IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Biogenic Green Synthesis Of Copper Oxide Nanoparticles With The Using Grown Through Biofertilizers And Mangampet Barytes Mine Waste Of *Vitex Negundo* Characterization And Evaluation Of Its Antibacterial Efficasy

Ganta Nalini and Tartte Vijaya

Department of Botany, Sri Venkateswara University, Tirupati 517502, Andhra Pradesh, India.

ABSTRACT

Metal nanoparticles (MNPs) and Metal Oxide nanoparticles (MONPs) used in diverse fields due to their capping and reducing properties. Plants are contain rich phytoconstituent bioactive compounds served as a good source of medicine. Hence, more recently nanoparticles are attracted to green nanotechnology to find the efficiency of biological activity by eco-friendly approach. To bring about the phenomenon present work was aimed to synthesis copper oxide nanoparticles by using nourished by biofertilizers and mangampet barytes mine waste of *Vitex negundo* aqueous leaves extract was characterized above mentioned NPs charcterized by using recent advanced instruments and to explicate preeminence antibacterial and antioxidants activities. Synthesized particles showed color change pattern revealed broad peak at 260 nm which was analyzed by UV-vis spectroscopy range from 190 to – 750 nm. DLS and Zeta Potential analysis affirmed that the size and appreciable stability of the NPs. FT-IR results revealed that the proteins, aamides, flavonoids and tannins are mainly involved in capping and stabilyzation of nanoparticles. XRD studies showed crystallographic nature of the nanoparticles with 26.82nm average size. Shape, size and morphology of the NPs were analyzed using by TEM and most of the NPs sphericle in shape, poly dispersed and without any agglomeration. Antibacterial studies of CuONPs exhibited significant activity against sellected two gram positive and two gram negative bacteria and also anti-oxidant activity.

Key Words: *Vitex negundo*, CuONP, antibacterial and antioxidants.

INTRODUCTION

Metalic nanoparticles have aquired very much attention in the current decenniums due to their distinct capability to their electrical, optical, chemical and biological (natural precursors) properties due to their small size, large surface area-volume ratio and spatial confinement Sheny et al., 2011 and Yang et al., (2012). In the field of biology synthesized nanoparticles through medicinal plants as acting reducing agents and is a fascinating research field by synthesizing diverse types of nanoparticles i.e calcium, copper, gold, iron, pelladium, silver and zinc Kumar *et* al., (2016). The greener approached nanoparticles are the quintessential solution to reduce the negative effects in the production, application of the nanomaterials and reduce the nanotechnology riskiness Hutchison *et* al., (2008). Plenty of whole parts of the medicinal plants have been utilized for the biologically synthesis of CuONPs due to the presence of enormous number of bioactive compounds in plants. The extracts of medicinal plants have been competently applied for this purpose. Synthesis of CuONPS has been synthesized by the aqueous extracts from plant parts various plant species victoriously like *Azdirachta indica* leaf Ahmed *et* al., (2019) *Asparagus adscendens* Roxb root and leaf Thakur *et* al., (2018), *Zingiber officinale* stem Delma *et* al., (2016), *Citrus medica* Linn (Idilimbu) juice Shende et al., (2015) , *Ziziphus spins-christi* (L) Wild fruit (Khani et al., 2018), *Terminalia catappa* leaf Muthulakshmi *et al.*, (2017), *Punica granatum* Kaur *et* al., (2016), Acalypha indica Sivaraj *et* al., (2014), *Eclipta prostrata* leaf Chung *et* al., (2017) *Syzygium alternifolium* (Wt.) Walp Yugandhar et al (2017), and *Aloe vera* Vijay Kumar et al., (2015). However, there is no report on Biological synthesis of CuO NPs using with *Vitex negundo* aswell as there is no evaluation of antibacterial activity against two gram positive and two gram negative selected bacteria. Hence, the present work has been taken on to synthesize CuO NPs and and to study their efficacy of antibacterial activity and antioxidant activity.

Vitex negundo plant widely available through out India and commonly called as Nirgundi, belongs to the family Verbenaceae. The plant is a large aromatic shrub or sometimes smaller slender tree with quadrangulars, densely whitish tomentose branchlets up to 4.5-5.5m in height. Bark thin and yellowish grey; leaves 3-5 foliate; lower surface of leaves appear silver in colour and upper surface of leaves are green; flower is blueish purple Prasad and Wahi (1965). The genus *Vitex* contains about 250 species of which 14 species are recognised in India and have importance as pharmaceutical and commercially.Roots are tonic, febrifuge, anti-rheumatic, diuretic, expectorant and are useful as a demulcent in dysentry, cephalalgia, otalgia, colic, uropathy, wound and ulcers. Bark is utilitarian in verminosis, odontolgia and optholmopathy. Leaves are aromatic and bitter using to treat astringent, anti- inflamatory, antipyretic orfebrifuge, anodyne, bronchial smooth muscle relaxant, anti arthritic antihelminthic and vermifuge. Flowere are cool, vermifuge, astringent, carminative and are using in haemorrhages and cardiac disorders. Fruit of the plant is cephalic, aphrodisiac, emmenagogue, vermifuge and nervine Chopra *et al.*, (1956).

