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ORIGINAL ARTICLE

Biofabrication of copper oxide nanoparticles using Andean blackberry (*Rubus glaucus* Benth.) fruit and leaf

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Abstract Biofabrication of copper oxide nanoparticles (CuO-NPs) of a desired size remains a significant challenge. In this report, CuO-NPs were fabricated by treating 10 mM copper nitrate with Andean blackberry fruit (ABF) and leaf (ABL); and evaluated its antioxidant activity. As-prepared NPs characterization were determined by UV–visible spectrophotometry, Dynamic Light Scattering (DLS), transmission electron microscopy (TEM) with selected area electron diffraction (SAED) and X-ray diffraction (XRD) analysis. UV–visible spectroscopy showed an electronic excitonic transition at 250–255 nm clearly reveals the formation of ABF and ABL CuO-NPs. DLS analysis demonstrated mean diameter of ABF CuO-NPs (43.3 nm) smaller than ABL CuO-NPs (52.5 nm). TEM with SAED confirmed the CuO-NPs are spherical and of partial crystalline nature. Furthermore, the antioxidant efficacy of ABF CuO-NPs showed 89.02%, 1 mM whereas ABL CuO-NPs 75.92%, 1 mM against 1,1-diphenyl-2-picrylhydrazyl. From the results obtained it is suggested that green CuO-NPs could be used effectively in future biomedical concerns.

1. Introduction

Nanotechnology with fabrication of metal nanoparticles (MNPs) using high-efficiency and low-cost biological sources are of interest to chemists, biologists and material scientists. Among all MNPs, copper oxide nanoparticles (CuO-NPs) have gained considerable attention in the past two decades due to their simplicity and exhibit a range of potentially useful physical properties, depending strongly on their shape, size, and composition [1]. They are found to be potentially useful in different materials, such as gas sensors, dye sensitized solar cells (DSSCs) [2], paints, plastics, filters and textiles, to provide antimicrobial properties [3,4], etc. In the medical field, CuO-NPs are of high interest due to the lower probability of microbial resistance against this type of biocide [5]. The production of metal based nanoparticles by chemical and physical methods is not ecofriendly [6,7]. Hence, ecofriendly methods for

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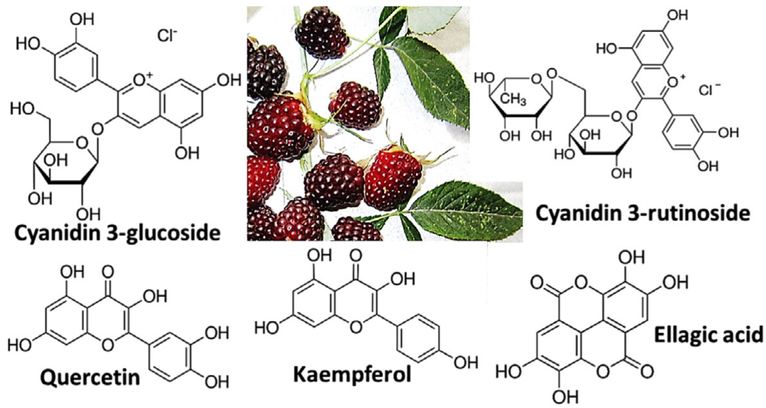

Figure 1 Major phytochemical of Andean blackberry fruit and leaf.

MNPs synthesis using the fruits [8], agricultural wastes [9], microorganisms [10], fungi extract [11], enzymes [12], leaf extracts [13], etc. are given much attention. In literature, biofabrication of CuO-NPs using magnolia leaf/plant extract [14], yeast, and fungi [15], algae [16], tea leaf and coffee powder [17] has been already reported.

Rubus species, commonly referred as the blackberry (Figure 1) has been used in day to day life because of their nutritional and medicinal importance [18]. The Andean blackberry (*Rubus glaucus* Benth.) is a naturally occurring dark-red color, juicy, and flavored fruit, native to the Andean region of South and Central America, mainly Ecuador and Colombia [19]. It is highly appreciated for food industry products such as jam, juice, frozen pulp and to a minor extent, wines. The main phenolic composition, consists of ellagitannins and anthocyanins [20,21]. The blackberry leaves have been used for their hypoglycemic activities, astringent, antidiarrheic, and as an anti-inflammatory agent for the mucous membrane of the oral cavity and throat [22]. It contains a notable amount of flavonoids, ellagic acid and tannins [23].

