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Biosynthesis, Characterization and Biological Studies of Copper Nanoparticles from Methanolic Extract of Bulb of *Zephyranthes citrina*

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ABSTRACT: This work reports the biosynthesis, characterization and biological studies of copper oxide nanoparticles using a methanolic extract of bulb of *Zephyranthes citrina*. The synthesised copper oxide nanoparticles were characterized by using various spectral techniques. The functional, structural and morphological studies were confirmed by FT-IR, XRD and SEM respectively. The elemental composition was determined by EDAX analysis. The various spectral data confirms the formation of copper oxide nanoparticles and is found to be orthorhombic, 18-50 nm of size and spherical in shape. The synthesised copper oxide nanoparticles were investigated for antibacterial activity against gram positive (*Staphylococcus aureus, Bacillus subtilis and Staphylococcus epidermidis*), gram negative (*Salmonella typhi, Escherichia coli and Klebseilla pneumoniae*) and antifungal (*Aspergillus niger, Candida albicans and Fusarium oxysporum*) activities with different pathogens. The results revealed that copper oxide nanoparticles exhibit excellent inhibition efficiency against representative microorganisms. The outcome of the study could be useful for the development of value added products from indigenous medicinal plants.

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INTRODUCTION

In recent years, Nanotechnology has been emerging as a rapidly growing field with numerous applications in science and technology for the purpose of manufacturing new materials. Nanotechnology has become one of the most promising technologies applied in all areas of science [1]. Nanotechnology is a field that is developing day by day, making an impact in all spheres of human life and creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology. The term 'nanoparticles (NPs)' are used to describe particle with size in the range of 1-100 nm [2]. NPs can be classified into different types according to their size, morphology, physical and chemical properties. Nanoparticles research is inevitable today not only because of its applications but also the way of synthesis [3]. The synthesis of NPs usually involves a chemical reduction reaction where toxic chemicals are used. For that reason, more sustainable methods, known as "Green Synthesis or Biogenic Synthesis" have been proposed. It is cost-effective, environmentally friendly, single step method for biosynthesis process and safe for human therapeutic use [4]. The plant or plant extract act as reducing agent as well as capping agent for the synthesis of NPs are more advantageous over other biological processes [5].

The types of NPs include carbon-based NPs, ceramic NPs, metal NPs, semiconductor NPs, polymeric NPs and liquid-based NPs [6]. Among other NPs, metal NPs have raised attention over the last few decades because they have larger surface area per weight or volume and many characteristics; biological, thermal, chemical, dielectric, electrical, physical, mechanical, electronic, magnetic and optical properties make them attractive tools for research work [7]. NPs of gracious metals like Pt, Au, Ag, Cu and Zn have received global attention due to extensive application in biomedical and Physiochemical fields [8].

Copper oxide nanoparticles (CuO NPs) are widely used and have been applied in many consumer products owing to their excellent properties, especially antimicrobial activity and biochemical detection. Natural products play an important role in unearthing and in the progress of copious drugs for the treatment of different types of diseases including cancer [9 and 10]. CuO NPs are less-toxic, high chemical and physical stability and long shelf-life, as compared to the organic antimicrobial agents [11]. CuO NPs received much attention on bioactive applications due to its versatile functions such as catalysis, photoconductivity, photothermal, antimicrobial and biocidal properties [12]. NPs with antimicrobial activity are very advantageous in reducing acute toxicity, lowering cost and overcoming resistance as compared with other prevalent antibiotics [13]. Several metallic materials, including gold, silver, iron and copper NPs are reported to be antimicrobial agents against various bacterial and fungal pathogens [11 and 14].

The main objective of the present study is to synthesis CuO NPs by a green biological route using methanolic extract derived from the bulb of *Zephyranthes citrina.* The synthesized CuO NPs was characterized by different techniques, such as, UV-Visible spectroscopy, fourier infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy dispersive X-ray spectroscopy (EDX or EDAX). Furthermore, the antimicrobial activity against bacterial and fungal species was also exhibited by biogenically synthesised CuO NPs.

