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Panchagavya mediated copper nanoparticles synthesis, characterization and evaluating cytotoxicity in brine shrimp.

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ABSTRACT

Nanotechnology is the most enabling technology that can be applied almost in all fields such as pharmaceutical, health care, food and feed, biomedical science and drug delivery. Synthesis of copper nanoparticle (CuNPs) with biological properties plays an important role in the development of scientifically valuable product. In this study, biosynthesis of Copper nanoparticles using Indian traditional medicine, Panchagavya is used which is eco-friendly, simple, cost effective and non-toxic. Copper sulphate (25 mM) solution was mixed with Panchagavya filtrate for the synthesis of Copper nanoparticles. The Nano metallic dispersion was characterized ultra violet spectrophotometer, Fourier transmission infrared ray spectroscopy, X-ray diffraction, Dynamic light scattering, Laser Raman, Scanning electron microscope, High resolution transmission electron microscope, X-ray fluorescent microscope. In addition, we studied the antioxidant and also cytotoxicity activity. The characterization results revels that the copper nanoparticles are round shaped in the size 98 nm (nm) with −34.4mv stability. Further, the toxicity of biosynthesized Copper nanopartilces was tested to evaluate the cytotoxic effect that displayed LC_{50} value of 95% confidence intervals.

1. Introduction

Nanotechnology is the development and manufacture of materials in the nanometer size range (at least one dimension less than 100 nm) and their application. Nanoparticles (NPs) have become a part of our daily life, in the form of cosmetics, drug delivery systems, therapeutics, and biosensors. However, little is known about their bio-distribution and bioactivity. The various interactions of NPs with fluids, cells, and tissues need to be considered, starting at the portal of entry and then via a range of possible pathways towards target organs. A discipline of nano-toxicology would make an important contribution to the development of a sustainable and safe nanotechnology. Developments in the organization of nanoscale structures into predefined superstructures ensure that nanotechnology will play critical role in many key technologies (Aboofazeli, 2010). Nanoparticles are of great interest due to their extremely small sizes and large surface to volume ratios. Metals have the ability to form oxides adopting a wide range of structural geometrics and exhibiting metallic, semi-conductor or insulating properties (Feng et al., 2013). The appropriate manipulation of these materials at the nanoscale and the production of metal oxide nanoparticles enabled the study of their interaction with biological system (KayalVizhi et al., 2016). Synthesis of Copper nanoparticles can be achieved through physical, chemical and biological routes. Biosynthesized nanoparticles will have enhanced stability, biocompatibility and reduced toxicity (Schrofel et al., 2014).

Copper nanoparticles have gained much attention due to its high electrical conductivity, high melting point, low electrochemical migration behaviour and it is a cheaper metal compared to other metals such as silver, gold, platinum and palladium. An added advantage of copper nanoparticles is that they oxidize and form copper nanoparticles, and are relatively stable in terms of both chemical and physical properties (Supriya et al., 2018). Apart from other applications, copper nanoparticles degrade DNA in a single oxygen-mediated fashion even in the absence of any external agents. This makes copper nanoparticles as an excellent candidate for targeted therapy. The use of copper nanoparticles as therapeutic agents could be in particular advantageous because human body has an efficient system to deal with metabolism of copper since it is a micronutrient (Jose et al., 2011).

Panchagavya is a term used in Ayurveda to describe five important substances obtained from cow namely urine, dung, milk, ghee and curd.

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Cow urine has a unique place in Ayurveda and has been described in Sushrita Sumhita and Ashtanga Sangraha to be most effective substances secretion of animal origin with innumerable therapeutic values. It has been recognized as water of life or Amrita (beverages of immortality) the nectar of the god (Rajesh and Jayakumar, 2013). Cow urine is believed to have therapeutic value and used in many drug formulations. Cow urine is used as disinfectant and for purification with an approximate shelf life of around five years, when compared to the synthetic chemicals these are currently available in the market (Mohanty et al., 2014).

Nanotoxicology research is applied to various fields including biology and pathology, but typically to pharmacology and to the use of nanomaterials (NMs) and nano-devices for diagnostic and therapeutic purposes. Therefore, a key goal for toxicologists is to identify in vitro and in vivo assays accurately reflecting the ability of nanoparticles to induce toxic effects in the humans and in the environment. In addition, standardized tests for both in vitro and in vivo studies are needed to develop better and more rapid screening techniques and to predict toxicity. The cytotoxicity effects of nanoparticles were investigated in a multitude of animal models by means of in vivo tests employing the typical nanoparticles exposure routes, i.e., pulmonary, oral, dermal, and injection based. The cost and labor intensiveness of the in vivo studies have led researchers to the use of in vitro methods for assessment of nanoparticles cytotoxicity.

Brine shrimp is zooplankton that is used to feed larval fishes (Sorgeloos, 1980). *Artemia* present one common characteristic, that is, their strong adaptability to hyper saline environments, such as permanent salt lakes, coastal lagoons, and man-made salt pans. They play an important role in the energy flow of the food chain in marine environment (Vanhaecke and Persoone, 1981; Sanchez-Fortun et al., 1997;Nunes et al., 2006a,b; Kanwar, 2007). *Artemia* use in toxicology poses a reasonable number of answerable questions, namely, practical considerations of laboratory culture and attainment of cyst, ecological relevance, systematic use, and practical conditions of maintenance and sustainability of laboratory conditions of animal model, thus making achievable a sustainable development of *Artemia* based bioassays.