MATERIAL AND METHODS

Collection of plant material

Fresh leaves of *Vitex negundo* collected kapilatheertham area of Tirumala hills, Chittor District, Andhra Pradesh, India. Leaves were washed thrice running tap water and followed by milli-Q water. They dry up to 14-21 days under shade conditions to evoperate residual moisture content and eventually ground fine powder with electric blender for further usage.

CHEMICALS

CuSO4. 5H2O (Sisco research laboratories pvt. Ltd., India), Nutrient agar (Sigma Aldrich, Banglore, India), NaOH, Streptomycine and Deionized Milli Q water (Merck Water Solutions, France) were used for this work.

SYNTHESIS OF NANOPATRTICLES

10 g of finely grounded plant leaves powder was added to 200 ml of Milli Q water in 500 ml steriled conical flask, then it was extracted with the help of Whatmann no.1 filter paper after 30 boiling on water bath. 20 ml of aqueous plant extract was titrated with 200 ml of 5 mM CuSO4 5H2O slowly dropwise with constant stirring for reduction of CuONPs at 60^0C for 2 h. Obtained reaction mixture was centrifuged at 15000 RPM for 15 min to separate agglomerated large sized particled and as well as admixtures of the plant.

CHARACTERIZATION

The biosynthesized CuO NPs characterization was done by diverse advanced spectroscopic and microscopic tools. Primary confirmation of the nanoparticles was analysed by UV-vis spectroscopy in the wavelength range 190 to 750 nm. To perceive average size and stability of nanoparticles were analysed by Dynamic light scattering (DLS) and Zeta potential (Nanoparticle analyser, Horiba SZ 100, Japan). The Fourier Transform Infrared Spectra (FT-IR ALPHA interferometer, ECO-ATR, Bruker, Ettlingen, Karlsruhe, Germany) results revealed to grasp the phytochemicals act as capping and stabilization of nanoparticles. Crystalline nature and to calculate the average size of particles done by the XRD (Shimadzu, XRD-6000). Microscopic analysis was done through Transmission Electron Microscopy (TEM) tool to examine the size, shape, agglomeration pattern and dispersed nature of the nanoparticles.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of biosynthesized CuO NPs was carried out against following four bacteria were used in this study viz. *Echerichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Staphylococcus aureus*. Disc diffusion method was executed by using standard protocol Anonymous (1996). 50 µg/ ml of CuO NPs concentrations showed growth of inhibition devastated toxic to four bacteria. For this 20 µl of 50 µg/ml concentration of plant extract, 5mM CuSO4.H2O solution, Amoxicillin and synthesized CuO NPs solutions applied on separate sterile What man no.1filter paper discs with 7 mm diameter and allowed to dry before being placed on the agar medium. It was incubated at 37^0 C for 4 h. in incubation chamber. 50 µl of microbial broth cultures were used for swabbing on nutrient agar medium Petri plates. Triplicates of all the experiments were executed under sterile conditions and were incubated at 37^0C for 24 hours. The diameter zone of inhibition measured in centimetres (cm) with the help of scale and results were tabulated.

ANTIOXIDANTS ACTIVITY

Antioxidant activity of VN-CuO NPs was assessed by DPPH- (2, 2-Diphenyl-1-picry Hydrazyl radical Scavenging activity) [Subramanian *et al* (2013). For the assay, 1mM DPPH stock solution was prepared by adding 4 mg DPPH in 100 mL of methanol. 2 mL of DPPH stock solution was added to 1 mL of methanolic solution of VN-CuO NPs consisting vary concentrations of VN-CuO NPs (25, 50, 75 and 100 µg). The reaction mixture was incubated for 45 min in the dark room at room temperature. Later incubation absorbance values were recorded at 518 nm. DPPH activity of the VN- CuO NPs was calculated using with the following formula % of inhibition= [(Absorbance of control- Absorbance sample)/ Absorbance of control] X 100. And the concentration of inhibition of 50% free radicals (IC_{50}) was calculated by regression coefficient (R_2=0.9).

RESULTS AND DISCUSSION

ULTRAVIOLET – VISSIBLE (UV-Vis) SPECTROSCOPY

The reduction of bio-inspired copper oxide nanoparticles primarily observed by visualization method of colour change pattern of the solution and it was changed brown in to deep brown colour, later process of synthesis; then this solution was brought to analysis, and it was performed by the using of **UV**-Vis Spectroscopy range from 190 to 750 nm Nano drop. The absorbance peak was obtained at 260 nm which was further confirmation the reduction nanoparticles are copper oxide (CuO NPs) (Fig.1). This respective peak shown because of the Surface Plasmon Resonance (SPR) phenomenon in the reaction solution. Similar kind of results was observed copper oxide nanoparticles fabricated from *Punica granatum* [Ghidana et al., (2016).