The aims of this study were to investigate the biofabrication of CuO-NPs using Andean blackberry fruit (ABF)/leaves (ABL) and *in vitro* evaluation of its antioxidant efficacy against 1, 1-diphenyl-2-picrylhydrazyl (DPPH·). The results would help to utilize as synthesized CuO-NPs effectively in future biomedical concerns.

2. Materials and methods

2.1. Synthesis of copper oxide nanoparticles

All chemical reagents were used without any purification. Copper Nitrate ($Cu(NO_3)_2$. $3H_2O$, 99.0%) was purchased from Spectrum (USA) and ABF/ABL were collected from the open market near Universidad de las Fuerzas Armadas-ESPE, Sangolqui, Ecuador. 1, 1-diphenyl-2-picrylhydrazyl (DPPH, >99.5%) was purchased from Sigma Aldrich, USA and Milli-Q water was used in all experiments. The collected fresh Andean blackberry fruit (5 g) and leaves (5 g) were washed thoroughly with Milli-Q water; heated (65–70 °C) in 50 mL of deionized water for 60 min. After cooling, the pinkish red (ABF) and green (ABL) color extract was filtered using Whatman paper No.1. The filtrate was collected in a 50 mL Erlenmeyer flask and stored at 4 °C for further use. For green synthesis, 0.3, 0.5, 1.0 and 1.5 mL of ABF/ABL filtrate was mixed with 10 mL of 10 mM copper nitrate solution and stirred for 6 h at 75–80 °C. The synthesized CuO-NPs were further subjected for characterization studies.

2.2. Evaluation of antioxidant activity

The free radical scavenging activity of the CuO-NPs was measured by using DPPH·as a free radical model and a method adapted from Kumar et al. (2014) [13]. An aliquot (1.0–0.2 mL) of CuO-NPs (10 mM) or control and (1.0–1.8 mL) of H_2O was mixed with 2.0 mL of 0.2 mM DPPH·in absolute methanol. The mixture was vortexed vigorously and allowed to stand at 22–25 °C for 30 min in the dark. Absorbance of the mixture was measured spectrophotometrically at λ_{max} 517 nm, and the free radical scavenging activity was evaluated using Eq. (1):

$$\text{Scavenging effect (\%)} = [1 - \{\text{absorbance of sample/absorbance of control}\}] \times 100 \quad (1)$$

The scavenging percentage of all samples were plotted. The final result was expressed as % of DPPH free radical scavenging activity (mM).

2.3. Characterization of copper oxide nanoparticles

The Andean blackberry fruit and leaf extract mediated CuO-NPs were confirmed with UV–visible, single beam spectrophotometer (Thermo Spectronic, GENESYS™ 8, England, Quartz Cell, path length 10 mm and graph plotted on the Origin 6.1 program). The hydrodynamic size distributions of nanoparticles were determined using the HORIBA, Dynamic Light Scattering (DLS) Version LB-550 program. Morphology and selected area electron diffraction (SAED) pattern of nanoparticles are studied on transmission electron microscopy, TEM (FEI, TECNAI, G2 spirit twin, Holland). X-ray diffraction (XRD) studies on thin films of the nanoparticle were carried out using a PANalytical brand θ–2θ configuration (generator–detector) X-ray tube copper λ = 1.54 A° and EMPYREAN diffractometer. The FTIR-ATR spectra were collected in the transmission mode (4000–650 cm^{-1}) using a Perkin Elmer spectrophotometer (FTIR Spectrum Two).

3. Results and discussion

3.1. UV–vis study

The CuO-NPs were efficiently synthesized from ABF and ABL extract with visual observation of color change (disappearance of blue color). In Figure 2a, ABF extract shows two peaks of maximum absorption at 240 nm and 510 nm characteristic of flavonoids, ellagitannins and anthocyanins [20,21] that are very abundant in the berry fruits whereas ABL extract (Figure 2b), shows peaks at 240 nm and 360 nm which are attributed to the presence of flavonoids, ellagic acid and tannins in berry leaves [23]. Figure 2 illustrates the effect of different compositions of ABF/ABL extract on the formation of CuO-NPs. When the composition of extract increases, the absorption peaks become stronger and the maximum absorption of CuO-NPs is shifted from 250 to 255 nm. The green synthesized CuO-NPs showed absorbance spectra at 250–255 nm in UV–visible spectroscopy, which are attributed to the formation of cuprous oxide (Cu_2O)/cupric oxide (CuO) nanoparticles [16] and there was no absorption peak of ABF/ABL extract observed in this range. The UV–visible spectra did not change even after a prolonged storage. The spectra clearly agree with the result enlightens that the synthesized nanoparticles were found to be symmetrical, with spherical in nature [16,17,24].

