Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(2), 2273-2278 | ISSN:2251-6727

Green Synthesis, Characterization and Antimicrobial Activity of Copper Oxide Nanoparticles

K. Rajalakshmi,
Department of Chemistry, Shrimati Indira Gandhi College, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamilnadu.
J. Albino Wins,
Department of Botany, Holy Cross College (Autonomous), Nagercoil, Kanyakumari District, Tamilnadu.
M. Maria Caroline Rebellow,
PG and Research Department of Biochemistry, Dwaraka Doss Goverdhan Doss Vaishnav College (Autonomous), Chennai, Tamilnadu.
S. Carmel Punitha,
Department of Biochemistry, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Chennai, Tamilnadu.
R. Mahalakshmi,
Department of Chemistry, Kumaraguru College of Technology (Autonomous), Coimbatore, Tamilnadu.
Naga Prashant Koppuravuri,
Department of Pharmaceutical Chemistry, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B. G Nagara, Karnataka.

(Received: 07 January 2024 Revised: 12 February 2024 Accepted: 06 March 2024)

KEYWORDS
Biosynthesis, Nanoparticles, Characterization, Anti-bacterial activity.

Abstract
The objective of this study was to examine the impact of biologically produced CuO-NPs (Copper oxide nanoparticles) on the growth of bacterial strains. The physico-chemical properties of CuO-NPs were analyzed using a UV-Vis spectrophotometer, X-ray diffraction (XRD), field emission scanning electron microscope (FE-SEM), and energy-dispersive X-ray spectroscopy (EDS). The disc plate diffusion experiment was employed to assess the antibacterial efficacy of CuNPs. The work has demonstrated the potential antibacterial effects of CuO-NPs generated through biological means. These effects were observed at various doses, ranging from 10 to 100 µg/ml, against Escherichia coli and Staphylococcus aureus germs. Nanoparticles, or NPs, are particles with sizes ranging from 1 to 100 nm. These particles exhibit enhanced physical and chemical capabilities as a result of their large surface area. The current study demonstrates the potential use of biosynthesized CuO NPs for treating bacterial infectious diseases in the near future.

Introduction

Nanotechnology refers to the application of materials at the nanoscale, which is between 1 and 100 nanometers in size. These materials have unique physical, chemical, and biological properties that can be harnessed for the sake of humanity. Presently, endeavors are underway to cultivate environmentally acceptable techniques for the manufacturing of nanoparticles (NPs). The utilization and recognition of plants and microbial-based biogenesis of nanoparticles (NPs) are widely embraced and valued throughout several scientific disciplines. Copper oxide nanoparticles (CuO-NPs) belong to a group of diverse nanoparticles that exhibit a wide range of biological activities and therapeutic benefits. The utilization of green synthesis for CuO-NPs has the potential to address several limitations associated with conventional chemical synthetic approaches. The biological synthesis of Copper oxide nanoparticles yields a higher quantity compared to the chemical synthesis approach. The production of CuO-NPs has been documented using different plant-derived extracts such as Tridax procumbents, Bifurcaria bifurcate, Aloe barbadensis, soybeans, Magnolia, Euphorbia nivulia, and Punica granatum [1-5].

The current investigation involved the utilization of Fenugreek (Trigonella oenum graecum) and Indian cherry (Malpighia emarginata), which are ancient medicinal plants found in different regions worldwide, for the eco-friendly production of CuO-NPs. In previous studies, researchers have observed that Fenugreek and West Indian cherry had the capacity to act as reducing agents in the synthesis of environmentally benign silver and gold nanoparticles. Recent studies have demonstrated that Fenugreek and West Indian cherry can be used as resources for synthesizing CuO-NPs. These

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(2), 2265-2272 | ISSN:2251-6727

investigations have also revealed that these nanoparticles possess various features, including anti-tumor activity. The annual mortality rate caused by various bacterial illnesses is on the rise. An inherent challenge with current medicines is the emergence of resistance, leading to therapeutic failure. Hence, it is imperative to investigate innovative antibacterial methods that specifically target microbial cells. The current study aimed to produce copper oxide nanoparticles (CuO-NPs) utilizing extracts from fenugreek leaves and West Indian cherry fruits. The objective was to assess the antibacterial properties of these nanoparticles [6-12].