ZETA POTENTIAL AND PARTICLE SIZE STUDIES (DLS)

DLS is the modern instrument to monitor distribution and size of synthesized nanoparticles. For this, the eco-synthesized nanoparticles were mixed in 10 mL of distilled water. By the Brownian movement in the nanoparticles, illumination is distributed at diverse intensities. By this dispersed illumination intensities, the DLS (Dynamic Light Scattering) can be used to find the average size of nanoparticles (NPs). The Zeta potential is used to estimate the stability, dissemination and aggregation levels of green synthesized nanoparticles through repulsion effects around through fluctuations in charge densities. Here bio synthesized VN- CuONPs in the present research work exhibited 28.0 nm z- average size (Fig) and -8.7 mV of zeta potential value (Fig. 2 a and b). This is revealed that the nanoparticles were competently settled in poly-dispersed juncture. These results CuONPs elucidated in the figure. Same type of results were seen in CuO NPs synthesized from *Cassia auriculata* leaf extract [Shi *et al.*, (2017)

FTIR (FOURIER TRANSFORM INFRA-RED)

Biologically synthesized nanoparticles were analysed by Fourier transform infra-red (FT-IR) spectra with scan range of 4000-500 cm^{-1} (ALPHA interfero meter ECO-ATR, Bruker Ettlingen, Karlsruhe Germany) was used to know the expedient phytoconstituents accountable for capping and stabilization. By the FT-IR spectrum broad peaks obtained from leaf aqueous extract of VN at 3275 cm^{-1} corresponds to strong sharp C-H stretching alkene, 2917 cm^{-} corresponds to N-H stretching amine salt, ^{1}2369 cm^{-1}, 1606 cm^{-1} corresponds to medium – C=C stretching conjugated alkene, 1516 cm^{-1}belongs to strong N-O stretching nitrocompound, 1013 cm^{-1} indicated phosphate ion (common inorganic ion) and 527 cm^{-1} corresponds to C-I stretching aliphatic iodo compounds (Aliphatic organo halogen compound). And from synthesized nanoparticles shown peaks at 3854 cm^{-1}, 1633 cm^{-1} corresponds to C=O stretching vibration of amide (proteins), 1425 cm^{-1} corresponds to – C- H bending, 1131 cm^{-1} , 875 cm^{-1} belongs to bending –C=O stretching inorganic carbonate. Based on these results confirmed that the amines and phenols of the plant extracts were principally responsible for capping and stabilization of CuO NPs (Fig. 3 a and b). These proteins have strong ability to bind CuO NPs, act as capping agents thus provided the stability to them. [Vasudeva Reddy and Vijaya]. Similar kind of results was acquired from entire plant of *commelina nudiflora* Kuppuswamy *et al.*, (2017).

XRD

X-ray diffraction (Shimazdu XRD-6000) was analysed to confirm the crystalline nature and average size of the VN- CuONPs. The peaks obtained at 2θ of X-axis 31.88^{0}, 35.35^{0}, 38.65^{0}, 48.62^{0} 61.410 corresponds to 111, 200, 202, 220 and 311 Bragg reflections of Y- axis respectively which may be indexed based on the end- centred monocrystalline structure of copper. Based on the X-ray diffraction reports clearly indicating that the formed particles are copper nanoparticles (Fig. 4). This kind of results were observed in leaf extract mediated CuONPs of *Aloe vera* Vijay Kumar *et al.*, (2015). The highest Bragg reflection was obtained at 2θ of 38.65 to predict FWHM (Full Width Half Maxima) value, e.g.26.82 and 28.64 nm average sizes of the nanoparticles by using the Debye-Scherrer equation:

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

Where D is diameter of NPs, k is the Scherrer constant, λ is the wave length of X-ray radiation source, β is full width half maximum value of XRD diffraction lines and θ is the half diffraction angle-Bragg angle.

TEM (TRANSMISSION ELECTRON MICROSCOPY)

For the TEM analysis of VN- CuO NPs performed by Hitachi advanced contain 300kV. TEM contain abundant resolution is possible to study of morphology of nanoparticles up to 2 nm by the provided focussing of 300 kV electron beam energy. With HR-TEM is carried out to facilitate meticulous resolution study of the biogenic synthesized nanoparticles. For the TEM analysis VN- CuO NPs coated on copper grids. At 20 nm resolution studies exhibited that the nanoparticles are predominantly spherical in shape and poly dispersed (Fig. 5.a and b). These tiny sized particles were show no physical contact and not seen any agglomeration. The result is consistent shape and uniformity of the copper oxide nanoparticles Khatami *et al.*,(2017). TEM results showed similar to previous reports Sulaiman *et al.*, (2018).

ANTIBACTERIAL STUDIES

Biologically synthesized VN-CuO NPs has been exhibited significant zone of inhibition on selected two gram negative bacteria like, *Escherichia coli, Salmonella typhi*; two gram positive bacteria like *Bacillus subtilis and Staphylococcus aureus* [Fig. 6, Fig. 7 and Table.1]. Among the activity zone of inhibition was observed lesser in gram positive bacteria when compared with gram negative bacteria due to possessing thick layer of peptidoglycans (together with polypeptides contains proteins) Yugandhar and Savithramma (2016a). Owing to this, the penetration of CuO- NPs by cell membrane is not easy in case of inhibition growth not possible than gram negative bacteria it leads the cell death. Copper oxide nanoparticles (CuONPs) acts as inhibitory substances on microorganisms through the action of destroying enzymes of cell membranes Ren *et al*., (2009). Maximum zone of inhibition was observed in *Escherichia coli* (gram negative bacteria) followed by *Staphylococcus aureus* (gram positive bacteria) belongs to this activity Meghana *et al.*, (2015). This as it may be the spherical shape and small sized CuO NPs have a high surface to volume ratio to interact with the cell membranes of microorganisms to show the indications of growth inhibitory results [Agnihotri et al., 2014]. In this study it was observed CuO NPs were synthesized by *Vitex negundo* aqueous leaf extract exhibit lean sized particles contain with spherical shape, this is due to the presence of diverse phytochemicals in the plant especially proteins, flavonoids and phenols. Similar type of results was observed in previous reports like *A. indica* leaf mediated copper oxide nanoparticles. 25-35 nm sized spherical shaped nanoparticles showed significant antibacterial activity against selected two gram negative and two gram positive bacteria respectively Sivaraj *et al.*, (2014).

