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Green synthesis and characterization of copper nanoparticles using *Parthenium hysterophorus* extract: Antibacterial and antioxidant activities evaluation

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In view of green route simplicity, cost effectiveness and eco-benign approach, this study focusses on the fabrication and characterization of copper nanoparticles (Cu NPs) using leaf extract (aqueous) of *Parthenium hysterophorus* (Gajar Booti). Characterization of *P. hysterophorus* mediated Cu NPs was performed by UV-Visible, Fourier-transform infrared, X-ray powder diffraction and scanning electron microscopy techniques. The UV-Vis characteristic peak was observed at 563 nm along with change in variation in color from light brown to dark green confirm the formation of Cu NPs. The Cu NPs was semi-spherical with average particle size of 15.48 nm. These green mediated Cu NPs was analyzed for their antibacterial activity by disc diffusion and MIC analysis and antibacterial activity was promising against *Escherichia coli, Pasteurella multocida, Staphylococcus aureus* and *Bacillus subtilis* strains. The Cu NPs also showed excellent antioxidant activity evaluated by DPPH free radical scavenging. In view of promising bioactivity, the Cu NPs prepared using *P. hysterophorus* extract could be used for biomedical applications.

Capsule Summary: The Cu NPs was prepared via a green route using leaf extract (aqueous) of *Parthenium hysterophorus* and NPs prepared by green route showed promising antioxidant and antimicrobial activities.

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INTRODUCTION

Nanotechnology is an important and dynamic field of investigation in recent material sciences. The NPs typically incorporate particles having size ranging in 1-100 nm with respect to one dimension, exhibiting entirely new or enhanced properties with respect to particular characteristics (Elsakhawy et al., 2022; Herrera-Barros et al., 2022). Nano-materials offer way out to environmental, technical and industrial problems in the fields of catalysis, medicine, solar energy transformation and water-treatment (Ameh and Sayes, 2019). Metallic nanomaterials have attained much more significance as they normally exhibit distinctive, unique and modified characteristics corresponding to bulk materials (Dzimitrowicz et al., 2019; Li et al., 2021). In nanotechnology field, the growth in the advancement of ecofriendly methods to synthesize metal nanoparticles having fine size, shape and structure is a huge challenge. It is broadly acknowledged that the unique properties of NPs are often governed by their shape, size, crystalline structure, composition, and arrangement (Mohammadi et al., 2016).

Copper-based materials are considered one of the most significant resources, among many metals such as Au, Ag, Pd, Pt. Metallic Cu has a huge role in recent electrical and microelectronic circuits for its outstanding conductivity and inexpensive nature (Bashir et al., 2015). Copper nanoparticles are difficult to prepare owing to the fact that the prepared nanoparticles are liable to be oxidized in air as compared to the noble metals like silver, gold and platinum (Kawamura et al., 2015).

Physical and chemical methods usually costly and utilize hazardous chemicals. To overcome these problems, biological synthesis has been designed and applied in many areas like electronic industries and biomedical sciences (Al Banna et al., 2020; Awwad and Amer, 2020; Awwad et al., 2020; Nwamezie, 2018). Viruses, bacteria, algae, fungi and plants are considered as a green source for the preparation of NPs. These biological systems have been suggested as substitute of chemical and physical methods to produce eco-friendly nanoparticles (Iqbal et al., 2020a; Iqbal et al., 2020b; Naseer et al., 2020; Yasmin et al., 2020). Practice to usage of plant extracts for nanoparticles synthesis is beneficial as compared to other environment friendly biological routes since this eradicates detailed cell culture process. The real benefit in exercising plant life to synthesize nanoparticles is due to reason that these are readily obtainable, secure in handling and acquire a wide range in metabolite bio-molecules that cause production of nanoparticles. Biological molecules resembling flavonoids, proteins and phenols cause the reduction of metal ions to the NPs, furthermore take part in the capping of the nanoparticles (Amer and Awwad, 2021; Lv et al., 2018; Shammout and Awwad, 2021; Ukpaka and Neo, 2021).