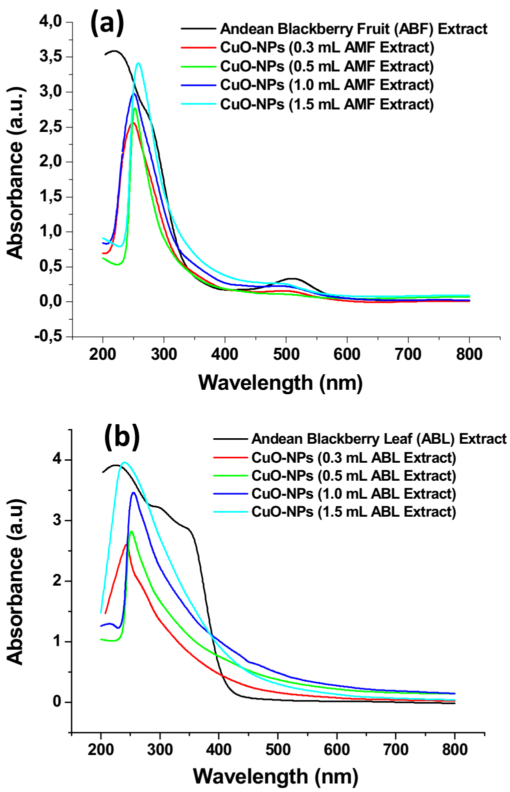

Figure 2 UV–Vis spectra of CuO-NPs using (a) ABF and (b) ABL.

3.2. DLS study

The DLS analysis was used to find out the size of nanoparticles. Figure 3a and b shows green synthesized CuO-NPs using ABF and ABL average particle size distribution were 43.3 and 52.5 nm, respectively. It clearly indicates that the ABF extract was more efficient than the ABL for the synthesis of smaller CuO-NPs.

3.3. TEM-SAED study

The morphology and size of the resultant CuO-NPs prepared using ABF and ABL were elucidated with the help of TEM as shown in Figure 4a–f. Nanoparticles observed from the micrograph majority are well organized and spherical with a mean size of 45 and 53 nm, respectively. The corresponding

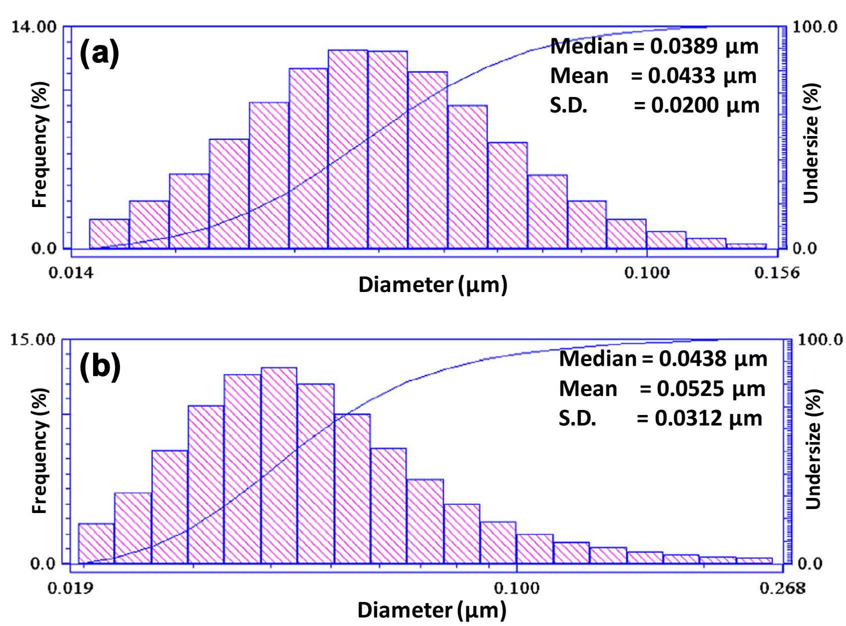

Figure 3 Histogram of the particle size distribution of CuO-NPs synthesized from the (a) ABF and (b) ABL.