MATERIALS AND METHODS

Materials

Copper sulphate penta hydrate is of analytical grade and was procured from Merck, India. Representative microorganisms of Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis and Staphylococcus epidermidis*) and Gram-negative bacteria (*Salmonella typhi, Escherichia coli and Klebseilla pneumonia*) as well as fungal species (*Aspergillus niger, Candida albicans and Fusarium oxysporum*) were used to evaluate the antimicrobial activity of prepared CuO NPs. All the microorganisms were purchased from Acme ProGen Biotech (India) Private Limited, Salem, Tamil Nadu, India for antimicrobial investigation. The bacterial strains were maintained on Nutrient Broth (NB) at 37˚C and fungi were maintained on Potato Dextrose Agar (PDA) at 28˚C.

Collection, authentication and extraction of Plant material

The plant was collected from the host area of Institution, Paramathi Velur, Namakkal District, Tamil Nadu, India. The plant was identified and authenticated in the Botanical Survey of India (Voucher No.: BSI/SRC/5/23/2018/Tech/1113), Southern Regional Centre, Department of Botany, Agricultural University, Coimbatore. The bulbous plant material was cut into pieces, dried under shade for 15 days, coarsely powdered and stored in air tight container. The powdered sample of bulb of ***Zephyranthes citrina*** was successfully extracted in methanol using a Soxhlet apparatus.

Biosynthesis of copper oxide nanoparticles (CuO NPs)

The procedure reported by Mangesh S. Jadhav *et al.*, [15] was followed to synthesis CuO NPs. 100 mL, 1 mM solution of copper sulphate was freshly prepared and stored in an amber color bottle. Then the methanol extract of the bulb was added to 1 mM copper sulphate solution and was kept aside for 24 hrs without shaking. The colour change of the solution from bluish green to blackish brown indicated the formation of CuO NPs and was also confirmed by using UV-Visible spectroscopy.

Characterization of synthesized CuO NPs

The reduction of metal ions was monitored as a function of wavelength using UV-Visible spectrophotometer (UV-2450, Shimadzu). FT-IR spectroscopy was used to identify the functional group present in the nanoparticles (Thermo Nicolet-nexus 670 spectrometer of resolution 4 cm^{-1}). The nature of naoparticles can be studied by X-ray diffraction analysis using Shimadzu XRD-6000/6100 model with 30 kV, 30 mA with CuKα radiations at 2θ angle. Morphological analysis of the samples was carried out by using Hitachi S-4500 SEM machine. Elemental analysis of the nanoparticles was performed by a Hitachi S-3400N SEM instrument equipped with thermo EDX attachments.

Antimicrobial Activity of synthesized CuO NPs

Preparation of Inoculum

The growth method was performed as follows,

At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop and the growth was transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth. The broth culture was incubated at 35ºC until it exceeded the turbidity of 0.5 McFarland standard [16] (usually 2 to 6 hours).

Well Diffusion Method – Antimicrobial activity

The antibacterial and antifungal activity of a methanolic bulb extract of *Zephyranthes citrina* was determined by disc diffusion method [17]. Muller- Hinton agar plates were prepared optimally within 15 minutes after adjusting the turbidity of the inoculums suspension. A sterile cotton swab was dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two or more times, rotating the plate approximately 60º each time to ensure an even distribution of inoculums. The rim of the agar was swabbed and sterile 6mm filter paper was placed on the plates and immediately the test sample was added which was dissolved in water or DMSO. The plates were left for 30 min at room temperature to allow diffusion and were incubated at 35˚C for 24 hrs [18 and 19].

RESULTS AND DISCUSSION

UV-Visible Spectroscopy

Green synthesis of copper oxide nanoparticles was characterized by various techniques as reported earlier [20]. The absorption of crude methanolic extract is observed at 298 nm in figure 1. Generally, the metal nanoparticles have free electrons, which give the absorption band due to combined vibration of electrons of metal NPs with light waves. The CuO NPs was synthesized using copper sulphate to initiate the reaction using a crude methanolic extract of bulb of *Zephyranthes citrina*. The colour of the reaction mixture started changing blackish brown after 2 hrs, indicating the generation of CuO NPs which is due to reduction of copper salts during exposure to plant extract, similar to results observed in different extracts from plant sources [21]. The colour change is due to excitation of Surface Plasmon Resonance (SPR). A characteristic and well defined SPR band for CuO NPs is observed at 279 nm. These absorption peaks confirm the formation of corresponding metal nanoparticles of methanolic extract of bulb of *Zephyranthes citrina* and the graph obtained is represented in **Figure 1**.

