

BIOGENIC COPPER NANOPARTICLES FROM MEDICINAL PLANTS AS NOVEL ANTIDIABETIC NANOMEDICINE

Tanay R. Bhagwat¹, Komal A. Joshi², Vijay Singh Parihar³, Adersh Asok⁴, Jayesh Bellare⁵, Sougata Ghosh^{6*}

¹Department of Microbiology, Modern College of Arts, Science and Commerce,
Ganeshkhind, Pune-411016, India.

²Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University, Pune-
411007, India.

³Department of Biomedical Sciences and Engineering, Bio Medi Tech, Tampere University
of Technology, Korkeakoulunkatu 10, 33720, Tampere, Finland.

⁴National Institute for Interdisciplinary Science and Technology, Council of Scientific and
Industrial Research, Thiruvananthapuram-695019, India.

⁵Department of Chemical Engineering, Indian Institute of Technology, Bombay, Powai,
Mumbai-400076, India.

⁶Department of Microbiology, RK University, Rajkot, Gujarat-360020, India.

Article Received on
31 Dec. 2017,
Revised on 21 Jan. 2018,
Accepted on 11 Feb. 2018
DOI: 10.20959/wjpr20184-10773

*Corresponding Author

Dr. Sougata Ghosh

Department of
Microbiology, RK
University, Rajkot, Gujarat-
360020, India.

ABSTRACT

In an attempt to design rapid, efficient and eco-friendly process to fabricate nanoparticles with potent biomedical applications, herein we report for the first time synthesis of copper nanoparticle (CuNPs) using three medicinal plants, *B. prionitis* leaf extract (BPLE), *L. chinensis* peel extract (LCPE) and *P. orientalis* leaf extract (POLE). The synthesis was completed within 5 h indicated by visible colour change and UV-visible spectroscopy. High resolution transmission electron microscopy revealed that the bio-reduced CuNPs were very small. CuNPs synthesized by BPLE were between 11 to 15 nm while for POLE they were between 90 to 200 nm. LCPE synthesized CuNPs in a

size range between 10 to 100 nm. Further the CuNPs were characterized using energy dispersive spectroscopy (EDS), dynamic light scattering (DLS) and Fourier transform infrared spectroscopy (FTIR). Both α -amylase and α -glucosidase were found to be inhibited by the CuNPs providing the strong rationale for their further use in clinical studies as candidate

nanomedicine against Type II Diabetes Mellitus.

KEYWORDS: *Barleria prionitis*, *Litchi chinensis*, *Platanus orientalis*, copper nanoparticle, α -amylase, α -glucosidase.

INTRODUCTION

Nanomedicine is one of the most emerging applications of material science and nanotechnology. One of the major challenges is biocompatibility as most of the synthesis processes involve hazardous and corrosive chemicals leading to toxicity.^[1] Hereby, there is a growing need to design environmentally benign routes for synthesis of biocompatible nanoparticles with potent medicinal importance.^[2] Among various metal nanoparticles like silver, gold, platinum, palladium, etc copper nanoparticles have gained considerable attention owing to its diverse applications as anticancer, antibacterial and antifungal agents addition to their catalytic, optical, and electrical properties.^[3] Various nanomedicines are being explored and evaluated for their efficacy to treat diabetes mellitus which is considered to be a multifactorial metabolic disorder responsible for significant morbidity and mortality due to its pathophysiological implications that include retinopathy, neuropathy, nephropathy, heart attack, stroke and peripheral vascular diseases. Type II diabetes or noninsulin-dependent diabetes mellitus (T2DM or NIDDM), is the most prevalent form of the disease, which according to World Health Organization is likely to increase up to 300 million or more by the year 2025.^[4-7] Although currently available therapies include sulfonylureas, biguanides and glinides, the associated severe adverse effects have encouraged exploration of more effective and safer hypoglycemic agents. Traditional complementary and alternative medicines from medicinal plants offer great potential for the discovery of such novel antidiabetic drugs. Targeting α -amylase and α -glucosidase is considered to be a powerful strategy for designing antidiabetic drugs.^[8-13] The spectacular success of our earlier research on fabrication of various antidiabetic nanomaterials have enabled us to design more effective, rapid, environmentally friendly methods for synthesis of such nanodrugs.

