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PHYSICO-CHEMICAL AND BIOLOGICAL ACTIVITIES OF *CARICA* PAPAYA PEEL EXTRACT MEDIATED BIOSYNTHESIS OF COPPER OXIDE NANOPARTICLES

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ABSTRACT

Nanotechnology, one of the scientific fields and direct most active area of research in all the branches of science. Generally it coalesce the information of chemistry, physics, biology and medicine. Nanotechnology deals productions and stabilizations of various nanoparticles having a size of 1-100 nm in one dimension acting a major role. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to developing interest in biological approaches which are free from the use of toxic chemicals. In this study, simple technique was applied for the synthesis of CuO nanoparticles using *carica papaya* peel extract. The plant extract acts as reducing, stabilizing and capping agent. Phytochemical test was carried out to identify the compounds responsible for reduction of copper ions. UV-Visible, FTIR and XRD spectral studies were taken to characterize CuO nanoparticles. The prepared nanoparticles was screened for *in-vitro* antibacterial, antifungal, antioxidant and larvicidal activity.

KEYWORDS: CuO nanoparticles, *Carica* papaya peel extract, Phytochemicals, Antioxidant, Antimicrobial and larvicidal activity.

INTRODUCTION

Reduction of metal ions results with nanoparticles through plant extract is a single step green synthesis methods. This biogenic reduction takes place between metal ion to nanoparticls

achieved by biomolecules present in extracts of plants. The plant components contains alkaloids, terpenoids, flavonoids, phenolic compounds perform as reducing agents as well as stabilizing agents for the production of nanoparticles.[1] One of the best way for the preparations of nanoparticles to be biological method such as fungi, bacteria and plants mediated synthesis as it is simple and inexpensive technique in addition the main aspect of nanotechnology is the development of eco-friendly production of nanoparticles. A better defined structure, morphology and size of nanoparticles obtained through green way than other physical and chemical methods.[2] Today a massive part of research based on nanotechnology. The size of the metal nanoparticles acting a fundamental job in the field of physiochemical and optical properties of metal. This technique mainly deals the design, synthesis and manipulation structure of particles with dimension smaller than 200 -800 nm. Nanomaterials gives solution to the areas of pharmacological, solar energy conversion, catalysis and waste water treatment.[3,4]

Among the various nanoparticles, copper and copper-oxide nanoparticles are in particular attractive owing their unique physical and chemical properties and good biological properties like antimicrobial, antioxidant and larvicidal activity.[3,4] Copper nanoparticles have great applications as heat transfer systems,[5] antimicrobials,[6] superstrong materials, sensors[7] and catalysts.[8] CuO nanoparticles can be used in paint or plaster as a bactericide agent to coat hospital equipment further it is also used for the crop protection and agriculture, food packing microbial loads and medicine. Based on this backdrop, the present research work deals the synthesis of CuO nanoparticles using *Carica Papaya* peel extract.

MATERIALS AND METHODS

Materials

All chemicals and reagents used for the research work were of analytical grade (AR) and of the highest purity available. Fresh *Carica* papaya fruits were collected locally in the market. *Culex.quinquefasciatus* larvae were procured from Zonal Entamological Unit, Velapadi, Vellore. Bacteria and Fungi strains were received from Department of Microbiology, D.K.M College for Women, Vellore.

Ultraviolet-Visible spectroscopy

The Ultraviolet-Visible spectra recorded on a SYSTRONICS 2201 spectrometer using DMSO in the wavelength range of 800-200 nm.

FTIR Spectroscopy

The FTIR spectrum was taken using SHIMADZU spectrometer in 4000 - 400 cm^{-1} using KBr pellet.

X-Ray Diffraction analysis

X-ray diffractogram of samples were obtained using XRD-SHIMAZUXD-D1, Ni-filter and Cu Ka radiation source.