Materials and Methods

Materials

Fenugreek leaves and West Indian cherry fruits were acquired from local fruit vendors in Ambala, Haryana, India. The experimental chemicals were obtained from Sigma Aldrich Chemicals, India and E-Merck India. The bacterial cell cultures were obtained from the Microbial Type Culture Collection (MTCC) located in Chandigarh, India.

Preparation of reducing extract

The phenolic-rich extract from both plant sources was made using the method described by Kim and Lee (2002), with appropriate modifications. In summary, 100 grams of recently harvested fenugreek leaves and the edible part of the fruit were combined with 100 milliliters of methanol individually. The pre-prepared components were placed into a blender and blended at a high speed for 3 minutes while maintaining a controlled temperature. The pulverized substances were subjected to sonication in a 50 ml solution of 80% methanol (aqueous) for a duration of 20 minutes. In addition, both mixes were filtered using two strainers with different pore diameters. The accumulated residues were further extracted in 100 ml of methanol and subsequently filtered using Whatman no. 2 filter paper. The filtrates from both plant sources were combined and then transferred to a rotary evaporator with a capacity of 1000 ml. 80 ml of methanol (Aq.) was added to the rotary evaporator. Methanol was evaporated under vacuum, and the concentrated aqueous extract was then resuspended in 100 ml of deionized water. The mixture was stored at -20 °C for further testing [13].

Synthesis of CuO NPs

A 0.1 M solution of CuSO4 in 30 ml of deionized water was subjected to treatment with 25 ml of a reducing plant extract. The solution was thoroughly blended, after which 10 ml of NaOH (0.1 M) was added. The mixture was agitated incessantly at a temperature of 55 °C for a duration of 2 hours. Subsequently, it was subjected to centrifugation and the resulting pellet was dried using ambient air. A jet-black pigment was stored in a sterile environment.

Analysis of CuO NPs

The produced nanoparticles were first analyzed using a Perkin Elmer Lambda 20 UV-visible spectrophotometer to obtain their UV-visible absorption spectra in the range of 250-800 nm. To determine the phase purity of the produced nanoparticles, we conducted XRD analysis using a PAN analytical X'Pert Pro diffractometer. The examination was performed with Cu Kα (λ= 1.5406 Å) radiation at 45 kV and 40 mA, covering a 2 θ range of 30-80 °. In addition, the diameters of the crystallites (D) in the sample were determined using the Scherrer formula (equation 1). The morphological and size-related characteristics of the produced nanoparticles (NPs) were analyzed using a Field Emission Scanning Electron Microscopy (FE-SEM) instrument called Sigma from Carl Zeiss. The instrument was equipped with an Energy Dispersive Spectroscopy (EDS) setup.

Assessment of anti-bacterial activity Bacterial Cell culture and disc diffusion assay

The antibacterial efficacy of the produced copper nanoparticles was examined against Gram-negative bacteria (Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus). The microbiology labs cultivated these germs that are harmful to humans. Every bacteria was grown separately on individual Petri dishes. Additionally, the bacteria were cultured on a nutrient agar slant (stationary culture) for 24 hours at a temperature of 37 degrees Celsius. In order to utilize bacterial cell culture for the experiment, the cultures were introduced into a new nutrient agar medium and left to incubate overnight in a shaker incubator. Subsequently, I put a volume of 1 milliliter from each of these bacterial strain cultures onto nutritional agar that had hardened. After the plates were prepared, discs containing different concentrations (20, 40, 60, 80, and 100 µg) of CuONPs were placed on top of the Nutrient agar plates. The plates were placed in an incubator at a temperature of 37 °C for a duration of 24 hours to allow for the diffusion of the sample. After the incubation period, the diameter of the zones of inhibition developed around the disc was measured [14-19].

Results and Discussions

Analysis (UV-Vis, XRD, FESEM and EDS) of CuO NPs

Figure 1 displays the UV-visible spectrum of CuO-NPs generated using green methods. The CuO-NPs that were

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(2), 2265-2272 | ISSN:2251-6727

produced through biosynthesis exhibit a single absorption peak at 265 nm and a second, less intense but broader resonance peak at approximately 670 nm. These characteristics suggest the successful creation of CuO-NPs. The peak observed at 265 nm is a result of inter-band transition of the core electrons of copper metal. On the other hand, the peak around 670 nm corresponds to the band edge transition of CuO.