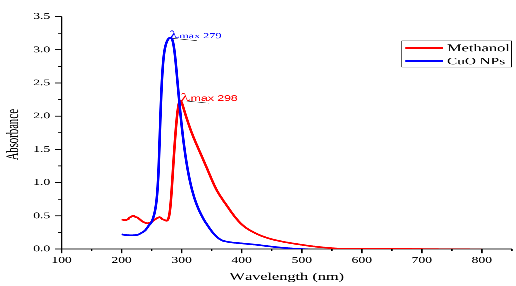

Figure 1 UV- Visible spectrum of crude mathanolic extract and CuO NPs of bulb of *Zephyranthes citrina*

FT-IR Spectral Analysis

FT-IR measurements were carried out to identify the major functional group of the methanolic extract of bulb of *Zephyranthes citrina* and their possible involvement in the synthesis and stabilization of copper oxide nanoparticles. FT-IR spectra of crude methanolic extract of bulb of *Zephyranthes citrina* and the synthesised CuO NPs from plant extract are shown in **Figure 2**. The spectral data for crude methanolic extract showed several peaks indicating the complex nature of the biological material and also proved that the synthesized nanoparticles are surrounded by plant metabolites [22 and 23].

The characteristics of CuO NPs at 3340.91 cm^{-1}, a broad peak shows the presence of N-H stretching from heterocyclic amine group. The peak at 1662.25 cm^{-1} is due to the presence of C=C stretching of alkenyl group in copper oxide nanoparticles. The sharp peak at 1096.19 cm^{-1} shows C-N stretching of primary amine in CuO Nps. The absorption peaks disclose to 775.18 cm^{-1} indicates the vibration modes of nanoparticles that are reduced and reacts with relevant phytochemicals from the extracts. The peak at 623 cm^{-1} indicates the presence of aliphatic C-Halogen compound.

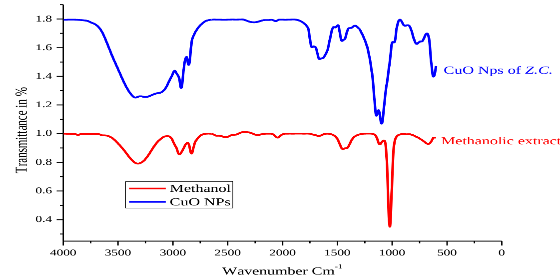

Figure 2 FT-IR spectra of crude mathanolic extract and CuO NPs of bulb of *Zephyranthes citrina*

XRD Analysis

X-ray diffraction (XRD) is one of the most significant and the easiest tool to determine the crystallite characteristics for any compound. The XRD spectrum of CuO NPs is illustrated in **Figure 3**. The d-spacing (interplanar spacing between the atoms) is calculated using Bragg's equation, $n\lambda = 2d \sin\theta$. The average crystallite size (D) is calculated by Debye-Scherrer formula and is found to be 20 nm for the most intensive planes of copper oxide NPs.

$$D = K\lambda / \beta \cos\theta$$

where, K is a numerical constant factor (0.89), λ is the wavelength of X-ray used (1.50406 X 10^{-10} m), β is the full width at half maximum and θ is the angle of diffraction.

Based on the XRD spectrum obtained for CuO NPs, the particles are crystalline in nature with a face centered cubic structure. For CuO NPs the major identical peak at 2θ values 21.89, 24.75, 30.51 and 38.38 with respect to (004), (220), (115) and (234) planes of fcc confirms the formation of CuO NPs (JCPDS file No. 771898). The lattice cell parameters are a=9.740, b=10.58, c=16.200 for end centered with orthorhombic system.