Preliminary phytochemical analysis

Prepared *carica papaya* peel extract was subjected for phytochemical study as per the standard methods.[9,10]

Preparation of peel extract

The fresh *carica papaya* fruits were collected and peeled off. The papaya peel were thoroughly washed several times using normal water and then followed by distilled water to remove impurities, the cleaned peels were subsequently dried under sunshade to remove moisture completely. Peels were powdered by using mechanical grinder and then stored. 5 g of powdered plant peel extract were taken in beaker along with 100 mL of double distilled water and allowed to boil at 60°C for ½ h then it was cooled down to room temperature. The prepared solution was filtered through Whatman No.1 filter paper to get clear solution and filtrate was stored at 4°C for further works.

Preparation of copper sulphate solution

Accurate amount of 1mM copper sulphate solution can be prepared by dissolving 0.0499 g of $CuSO_4$ in 200 mL of double distilled water and stored in clean, dried standard flask.

Synthesis of CuO Nanoparticles

Synthesis of CuO nanoparticles were done by the standard procedure.[11,12,13] 25 mL of papaya peel extract was introduced drop wise in 60 mL of 1 mM copper sulphate under continuous stirring. The initial colour change occurs from sky blue colour to light green colour. After the complete addition of the peel extract, the reaction mixture was kept for stirring for about 3 h. The colour change occurs from blue to dark green colour.

Antimicrobial studies (*in vitro*)

In vitro antimicrobial studies against bacterial strains such as *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and fungal strains such as *Aspergillus*

niger and Pencillium notatum were studied for the prepared nanoparticles by agar well diffusion method. The microorganisms were inoculated on Muller Hinton Agar and spread uniformly using sterile spreader in petri plates. 50 µL and 100 µL of the freshly prepared CuO nanoparticles (1 mg / mL) in DMF were used as positive control and DMF alone taken as negative control. The plates were allowed to stand for 1 h at room temperature for the diffusion of the substances. Before the growth of organism commenced, the plates were incubated at 37°C for 4 h and inhibition of microbes was determined by measuring the diameter of zones.

Antioxidant activity

Oxidation is chemical reactions which produce free radicals results with many diseases. A substance that inhibits oxidation of other molecules is called antioxidant. Hydrogen peroxide scavenging activity is one of the best method to study the antioxidant property.[14,15] A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. 2 mg/mL in DMF of prepared CuO nanoparticles were added to hydrogen peroxide and absorbance at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide act as control. The percentage of hydrogen peroxide scavenging activity is calculated.

scavenging activity = (Ac-As) / Ac x 100

Ac - absorbance of control, As - absorbance of sample.

Larvicidal bioassay

The larvicidal activity was done by the procedure of WHO guide lines with some modification.[16] The eggs and egg rafts of *C. quinquefasciatus* were dipped into a plastic bottle containing 500 mL of dechlorinated water for 30-40 min to hatch out larvae. They were maintained in the laboratory as per literature.[17] Mosquito larvae were fed with powdered nutrient broth once a day. After 4 days the hatched larvae turned into larvae in early fourth stage and were subjected for further experiment. A total of 20 reared mosquito larvae was placed in 200 mL of double distilled sterilized water containing various concentrations (4 mg, 2 mg, 1 mg, 0.5 mg) of synthesized nanoparticles. A set up with sterile distilled water is considered as negative control. Percentage of mortality was assessed after 24 h of incubation. A number of dead larvae in each batch were counted every hour for 24 h exposure period.

The treated larvae was mounted on a slide and examined under a microscope for image capture.