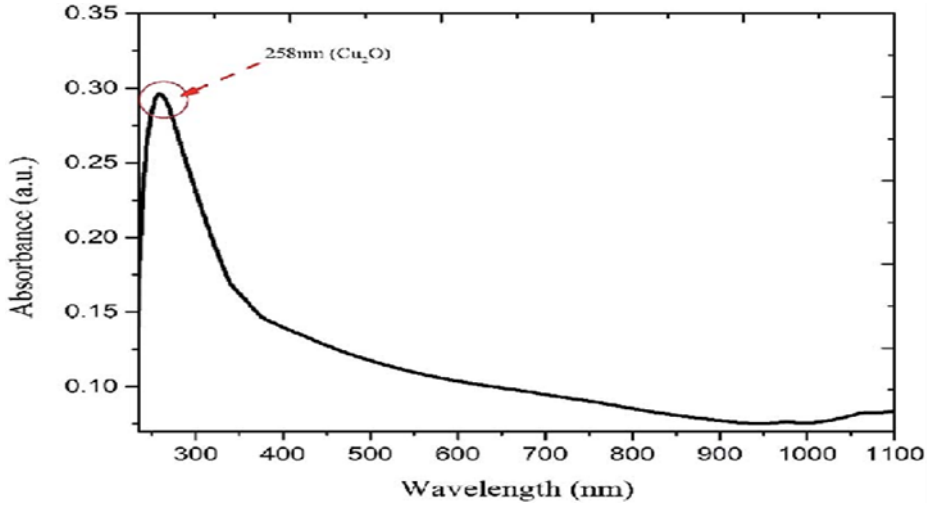

Fig. 1: UV-visible absorption spectrum of the as-synthesized copper oxide

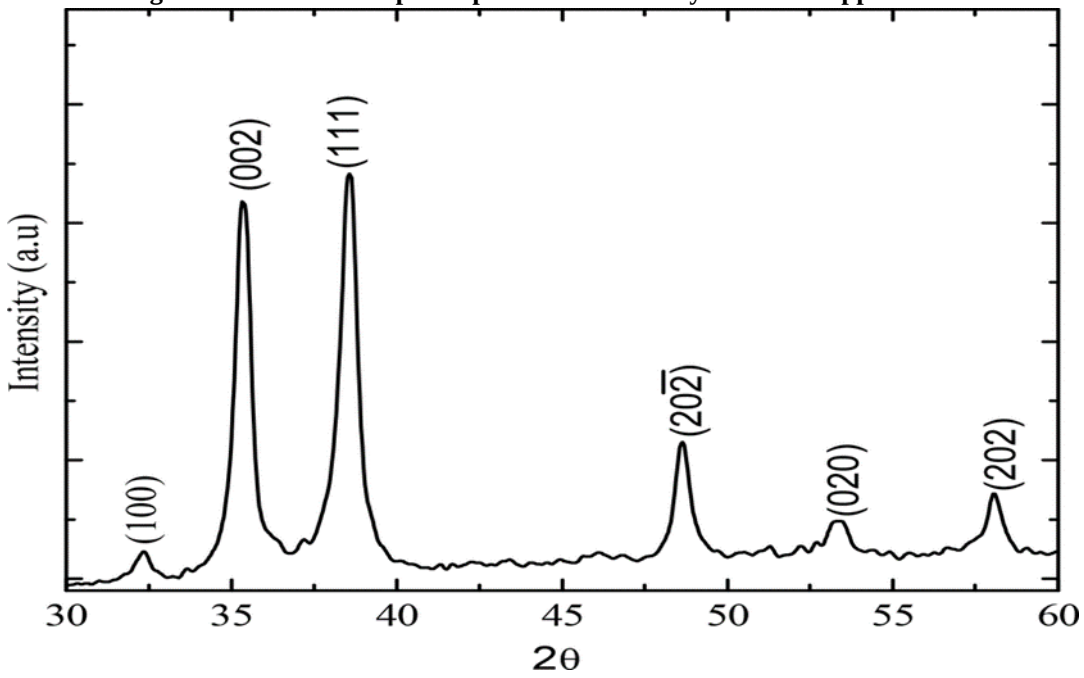

Fig. 2: XRD spectrum of CuO-NPs.