ANTI-OXIDANT- DPPH- (2,2-Diphenyl-1-picry Hydrazyl) RADICAL SCAVENGING ACTIVITY

Anti-Oxidant activity of VN-CuO NPs was evaluated by DPPH (2, 2-Diphenyl-1-picry Hydrazyl) assay. Among the activity exhibited concentration dependant scavenging activity against DPPH scavenging. In which concentration of VN-CuONPs was increased from 25-100 µg/ml (Fig). Therefore, the activity was showed by increased from 28.12±1.40 µg/ml to 64.6±1.43 µg/ml. IC_{50} of VN-CuO NPs against DPPH was found to be 43.58 µg/ml, this inhibition percentage and IC_{50} values clearly indicated that VN-CuONPs are significantly exhibited antioxidants activity (Table. 2 and Fig 8). The activity of antioxidants was showed because of high amount flavonoids, proteins, tannins and polyphenols presence and interacted in the bio reduction as well as stabilization of VN-CuONPs Abdel-Aziz *et al.*, (2014).These findings concurrence competently with earlier reports signing that leaves of the *Ziziphus genus* showed the significant antioxidant activity Hossain *et al.,* (2016). The disproportion between antioxidants and oxidative system results in the manufacturing of oxidative stress. Oxidative stress is accompanying with diverse disorders including hypertension, atherosclerosis, cardiovascular, neurodegenerative disorders, diabetes, Cancer and aging Birben *et al.,* (2012). Therefore it is needed to remediate oxidative stress by these antioxidants.

CONCLUSION

The present study we have reported simple, low-cost, eco- friendly and cost- effective method to produce bioinspired stable copper oxide nanoparticles from *Vitex negundo* traditional medicinal aqueous leaf extract as reducing agent. The color change pattern results and SPR spectra of UV-Vis data (260 nm) confirms the presence of copper oxide nanoparticles in the reaction mixture. By the FT-IR data revealed that the phenols, proteins, flavonoids, amides and tannins are mainly accountable for the reduction, capping and stabilisation of the CuONPs. 28.0 nm z- average size and -8.7 mV of zeta potential value clearly indicated they highly stable. XRD pattern confirmed their crystalline nature. Advanced microscopic analysis by TEM tool showed spherical shaped tiny sized CuONPs were recognised, particles are well settled and without any agglomeration, poly- dispersed in condition and average size of the nanoparticles

28.64nm. Biologically synthesized VN-CuONPs significantly shown against two gram positive and gram negative bacteria. The bio-inspired VN-CuONPs exhibited magnificently antioxidant activity. For the production of stable metal nanoparticles with the using of the *Vitex Nigundo* we followed by environmentally benign and safe approach. *Vitex negundo* traditional medicinal plant widely used to treat various diseases. Roots are tonic, febrifuge, anti-rheumatic, diuretic, expectorant and are useful as a demulcent in dysentry, cephalalgia, otalgia, colic, uropathy, wound and ulcers. Bark is utilitarian in verminosis, odontolgia and optholmopathy. Leaves are aromatic and bitter using to treat astringent, anti-inflamatory, antipyretic orfebrifuge, anodyne, bronchial smooth muscle relaxant, anti arthritic antihelminthic and vermifuge. Flowere are cool, vermifuge, astringent, carminative and are using in haemorrhages and cardiac disorders. Fruit of the plant is cephalic, aphrodisiac, emmenagogue, vermifuge and nervine.

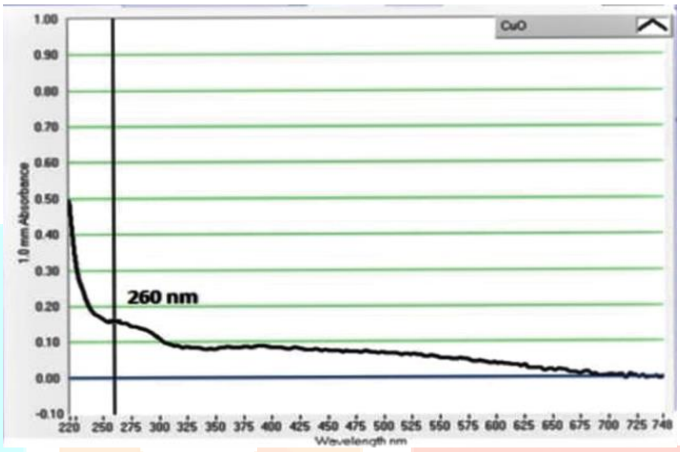

Fig.1 UV-Vis analysis of synthesized CuONPs showing SPR peak at 260 nm.