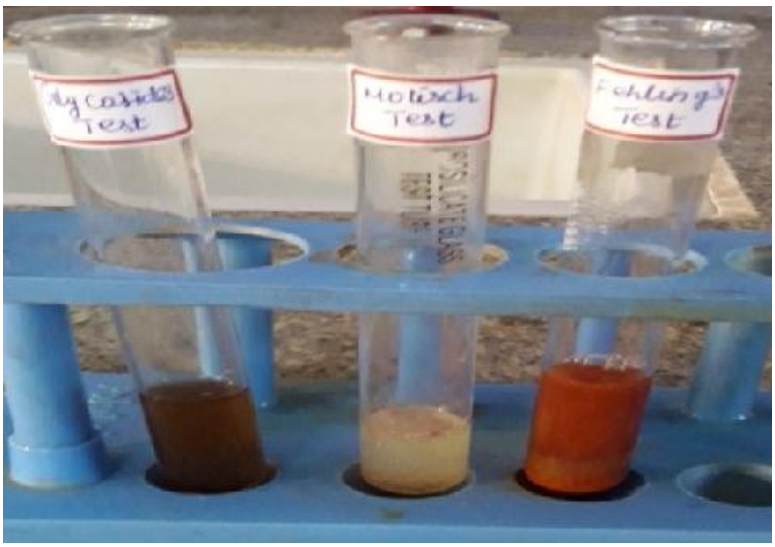
RESULT AND DISCUSSION

Phytochemical Screening Test

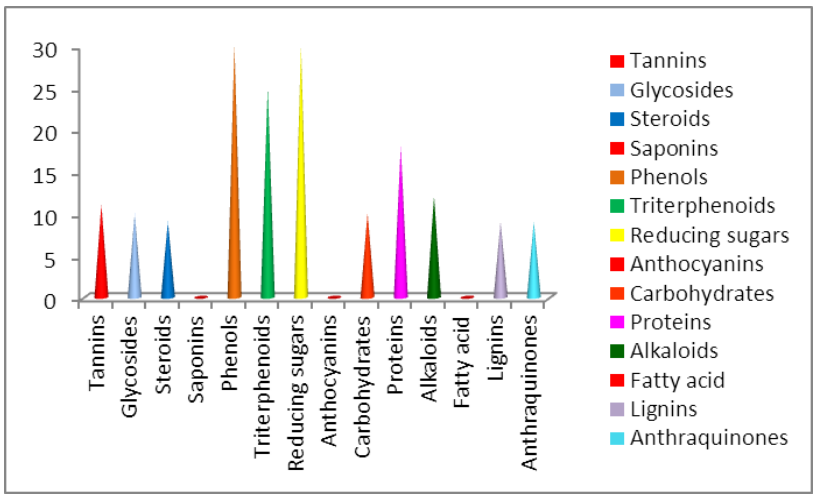
A modern techniques of plant analysis was adopted to study phytochemicals present in the *C.Papaya* peel extract.[18,19] These phytochemicals are responcible for the immiediate reduction of ions and formation of nanoparticles. The chemical reactions, which proceed in the aqueous exract may be as follows.[20]

Metal solution + Plant aqueous extract → $[Metal]^+$ + byproduct

Biomolecules of phenols + $[Metal]^+ + e^-$ →[Metal]{ [Reduction]}



"Fig. 1" Phytochemical Screening Test.



"Fig. 2" Graphical representation of phytochemical analysis.

UV-VISIBLE SPECTRA

UV-Visible Spectra of Peel extract

Plant extract shows maximum absorption at 280 and 350 nm with approximately absorbance values of 0.8 and 0.3 respectively. The absorption maximum 230-290 nm corresponds to phenolic compounds (tannins) and flavonoids[17,18] and the range 330-420 nm corresponding to phenolic acids and their derivatives.[17,18] Hence, the result of UV-VIS spectroscopic analysis confirms the presence of phenolic compounds and flavonoids in the aqueous peel extract of C. Papaya which are capable of acting as reducing agent for the conversion of metallic state to nanoparticles.

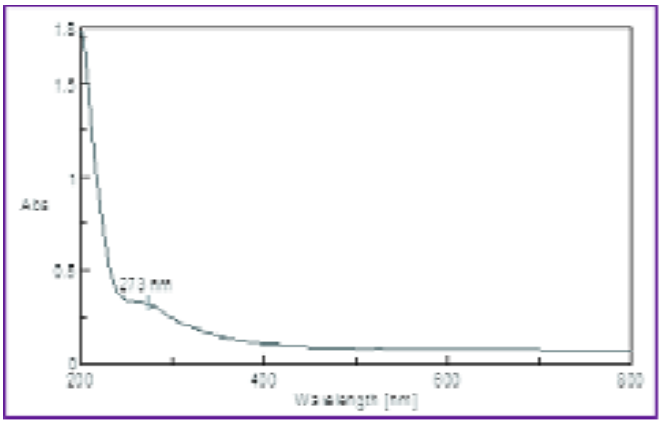
UV-Visible spectra of Cu-O Nanoparticles