The cytotoxicity assays are often tedious and expensive, and there is a lack of a simple and rapid screening procedure. Nowadays, brine shrimp lethality assays are extensively used in research and applied toxicology (Costa-Lotufo et al., 2005). There is a tendency to use a Brine shrimp assay in toxicological tests that screen a large number of extracts for drug discovery in medicinal plants (Kheiri Manjili et al., 2012; Ramazani et al., 2010a, 2010b; Sangian et al., 2013). This is because in this case, aseptic techniques are not required, and thus Brine shrimp assays could replace the more ethically challenging MTT assay that requires animal serum (Mclaughlin et al., 1998). This assay was proposed by Michael and coworkers in 1959 and was later adopted by many laboratories as a method for preliminary estimation of toxicity (Insanu et al., 2012). Brine shrimp is one of the most valuable test organisms available for ecotoxicity testing, and the available research suggests that several applications of Brine shrimp to toxicology and ecotoxicology will continue to be used widely (Nunes et al., 2006a,b). Because of the rapidity, convenience, and low cost of Artemia-based assays, we decided to evaluate the Brine shrimp test in comparison with the MTT assay in the assessment of cytotoxicity of different classes of NPs.

In this research, well stabilized Copper nanoparticles were green synthesized using Panchagavya as reducing agent. Panchagavya is an organic product blended from five different cow product. Synthesized copper nanoparticles were characterized by UV, FTIR, XRD, DLS, SEM, HRTEM, XRF and Laser Raman spectroscopic studies. Additionally, some biological activities including Anti-oxidant, Antibacterial and cytotoxicity assay of the green synthesized CuNps were evaluated.

2. Materials and methods

2.1. Panchagavya preparation

2.1.1. To prepare 20 L of Panchagavya following ingredients is as follows

Cow dung slurry- 4 Kgs, Indigenous cow dung- 1 Kg, Cow urine – 3 ltrs, Indigenous cow milk – 2 ltrs, Sour Curds – 2 ltrs, Ghee from cow – 1 Kg, Sugar cane Juice – 3 ltrs, Tender Coconut water – 3 ltrs, Over ripe banana-12 nos.

All the above items was added to a wide – mouthed mud pot in the order specified above. The container should be kept open but in the shade. The contents were stirred twice a day morning and evening till seventh day (web portal). But we have prepared for 5 lit for our research in our university campus. The Panchagavya was filtered in Whatmann No.1 filter paper and used for future studied.

2.2. Synthesis of copper nanoparticles

15 ml of Panchagavya was mixed with 85 ml of 25 mM $CuSo_4$ solution and the resulting mixture was incubated at 37 °C for 24 h at magnetic stirrer. Reduction of cu + ion to cu was monitored by the colour change in the reaction mixture from blue to blackish green. The Synthesized nanoparticle was characterized using some spectroscopic methods.

2.3. Characterization of synthesized copper nanoparticles

The reduction of copper sulphate to copper nanoparticles was monitored using UV–vis spectrum. The UV–Vis spectrum was recorded on a Shimadzu (model UV-2450) spectrophotometer. X-ray diffraction is a versatile, non-destructive analytical method for the detection and quantitative determination of various crystalline phases, powder XRD data were collected Via Bruker, JOEL Model JDX – 8030 diffractometer with Cu Kα radiation ($\lambda = 1.5406\ A^0$)operated at 45 kV; 30 mA. FTIR spectra were obtained with Bruker, Tensor Model 27 spectrometer using KBr pellets within the range of 400–4000 cm^{-1}. The aqueous suspension of the synthesized nanoparticles was filtered through a 0.22 μm syringe driven filter unit, and the size and distribution of the nanoparticles were measured using dynamic light scattering technique (Nanopartica, HORIBA, SZ-100). The size, shape and morphology of the nanoparticles was examined by Scanning Electron microscopy and Transmission electron microscopy (High Resolution Transmission Electron Microscopy (HRSEM-HRTEM – FE8121-H).

The sample's nature at different temperature were detected by Laser Raman spectroscopy (Renishawinvia Raman Microscope) from the wavelength of 400–4000 cm^{-1} with exposure time of 25s (100% intensity). X-Ray Fluorescence was used to analyze the presence of the element in the sample. In this study X-ray fluorescent microscope (XGT-2700, Horiba, Japan) was used.

2.4. Bio assays of copper nanoparticles

2.4.1. DPPH free radicals scavenging antioxidant activity

The antioxidant activity of the CuNPs were evaluated using DPPH (2, 2 diphenyl-1 picrylhydrazyl) by the method of Blois (1958) 0.1 M solution of DPPH in ethanol was prepared and 2.96 ml of this solution was added to 0.4 ml of various quantities and the reference compound, after 30 min the absorbance was measured. BHA was used as a reference material. Percentage of inhibition was calculated by comparing the absorbance values of control and samples.

$$\% \text{ inhibition} = A \text{ control} - A \text{ test} / A \text{ control} \times 100$$

2.4.2. Cytotoxicity assay (Brine shrimp)

Brine shrimp eggs were purchased from the New Aqua Laboratory in

Thampanoor, Thiruvananthapuram. Dried cysts were placed in a bottle containing artificial sea water which was prepared by dissolving 35 g of sodium chloride in 1 L of distilled water. After 48 h incubation at room temperature 37 °C under conditions of strong aeration and continuous illuminations, the larvae (nauplii) hatched within 48 h. The evaluation of cytotoxicity of CuNPs in Brine shrimp was performed according to the previous methods.[13,16] The assay was carried out on larvae of brine shrimp (A. salina Leach.). When the shrimp larvae are ready, 5 mL of the artificial seawater and 5 mL of nanoparticles solution was added to each test tube and 10 brine shrimps were introduced into each tube. Thus, there were a total of 30 shrimps per dilution. The artificial seawater up to 10 mL per test tube is control. The test tubes were left uncovered under the lamp. The number of surviving shrimps were counted and recorded after 24 h. Using probit analysis, the lethality concentration (LC_{50}) was assessed at 95% confidence intervals. LC_{50} of less than 100 ppm was considered as potent (active). As mentioned by Meyer and others, LC_{50} value of less than 1000 µg/mL is toxic while LC_{50} value of greater than 1000 µg/mL is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number, and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the compounds present in the nanoparticles.