Under this particular study, nanoparticles of copper were synthesized by means of *Parthenium hysterophorus* plant leaf aqueous extract as reducing and capping resources. *Parthenium hysterophorus* belongs to Asteraceae family. Cu NPs perform as exceptional germicide because of their high average surface area and tiny size (Babushkina et al., 2017). Antibacterial effect due to Cu NPs is caused in many ways like manufacture of ROS, proteins oxidation, lipids peroxidation and DNA degradation of bacterial cell. Antibacterial activity reduces due to presence of EDTA which forms chelates with copper ions thus inhibiting degradation of DNA. The Bio-synthesized Cu NPs have both therapeutic as well as diagnostic applications (Mukhopadhyay et al., 2018; Yaqub et al., 2020).

The main objective of this research work was to bio-synthesize Cu NPs utilizing aqueous leaf extract of *P. hysterophorus* without use of any toxic chemicals. These synthesized Cu NPs were categorized using different advanced techniques including FTIR, EDX, XRD and SEM. The NPs were appraised for their antioxidant and antimicrobial activities.

MATERIAL AND METHODS

Chemicals, reagents and samples preparation

All the salts and solvents were of high purity grade and were purchased from Sigma Aldrich. The leaves of *Parthenium hysterophorus* were acquired from grass land of Alhar Tehsil Pasrur, Sialkot. Leaves of *Parthenium hysterophorus* were comprehensively cleaned with tap water followed by washing with distilled water to eradicate dirt particles. The leaves were dried under shade and stored in sealed containers. The dried leaves were made into fine powder form using pestle mortar. 20 g of powder was taken along with 200 cm^3 of water followed by heating with the help of hot plate at 75-85°C for 40 min with constant stirring via magnetic stirrer. This mixture was then sieved. The extract obtained was gently centrifuged at 6000 rpm for 5 min to eliminate any suspended materials. The clear extract thus obtained was stored at very low temperature (4 °C) to make it use in future.

Green synthesis of copper nanoparticles

Fabrication of Cu NPs was done by drop wise addition of 50 mL of Plant extract to 150 mL of 0.01 M $CuSO_4$ solution at 70 °C with continual stirring. The color of copper sulphate solution immediately changed from blue to dark greenish indicative of development of copper NPs. The mixture was further agitated for 60 min to enhance the development of Cu NPs. The obtained suspension was further kept for 2 h at room temperature for the process of nucleation of NPs. Afterwards the centrifugation of suspension was done at 6000 rpm for 20 min. The supernatant fluid was poured out and remainder was recurrently washed three to four times with distilled water followed by methanol to remove suspended and dissolved impurities. The subsequent precipitates were dehydrated at 90°C for 5 h. Green fabricated Cu NPs were crushed to fine powder and kept for further study.

Characterization

FTIR spectroscopy is an analytical technique in which the sample is irradiated with Infra-Red radiations due to which various functional groups of compounds present in sample show absorbance in range from 4000 to 400 cm^{-1}. FTIR spectroscopy was carried out to recognize biological molecules that are tangled in phenomenon of bio-capping and reduction of copper NPs. FTIR is sensitive to small absorbance variations, which leads separation of even weak absorption peaks of active compounds from those of the whole extract.