Recently, we have also demonstrated that bio-reduced copper nanoparticles exhibits potent antidiabetic activity.^[14] Hereby, it is of utmost importance to explore novel reproducible methods for synthesis of CuNPs with specific size, well defined surface composition, isolable and redispersable properties which is a very challenging task in chemical synthesis. Very little work has been carried out on the biological synthesis of CuNPs. Although bacteria, fungi, algae and plants are widely used for synthesis of various metal nanoparticles, there are

very few reports on synthesis of CuNPs using medicinal plants.^[15-19] Owing to the spectrum of various bioactive principles, medicinal plants like *Dioscorea bulbifera*, *Dioscorea oppositifolia*, *Gloriosa superba*, *Barleria prionitis*, *Gnidia glauca*, *Plumbago zeylanica*, etc are considered to be a rich source of reducing and stabilizing agents towards nanoparticle synthesis.^[20-28]

Medicinal plants like *Barleria prionitis*, *Litchi chinensis* and *Platanus orientalis* have immense significance in the traditional alternative and complementary medicine owing to their rich phytochemical composition. *B. prionitis* is known to possess antimicrobial, anthelmintic, antifertility, antioxidant, antidiabetic, anti-inflammatory, anti-arthritic, cytoprotective, hepatoprotective activity.^[20] *P. orientalis* is known to be used for treatment of opthalmia, dysentery, toothache. It is also antiseptic, antimicrobial and anticancer.^[29] Similarly, *L. chinensis* is reported to possess antioxidant, anticancer, antiinflammatory, antimicrobial, antiviral, antidiabetic, antiobesity, hepatoprotective and immunomodulatory activities.^[30,31] However, there are no reports showing their potential to synthesize CuNPs.

In view of the background, herein we have attempted the synthesis of CuNPs using three medicinal plants namely, *B. prionitis*, *L. chinensis* and *P. orientalis*. In this paper, we have characterized the bio-reduced nanoparticles followed by evaluation of antidiabetic potential by inhibition of α -amylase and α -glucosidase.

MATERIALS AND METHODS

Plant material and preparation of extract

B. prionitis leaf, *P. orientalis* leaf and *L. chinensis* peel were collected and dried in shade at room temperature for 2-3 days. The dried plant materials were pounded into fine powder in an electric blender. *B. prionitis* leaf extract (BPLE), *P. orientalis* leaf extract (POLE) and *L. chinensis* peel extract (LCPE) were prepared by boiling 5g of corresponding powder in 100 mL distilled water for 5 min. Supernatant was filtered through Whatman No.1 filter paper after centrifugation at 3000 rpm for 10 min. The filtrate was collected and stored at 4 °C for further use.^[20]

Synthesis and characterization of copper nanoparticles

5 mL of BPLE, POLE and LCPE were added separately to 95 mL of 1 mM aqueous $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution and kept under shaking condition at 150 rpm in the dark at 40°C. Visible colour change along with UV-visible spectroscopy at regular intervals on a

spectrophotometer (SpectraMax M5, Molecular Devices Corporation, Sunnyvale, CA) operated at resolution of 1 nm were used to monitor the reduction of Cu^{2+} ions to CuNPs. Bio-reduced CuNPs were characterized employing high resolution transmission electron microscopy (HRTEM), energy dispersive spectroscopy (EDS), dynamic light scattering, X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR) as per our earlier reports.^[18]

Glycosidase inhibitory activity

Porcine pancreatic amylase inhibition assay

Chromogenic 3,5-dinitrosalicylic acid (DNSA) method was used as per our earlier report to evaluate α -amylase activity.^[5] Bio-reduced CuNPs (10 $\mu\text{g}/\text{mL}$) were incubated with 50 $\mu\text{g mL}^{-1}$ of porcine pancreatic α -amylase at 37°C for 10 min followed by addition of 1% starch solution which was used as substrate. Appropriate controls were kept. Reducing sugar was estimated using DNSA assay at A 540 nm and the inhibitory activity was calculated by using the formula.

$$\% \text{ Inhibition} = (\text{A540}_{\text{Control}} - \text{A540}_{\text{Test}}) / \text{A540}_{\text{Control}} \times 100$$