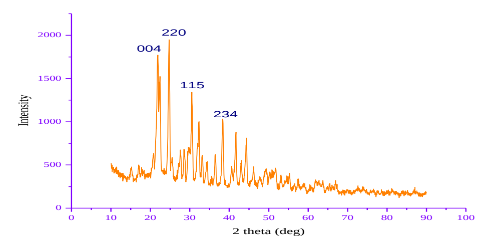

Figure 3 XRD spectra of CuO NPs

SEM-EDAX Analysis

The SEM images provided further insight into the morphology and size details of the nanoparticles. SEM images of CuO NPs are as shown in **Figure 4**, which is obtained from *Zephyranthes citrina* bulb extract. The formation of copper oxide nanoparticles as well as their morphological structure was observed by SEM images. The average particle size of CuO NPs is 18-50 nm calculated by pixel ruler software. The shapes CuO NPs are identified as spherical.

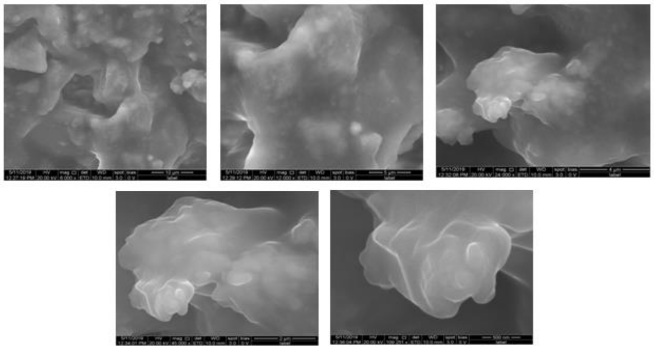

Figure 4 SEM images of CuO NPs from methanolic extract of bulb of *Zephyranthes citrina*

EDAX spectrum of nanoparticles helps to identify the elemental composition of the sample. EDAX spectra for synthesized CuO NPs from the methanolic extract of bulb of *Zephyranthes citrina* are shown in **Figure 5**. The report provides information about presence of S, C, O, Cu, In, Hf, K and N from synthesized copper oxide nanoparticles with different percentages. The EDAX spectrum of CuO NPs showed that the presence of copper oxide nanoparticles. From the spectral information, it is concluded that the CuO NPs is reduced by *Zephyranthes citrina* and the presence of carbon content is obtained from phytochemicals which acts as a stabilizing agent.

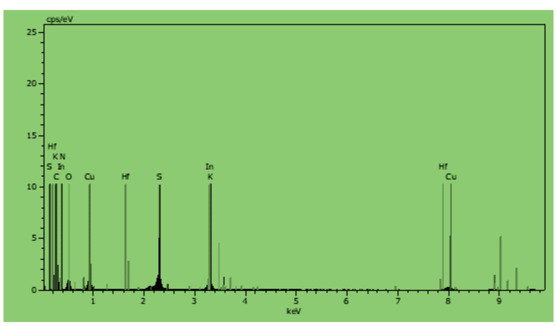

Figure 5 EDAX spectra of CuO NPs

Antibacterial activity of synthesized CuO NPs

The antibacterial activities of CuO NPs was investigated with different pathogens of Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis* and Gram-negative *Salmonella typhi*, *Escherichia coli*, *Klebseilla pneumonia* bacteria. **Figure 6** shows the zone of inhibition on bacteria against CuO NPs at different concentrations. The present study provides the zone of inhibition measured in millimeter with different concentrations (25µl, 50µl, 75µl, 100µl) of synthesized nanoparticles. The nanoparticle inhibition activities measured by the radial diameter of zone are shown in **Table 1**, methanol is used as negative control and Tetracyclin (10mg/ml) as a standard.

The results revealed that increasing the concentrations of CuO NPs, the zone of inhibition also increased against the test bacteria. The NPs attached to the negatively charged cell surface alter the physical and chemical properties of the cell membranes and cell wall and disturb the important functions [24]. Some carboxyl and amine groups present in the nanoparticles can react on the cell surface of gram positive and gram negative bacteria [25]. The control (Methanol) shows nil activity against the tested organisms.