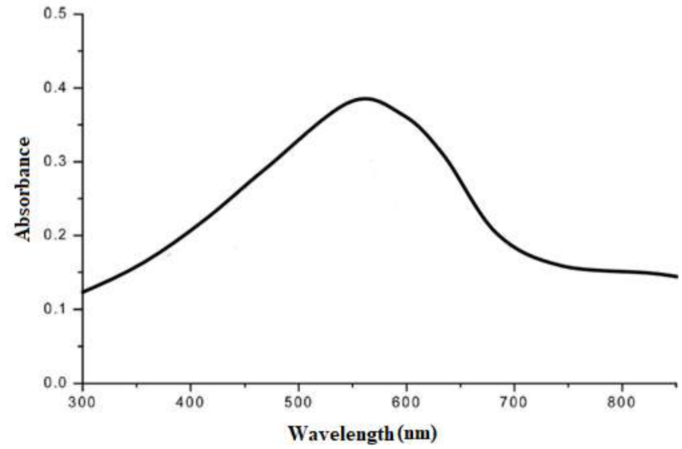

Fig. 1: UV-Visible spectrum showing the λ_{max} of Cu NPs

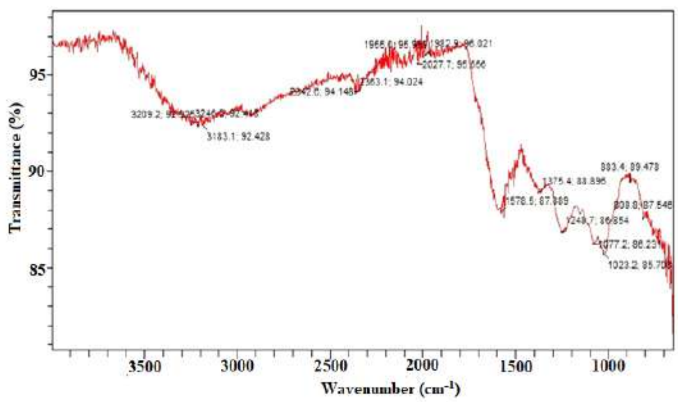

Fig. 2: FTIR spectrum of *Parthenium hysterophorus* mediated Cu NPs

SEM is an imaging technique that utilizes beam of electrons and is used to describe morphology and shape of Cu NPs (Mohammadlou et al., 2016). The morphology of Ag NPs was appraised using SEM (JEOL-JSM-5910, USA). It was performed by placing a thin covering of NPs on carbon treated Cu grating. This copper lined grid was dried out using heat from mercury lamp for the duration of 5 min. At the end, an electron microscope was employed for observation of Cu NPs at 150X, 500X, and 1000X magnification. We can detect any kind of impurity or contaminant in our samples using EDX in conjugation with SEM. Energy-Dispersive X-Ray Spectroscopy (INCA-200, UK) can verify the elemental composition of metallic nanoparticles.

XRD examination is utilized to get information about crystalline structure of NPs. When the crystal is hit by X-ray light, the diffraction occurs and properties of crystals are shown due to these observed diffraction patterns of crystal. Before XRD analysis, calcination of desiccated sample of the prepared Cu NPs was done at 450°C for 5 h in electric furnace. XRD system (BRUKER D8 Advanced XRD, Germany) which could produce Cu Ê (á) emission radiation of 1.54067 nm wavelength. Instrument is powered with 30 kV voltage and 30 mA current and Ni gauze is utilized as filter. (Rath et al., 2014). To carry out the XRD studies, a thin film was drawn on to the glass plate using dipping technique. The sample was then scanned over 2 θ value range of 4°-80° at 0.02 min^{-1} and at 1 second time constant. The size of crystalline particle was calculated with the help of Scherer formula (Eq. 1).

$$D = \frac{K\lambda}{\beta Cos\,\theta} \quad (1)$$

Antimicrobial activity evaluation

Antibacterial action of the green produced Cu NPs of *Parthenium hysterophorus* was explored for a panel of gram +ive & gram -ive bacteria. For example, *Escherichia coli, Pasteurella multocida, Staphylococcus aureus and Bacillus subtilis* were used as a test organism. Different techniques including minimum inhibitory concentration and agar disc diffusion method were used to record the antibacterial potential of green synthesized NPs. The fresh culture in nutrient broth was prepared using standard procedure. Furthermore, the cultures were tested and were stored at very low temperature of 4℃ Significant amount of cell culture (1×10^8 CFU/mL) was used for disc diffusion and minimum inhibitory concentration method. Similarly, the prepared Cu NPs were also tested for antibacterial activity using agar disc diffusion method. Common growth media was prepared, autoclaved to avoid adulteration. The prepared discs were sterilized and autoclave media after mixing with tested microorganism, left for 30 min to harden. On solidification, the sample of NPs was absorbed on every disc. The standard antibacterial agent for the process was Rifampicin. After 30 min, the disc was placed on petri plate and incubated at 37℃for 24 h. The zone of inhibition was measured in millimeter and was documented using zone reader (Riaz et al., 2012).

Minimum inhibitory concentration of every tested sample was measured using Well plate method. The test sample was diluted in downward order using sequential dilution method and in the last well, test sample was released. In every well, 10 µL resazurin and 10 µL of tested microorganism culture (5×10^6 CFU/mL) was added. The positive and negative control was run for every plate. Finally, to avoid dehydration, aluminum foil was enveloped around each plate. These plates are incubated and absorbance was recorded at 500 nm (Sarker et al., 2007).

Total phenolic content was measured by Folin-Ciocalteu reagent method as illustrated by Ainsworth and Gillespie, (Ainsworth and Gillespie, 2007). The absorbance was recorded at 765 nm. Calibration curve of Gallic acid was used as standard to calculate TPC amount. The results were expressed as Gallic acid equivalent (GAE). The total flavonoids contents was determined following the method described in Sakanaka et al. (Sakanaka et al., 2005). The absorbance for flavonoids was recorded at 510 nm. TFC amounts were presented as catechin equivalents.