α -glucosidase inhibitory assays

CuNPs were checked for α -glucosidase inhibitory activity as reported earlier.^[4] 100 μL of α -glucosidase (0.1 unit / mL) was mixed with 200 μL of CuNPs (100 $\mu\text{g}/\text{mL}$) and incubated for 1 h at 37°C. Addition of 10 mM *p*-nitrophenyl- α -D-glucopyranoside in 100 mM phosphate buffer of pH 6.8 marked the initiation of enzyme activity which was stopped by addition of 2 ml Na_2CO_3 (0.1 M) after an incubation of 10 min at 37°C. α -Glucosidase activity was determined by measuring absorbance of the *p*-nitrophenol released from *p*NPG at 420 nm. One unit of glucosidase activity is defined as the amount of enzyme that hydrolyzed 1 μM of *p*-nitrophenyl pyranoside per minute under assay condition.

$$\% \text{ Inhibition} = (\text{A420}_{\text{Control}} - \text{A420}_{\text{Test}}) / \text{A420}_{\text{Control}} \times 100$$

RESULTS

UV-visible spectroscopy

Synthesis of CuNPs was confirmed by gradual colour change on addition of plant extracts. Initially the light blue colour changed to faint yellow which later developed into intense brown colour. Simultaneous monitoring using UV-visible spectroscopy showed the increase in the intensity of the spectra with time at 40°C. In case of BPLe the synthesis was completed almost at 1 h as no significant increase in the intensity was observed between 1 to

5 h (Fig. 1A). Rate of synthesis with POLE was found to be comparatively faster as maximum synthesis was completed within 0.5 h (Fig. 1B). A significant increase in the synthesis of CuNPs was observed till 2 h with LCPE as evident from the spectra while beyond 2 h no more increase in the spectral intensity was noticed (Fig. 1C).

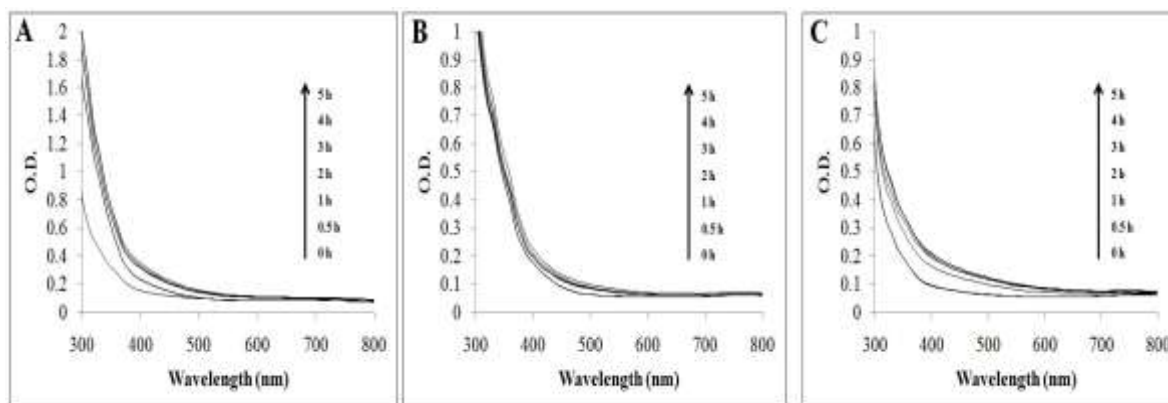


Figure 1: UV-vis spectra recorded as a function of reaction time for CuNPs formation at 40°C using (A) BPLe, (B) POLE and (C) LCPE.

HRTEM, EDS, DLS analysis

CuNPs synthesized by BPLe were found to be monodispersed and spherical in shape. The size varied between 11 to 15 nm (Fig. 2A). The particles were found to be very small and free from any agglomeration. POLE mediated bio-reduced CuNPs were found in small clusters of irregular morphology ranging between 90 to 200 nm (Fig. 2B). LCPE synthesized CuNPs were found to vary from 10 to 100 nm in size as long dispersed stretches of spherical nanoparticles (Fig. 2C). EDS analysis confirmed the presence of elemental copper in the biogenic nanoparticles synthesized using BPLe, POLE and LCPE (Fig. 3). Particle size distribution indicated in DLS was found to be in well agreement with the HRTEM analysis (Fig. 4). Widely distributed particle dimensions show the initial synthesis of smaller CuNPs which with time might grow bigger by deposition of more copper on its surface. Later, such larger aggregates were formed due to surface attraction and agglomeration of numerous smaller CuNPs.