The synthesised CuO NPs show good antibacterial activity with efficient inhibition against the *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Escherichia coli*, *Klebseilla pneumonia*. The liberated Cu^{+} ions from nanoparticles are connected with bacterial enzymes which lead to rupture of the bacteria. This is the main reason for the CuO NPs to possess high potential to kill the bacteria. Based on the result obtained, it is concluded that CuO NPs exhibit good inhibition activity against gram negative and gram positive bacteria on the culture plates and the graph is shown in **Figure 7**.

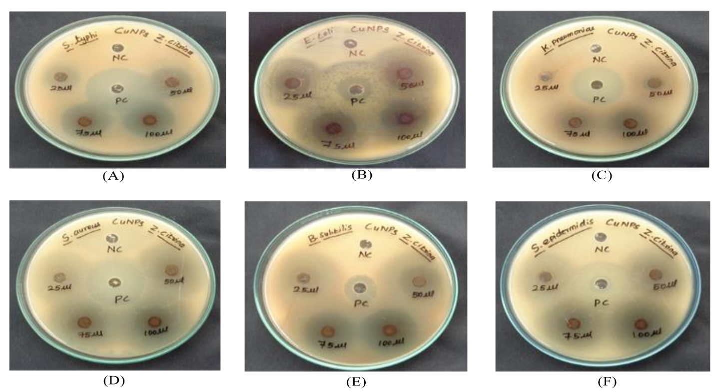

Figure 6 The inhibition zone of CuO NPs with different concentrations against bacteria

Table. 1: Antibacterial activity of CuO NPs from *Zephyranthes citrina*

S.No.	Test organisms	Zone of inhibition (mm) in different concentrations				
		25 µl	50 µl	75 µl	100 µl	Standard (10 mg/ml)
1.	*S. typhi*	8	10	12	13	15
2.	*E. coli*	8	9	9	10	11
3.	*K. pneumoniae*	4	6	7	8	14
4.	*S. aureus*	7	10	11	12	15
5.	*B. subtilis*	7	8	9	10	10
6.	*S.epidermidis*	5	7	8	9	15

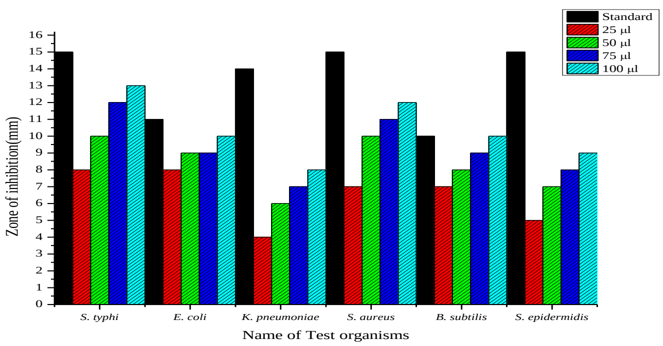

Figure 7 Zone of inhibition efficiency of CuO NPs

Antifungal activity of synthesized CuO NPs

The fungal activities of CuO NPs were investigated with *Aspergillus niger, Candida albicans* and *Fusarium oxysporum.* In the present study, the antifungal activity of the CuO NPs synthesized from the methanolic extract of bulb of *Zephyranthes citrina* and the zone of inhibition efficiency of CuO NPs is shown in **Figure 8** and the activities are reported in **Table 2**. Antifungal activities of CuO NPs are illustrated in the graphical **Figure 9**.

The CuO NPs are highly reactive against the fungus. The antifungal activities of the synthesized metal nanoparticles were measured at different concentrations (25µl, 50µl, 75µl, 100µl). Methanol was used as a negative control and Kanamycin used as a standard. The control (Methanol) shows nil activity against the fungal pathogens tested. *Aspergillus niger* showed nil efficiency at 25µl concentration, whereas *Fusarium oxysporum* and *Aspergillus niger* indicates 7 mm zone of inhibition at 100µl. From the results obtained, it is shown that the *Candida albicans* show highest inhibition efficiency of 11 mm.