Figure 2 displays an XRD diffractogram of CuO-NPs, exhibiting distinct and intense peaks at 2θ values of 32.10, 35.40, 38.20, 4840, 53.50, 58.10, 61.20, 65.50, and 67.30, corresponding to the marked indices of (110), (002), (111), (202), (020), (202), (113), (022), and (113) correspondingly. The data clearly demonstrate the presence of CuO-NPs with a high degree of crystallinity. The field emission scanning electron microscopy (FESEM) image of the produced CuO-NPs (figure 3) displayed the presence of slightly clustered spherical nanoparticles. The NPs have a diameter ranging from 20 to 80 nm. The energy dispersive X-ray examination (EDX) of CuO-NPs (fig. 4) revealed distinct peaks corresponding to the elements Cu and O, thereby confirming the synthesis of CuO-NPs. An increase in carbon levels was also detected as a result of using carbon tape to attach the sample to an aluminum stub prior to examination.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(2), 2265-2272 | ISSN:2251-6727

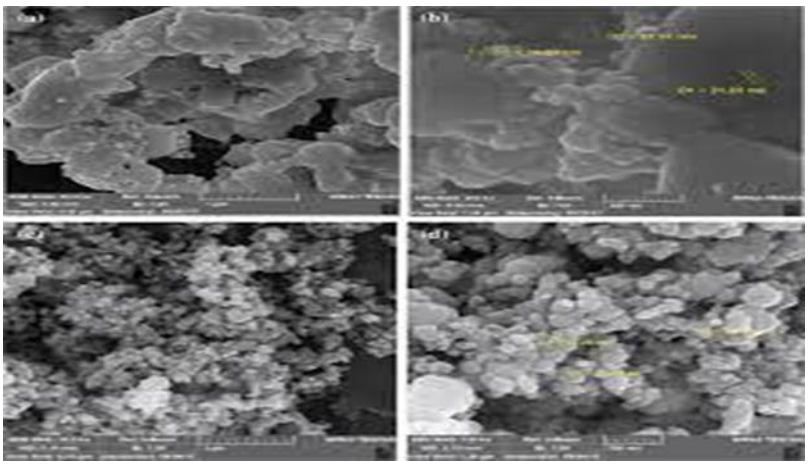

Fig. 3: FE-SEM images of CuO-NPs.

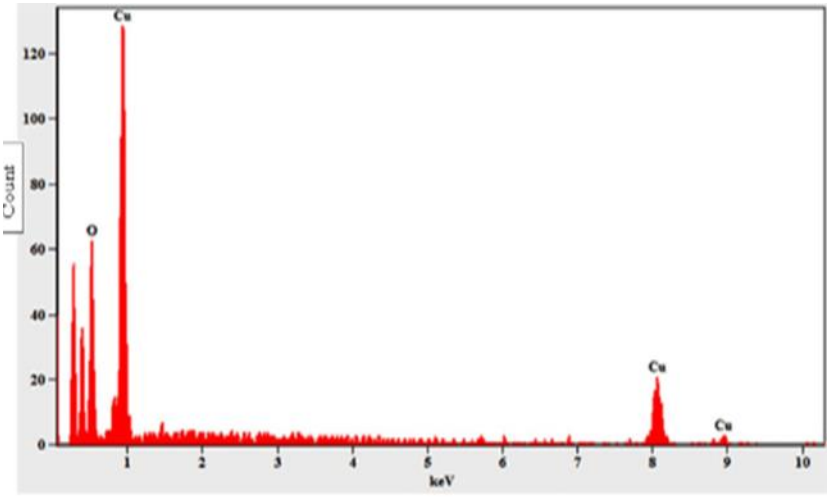

Fig. 4: EDX spectrum of CuO-NPs.

Antibacterial activity of copper oxide nanoparticles

The bacterial cells were subjected to various doses of produced CuO-NPs for a duration of 24 hours, whereas the control group was not exposed to the drug. An anti-bacterial action of CuO-NPs was detected on both bacterial strains, with the concentration required to inhibit 50% of bacterial growth (IC50) being 56 µg/ml for E. Coli and 46 µg/ml for S. aureus. The highest level of growth inhibition caused by CuO-NPs was observed at a dose of 100 µg/ml [20].