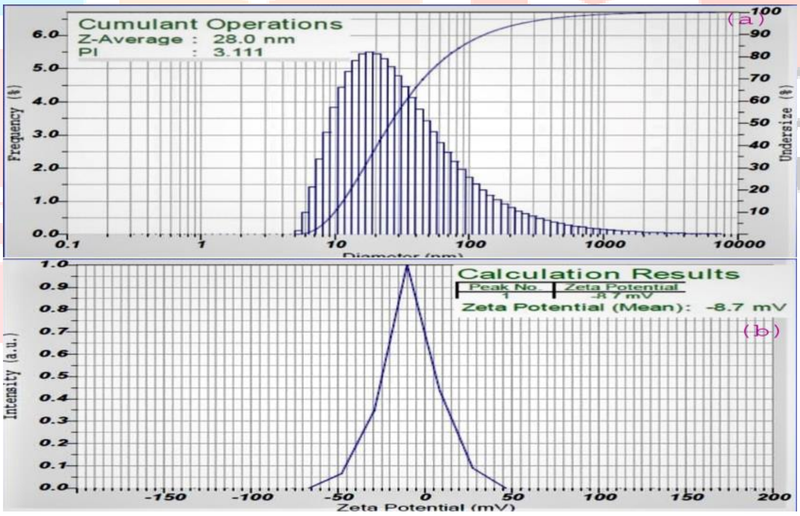

Fig. 2 a). Particle size analysis of biosynthesized VN-CuO NPs **b).** Zeta potential analysis of biosynthesized VN-CuO NPs

a). FT-IR results VN- Extract	**b). FT-IR results VN-CuONPs**

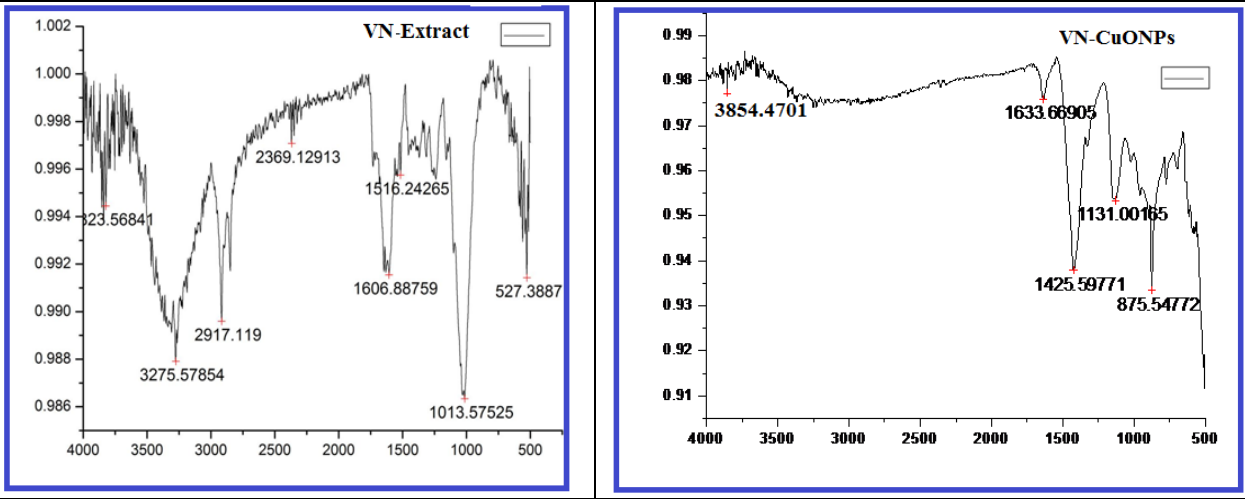

Fig.3 FT-IR pattern analysis of **a).** VN- aqueous Extract and **b).** biosynthesized VN- CuO NPs