Figure 8 The inhibition zone of CuO NPs with different concentrations against fungi

Table 2: Antifungal activity of CuO NPs from *Zephyranthes citrina*

S.No.	Test organisms	Zone of inhibition in (mm) different concentration				
		25 µl	50 µl	75 µl	100 µl	Standard Kanamycin
1.	*Aspergillus niger*	1	4	7	7	8
2.	*Candida albicans*	6	9	10	11	16
3.	*Fusarium oxysporum*	4	5	6	7	8

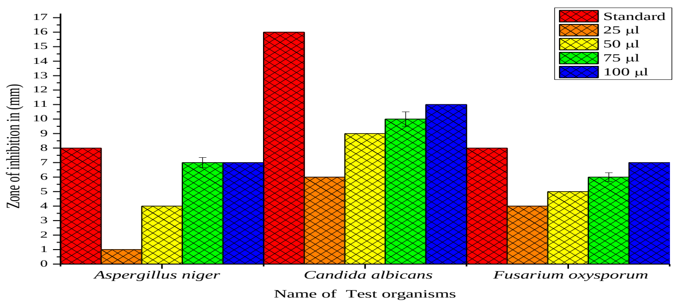

Figure 9: Zone of inhibition efficiency of CuO NPs

CONCLUSION

The methanolic extract of bulb of *Zephyranthes citrina* was successfully utilized for the green synthesis of CuO NPs. The biosynthesised CuO NPs from plant material were characterized by various spectral techniques and confirmed that CuO NPs is orthorhombic in nature. The particles are identified with spherical shape and high stability. The biosynthesised CuO NPs are also investigated by antibacterial activity for Gram-positive and Gram-negative bacteria and antifungal activity. The results revealed that the CuO NPs show potential antimicrobial activity against the selected microorganisms. This biosynthesis approach appears to be a cost effective, non-toxic, eco-friendly and alternative to the conventional microbiological, physical and chemical methods. Hence, the synthesised CuO NPs may be used as prospective for biological mediator on microorganism.

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Not declared.

CONFLICT OF INTEREST

There is no conflict of interest regarding the research article.

DATA AVAILABILITY

Not declared.

FUNDING SOURCE

No external funding source has been declared.

REFERENCES

1. Sasikala, A., Linga Rao, M., Savithramma, N. and Prasad, TNVKV. Synthesis of silver nanoparticles from stem bark of *Cochlospermum religiosum* (L.) Alston: an important medicinal plant and evaluation of their antimicrobial efficacy. Appl. Nanosci., 2014; 5: 827-835.
2. Parida, U., Bindhani, B. and Nayak, P. Green synthesis and Characterization of Gold nanoparticles using Onion *(Allium cepa)* extract. World Journal of Nano Science and Engineering, 2011; 1(4): 93-98.
3. Gopinath, V., Mubarak Ali, D., Priyadarshini, S., Priyadharsshini, NM., Thajuddin, N. and Velusamy, P. Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity, a novel biological approach. Colloids and Surfaces. B: Biointerfaces, 2012; 96: 69-74.
4. Haytham, MM. Ibrahim. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. Journal of Radiation Research and Applied Sciences, 2015; 8(3): 265-275.
5. Valli, JS. and Vaseeharan, B. Biosynthesis of silver nanoparticles by *Cissus quadrangularis* extracts. Materials Letters, 2012; 82:171-173.
6. Joao Conde., Goncalo Doria. and Pedro Baptista. Noble metal nanoparticles Application in Cancer. Journal of Drug Delivery, 2012; 751075: 1-12.
7. Khan, I., Saeed, K. and Khan, I. Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry, 2017; 12(7): 908-931.
8. Ahmadi, SJ., Outokesh, M., Hossseinpour, M. and Mousavand, T. A simple granulation technique for preparing high-porosity nano copper oxide (II) catalyst beads. Particuology, 2011; 9(5), 480-485.
9. Olaku, O. and White, JD. Herbal therapy use by cancer patients: a literature review on case reports. Eur.J.Cancer, 2011; 47: 508-514.
10. Dias, DA., Urban, S. and Roessner, U. A historical overview of natural products in drug discovery, Metabolites, 2012; 2: 303-36.
11. Das, D., Nath, BC., Phokon, P. and Dolui, SK. Synthesis and evaluation of antioxidant and antibacterial behavior of CuO nanoparticles. Colloids and Surfaces. B: Biointerfaces, 2013; 101: 430-433.
12. Perreault, F., Melegari, SP., Da Costa, CH., De Oliveira Franco Bossetto, AL, Popovic, R. and Matias, WC. Genotoxic effects of copper oxide nanoparticles in Neuro 2A cell cultures. Sci. Total Environ., 2012; 441: 117-124.
13. Weir, E., Lawlor, A., Whelan, A. and Regan, F. The use of nanoparticles in antimicrobial materials and their characterization. Analyst, 2008; 133(7): 835-845.
14. Dhayalan, M., Dension, MIJ., Ayyar, M., Gandhi, NN., Krishnan, K. and Abdulhadi, B. Biogenic synthesis, Characterization of gold and silver nanoparticles from *Coleus forskohlii* and their clinical importance. J. Photochem. Photobiol. B. Biol., 2018; 183: 251-257.
15. Mangesh S Jadhav., Sameer Kulkarni., Prasad Raikar., Delicia A Barretto., Shyam Kumar Vootla. and Raikar, US. Green