3. Results

Biological synthesis of copper nanoparticles was synthesized from the aqueous copper sulphate and Panchagavya filtrate at 37 °C. Panchagavya filtrate containing 25 mM copper sulphate solution starts color change after 24 h of incubation and turn dark brown. From this visual observation, it was confirmed that Panchagavya might act as reducing and stabilizing agent.

3.1. UV–vis spcectroscopy

UV- Visible spectrum of panchakavya extract mediated synthesis of copper nanoparticles shows specific Signals at 360 nm (Fig. 1) and it has confirmed the reduction of copper nanoparticles. This peak can be assigned to the absorption of copper nanoparticles.

3.2. Fourier transform infrared spectrophotometry (FT-IR)

The FT-IR measurement of the Panchagavya sample and copper nanoparticles are shown in the Fig. 2 respectively. The Panchagavya filtrate showed at the peak 3398,1590, 1424,1124,870,1045,780 cm^{-1}. The peak at 3398 was due to the presence of O-H stretching of alcohol/phenols. The peak at 1590 cm^{-1} indicates the aromatic C=C bending. The peak at 1424 showed the C-C stretching of aromatics. The peak at 1124 cm^{-1} was due to presence of C-N stretch of aliphatic amines. The peak at 870 cm^{-1} corresponds to C-H stretching of aromatics. C-N stretching of aliphatic amines were obtained at the peak 1045 cm^{-1} the peak at 780 cm^{-1} showed C-cl stretch of alkyl halides. The FT-IR spectrum of synthesized copper nanoparticles at the peaks

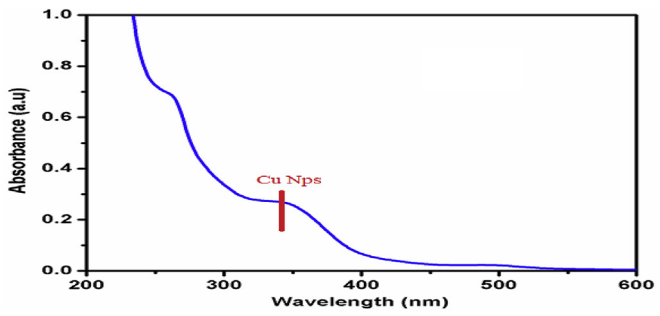


Fig. 1. UV–Visible spectroscopy of Copper nanoparticles.

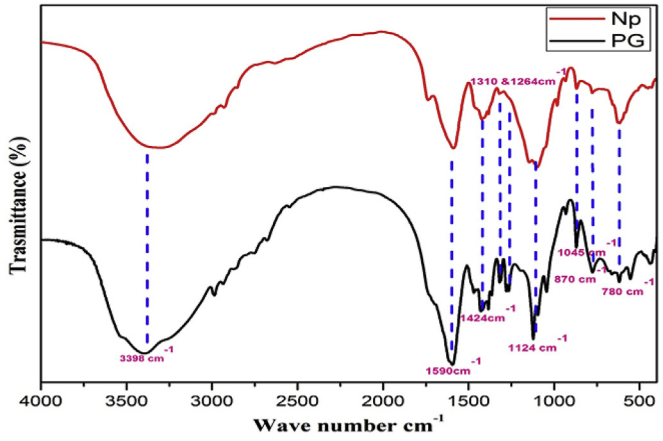


Fig. 2. FTIR spectrum of Copper Nanoparticle (NP) & Panchagavya (PG).

3398,1590,1310,1264,780 cm^{-1}. The peak at 3398 cm^{-1} correspond to O-H stretching of alcohol/phenols.The peak at 1590 showed the aromatic C=C bending. The peak at 1310 cm^{-1} was due to the presence of N-O symmetric strech of nitro compounds. The peak at 1264 indicates C-H wag of alkyl halides. The peak at 780 cm^{-1} showed C-cl strech of alkyl halides. The molecules present in the Panchagavya filtrate were responsible for the reduction of copper ions to copper nanoparticles by their reducing and capping agent.

3.3. X-Ray diffraction (XRD) analysis

The crystalline nature of Copper nanoparticles was confirmed by the analysis of XRD pattern. Fig. 3 shows three distinct peaks at 2θ degree at 28.72°, 47.59°, and 56.6°corresponding lattice indexed at (111), (200) and (220) of face centered cubic (FCC) structure of Copper nanoparticles respectively. The peaks match with the Joint Committee of powder Diffraction Standards (File No. 089–2838), which further proves the formation of crystals of copper nanoparticles.

3.4. Laser Raman

In the laser Raman spectrum the major peak was raised from the 480–495 cm^{1}. The linear peak appeared at 485 cm^{1} and 493 cm^{1} which confirms the presence of Cu Nps Fig. 4.