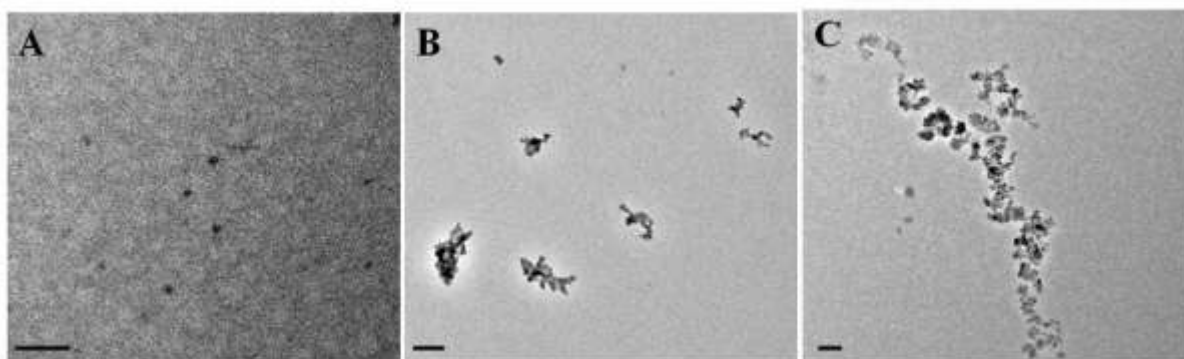


Figure 2: High-resolution transmission electron micrographs of CuNPs synthesized. (A) CuNPs bio-reduced by BPLE, inset bar representing 100 nm; (B) CuNPs bio-reduced by POLE, inset bar representing 200 nm; (C) CuNPs bio-reduced by LCPE, inset bar representing 50 nm.

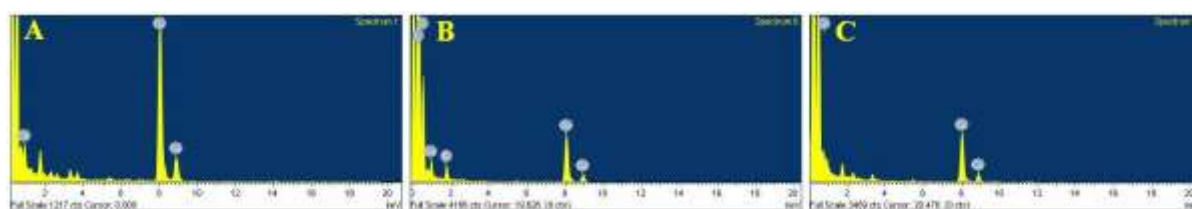


Figure 3: Representative spot EDS profile of CuNPs synthesized by (A) BPLE, (B) POLE and (C) LCPE.

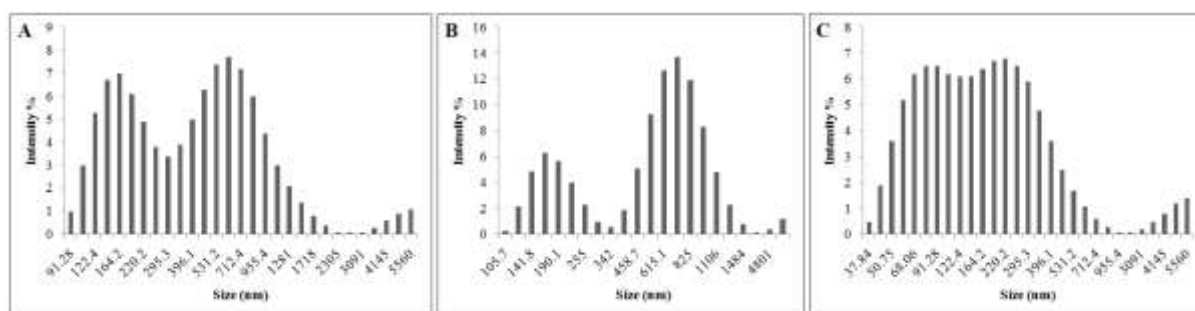


Figure 4: Histogram of size distribution of CuNPs synthesized by (A) BPLE, (B) POLE and (C) LCPE.