Discussion

The objective of this study was to manufacture CuO-NPs and evaluate their antibacterial properties against both Gram-positive and Gram-negative bacterial cultures. The CuO-NPs were effectively synthesized from extracts of Fenugreek and West Indian cherry. Several medicinal benefits of Fenugreek and West Indian cherry have already been documented. The use of Fenugreek Leaves and West Indian cherry extract has demonstrated a promising capacity as reducing agents in the synthesis of gold and silver nanoparticles. Hence, this article represents our initial attempt to produce physiologically active CuO-NPs by utilizing fenugreek leaves and West Indian cherry extract, to the best of our understanding. The analytical findings of the produced nanoparticles were consistent with previously published publications. Recent reports indicate that metallic copper nanoparticles, which are enveloped by copper oxide shells, exhibit an absorption peak at 670 nm. Furthermore, the d-spacing values of the X-ray diffraction planes correspond precisely to the Cu (JCPDS no. 71-4610) structures [5, 20]. An extensive diffraction peak of cuprite (111) was detected at a diffraction angle of 36.3°.

Moreover, the investigation of the cytotoxicity activity of nanoparticles (NPs) such as silver (Ag), gold (Au), copper (Cu), and zinc (Zn) has already garnered significant attention as potential therapy possibilities.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(2), 2265-2272 | ISSN:2251-6727

The produced CuO-NPs have significant antibacterial properties in terms of biological activities. Statistics have proved that infectious diseases are increasing extensively, and there is an urgent need for new medications to decrease the mortality rate. The CuO-NPs had a substantial inhibitory impact on bacterial cell cultures up to a concentration of 100µg/ml. The study found that CuO- NPs disrupt the bacterial cell membrane, leading to the demise of the bacterial cells. The mechanism of action of many nanoparticles is known to involve causing damage to the bacterial cell membrane. Because of its reduced dimensions, a nanoparticle offers a larger surface area, which is linked to the production of reactive oxygen species (ROS) within cells. These ROS include the superoxide anion, hydroxyl radical, and hydrogen peroxide.

In addition, CuO-NPs are also recognized for their ability to suppress the activity of the B-lactamase enzyme, which is responsible for conferring antibiotic drug resistance to bacterial cells. Prior studies utilizing bacterial cell cultures have shown that the increased toxicity of nanoparticles (NPs) can also be attributed to their capacity to inhibit drug efflux pumps. CuO-NPs were recently produced utilizing Desmodium gangetieum root extract in a cost-effective and environmentally friendly manner. The XRD investigation has verified the crystalline nature of the material, specifically its cubic structure, and determined that it has an average diameter of 1.46 nm. A different study involved the synthesis of CuO-NPs using the sol-gel method, followed by their characterization using X-ray diffraction (XRD) and transmission electron microscopy (TEM) techniques. The interaction between CuO-NPs and bovine serum albumin is confirmed by fluorescence quenching. The current disc diffusion assay findings have demonstrated the wide range of antibacterial activity of CuO-NPs against E. coli and S. aureus strains. The outcomes of our anti-bacterial experiments were in line with the findings reported in prior publications [21-23].

Conclusion

The current investigation showcases the successful synthesis of CuO-NPs using extracts derived from Fenugreek Leaves and West Indian cherry. The findings indicated that the CuO-NPs produced through biological means exhibit strong antibacterial properties against harmful bacterial strains that affect humans. The inhibitory effect of CuO-NPs on growth may be attributed to their interaction with the bacterial cell membrane, leading to the inactivation of β-lactamases and efflux pumps. Thus, CuO-NPs can be regarded as a powerful and cost-effective antibacterial agent. Naturally, thorough research must be conducted prior to their implementation in a clinical environment.

References

1. Tuli HS, Kashyap D, Bedi SK, Kumar P, Kumar G, Sandhu SS. Molecular aspects of metal oxide nanoparticle (MO-NPs) mediated pharmacological effects. Life Sci 2015;143:71-9.
2. Guin R, Banu AS, Kurian AG. Synthesis of copper oxide nanoparticles using desmodium gangeticum aqueous root extract; 2015.
3. K Gopalakrishnan, C Ramesh, V Ragunathan, M Thamilselvan. Antibacterial activity of Cu2O nanoparticles on E. coli synthesized from tridax procumbens leaf extract and surface coating with polyaniline. Digest J Nanomaterials Biostructures 2012;7:833-9.
4. Y Abboud, T Saffaj, A Chagraoui, A El Bouari, K Brouzi, O Tanane, *et al.* Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (Bifurcaria bifurcata). Appl Nanosci 2014;4:571-6.
5. S Gunalan, R Sivaraj, R Venckatesh. Aloe barbadensis miller mediated green synthesis of mono-disperse copper oxide nanoparticles: optical properties. Spectrochim Acta Part A 2012;97:1140-4.
6. PV Kumar, U Shameem, P Kollu, R Kalyani, S Pammi. Green synthesis of copper oxide nanoparticles using Aloe vera leaf extract and its antibacterial activity against fish bacterial pathogens. BioNanoSci 2015;5:135-9.
7. MJ Guajardo Pacheco, J Morales Sanchez, J Gonzalez Hernandez, F Ruiz. Synthesis of copper nanoparticles using soybeans as a chelant agent. Mater Lett 2010;64:1361-4.
8. P Kumar, VS Kundu, S Kumar, B Saharan, V Kumar, N Chauhan. Hydrothermal synthesis of Cu-ZnO-/TiO 2-based engineered nanomaterials for the efficient removal of organic pollutants and bacteria from water. BioNanoSci 2017;7:574-82.
9. M Valodkar, RN Jadeja, MC Thounaojam, RV Devkar, S Thakore. Biocompatible synthesis of peptide capped copper nanoparticles and their biological effect on tumor cells. Materials Chem Physics 2011;128:83-9.