3.5. Scanning electron microscopic analysis (SEM)

SEM analysis is used to determine the size and shape of nanoparticles. The SEM images reveal that particles are well dispersed.

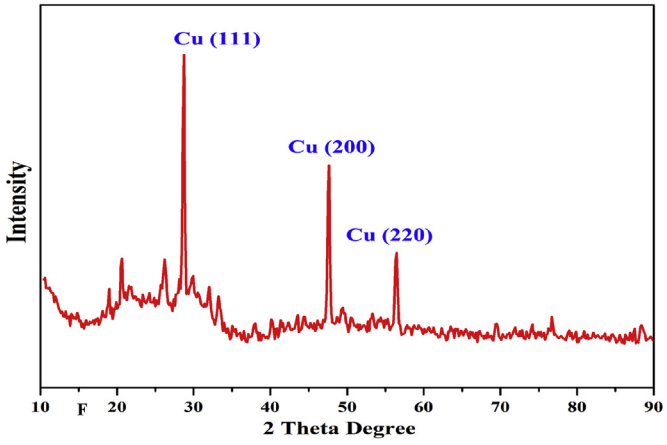


Fig. 3. XRD Patterns of copper nanoparticles.

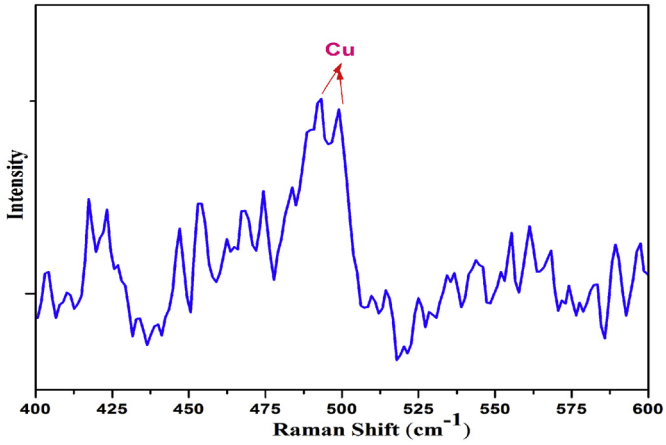


Fig. 4. Laser Raman spectrum of nanoparticles.

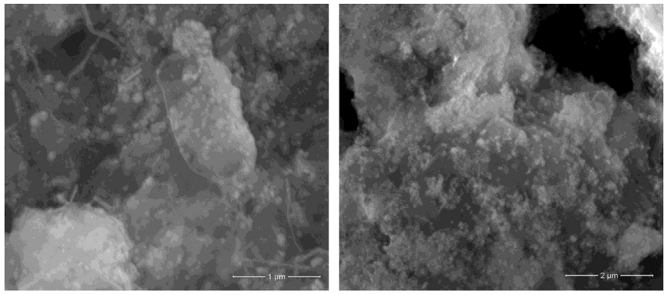


Fig. 5. SEM images of copper nanoparticle.

Structure of prepared copper nanoparticles is irregular and spherical. The images were recorded at the range of 1 µm–2 µm, The SEM image reveals that nanoparticles are not in physical contact but are separated by uniform inter particle distance, which was confirmed by microscopy visualizing under higher resolution (Fig. 5).

3.6. High Resolution Transmission Electron Microscopy (HR-TEM)

The morphology and size of the resultant copper nanoparticles prepared using Panchagavya were elucidated with the help of TEM as shown in (Fig. 6) Nanoparticles observed from the micrograph majority are well organized and spherical with a mean size of 20 and 50 nm, respectively. The corresponding size indicated in the TEM image is in good agreement with crystalline properties of the synthesized copper nanoparticles.

3.7. X-ray Fluorescence (XRF)

XRF presents the potential for a reliable uniformity compositional analysis of nano scaled particles deposited on the top of an optically flat substrate. The presence of Copper has shown very large peak due to the interaction of sample atoms with the radiation of the X-ray and some of the small disturbance peaks has seen it is due to the presence of proteins present in the Panchagavya extract. The presence of copper was confirmed in the peak shown in the (Fig. 7).

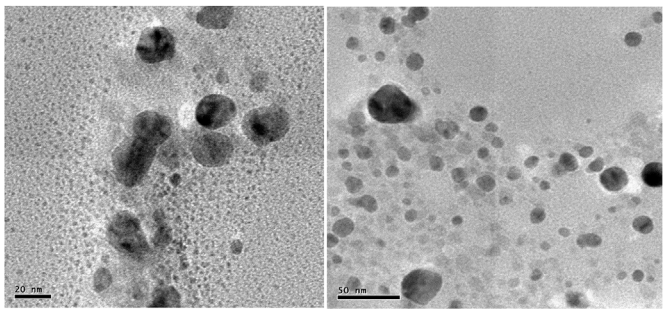


Fig. 6. TEM images of copper nanoparticles.

3.8. Dynamic light scattering (DLS)

The zeta potential spectra for the copper nanoparticles were recorded zeta potential verses intensity spectra with zeta potential (mV) on x-axis and intensity (a.u) on y-axis. Particle size of synthesized sample is 98.9 nm (nm) with zeta potential of −34.4 mV was recorded for the zinc oxide nanoparticles synthesized from bark extract (Fig. 8a and b) signifies the presence of repulsive electro-static forces among the synthesized copper nanoparticles, which leads to the monodispersity of the particles.