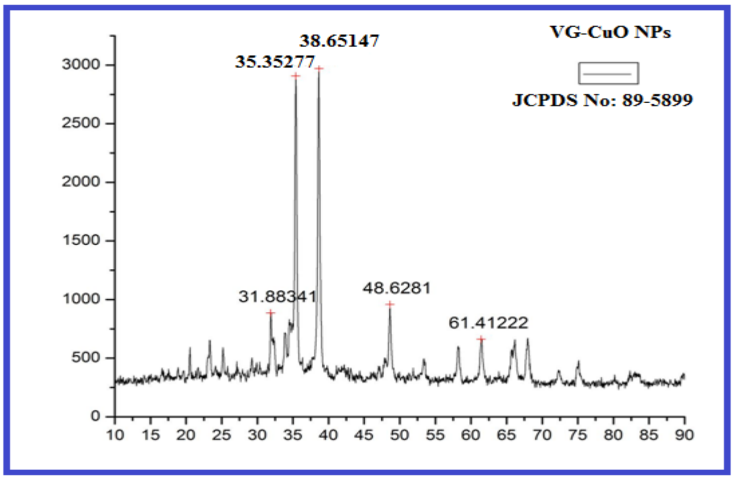

Fig.4 XRD pattern of biosynthesized VN-CuONPs

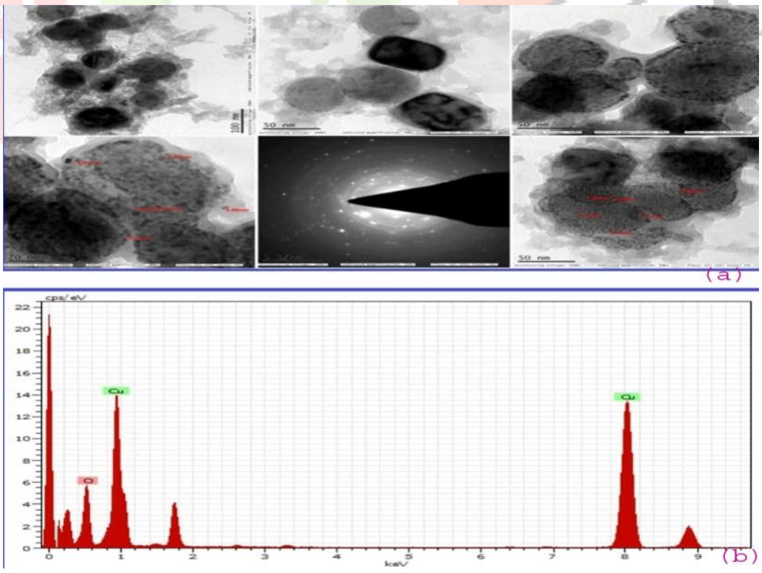

Fig. 5 a). TEM images of biosynthesized VN-CuONPs at different nanometres **b).**EDS pattern of VN-CuONPs

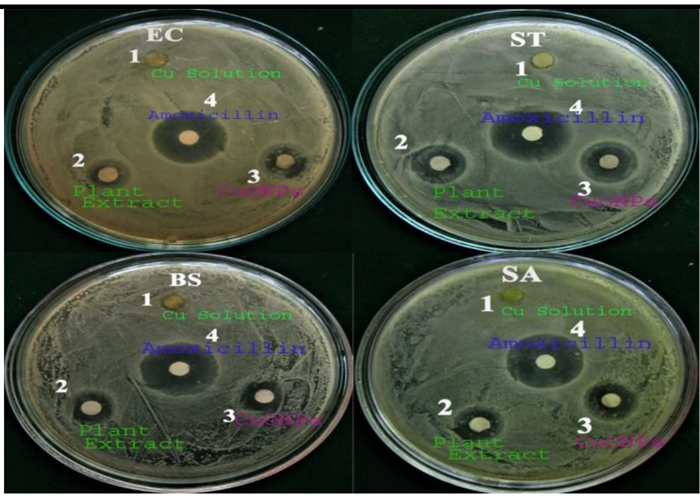

Fig. 6 Antibacterial studies of VN-CuONPs against four selected bacteria

1.Cu-solution 2. Plant extract 3. VN-CuO NPs 4. Amoxicillin

Zome of inhibition (mm)				
Name of the Bacteria	**Extract(μg/ml)**	**Cu (μg/ml)**	**CuONPs(μg/ml)**	**Amoxicillin**
Escherichia coli	8±0.41	14.5±0.65	17.75±0.48	24.5±0.29
Salmonella typhi	7.75±0.48	15.5±0.50	17.5±0.50	23.5±0.29
Bacillus subtilis	7.25±0.25	13.75±0.63	15.25±0.48	23±0.41
Staphylococcus aureus	8.5±0.65	14.25±0.63	16.5±0.29	22.75±0.25

Table. 1 Zone of inhibition (mm) of VN-CuONPs on selected four bacteria species with copper solution, plant aqueous extract and Amoxicillin.

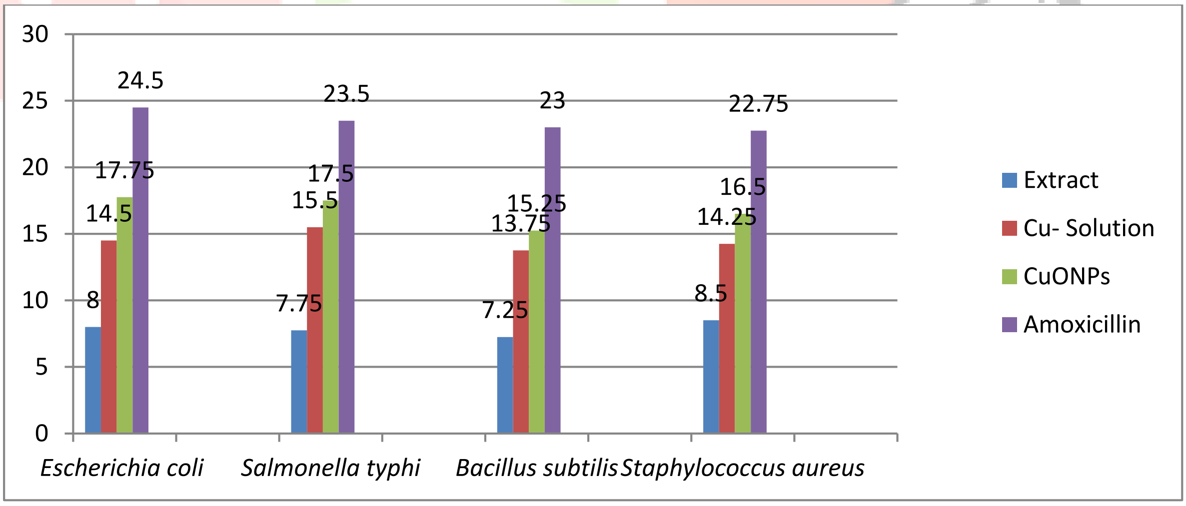

Fig. 7 Graphical representation of antibacterial studies of VN-CuO NPs

Constituents	**25 μg/ml**	**50 μg/ml**	**75 μg/ml**	**100 μg/ml**	**IC50**
CuONPs	28.12±1.40	40.46±1.80	57.36±1.42	64.6±1.43	43.58
Plant sample	25.35±0.60	30.28±0.56	40.64±0.78	53.40±0.48	93.63
Ascorbic Acid	44.44±1.40	57.64±0.47	65.65±1.50	77.54±0.60	28.12

