, pH, availability of molecular ligands and others may play role in toxicity in biological system (Muñoz-Escobar *et al*., 2020). Further, research is required to investigate the mechanism by which nanoparticles display antifungal activity.

Conclusion: CuO NPs were extracted from *A. javanica* leaf extract which is eco-friendly and cheap method. UV-Vis, XRD, FTIR confirmed the biological synthesis of nanoparticles with suitable size and structure. SEM-EDXS exhibited the morphology of CuO NPs. Additionally, we documented the physicochemical characterization results of CuO NPs supplemented along in vitro antibacterial and antifungal studies. Therapeutic ability of CuO NPs against *P. aeruginosa* and *A. niger* is remarkably higher than *S. aureus* and *C. albicans*. This study is reported first time using *A. javanica* leaf extract. Green synthesised CuO NPs could be used to flourish targeted remedies against fungi, bacteria and viruses. In comparison to other nanoparticles, very few reports on interaction of pathogens with CuO NPs have been documented. So, further investigating studies are required to explore the processes of interaction of CuO NPs on extended antimicrobial efficiency.

Disclosure: The authors state no conflict of interest.

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