3.9. Antioxidant activity

The antioxidant activity of CuNPs synthesized by using Panchagavya extract was evaluated by DPPH scavenging assay. The values were increased in a dose dependent manner. The results of DPPH assay at different concentrations of 20,40,60,80,100 µg/ml of CuNPs were determined and compared standard antioxidant ascorbic acid. The mean and standard of the obtained results were plotted in the graph and the inhibitory concentration of fifty percent (IC_{50}) is determined at 66.76 µg/ml (Fig. 9).

3.10. Cytotoxicity assay

The copper nanoparticles tested showed poor brine shrimp larvicidal activity. The lethality concentration (LC_{50}) of copper nanoparticles was 30ppm (µg/mL), 40ppm, and 50ppm respectively (Fig. 10). The degree of lethality was directly proportional to the concentration of the nanoparticles. Minimum mortalities (20%) were observed at a concentration of 30ppm while that of 40 and 50ppm there were no mortality. Based on the results, the brine shrimp lethality of the three different ppm of Cu was found to be concentration-dependent. The observed lethality of the Cu to brine shrimps indicated the presence of potent cytotoxicity. According to these results the CuNPs are slightly toxic (active) if it has an LC_{50} value of less than 1000 µg/mL while non-toxic (inactive) if it is greater than 1000 µg/mL. However, these effects were most likely due to the lack of food uptake since the guts were completely filled with the aggregates of copper nanoparticles.

4. Discussion

Panchagavya is the combined formulation of five products from cows: Milk, curd, ghee, urine and dung. It is a mixture of microorganisms, including Azospirillum Sp., Azatobacter Sp., Pseudomonas Sp., and many other beneficial organisms. Moreover, it also contains proteins, lipid, carbohydrates, micronutrients and antioxidants (Ganesh Kumar et al., 2006). The initial characterization of synthesized Copper nanoparticles were carried out by UV–Visible spectroscopy. The absorption band at 360 nm was indicative the formation of copper nanoparticles. Similar result was observed in the previous study (Niasari and Davar, 2009). The bioactive molecules present in Panchagavya filtrate reduce precursor and the formation of copper nanoparticles were confirmed by FTIR. The proteins present in the biological materials are involved in the reduction and stabilization of the copper nanoparticles (Govarthanan et al., 2014). XRD is commonly used for determining the chemical composition and crystal structure of a material. The XRD results shows the synthesized Copper nanoparticles were crystalline in nature. Apart from the Major peaks, there were also some other unassigned peaks seen, which might due to the existence of the phytochemicals present the sample (Rajaram et al., 2014). Laser raman

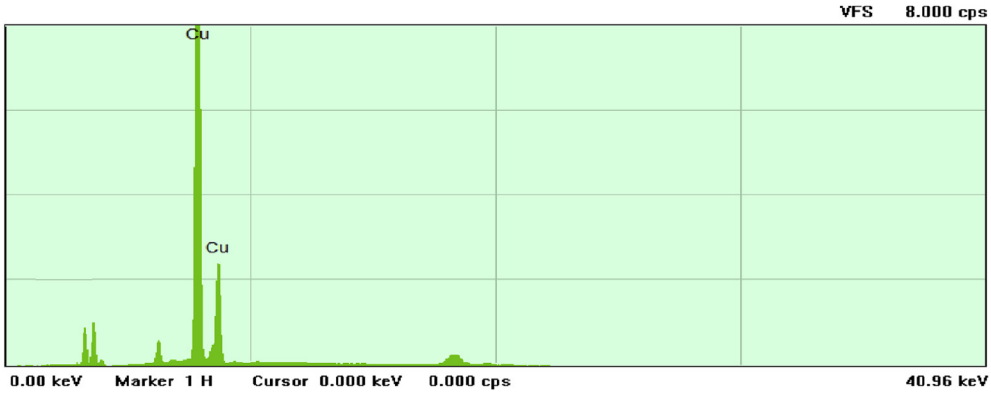
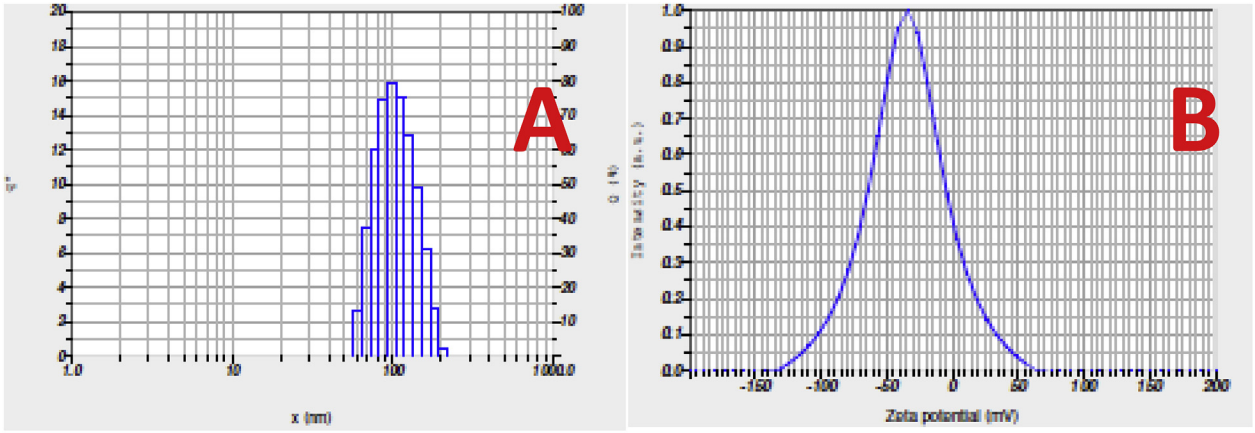


Fig. 7. XRF spectrum of copper nanoparticles.



Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	107.8 nm	29.9 nm	98.9 nm
2	—	-- nm	-- nm	-- nm
3	—	-- nm	-- nm	-- nm
Total	1.00	107.8 nm	29.9 nm	98.9 nm

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-34.4 mV	-0.000266 cm2/Vs
2	— mV	— cm2/Vs
3	— mV	— cm2/Vs

Zeta Potential (Mean) : -34.4 mV
Electrophoretic Mobility mean : -0.000266 cm²/Vs

Fig. 8. (a) Particle size analyzer. (b) Zeta potential of copper nanoparticles.

spectroscopy is one of the useful technique for the study of phases and structure of metal oxide systems. The raman shift and the bandwidth change with decreasing particle size (Nagajyothi et al., 2017). Size of the particles and zeta were determined by DLS and morphology were concluded by the SEM and TEM results, Raman intensity is related to the size of the particles, when the particle size increases the peaks become stronger and sharper (Xu et al., 1999). The XRF spectra for Copper nanoparticles gives clear evidence of the presence of copper nanoparticles. DPPH is a stable long lived nitrogen centered free radical that can readily accept an electron or hydrogen from any antioxidants

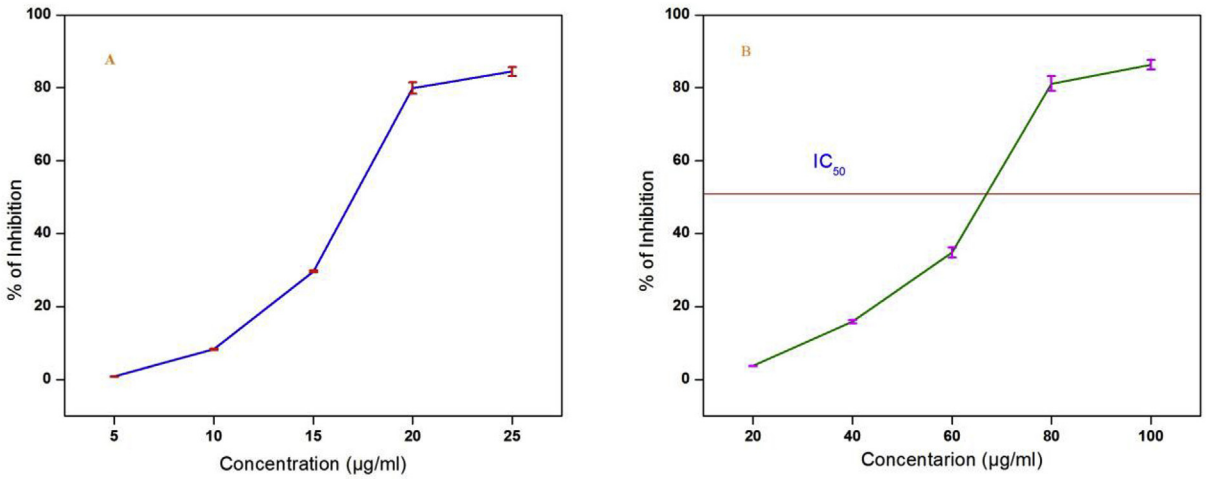


Fig. 9. DPPH assay graphs. (A).Standard Ascorbic graph. (B) Copper nanoparticle activity graph.