UV-Visible spectra for synthesized nanoparticle exhibit a strong absorbance between 250-300 nm suggesting the formation of copper oxide nanoparticles.[19]

"Fig. 3" Plant extract

"Fig. 4" Copper sulphate

"Fig. 5" Copper nanoparticles

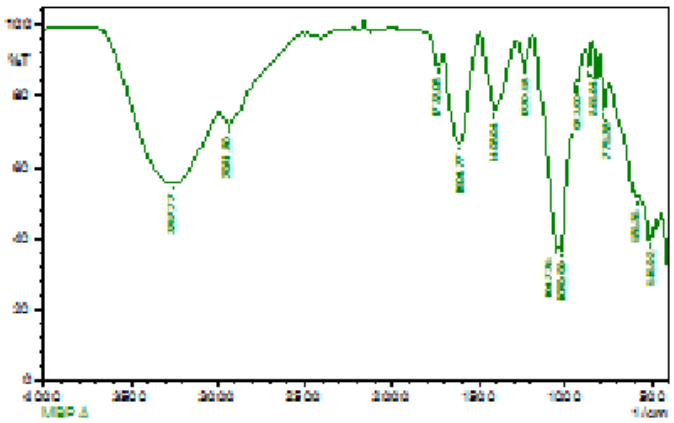


"Fig. 6" UV-Visible spectra for Cu-O nanoparticles.

FT-IR SPECTRA

FT-IR spectra of peel extract

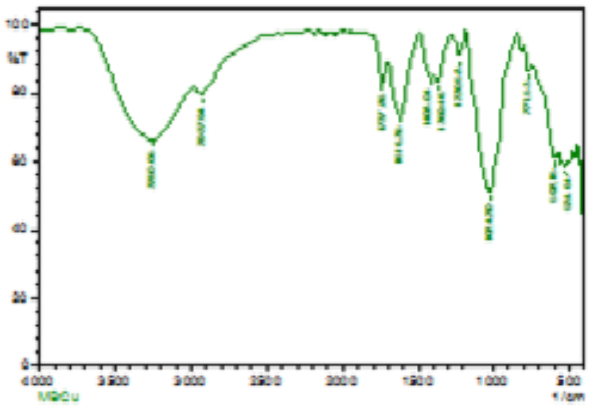
The absorption band obtained for the plant extract was compared with the prepared nanoparticles.



"Fig. 7" FT-IR of C.papaya peel extract.

Aqueous leaf extract showed the strong absorption band at 3257.77 cm^{-1} suggests O-H stretching vibrations of the hydroxyl group, H-bonded alcohols and phenols.[21,22] The strong band observed at 2931.80 cm^{-1} corresponds to C-H stretching of alkanes. The band at 1732.08 cm^{-1} was due to presence of C=O stretching, α, β - unsaturated aldehydes and ketones.[23] A band at 1604.77 cm^{-1} corresponds to N-H bonding of primary amines. The absorption band observed at 1408.04 cm^{-1} assigned to O-H band indicates carboxylate functionalized nanoparticles. The bands at 1047.35 cm^{-1} and 1029.99 cm^{-1} correspond to C-N stretching of amines. The peak at 866.04 cm^{-1}, 775.38 cm^{-1} assigned to C-H stretching.[22]

FT-IR spectra of Cu-O Nanoparticles



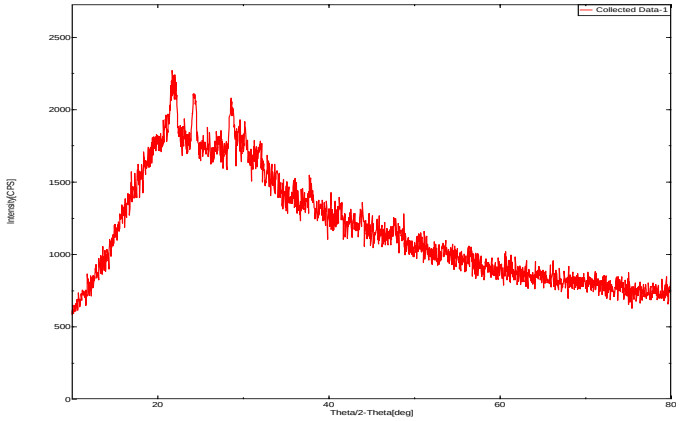
"Fig. 8" FT-IR of *C.papaya* peel extract.