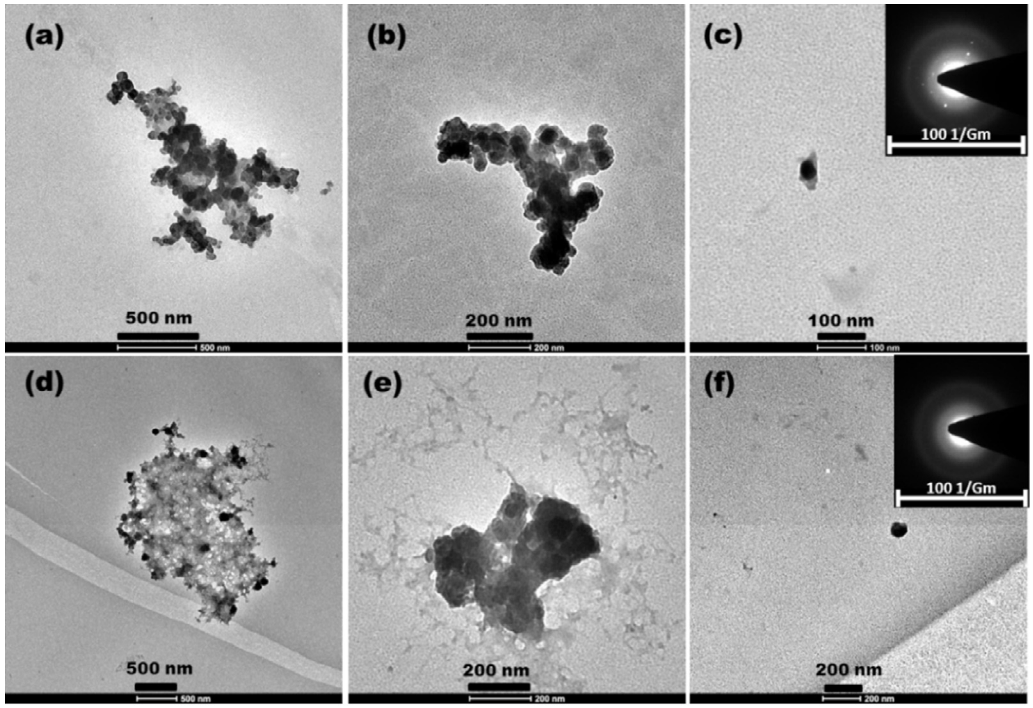

Figure 4 TEM-SAED images of synthesized CuO-NPs using ABF (a–c) and ABL (d–f).

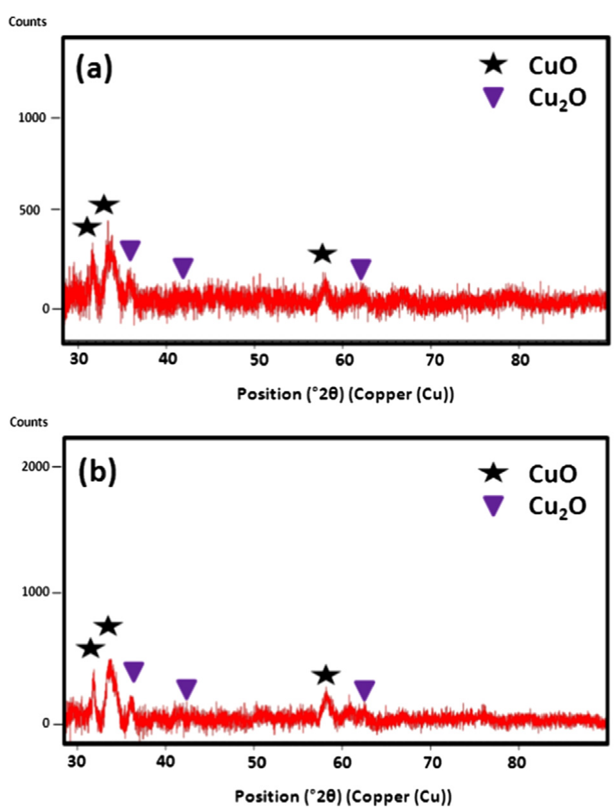

Figure 5 XRD spectrum of CuO-NPs synthesized from the (a) ABF and (b) ABL.

size indicated in the TEM image is in good agreement with the DLS size (Figure 3a and b). The presence of SAED pattern (Figure 4d and f) indicated the partial crystalline properties of the synthesized CuO-NPs. These properties could enhance the diffraction contrast due to their orientation with respect to the electron beam.