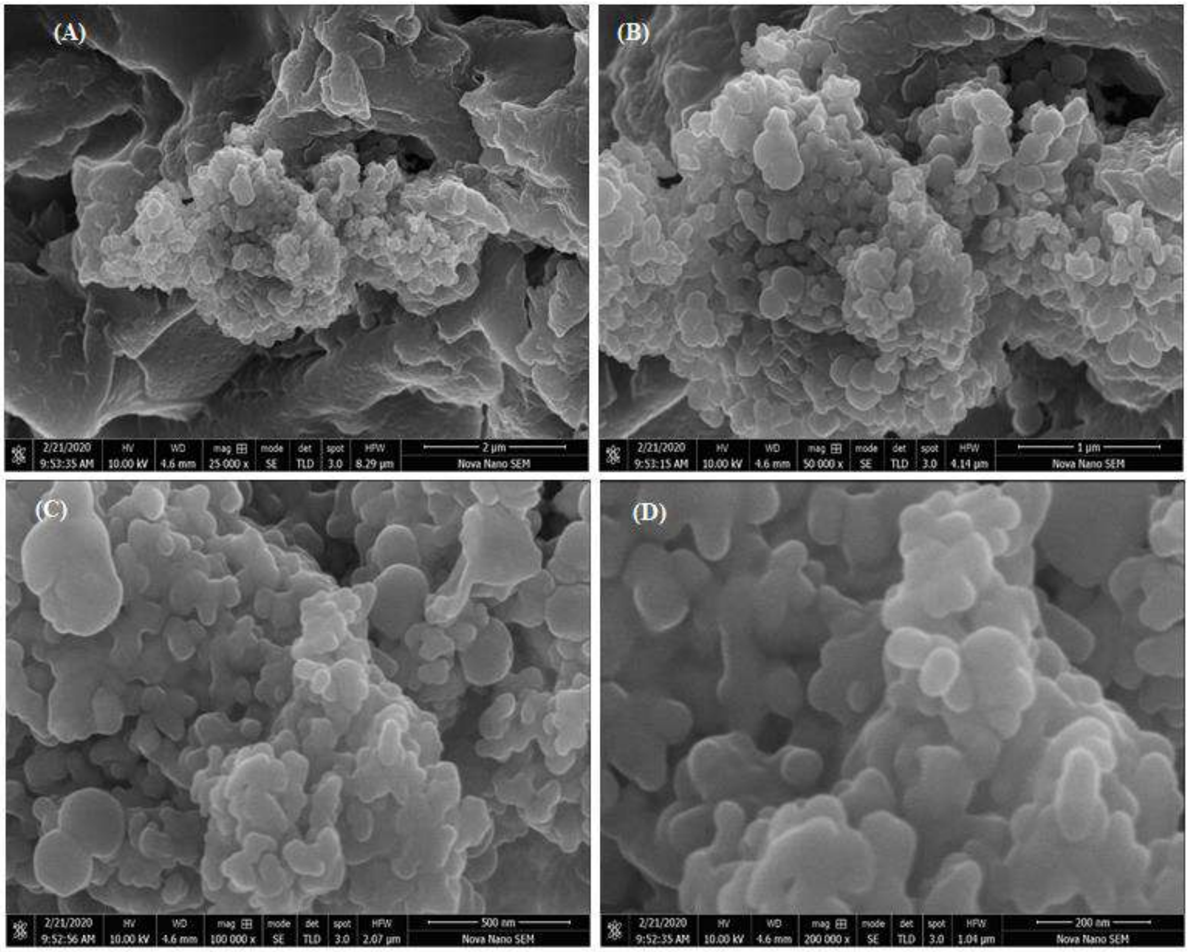

Fig. 1: SEM analysis of synthesized Cu NPs at different magnifications

The DPPH analysis was performed as described by (Bozin et al., 2006). The antioxidant effects of bio-synthesized NPs were appraised using their scavenging capacities against 2, 2-diphenyl-1-1-picrylhydrazyl radical. The absorbance was recorded at 517 nm. The percent inhibition was determined using Eq. 2.