Table 2. DPPH activity

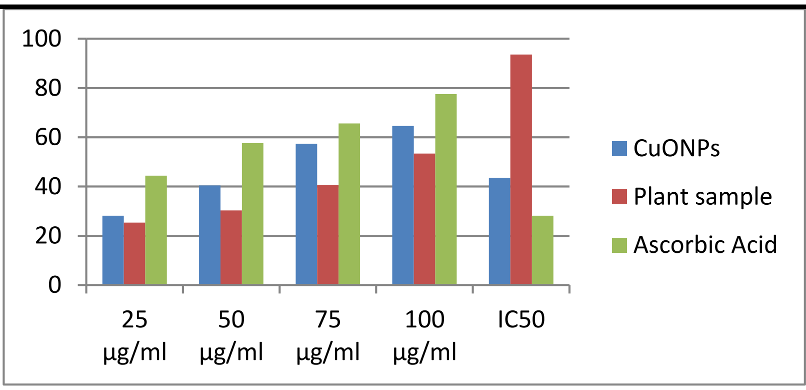

Fig. 8 Graphical representation of VN-CuO NPs DPPH activity

References:

Sheny DS, Mathew J, Philip D. Phytosynthesis os Au, Ag and Au-Ag bimetallic nanoparticles using aqueous extract and dried leaf of Anacardium occidentale. Spectrochim Acta A Mol Biomol Spectrose. 2011;79(1):254-262.

Guang Yang, Jianjia Xie, Feng Hong. Antimicrobial activity of silver nanoparticles impregnated bacterial cellulose membrene: Effect of fermentation carbon sources of bacterial cellulose. Carbohydrate polymers. 2012;87(1):839-845.

Kumar CMK, Yugandhar P, Savithramma N (2016) Biological synthesis of silver nanoparticles from Adansonia digitata L. fruit pulp extract, characterization, and its antimicrobial properties. J Intercult Ethnopharmacol 5:79–85. doi:10.5455/jice. 20160124113632.

Hutchison JE. Greener nanoscience: a proactive approach to advancing applications and reducing implications of nanotechnology. ACS Nano. 2008; 2(3):395-402. 10.1021/nn800131j.

S. Ahmed, M. Saifullah, B. Ahmad, L. Swami, and S. Ikram, "Green synthesis of silver nanoparticles using Azadirachta indica queous leaf extract," journal of Radiation research and applied Science, vol.9, no.1, pp.1-7, 2019.

S. Thakur, S. Sharma, S. Thakur and R. Rai "Green synthesis of copper nano-particles using Asparagus adscendens Roxb. Root and leaf extract and their antimicrobial activities" International Journal of current Microbiology and applied Sciences, vol. 7, no.4, pp. 683-694, 2018.

M. T. Delma and M. Jaya Rajan " Green synthesis of copper and lead nanoparticles using *Zingiber officinale* stem extract" International journal of scientific and research publications, vol. 6 no.11 pp134- 137, 2016.

S. Shende, B. A. P. Ingle, A. Gade and M Rai, "Green synthesis of copper nanoparticles by Citrus medica Linn. (Idilimbu) juice and its antimicrobial activity", World journal of microbiology and biotechnology, vol.31, no.6, pp.865-873, 2015.

R. Khani, B. Roostaei, and G. Bagherzade, and M. Moudi, "Green synthesis of copper nanoparticles by *Ziziphus spins-christi* (L) Wild.: application for adsorption of triphenylmethane dye and antibacterial assay" Journal of Molecular Liquids, vol. 255, , no.541-549, 2018.

L. Muthulakshmi, N. Rajini, H. Nellaiah, T. Kathiresan, M. Jawaid, and A. V. Rajulu, "Preparation and properties of cellulose nanocomposite films with in situ generated copper nanoparticles using Terminalia catappa leaf extract" International journal of Biological Macromolecules, vol.95, pp.1064-1071, 2017.

P. kaur, R. Thakur and A. Choudary, "Biogenesis of copper nanoparticles using peel extract of Punica granatum and their antimicrobial activity against opportunistic pathogens" Green Chemistry letters and reviews, vol.9, no.1, pp. 33-38, 2016.

Sivaraj R, Rahman PK, Rajiv P, Narendhran S, Venckatesh R (2014) Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. Spectrochim Acta A Mol Biomol Spectrosc. 14:255–258. doi:10.1016/j.saa.2014.03.027.

Chung I, Abdul Rauman A, Marimuthu S et al., "Green synthesis of copper nanoparticles using Eclipta prostrata leaves extract and their antioxidants and cytotoxic activities," vol.14, no.1 pp. 18-24, 2017.