3.4. XRD study

Figure 5 shows the XRD patterns of the as-prepared CuO-NPs using ABF and AML extract. XRD analysis revealed small distinct diffraction peaks at 31.85°, 34.06° and 58.57° for the CuO. The weak diffraction peak at 36.21° and 42.28° indexed the planes (1 1 1) and (2 0 0) of the face-centered-cubic structure of Cu_2O nanoparticles. The present experimental results were found to be in agreement with the reported diffraction patterns of CuO [25] and Cu_2O [26] nanoparticles.

3.5. FTIR study

FTIR analysis was carried out to determine the functional groups present in ABF/ABL extract. In Figure 6, the FTIR peaks at around 3270 and 3230 cm^{-1} correspond to O–H stretching vibrations, whereas 2933 cm^{-1} attributed to the symmetric and asymmetric C–H stretching vibration of flavonoids/phenolic, respectively [27]. The peaks at 1642 cm^{-1} correspond to the C=O whereas 1588 cm^{-1} corresponds to C=C stretch in aromatic rings. The peaks at 1408 and 1390 cm^{-1} correspond to the O–H bend of polyphenol, confirm the presence of an aromatic group [28]. Subsequently, the absorption peaks at 1013 and 1044 cm^{-1} were assigned for C–O–C and secondary –OH of the phenolic

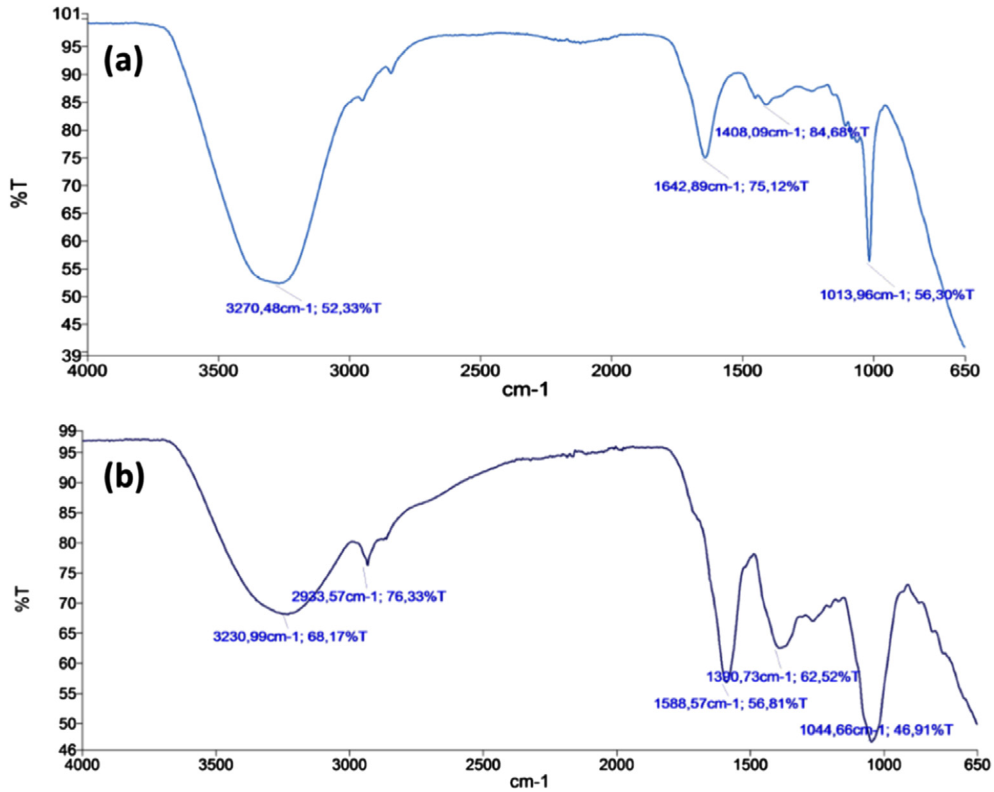

Figure 6 FTIR spectrum of the (a) ABF and (b) ABL extract.

group. It is clearly hypothesized that the O–H and C=O functional groups of the ABF/ABL may participate in the reduction process.