$$IC_{50}\,(\%) = \frac{\text{Sample absorbance} - \text{Blank absorbance}}{\text{Blank absorbance}} \times 100 \qquad (2)$$

RESULTS AND DISCUSSION

UV-Visible Spectroscopy

Color transformation in $CuSO_4$ aqueous solution from blue to dark greenish coloration and formation of brownish precipitates in reaction container indicates successful creation of Cu NPs subsequent to addition of suitable quantity of leaf extract of *Parthenium hysterophorus*. Result was comparable with earlier as synthesizing copper nanoparticles utilizing *Punica granatum* seed extract. Color development is owing to electronic excitation of Cu NPs exterior Plasmon resonance. Formation of copper NPs was due to reduction process of Cu^{+2} ions and afterwards nucleation development occurred. Cu NPs displayed absorption maxima at 563 nm (Fig. 1). The process of surface Plasmon of Cu NPs is foundation of establishment of this absorption maxima. The strong plasma resonance may exist due to the construction of pure metallic Cu NPs. Peak of maximum absorbance for Cu NPs have early been described to be found in the array of 550–700 nm (Kazuma et al., 2011). It is therefore confirmed from the UV-Visible study that the *Parthenium hysterophorus* leaf extract can convert copper ions into copper nanoparticles.

FTIR spectroscopy

FTIR analysis is utilized for identifications of bio-functional groups causing reduction and stability of copper nanoparticles. Meva *et al*. (2019) have done plant mediated manufacturing of copper nanoparticles and carried out FTIR for analysis of biochemistry of functional groups contributing Cu NPs preparation. Fig. 2 shows FTIR spectrum of copper nanoparticles. In vibrational spectrum, broad band at 3183 cm^{-1} appears to be due to stretching frequency of hydroxyl group (−OH) and points toward existence of phenol or alcoholic functional groups. The emergence of sharp peak at 2027 cm^{-1} is due to the reason that carbonyl group (>C = O) of aldehydes, carboxylic acids and ketones vibration occurs. The peak at 1578 cm^{-1} is shown by amide group doing its –NH bending vibration while the band at 1248 cm^{-1} is allocated to carbonyl (>C=O) stretch of amide group. The sharp peak originated at 1375 cm^{-1} is due to distinctive vibrational bend of C−H bond. Presence of sulfoxide functional group is evident due to stretching of S=O, seen as sturdy band at 1023 cm^{-1}. So it can be summarized that various functional groups like alcohols, phenols, aldehydes, ketones, carboxylic acids, amides and sulfoxides are contributing in green-synthesis of Cu NPs.

Scanning electron microscopy

The SEM technique can be utilized to find out the size and shape of Cu NPs. It presents detailed surface patterns by scanning the nanoparticles surfaces utilizing electron beam. Interaction of electrons with surface atoms of the sample presents insight view of surface structure. Fig. 3 display images from SEM in different magnifications of synthesized copper NPs using extract obtained from *Parthenium hysterophorus*. Results of SEM images demonstrate the shape of synthesized copper NPs is semi- spherical. It is also evident from the SEM analysis that the prepared nanoparticles are somewhat agglomerated as shown by Fig. 3d at range of 200 nm with resolution of 200000X. It is due to the nucleation caused by the plant materials acting as capping and reducing agents as studied earlier (Kimber et al., 2018). SEM results also validate the presence of different peaks in FTIR data corresponding to the existence of biomolecules on the nanoparticles surface.

EDX analysis

Analysis of elements and hence Cu NPs composition prepared from aqueous extract of *Parthenium hysterophorus* was investigated with the help of EDX. It is reported earlier that when Cu NPs were prepared using plant sources and their characterization was done using EDX, peaks were observed which indicates the presence of different elements on the sample matrix (Dayakar et al., 2017). It is evident from the Fig. 4 that elements like carbon and oxygen are also present along with copper in significant percentage. It is possibly attached to the plant materials adhering on the prepared nanoparticles surface.

XRD analysis

The size and crystal structure of synthesized copper nanoparticles are investigated by XRD. It has been demonstrated in Fig. 5. The spectrum elaborates three peaks at 2θ values of 43.20°, 50.42°, and 74.13°, index with (111), (200) and (220) planes of copper correspondingly. The outcomes are found in accordance with the standard JCPDS card number 85-1326 (Biçer and Şişman, 2010). It has been declared that synthesized Cu NPs are of crystalline with face centered cubic symmetry. Most notably, it is seen that oxidation of the Cu NPs does not happen significantly but only a little peak was observed at 32.1° due to formation of CuO under the influence of atmospheric oxidation. Also, it is revealed form the spectrum that reduction of Cu^{+2} ions was complete. We have calculated the crystallite size utilizing Debye–Scherer equation (Eq. I). For that purpose, the XRD plane (111) has been employed. The size of Cu NPs was found 15.48 nm using this technique.