The FTIR spectra of CuO nanoparticles showed the characteristic absorption bands at 3250 cm^{-1}, 2927.94 cm^{-1}, 1737.86 cm^{-1}, 1616.35 cm^{-1} and 1024.20 $cm^{-}1$. The strong band observed at 1616.35 cm^{-1}, 3250 cm^{-1} corresponds to C=O stretching of amides and O-H stretching of phenolic compound respectively. The existence of band at 437 cm^{-1} recognized to the vibration of CuO certified the formation of copper oxide nanoparticles.[24,25] Moreover an approval of Cuprus oxide (Cu_2O) peaks 605 to 660 cm^{-1} not detected in FT-IR spectra. The FT-IR analysis of CuO suggested that are surrounded by any of their organic molecules such as polyphenols, terpenoids and alkaloids. Hence, the chemical constituent present in papaya peel extract such as flavonoids, phenols, alkaloids and fatty acids are responsible for the reduction of copper ion to copper oxide nanoparticles due to their capping and reducing capacity.

X-Ray Diffraction analysis

X-Ray Diffraction analysis is the most useful method by which X-Rays of a known wavelength are passed through a sample to identify the crystalline structure. The average size of particle was calculated by applying full width at half maximum (FWHM) and the value of 2θ of characteristic peak of the experimental XRD powder pattern using the Debye–Scherer equation. $D = k\lambda/ \beta \cos \theta$ (1) Where D is the average size, K is a constant (ca. 0.9), λ is the wavelength of Cu Kα, β is the full width at half maximum (FWHM) of the diffraction peak and θ is the Bragg's angle.

The XRD pattern exposed that synthesized CuO nanoparticles are crystalline in nature. According to Debye–Scherer equation the average particle size was found to be 4.39 nm and the lattice strain was calculated as 0.0902. The percentage of crystalline was determined as 8.658%. The 2θ values for the CuO nanoparticles are as follows 21.88, 24.342, 28.628, 30.211, 31.964, 37.890, 41.282 and 47.682.



"Fig. 9" XRD analysis of CuO nanoparticles.

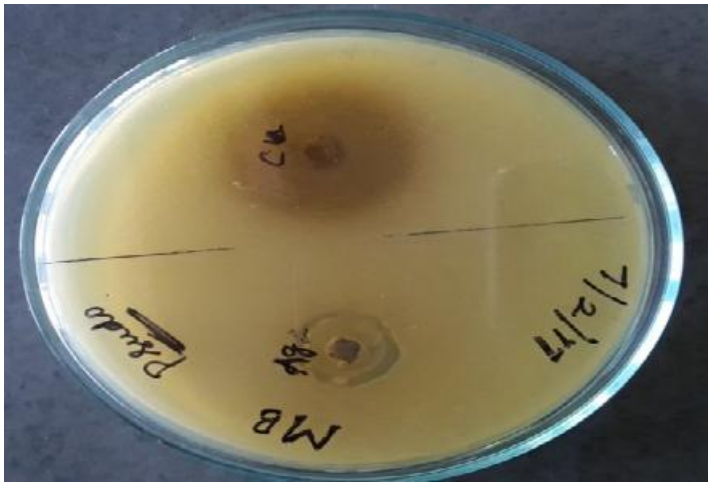
BIOLOGICAL PROPERTIES OF PREPARED CuO NANOPARTICLES

Antibacterial Activity

Antibacterial test was performed for aqueous peel extract and synthesized copper oxide nanoparticles. The zone of inhibition values are measured in mm. The zone of inhibition value less than 10 mm is considered as resistant towards the corresponding microorganism. The measured values were compared with standard Ampicillin used as positive control.

Table 1: Antibacterial activity of prepared nanoparticles.