3.6. Evaluation of antioxidant activity

In the present study, the green synthesized CuO-NPs antioxidant potential was evaluated against DPPH with different concentration (0.5–2.5 mM) as shown in Figure 7. It was found that, scavenging activity responds to CuO-NPs with dose dependent concentration and the antioxidant efficacy of CuO-NPs synthesized by ABF is higher than ABL against DPPH·assay. The DPPH·radical scavenging activities were found to be maximum of 89.02% in 1 mM for ABF CuO-NPs whereas 75.92% in 1 mM for ABL CuO-NPs. The improved antioxidant efficacy may be due to the presence of more bioactive molecules in ABF than ABL extract, which play a role as an encapsulating agent in CuO-NPs and the CuO-NPs are spherical in shape with larger surface area. The highest antioxidant efficacy of CuO-NPs against DPPH· is probably derived, through the electrostatic attraction between negatively charged bioactive compounds (COO^-, O^-) and neutral or positively charged nanoparticles. CuO-NPs bound to the phytochemicals and their bioactivity increase synergistically. The effect of activity depends on the site of attachment of the metals and its consequent impact on the activity of the antioxidant agent [13,29].

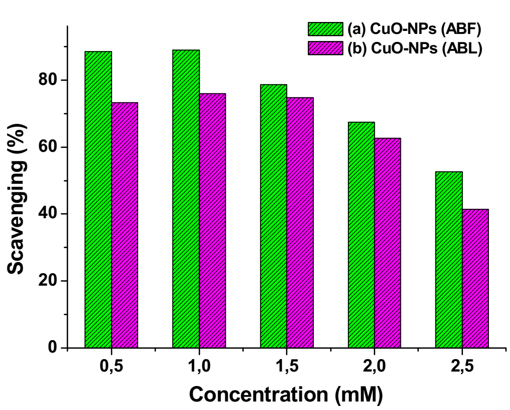

Figure 7 Antioxidant activity of synthesized CuO-NPs.

4. Conclusion

In this report, a novel method for fabrication of Cu-ONPs using non-toxic/biodegradable Andean blackberry fruit and leaf material is presented. The formation of CuO-NPs was characterized by UV–Vis, DLS, TEM and XRD. It was found that the mean diameter of CuO-NPs, synthesized by using Andean blackberry fruit (43.3 nm) was smaller than the leaf (52.5 nm). The improved antioxidant efficacy of CuO-NPs (89.02% and 75.92% for 1 mM) is observed due to encapsulation of phytochemicals and spherical shape.

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References

[1] J.M. Tranquada, B.J. Sternlieb, J.D. Axe, Y. Nakamura, S. Uchida, Evidence for stripe correlations of spins and holes in copper oxide superconductors, Nature 375 (1995) 561–563.

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

[3] G. Ren, D. Hu, E.W.C. Cheng, M.A. Vargas-Reus, P. Reip, R.P. Allaker, Characterisation of copper oxide nanoparticles for antimicrobial applications, Int. J. Antimicrob. Agents 33 (2009) 587–590.

[4] R. Dastjerdi, M. Montazer, A review on the application of inorganic nanostructured materials in the modification of textiles: focus on antimicrobial properties, Colloids Surf. B Biointerfaces 79 (2010) 5–18.

[5] A.J. Huh, Y.J. Kwon, Nanoantibiotics: a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era, J. Control Release 156 (2011) 128–145.

[6] J. Zhu, D. Li, H. Chen, X. Yang, L. Lu, X. Wang, High dispersed CuO nanoparticles prepared by a novel quick-precipitation method, Mater. Lett. 58 (2004) 3324–3327.

[7] R.V. Kumar, R. Elgamiel, Y. Diamant, A. Gedanken, J. Norwig, Sonochemical preparation and characterization of nanocrystalline copper oxide embedded in poly(vinyl alcohol) and its effect on crystal growth of copper oxide, Langmuir 17 (2001) 1406–1410.

[8] B. Kumar, K. Smita, L. Cumbal, A. Debut, Biogenic synthesis of iron oxide nanoparticles for 2-arylbenzimidazoles fabrication, J. Saudi Chem. Soc. 18 (2014) 364–369.

[9] B. Kumar, K. Smita, L. Cumbal, A. Debut, Sacha inchi (*Plukenetia volubilis* L.) shell biomass for synthesis of silver nanocatalyst. J. Saudi Chem. Soc. 21 (2017) S293–S298.

[10] V. Bansal, A. Bharde, R. Ramanathan, S.K. Bhargava, Inorganic materials using 'unusual' microorganisms, Adv. Colloid Interface Sci. 179–182 (2012) 150–168.