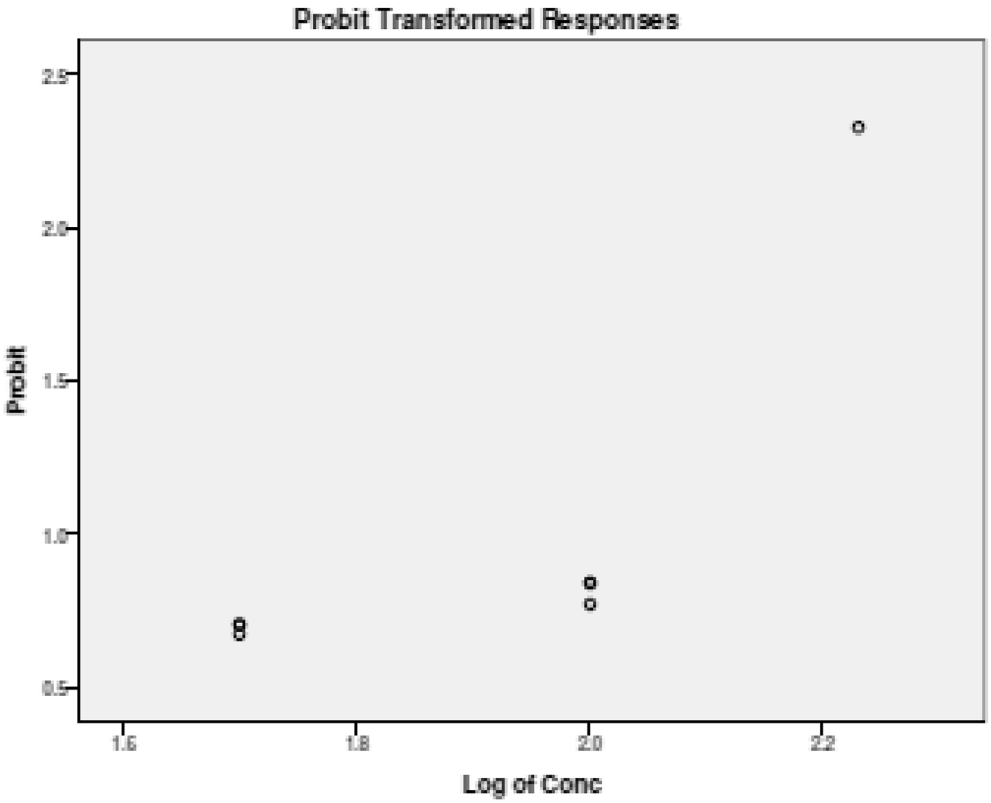


Fig. 10. LC_{50} (median lethal concentration) values were calculated using the regression line obtained by plotting the concentration against the death percentage on a probit scale, and the results were evaluated with probit analysis (SPSS 13.0).

to form non-radical DPPH. Based on this assay, a good free radical scavenging potential was observed for the biosynthesized copper nanoparticles. It was noted that Copper nanoparticles shows DPPH radical scavenging activity in a dose dependent manner (Korbekandi et al., 2012). Antioxidant activity was due to the redox potential of phytochemicals, which play an important role in quenching singlet and triplet oxygen, decomposing the peroxides or neutralizing the free radicals. The higher antioxidant activity of nanoparticles might be due to the preferential adsorption of the antioxidant material from the extract onto the surface of the nanoparticles. It is reported that Copper nanoparticles attached to the surface of the cell membrane, disturbs its function and penetrates directly with the bacterial outer membrane and release Copper ions. Where, as Brine Shrimp Lethality Assay shown very less cytotoxic results based on this we can the bio synthesized metal copper oxide nanoparticles has emerged as a low cost, simpler and better choice than physical and chemical methods. The eggs of Brine shrimp are readily available at low cost and remain viable for years in dry storage. The assay easily accommodates a large number of nauplii for statistical validation and no special equipment is needed. Moreover, this assay does not require animal serum and thereby it prevents unnecessary use of animals in scientific experiments. In summary, it is possible to measure cytotoxicity of nanoparticles using the brine shrimp lethality assay instead of the common in vitro cell culture assays (Supraja et al., 2016; Ambrosone et al., 2014).

5. Conclusion

The synthesis of copper nanoparticles have a great attention in the development of scientific product. In this present study, the synthesized copper nanoparticles are eco-friendly, simple and low cost approach. The characterization studies revealed the microstructure and crystallinity of the nanoparticles and also highlighted the role of stabilizing agents during interaction. The biosynthesized copper nanoparticles using Panchagavya extract showed significant effect on brine shrimp lethality assay can be used to study toxicity of nanostructures. Self-sufficiency and rapid results are important advantages of this method. Brine shrimp based toxicity assay of nanoparticles are cheap, continuously available, simple and reliable and are thus an important answer to routine needs of toxicity screening, for industrial monitoring requirements or for regulatory purposes. Our data are expected to facilitate pharmacological and nano-toxicological research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101132.

References

Aboofazeli, R., 2010. Carbon nanotubes: a promising approach for drug delivery. Iran. J. Pharm. Res. (IJPR) 9 (1), 1–3.

Ambrosone, A., Marchesano, V., Mazzarella, V., Tortiglione, C., 2014. Nanotoxicology using the sea anemone Nematostella vectensis: from developmental toxicity to genotoxicology. Nanotoxicology 8, 508–520. https://doi.org/10.3109/17435390.2013.802386.

Blois, M., 1958. Antioxidant Determinations by the use of a stable free radical. Nature 181, 199–1200.

Costa-Lotufo, L.V., Khan, M.T., Ather, A., Wilke, D.V., Jimenez, P.C., Pessoa, C., et al., 2005. Studies of the anticancer potential of plants used in Bangladeshi folk medicine. J. Ethnopharmacol. 99, 21–30. https://doi.org/10.1016/j.jep.2005.01.041.

Feng, T.Y., Jun, H.S., Shen, Y.S., Mo, M.L., 2013. Oxide magnetic semiconductors: material, properties and device. Chin. Phys. B 1–19 22 088505.

Ganesh Kumar, K., Kumaravelu, N., Sivakumar, T., Gajendran, K., 2006. Study on Panchagavya – an indigenous formulation and its effect on the growth promotion of crossbred pigs. Indian J. Anim. Res. 40, 158–160.

Govarthanan, M., Selvakumar, T., Manoharan, K., Rathika, R., Shanthi, K., Lee, K.J., Cho, M., Kamalakannan, S., Teak, B., 2014. Biosynthesis and characterization of silver nanoparticles using Panchagavya an indian traditional farming formulating agent. Int. J. Nanomed. 9, 1593–1599.

Insanu, M., Anggadiredja, J., Kayser, O., 2012. Curcacycline A and B–new pharmacological insights to an old drug. Int. J. Appl. Res. Nat. Prod. 5, 26–34.

Jose, G.P., Santra, S., Mandal, S.K., Sengupta, T.K., 2011. Singlet oxygenmediated DNA degradation by copper nanoparticles: potential towards cytotoxic effect on cancer cells. J. Nanobiotechnol. 9, 9.

Kanwar, A., 2007. Brine shrimp (*Artemia salina*) a marine animal for simple and rapid biological assays. J. Chin. Clin. Med. 2, 236–240.