Components	Zone of inhibition (mm)		
	E-coli	Pseudomonas aeruginosa	Staphylococcus aureus
Ampicillin (control)	30	25	43
Plant extract	12	14	12
Cu-O nanoparticles	28	18	35



"Fig. 10" Antibacterial activity of CuO nanoparticles.

The aqueous peel extract showed better antibacterial activity against selected bacterial strains also zone of inhibition increased in CuO nanoparticles and exhibited good antibacterial activity against Gram positive and Gram negative bacteria than aqueous peel extract.

Antifungal Activity

The prepared CuO nanoparticles and aqueous peel extract were assessed for their *in vitro* antifungal activity by well diffusion method against fungi strains. The standard Polymyxin B sulphate was used as positive control.

Table 2: Antifungal activity of CuO nanoparticles.

Components	Zone of inhibition(mm)	
	Aspergillus niger	Penicillium Notatum
Polymyxin B sulphate (control)	11	11
Peel extract	15	14
CuO nanoparticles	18	16

The synthesized CuO nanoparticles exhibited good antifungal activity against selected fungi. The zone of inhibition values were compared with the standard Polymyxin B sulphate.

ANTIOXIDANT ACTIVITY

Hydrogen peroxide method

Antioxidant scavenging activity of CuO nanoparticels (4 mg, 2 mg, 1 mg and 0.5 mg) was examined. α-tocopherol with 89.45% of scavenging activity is used as positive control and DMF is used as negative control. CuO nanoparticles exhibits moderate antioxidant scavenging activity when compared with standard α-tocopherol. This study proves that on

increasing the concentrations of CuO nanoparticles results with increase in scavenging activity.

Table 3: Scavenging activity of prepared nanoparticles.

Concentration mg /mL	CuO NPs Scavenging activity
4.0	91.82%
2.0	75.45%
1.0	45.22%
0.5	37.45%

LARVICIDAL ACTIVITY

The larvicidal activity of synthesized CuO nanoparticles was performed against Culex. quinquefasciatus. The highest mortality was found after 24 h of exposure period. Highest concentrations of CuO nanoparticles exhibit good larvicidal activity.

"Fig. 11" Photograph of larvicidal activity.

Table 4: Mortality activity of CuO nanoparticles.

Concentrations mg / 200 mL	CuONPs Mortality
4.0	78 %
2.0	59 %
1.0	38 %
0.5	24 %

CONCLUSION

In the present study CuO nanoparticles has been synthesized using *C.papaya* peel extract and characterized by various physico-chemical and spectral analyses like UV-Visible, FTIR and XRD. FTIR results shows that reduction of copper ions to stabilization of CuO nanoparticels occur through possible participation of peel extracts and others metabolites present in the peel

extracts. XRD spectral results confirmed the particle size 4.39 nm of prepared nanoparticles. The prepared CuO nanoparticles were screened to study their biological properties like *in*-vitro antimicrobial, antibacterial and antifungal antioxidant and larvicidal activity.

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REFERENCES

1. Kumar KM, Sinha M, Mandal BK, Ghosh AR, Kumar KS. Biobased green method to synthesise palladium and iron nanoparticles using *Terminalia chebula* aqueous extract. Spectrochim. Acta A, 2012; 91: 228–233.
2. Raveendran P, Fu J, Wallen SL. Completely green synthesis and stabilization of metal nanoparticles. J Am Chem Soc, 2003; 125(46): 13940-1.
3. Kalimuthu Kalishwaralal, Ramanathan Vaidyanathan, Venkataraman Deepak, Sureshbabu Ram, Kumar Pandian, Jeyaraj Muniyandi, Nellaiah Hariharan, Soo Hyun Eom. Biosynthesis, Purification and Characterization of Silver Nanoparticles using Escherichia coli. Colloids and Surfaces B: Biointerfaces, 2009; 74: 328-335.
4. Savithramma N, Sulochana C, Rao KN. Ethnobotanical survey of plants used to treat asthma in Andhra Pradesh, India. J Ethnopharmacol, 2007; 113(1): 54-61.
5. Eastman JA, Choi SUS, Li S, Yu W, Thompson LJ. Anomalously increased effective thermal conductivities of ethylene glycol-based nanofluids containing copper nanoparticles. Appl Phys Lett, 2001; 78(6): 718–720.
6. Esteban-Cubillo A, Marco JF, Moya JS, Pecharroman C. On the nature and location of nanoparticulate iron phases and their precursors synthetized within a sepiolite matrix. J Phys Chem C, 2008; 112: 2864–287.
7. Ahuja A, Dev K, Tanwar RS, Selwal KK, Tyagi PK. Copper mediated neurological disorder: Visions into amyotrophic lateral sclerosis, Alzheimer and Menkes disease. J Trace Elem Med Biol, 2015; 29: 11-23.
8. Kantam RRL, Rosaiah K, Rao GS. Acceptance sampling based on life tests, Log-logistic distribution, Journal of Applied Statistics, 2001; 28(1): 121–128.