[11] N. Vigneshwaran, N.M. Ashtaputre, P.V. Varadarajan, R.P. Nachane, K.M. Paralikar, R.H. Balasubramanya, Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*, Mater. Lett. 61 (2007) 1413–1418.

[12] I. Willner, R. Baron, B. Willner, Growing metal nanoparticles by enzymes, Adv. Mater. 18 (2006) 1109–1120.

[13] B. Kumar, K. Smita, L. Cumbal, A. Debut, Synthesis of silver nanoparticles using sacha inchi (*Plukenetia volubilis* L.) leaf extracts, Saudi J. Biol. Sci. 21 (2014) 605–609.

[14] H.J. Lee, G. Lee, N.R. Jang, J.H. Yun, J.Y. Song, B.S. Kim, Biological synthesis of copper nanoparticles using plant extract, Nanotechnology 1 (2011) 371–374.

[15] S. Honary, H. Barabadi, E.G. Fathabad, F. Naghibi, Green synthesis of copper oxide nanoparticles using *Penicillium aurantiogriseum*, *Penicillium citrinum* and *Penicillium wakasmanii*, Dig. J. Nanomater. Biostruct. 7 (2012) 999–1005.

[16] Y. Abboud, T. Saffaj, A. Chagraoui, A. El Bouari, K. Brouzi, O. Tanane, B. Ihssane, Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown algae extract (*Bifurcaria bifurcate*), Appl. Nanosci. (2013), http://dx.doi.org/10.1007/s13204-013-0233-x.

[17] P. Sutradhar, M. Saha, D. Maiti, Microwave synthesis of copper oxide nanoparticles using tea leaf and coffee powder extracts and its antibacterial activity, J. Nanostruct. Chem. 4 (2014) 86.

[18] A.V. Patel, J. Rojas-Vera, C.G. Dacke, Therapeutic constituents and actions of *Rubus* species, Curr. Med. Chem. 11 (2004) 1501–1512.

[19] M.E. Schreckinger, J. Lotton, M.A. Lila, E.G. de Mejia, Berries from South America: a comprehensive review on chemistry, health potential and commercialization, J. Med. Food (2013) 233–246.

[20] C. Mertz, V. Cheynier, Z. Günata, P. Brat, Analysis of phenolic compounds of two blackberry species (*Rubus glaucus* and *Rubus adenotrichus*) by high-performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry, J. Agric. Food Chem. 55 (2007) 8616–8624.

[21] C. Vasco, K. Riñen, J. Ruales, A. Kamal-Eldin, Phenolic compounds in Rosaceae fruits from Ecuador, J. Agric. Food Chem. 57 (2009) 1204–1212.

[22] B. Borkowski, J. Lutomski, E. Skrzydlewska, B. Zygmunt, Rosliny Lecznicze w Fitoterapii, Poznan, IRiPZ, 1994 (pp. 470–471).

[23] J. Gudej, M. Tomczyk, Determination of flavonoids, tannins and ellagic acid in leaves from *Rubu*s L. species, Arch. Pharm. Res. 27 (2004) 1114–1119.

[24] O. Mahapatra, M. Bhagat, C. Gopalakrishnan, K.D. Arunachalam, Ultrafine dispersed CuO nanoparticles and their antibacterial activity, J. Exp. Nanosci. 3 (2008) 185–193.

[25] V.V. Thekkae Padil, M. Černík, Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application, Int. J. Nanomed. 8 (2013) 889–898.

[26] U.S. Shenoy, A.N. Shetty, A facile one step solution route to synthesize cuprous oxide nanofluid, Nanomater. Nanotechnol. 3 (2013) (2013) (Art. 5).

[27] A.K. Mittal, J. Bhaumik, S. Kumar, U.C. Banerjee, Biosynthesis of silver nanoparticles: elucidation of prospective mechanism and therapeutic potential, J. Colloid Interface Sci. 415 (2014) 39–47.

[28] K.J. Rao, S. Paria, Green synthesis of silver nanoparticles from aqueous *Aegle marmelos* leaf extract, Mater. Res. Bull. 48 (2013) 628–634.

[29] B. Kumar, K. Smita, L. Cumbal, A. Debut, Green approach for fabrication and applications of zinc oxide nanoparticles, Bioinorg. Chem. Appl. (2014) (Article ID 523869, 7).