KayalVizhi, D., Supraja, N., Devipriya, A., Prasad, T.N.K.V., Babujanarthanam, R., 2016. Evaluation of antibacterial activity and cytotoxic effects of green AgNps against breast cancer cells (MCF- 7). Adv. Nano Res. 4 (2), 129–143.

Kheiri Manjili, H., Jafari, H., Ramazani, A., Davoudi, N., 2012. Anti-leishmanial and toxicity activities of some selected Iranian medicinal plants. Parasitol. Res. 111, 2115–2121. https://doi.org/10.1007/s00436-012-3059-7.

Korbekandi, H., Iravani, S., Abbasi, S., 2012. Optimization of biological synthesis of silver nanoparticles using Lactobacillus casei sub sp. casei. J. Chem. Technol. Biotechnol. 87, 932–937.

Mclaughlin, J.L., Rogers, L.L., Anderson, J.E., 1998. The use of biological assays to evaluate botanicals. Drug Inf. J. 32, 513–524.

Mohanty, I., Rajan Senapati, M., Jena, D., Palai, S., 2014. Diversified uses of cow urine. Int. J. Pharm. Pharm. Sci. 6 (3), 975–1491.

Nagajyothi, P.C., Muthuraman, P., Sreekanth, T.V.M., Hwan Kim, Doo, shim, Jaesool, 2017. Green synthesis: invitro anticancer activity of copper oxide nanoparticles against human cervical carcinoma cells Arabian. J. Chem. 10 (2), 215–225. https://doi.org/10.1016/j.arabjc.2016.01.011.

Niasari, M.S., Davar, F., 2009. Synthesis of copper and copper (I) oxide nanoparticles by thermal decomposition of a new precursor. Mater. Lett. 63, 441–443 2009.

Nunes, B.S., Carvalho, F.D., Guilhermino, L.M., van Stappen, G., 2006a. Use of the genus *Artemia* in ecotoxicity testing. Environ. Pollut. 144 (2), 453–462.

Nunes, B.S., Carvalho, F.D., Guilhermino, L.M., Van Stappen, G., 2006b. Use of the genus Artemia in ecotoxicity testing. Environ. Pollut. 144, 453–462. https://doi.org/10.1016/j.envpol.2005.12.037.

Rajaram, K., Aiswarya, D.C., Sureshkumar, P., 2014. Green synthesis of silver nanoparticle using Tephrosia tinctoria and its antidiabetic activity. Mater. Lett. 138, 251–254. http://dx.doi.org/10.1016/j.matlet.2014.10.017.

Rajesh, M., Jayakumar, K., 2013. Changes in morphological, biochemical and yield parameters of abelmoschus esculents (L.) moench due to Panchagavya spray. Int. J. Mod. Plant Anim. Sci. 1 (2), 82–95.

Ramazani, A., Sardari, S., Zakeri, S., Vaziri, B., 2010a. In vitro antiplasmodial and phytochemical study of five Artemisia species from Iran and in vivo activity of two species. Parasitol. Res. 107, 593–599. https://doi.org/10.1007/s00436-010-1900-4.

Ramazani, A., Zakeri, S., Sardari, S., Khodakarim, N., Djadidt, N.D., 2010b. In vitro and in vivo anti-malarial activity of Boerhavia elegans and Solanum surattense. Malar. J. 9, 124. https://doi.org/10.1186/1475-2875-9-124.

Sanchez-Fortun, S., Sanz, F., Santa-Marıa, A., et al., 1997. Acute sensitivity of three age classes of *Artemia salina* larvae to seven chlorinated solvents. Bull. Environ. Contam. Toxicol. 59 (3), 445–451.

Sangian, H., Faramarzi, H., Yazdinezhad, A., Mousavi, S.J., Zamani, Z., Noubarani, M., et al., 2013. Antiplasmodial activity of ethanolic extracts of some selected medicinal plants from the northwest of Iran. Parasitol. Res. 112, 3697–3701. https://doi.org/10.1007/s00436-013-3555 4.

Schrofel, A., Kratosov, G., Safar, Ik I., Safar ikova, M., Raska, I., 2014. Shor LM. Application of biosynthesized metallic nanoparticles – a review. Acta Biomater. 10 (10), 4023–4042. https://doi.org/10.1016/j.actbio.2014.05.022.

Sorgeloos, P., 1980. Availability of reference *Artemia* cysts. Mar. Ecol. Prog. Ser. 3, 363–364.

Supraja, N., Avinash, B., Prasad, T.N.V.K.V., 2016. Antimicrobial efficacy and safety analysis of Zinc Oxide nanoparticles against water borne pathogens. Adv. Nano Res. 5 (2), 127–140.

Supriya, A.P., Ryu, C.H., Kim, H.S., 2018. Synthesis and characterization of copper nanoparticles (Cu-Nps) using rongalite as reducing agent and photonic sintering of Cu-Nps ink for printed electronics. Int. J. Precis. Eng. Manuf. Green Technol. 5 (2), 239–245.

Vanhaecke, P., Persoone, G., 1981. Report on an intercalibration exercise on a short-term standard toxicity test with *Artemia* nauplii (ARC-test). INSERM 106, 359–376.

Web Portal http://agritech.tnau.ac.in/org_farm/orgfarm_pachakavya.html.

Xu, J.F., Ji, W., Shen, Z.X., Li, W.S., Tang, S.H., Ye, X.R., Jia, D.Z., Xin, X.Q., 1999. Raman spectra of Cuo nanocrystals. J. Raman Spectrosc. 30, 413–415.