9. Audu R, Yusri K, Muhammad Sukri S. Acquisition of employability skills in technical vocational education: Necessity for the 21st century workforce. Aust J Basic Appl Sci, 2013; 7(6): 9-14.
10. Roopashree TS, Dang R, Rani RHS, Narendra C. Antibacterial activity of antipsoriatic herb: Cassia tora, Momordica charantia and Calendula officinalis. Int J Appl Res Natu Pdts, 2008; 1(3): 20-28.
11. Siddiqui BS, Afshan F, Faizi GS, Naqui SNH, Tariq RM. Two insecticidal tetranortriterpenoids from Azadirachta indica. Phytochemistry, 2000; 53: 371–376.
12. Lalitha A, Subbaiya R, Ponmurugan P. Green synthesis of silver nanoparticles from leaf extract Azhadirachta indica and to study its anti-bacterial and antioxidant property. Int J Curr Microbiol App Sci, 2013; 2: 228–235.
13. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. J Nanoparticle Res, 2011; 13: 2981–2988.
14. Sreejayan N, Roa MNA. Nitric oxide scavenging by curcuminoids. J Pharm, 1997; 49: 105–107.
15. Cao G, Prior RL. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clin Chem, 1998; 44: 309-1315.
16. Kamaraj C, Rahuman AA, Bagavan A. Antifeedant and larvicidal effects of plant extracts against Spodoptera litura (F.), Aedes aegypti L. and Culex quinquefasciatus Say. Parasitol Res, 2008; 103(2): 325–331.
17. Parekh J, Chanda V. In vitro Antimicrobial activity and Phytochemical analysis of some Indian medicinal plants. J. Biol, 2007; 31: 53-58.
18. Shankar SS , Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J Colloid Interface Sci, 2004; 275(2): 496-502.
19. Ren G, Hu D, Cheng EW, Vargas-Reus MA, Reip P, Allaker RP. Characterization of copper oxide nanoparticles for antimicrobial applications. International Journal of Antimicrobial Agents, 2009; 33(6): 587–590.
20. Harborne JB. Phytochemical methods: A Guide to Modern Techniques of plant Analysis. 2002.
21. Anita Sagadevan Ethiraj, Dae Joon Kang. Synthesis and characterization of CuO nanowires by a simple wet chemical method. Nanoscale Research Letters, 2012; 20127: 70.

22. Hassan MS, Amna T, Yang OB, E1-Newehy MH, Al-Deyab SS, Khil MS. Smart copper oxide nanocrystals: synthesis, characterization, electrochemical and potent antibacterial activity. Colloids Surf B: Biointerfaces, 2012; 97: 201–206.
23. Xu JF, Ji W, Shen ZX. Preparation and characterization of CuO nanocrystals. J Solid State Chem, 1999; 147(2): 516–519.
24. Huang J, Chen C, He N, Hong J, Lu Y. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnol, 2007; 18: 105-104.
25. Gunalan S, Sivaraj R, Venckatesh. Aloebarbadensis Miller mediated green synthesis of mono disperse copper oxide nanoparticles: optical properties, Spectrochim. Acta. Mol. Biomol. Spectroscopy A, 2012; 97: 1140-1144.