Antibacterial potential

Antibacterial potential of copper nanoparticles was patterned utilizing designated microorganisms. The NPs expressed fair amount of antibacterial potential as inhibition zone against known bacteria using disk diffusion method as demonstrated by Fig. 6. Mohanta et al. (Mohanta et al., 2017) prepared Cu NPs from *Erythrina subserosa* aqueous leaf extract and demonstrated the antibacterial activities of prepared NPs. In this study, green synthesized copper nanoparticles showed analogous inhibitory activity at dose of 40 mg/mL with standard Rifampicin. We have also determined the Minimum inhibitory concentration of synthesized copper nanoparticles on *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pasteurella multocida*. The test was accomplished using different concentrations of microorganisms in the range of 10-40 mg/mL.

Antioxidant activity

The bioactivity of the NPs is due to bioactive components in the extract used for the synthesis of NPs. Total phenolic contents in synthesized copper NPs were measured using Folin-Ciocalteu reagent method. Kumar et al. (2019) checked TPC of prepared Cu nanoparticles synthesized from leaf extract of *H. integrifolia*. We have observed in our study that the concentration of TPC increases by increasing Cu NPs. The maximum phenolic contents were recorded at the nanoparticle's concentration of 40 mg/mL (Fig. 6). On similar grounds, the TFC were also checked and maximum value was observed at 40 mg/mL. So, there was a direct relation between the TFC, TPC and concentration of Cu nanoparticles. Free radical scavenging ability of NPs can be checked using Diphenyl picrylhydrazyl, a free-radical complex. Chung et al. (Chung et al., 2017) studied antioxidant activity of bio-synthesized copper NPs using DPPH assay. The DPPH scavenging activity was increased by increasing concentration of Cu NPs. Wu et al. (Wu et al., 2020) reported manufacturing of copper nanoparticles efficiently using *Cissus vitiginea* (Fig. 6).

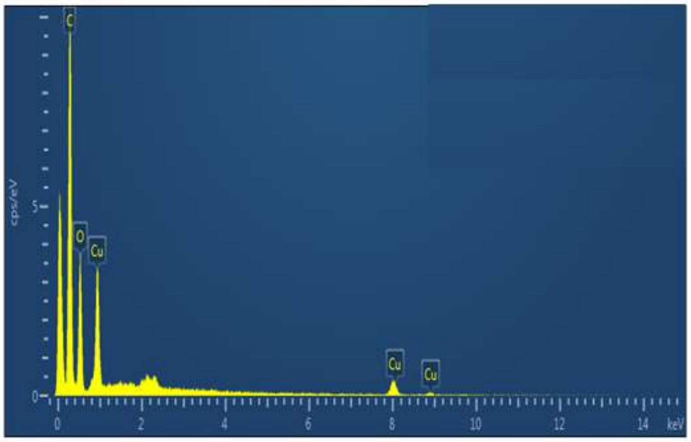

Fig. 4: EDX spectrum of the Cu NPs

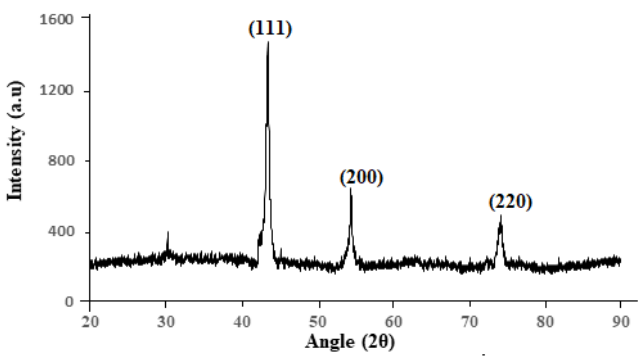

Fig. 5: XRD pattern of Cu NPs prepared using *Parthenium hysterophorus* leaves extract