10. P Kaur, R Thakur, A Chaudhury. Biogenesis of copper nanoparticles using peel extract of punica granatum and their antimicrobial activity against opportunistic pathogens. Green Chem Lett Rev 2016;9:33-8.
11. Demuth B, Farkas A, Pataki H, Balogh A, Szabo B, Borbas E, *et al.* Detailed stability investigation of amorphous solid dispersions prepared by single-needle and high speed electrospinning. Int J Pharm 2016;498:234-44.
12. El Bairi K, Ouzir M, Agnieszka N, Khalki L. Anticancer potential of Trigonella foenum graecum: cellular and molecular targets. Biomed Pharm 2017;90:479-91.
13. Alvarez Suarez JM, Giampieri F, Gasparrini M, Mazzoni L, Santos Buelga C, Gonzalez Paramas AM, *et al.* The protective effect of acerola (Malpighia emarginata) against oxidative damage in human dermal fibroblasts through the improvement of antioxidant enzyme activity and mitochondrial functionality. Food Function 2017;8:3250-8.
14. Belwal T, Devkota HP, Hassan HA, Ahluwalia S, Ramadan MF, Mocan A, Atanasov AG. Phytopharmacology of acerola (Malpighia spp.) and its potential as functional food. Trends Food Sci Technol 2018;74:99-106.
15. Hussein NH, Shaarawy H, Hawash S, Abdel Kader AE. Green synthesis of silver nano particles using fenugreek seeds extract. J Eng Appl Sci 2018;13:417-22.
16. Fragoon A, Frah L, Mamoun A. Biosynthesis of gold nanoparticles by fenugreek (Trigonella foenumgraecum) extract. Adv Sci Technol Eng Syst J 2016;1:50-5.
17. Acharyulu N, Dubey R, Swaminadham V, Kalyani R, Kollu P, Pammi S. Green synthesis of CuO nanoparticles using phyllanthus amarus leaf extract and their antibacterial activity against multidrug resistance bacteria. Int J Eng Res Sci Technol 2014;3:639-41.
18. Kim DO, Lee CY. Extraction and isolation of polyphenolics. Curr Protocols Food Anal Chem 2002;6:I1. 2.1-I. 2.12.
19. Kumar A, Saxena A, De A, Shankar R, Mozumdar S. Facile synthesis of size-tunable copper and copper oxide nanoparticles using reverse microemulsions. Res Adv 2013;3:5015-21.
20. Sankar R, Manikandan P, Malarvizhi V, Fathima T, Shivashangari KS, Ravikumar V. Green synthesis of colloidal copper oxide nanoparticles using carica papaya and its application in photocatalytic dye degradation. Spectrochim Acta Part A 2014;121:746-50.
21. Feris K, Otto C, Tinker J, Wingett D, Punnoose A, Thurber A, *etal.* Electrostatic interactions affect nanoparticle-mediated toxicity to gram-negative bacterium pseudomonas aeruginosa PAO1. Langmuir 2009;26:4429-36.
22. Hosseinkhani P, Zand A, Imani S, Rezayi M, Rezaei Zarchi S. Determining the antibacterial effect of ZnO nanoparticle against the pathogenic bacterium, Shigella dysenteriae (type 1). Int J Nano Dimens 2011;1:279-85.
23. Jayaraman R. Antibiotic resistance: an overview of mechanisms and a paradigm shift. Curr Sci 2009;96:1475-84.