FTIR analysis

As evident from the FTIR spectra, the peak at 3415 cm^{-1} corresponding to O-H stretch might be attributed by the polyphenols in BPLE, POLE and LCPE (Fig. 5). Upon reduction of the Cu^{2+} to CuNPs the -OH groups of polyphenols might be oxidizing itself, thereby showing the peak at 1740 cm^{-1} that represents C=O stretch. At the same time other functional groups were also observed to be present before and after bio-reduction like $\text{C}\equiv\text{C}$ (stretch) 2366 cm^{-1} , $\text{C}=\text{C}$

(stretch) or N-H (bending) 1646 cm^{-1} , C-H (bending) 1365 cm^{-1} , C-N (stretch) or C-O (stretch) 1223 cm^{-1} , C=C (stretch) or C-H (bending) 1082 cm^{-1} which might play a significant role in capping or stabilization of the bio-reduced nanoparticles.

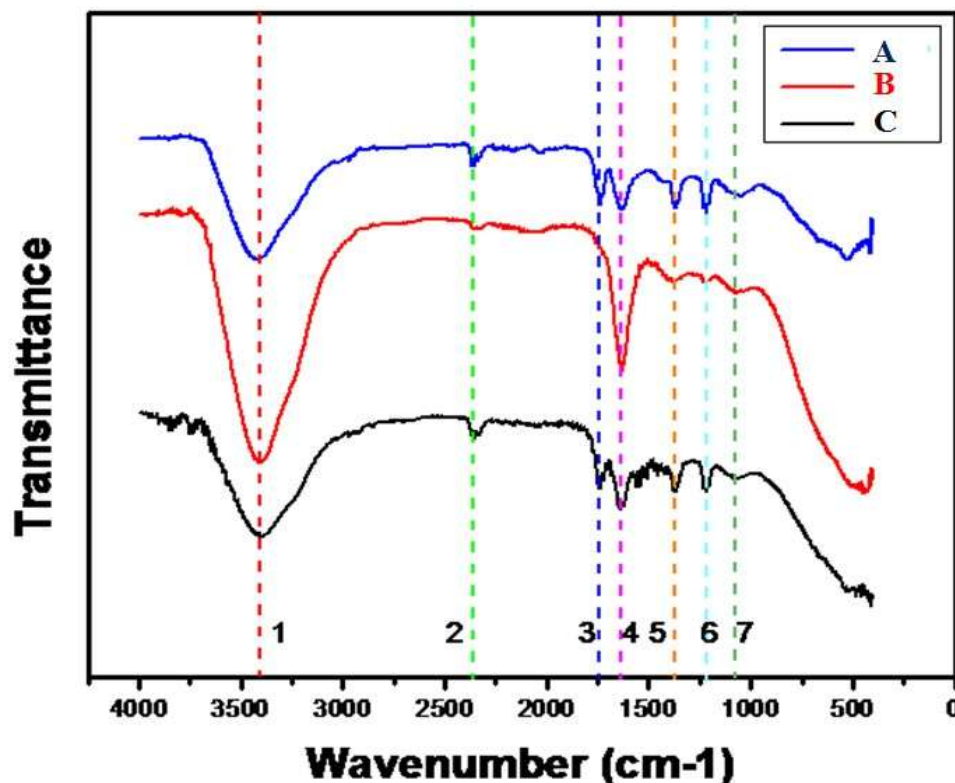


Figure 5 Fourier transform infrared absorption spectra after complete bio-reduction.

(A) LCPE, (B) BPPE and (C) POPE. The inset represents 1) O-H (stretch); 2) $\text{C}\equiv\text{C}$ (stretch); 3) C=O (stretch); 4) C=C (stretch) or N-H (bending); 5) C-H (bending); 6) C-N (stretch) or C-O (stretch); 7) C=C (stretch) or C-H (bending).