Biosynthesis of CuO and Ag-CuO nanoparticles from *Malus domestica* leaf extract and evaluation of antibacterial, antioxidant, DNA cleavage activities. New Journal of Chemistry, 2018; 42: 204-213.

16. McFarland, J. Standardization of bacterial culture for disc diffusion assay. J. Am. Med. Assoc., 1987; 49: 1176-1178.
17. Taylor, RSL., Manandhar, NP., Hudson, JB. and Towers, GHN. Screening of selected medicinal plants of Nepal for antimicrobial activities. J. Ethnopharmacol., 1995; 546: 153-159.
18. Alka Bameta., Anita Kumari. and Anushulika Upadhyaya. Phytochemical analysis and antimicrobial activity of *Nerium oleander* L. Inter. J. of Bio. Res., 2017; 2(3): 29-32.
19. Hira Sakha., Rejila Hora., Shilpa Shrestha., Shreeya Acharya., Dinesh Dhakal., Srijana Thapaliya. and Kamil Prajapati. Antimicrobial activity of Ethanolic extract of medicinal plants against Human Pathogenic bacteria. TUJM, 2018; 5(1), 1-6.
20. Tabassum, S., Asim, A., Khan, RA., Arjmand, F., Rajakumar, D., Balaji, P. and Akbarsha M.A. A multifunctional molecular entity Cu^{II}-Sn^{IV} heterobimetallic complex as a potential cancer chemotherapeutic agent: DNA binding/cleavage, SOD mimetic, topoisomerase 1α inhibitory and *in vitro* cytotoxic activities. R. Soc. Chem. Adv., 2015; 5(59): 47439–47450.
21. El-Trass, A., Elshamy, H., El-Mehasseb, I. and El-Kemary, M. CuO nanoparticles: Synthesis, characterization, optical properties and interaction with aminoacids. Appl. Surf. Sci., 2012; 258(7): 2997–3001.
22. Coates, J. Interpretation of infrared spectra a practical approach. Encycl. Anal. Chem., 2000; 10815–10837.
23. Linga Rao, M., Bhumi, G. and Savithramma, N. Green synthesis of silver nanoparticles by using *Allamanda cathartica* L. leaf extract and evaluation of their antimicrobial activity. Int J Pharm Sci Nanotechnol., 2013; 4: 2260–2268.
24. Marambio-Jones, C. and Hoek, EMV. A review of the antibacterial effects of silver nanoparticles and potential implications for human health and environment. Journal of Nanoparticle research, 2010; 12: 1531-1551.
25. Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M.I. and Sastry, M. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, Colloids and Surfaces B: Bio interfaces, 2003; 28: 313–318.