Pulicherla Yugandhar, Thirumalanadhuni Vasavi, Palempalli Uma Maheswari and Nataru Savithramma "Bioinspired green synthesis of copper oxide nanoparticles from Syzygium alternifolium (Wt.) Walp: characterization and evaluation of its synergistic antimicrobial and anticancer activity" Appl Nanosci (2017) 7:417–427, DOI 10.1007/s13204-017-0584-9.

Vijay Kumar PPN, Shameem U, Pratap K, Kalyani RL, Pammi SVN (2015) Green synthesis of copper oxide nanoparticles using Aloe vera leaf extract and its antibacterial activity against fish bacterial pathogens. BioNanoSci 5:135–139. doi:10.1007/ s12668-015-0171-z

Prasad Sand Wahi SP, Pharmacognostic study of leaf of *Vitex negundo* Linn.(Nirgundi), J Res Indian Med, 1965, 72, 208-211.& Industrial research, New Delhi.

Chopra RN, Nayar SL and Chopra IC, Glossary of indian medicinal plants (publications and information directorate, council of scientific) 1956, 256-257.

Anonymous, Pharmacopiea of India (the Indian Pharmacopiea) 3rd ed. Delhi: Ministry of Health and Family Welfare, 1996.

Subrmanian R, Subrmanian P, Raj V 2013 Antioxidant activity of the stem bark of Shorea roxburghii and its silver reducing power. Springerplus 2:28 https://doi.org/10.1186/2193-1801-2-28.

Ghidana AY, Al-Antarya T M, and Awwadb A M (2016). Environ. Nanotechnol. Monit. Manag. 6, 95–98.

Ghidana AY, Al-Antarya T M, and. Awwadb AM (2016). Environ. Nanotechnol. Monit. Manag. 6, 95–98.
Shi LB,. Tang PF, Zhang W, Zhao YP, Zhang L C, and Zhang H (2017). Trop. J. Pharm. Res. 16, 185–192.
Vijay Kumar PPN, Shameem U, Pratap K, Kalyani RL,Pammi SVN (2015) Green synthesis of copper oxide nanoparticles using Aloe vera leaf extract and its antibacterial activity against fish bacterial pathogens. BioNanoSci 5:135-139. doi:10.1007/s12668-015-0171z.

Vasudeva Reddy Netala, Venkata Subbaiah Kotakadi, Pushpalatha Bobbu, Susmila Aparna Gaddam, Vijaya Taette (2016): Endophytic fungal isolate mediated biosynthesis of silver nanoparticles and their free radical scavenging activity and anti microbial studies 3Biotech (2016) 6:132/ DOI 10.1007/s13205-016-0433-7.

Kuppuswamy P, Ilavenil S, Srigopalaram S, Maniam GP, Yusoff MM, Govinda N et al (2017) Treating of palm oil mill effluent using commelina nudiflora mediated copper nanoparticles as novel bio-control agent. J Clean prod 141:1023-1029. Doi:10.1016/j.jclepro.2016.09.176.

Khatami M, Heli H, Jahani PM, Azizi H, Nobre M A L (2017): Copper/coppe oxide nanoparticles synthesis using Stachys lavendulifolia and its antimicrobial activity, Iet Nanobiotechnol. 11 (2017) 709-713.

Sulaiman G M, Tawfeeq AT, and Jaaffer M D (2018).Biotechnol Prog. 34, 218–230.

Yugandhar P, Savithramma N (2016a) Biosynthesis, characterization and antimicrobial studies of green synthesized silver nanoparticles from fruit extract of *Syzygium alternifolium* (Wt.) Walp. an endemic, endangered medicinal tree taxon. Appl Nanosci 6:223–233. doi:10.1007/s13204-015-0428-4.

Ren G, Hu D, Cheng EWC, Vargas-Reus MA, Reipd P, Allaker RP (2009) Characterisation of copper oxide nanoparticles for antimicrobial applications. Int J Antimicrob Agents 33:587–590. doi:10.1016/j.ijantimicag.2008.12.004.

Meghana S, Kabra P, Chakraborty S, Padmavathy N (2015) Understanding the pathway of antibacterial activity of copper oxide nanoparticles. RSC Adv 5:12293–12299. doi:10.1039/ C4RA12163E.

Agnihotri S, Mukherji S, Mukherji S (2014) Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. RSC Adv 4:3974–3983. doi:10.1039/C3RA44507K.

Sivaraj R, Rahman PK, Rajiv P, Narendhran S, Venckatesh R (2014) Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. Spectrochim Acta A Mol Biomol Spectrosc. 14:255–258. doi:10.1016/j.saa.2014.03.027.

Abdel-Aziz MS, Shaheen MS, EI-Nekeety AA, Abdel-Wahhab Mosaad A (2014) antioxidant and antibacterial activity of Silver nanoparticles biosynthesized using Chenopodium murale leaf extract. J Saudi Chem Soc 18:356-363 https://doi.org/10.1016/j.iscs.2013.09.011.

Hossain A, Hamood A, Humaid MT. Comparative evaluation of total phenols content and antioxidant potential of leaves and fruit extracts of Omani Ziziphus jujube L. 2016; Pacific Science Review A; Nat. Eng. 18:78-87. https://doi.org/10.1016/j.psra.2016.09.001.

Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. World Aller Organ J 5:9-19. https://doi.org/10.1097/WOX.0b013e3182439613.