The prepared copper NPs has been employed to determine the antibacterial effectiveness of pathogens against UTIs. The resulting fabricated nanoparticles were categorized using UV spectroscopy at about 370 nm. The distribution of nanoparticles was shown by scanning electron microscopy results and particle sizes were found to be in the 5-20 nm range. Distinctive diffraction peaks for Cu NPs were detected in the X-ray diffraction spectrum at 2θ ranges of 35.5° and 43.2° matching to lattice planes (111) and (202), correspondingly. The XPS studies suggest that the chemical states of copper resulted in two different peaks of binding energy. The findings serve the indication that the Cu NPs may be the significant source to tackle UTIs induced by microorganisms. The whole inference seems to be that green synthesized Cu NPs found to effectively destroy it or substantially inhibit activity against UTIs pathogens.

Similarly, Zangeneh et al. (Zangeneh et al., 2019) reported simple green method to prepare Cu NPs from leaf extract of *Falcaria vulgaris*, a medicinal plant. Prepared Cu NPs were evaluated for their characterization by means of UV-Visible, FTIR, XRD, TEM and FESEM techniques. The outcomes of these advanced techniques authenticate the utilization of *F. vulgaris* leaf aqueous extract that produces Cu NPs with prominent antifungal, antioxidant, antibacterial and cutaneous wound curative ability. More experimental judgements are essential for affirmation of these restorative properties of Cu NPs in human. Gu et al. (Gu et al., 2018) described ultrasonic mediated preparation of CuO NPs utilizing *Cystoseira trinodis* extract. Study of characteristics of CuO NPs was carried out by means of EDX, TEM, FE-SEM, XRD, AFM, photoluminescence, FTIR and Raman spectroscopic analysis. Average particle size of 7.8 nm and sphere-shaped copper oxide nanoparticles was revealed by SEM studies. XRD analysis agreed upon the creation of uncontaminated monoclinic crystalline arrangement of copper oxide nanoparticles. These interpretations were recognized by transmission electron microscopic studies. Photo-catalytic experiments disclosed character of synthesized Copper oxide nanoparticles as a proficient catalyst used for methylene blue dye degradation under UV and Solar irradiation. Effect of various experimental factors for instance catalytic amount, pH, the concentration of dye was also studied. The outcomes of growth inhibition and minimal inhibitory concentrations test of copper oxide nanoparticles in contrast to cephalexin standard confirmed antibacterial action in opposition to several bacteria strains. CuO NPs also hinder DPPH free radical in its scavenging action.

Also, Rajesh *et al*. (2017) reported preparation of Cu NPs using *Syzygium aromaticum* bud extract. Structure, morphology and antimicrobial studies were carried out. XRD pattern proved FCC nature of Cu NPs. Energy dispersive spectroscopy revealed high intensity metallic peak of copper as well as lower peaks of carbon, oxygen chlorine and phosphorus elements, and showing bio-capping on Cu NPs by means of bud extract bio-molecules. Additionally, use of UV-Visible spectroscopy and FTIR analysis showed, characteristic absorption peak of Cu NPs and presence of various functional groups at different positions on Cu NPs respectively. Antimicrobial activity of Cu NPs showed positive results with zone of inhibitions of 8 mm and 6 mm against *bacillus spp.* and *Penicillium ssp.* respectively. Dayakar et al. (Dayakar et al., 2017) described development of carbon glassy electrode coated with copper nanoparticles for the detection of glucose. *Ocimum tenuiflorum* was used as bio-source to prepare pure Cu NPs and the nanoparticles were characterized using spectroscopic techniques, like XRD, EDX, SEM, FTIR, TEM and particle size (PSA) distribution. The produced Cu NPs demonstrated the electrochemical comeback of glucose and was measured by cyclic voltammetry and ampere meter. The outcomes showed that the customized bio-sensor electrode showed an extraordinary sensitivity, broad linear range, rapid response time < 3s and low detection limit. Consequently, the prepared Cu NPs via green route have ability to construct a reliable non-enzymatic biosensor. The results revealed that the *Parthenium hysterophorus* leaves extract contains bioactive agents that are responsible for the formation of Cu NPs, which could have promising application in biomedical field.

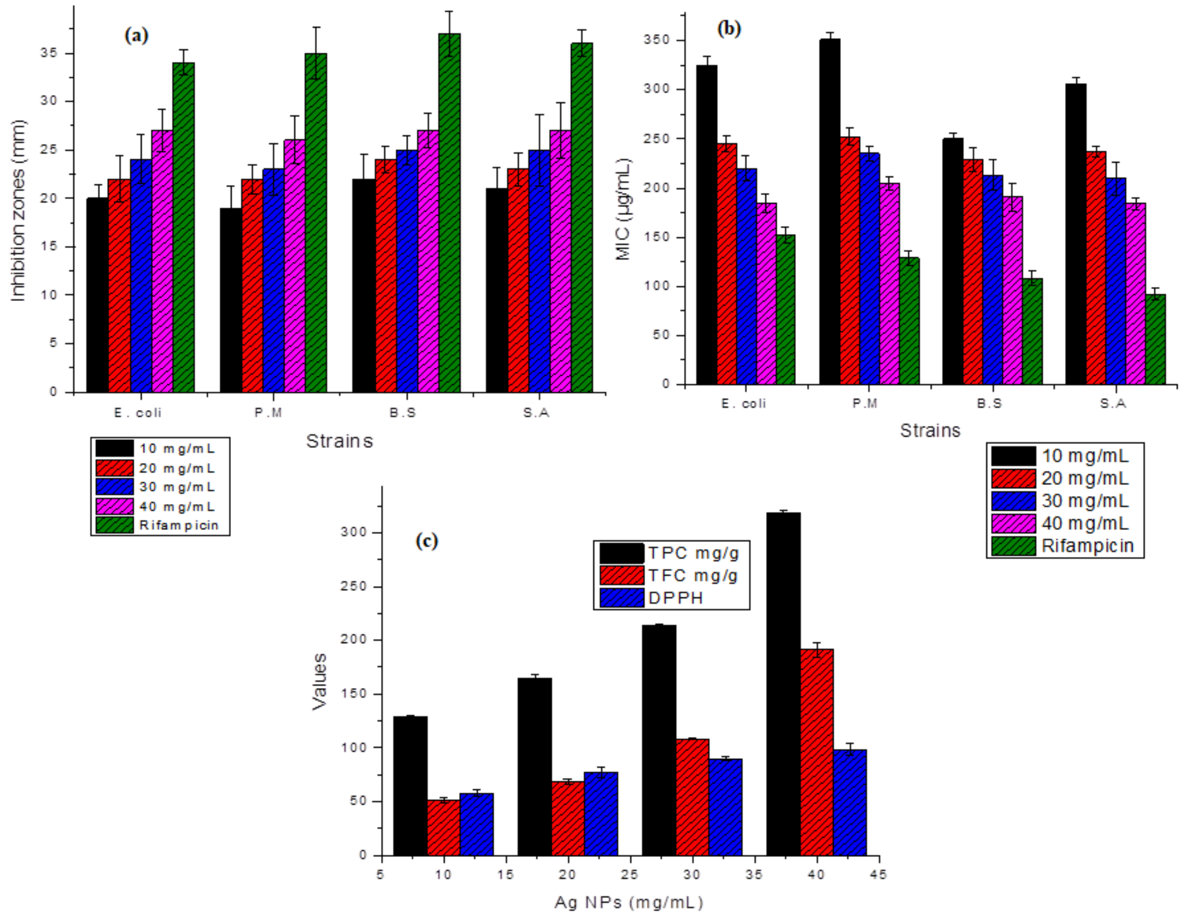

Fig. 6: Bioactivities of Cu NPs, (a) Zone of inhibition (mm), (b) MIC (μg/mL) and (c) antioxidant activity

CONCLUSIONS

Copper nanoparticles are synthesized from *Parthenium hysterophorus* extract. Plant extract mediated copper nanoparticles were typified by different spectroscopic techniques. UV-Visible spectrum showed λ_{max} at 563 nm indicating the establishment of Cu NPs. FTIR spectral data indicates the presence of residual bio molecules attached on Cu NPs surface. SEM photographs depict that copper nanoparticles are somewhat agglomerated having semi-spherical shape. XRD spectrum shows face centered cubic crystals of Cu NPs with 15.48 nm average particle size. Disc diffusion and MIC examination show that fabricated Cu NPs display noteworthy antibacterial action at 40 mg/L concentration against gram +ive and gram –ive bacterial strains as compared with standard Rifampicin. DPPH assay demonstrates antioxidant activity of Cu NPs. Further investigation in this regard may be proceeded to evaluate antibacterial activity against pathogenic bacteria in clinical trials.

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