Porcine pancreatic amylase inhibition assay

CuNPs synthesized by LCPE, BPPE and POPE showed variability in α -amylase inhibition. CuNPs synthesized by LCPE showed the highest inhibition upto $25.92\pm 1.9\%$ followed by CuNPs synthesized by BPPE and POPE showing an inhibition of $14.28\pm 1.58\%$ and $13.22\pm 0.52\%$, respectively (Fig. 6). Acarbose showed $77.24\pm 0.52\%$ inhibition.

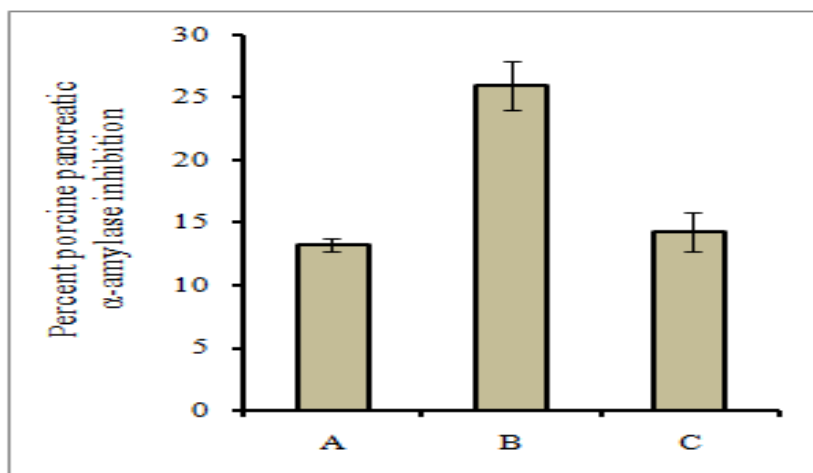


Figure 6: Percent porcine pancreatic α -amylase inhibition by CuNPs synthesized using (A) POLE, (B) LCPE and (C) BPLE.

Glucosidase inhibition assay

Glucosidase inhibition exhibited by the bio-reduced CuNPs also showed variability (Fig.7). CuNPs synthesized by POLE exhibited maximum α -glucosidase inhibition of $89.31 \pm 0.17\%$ followed by CuNPs synthesized using BPLE ($80.09 \pm 1.41\%$). However, CuNPs synthesized by LCPE exhibited considerably low α -glucosidase inhibition ($27.37 \pm 5.9\%$) which was comparable to its α -amylase inhibition. Acarbose showed $98.79 \pm 0.18\%$ inhibition.

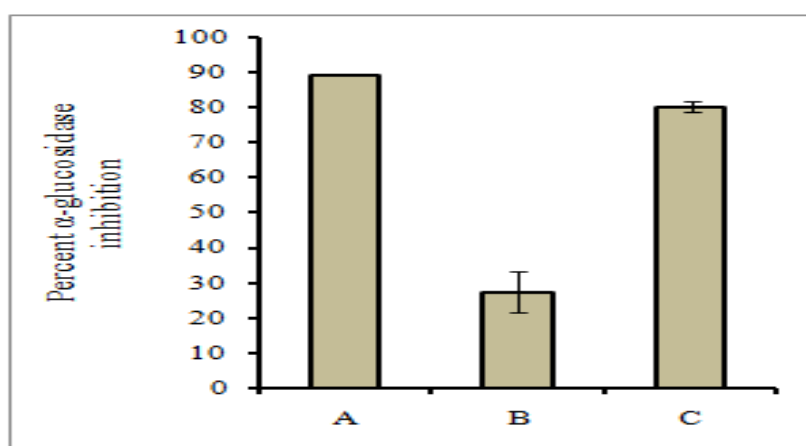


Figure 7: Percent α -glucosidase inhibition by CuNPs synthesized using (A) POLE, (B) LCPE and (C) BPLE.

DISCUSSION

Medicinal plants are considered to be treasures of diverse groups of phytochemicals that might lead to rapid synthesis of biogenic nanoparticles and thereafter their stabilization. Our earlier reports have demonstrated the potential of various medicinal plants to synthesize

AuNPs, AgNPs, PtNPs and PdNPs. However, synthesis of CuNPs is less common owing to its difficulty for reduction and stabilization. In this report, we have shown the potential of POLE, LCPE and BPLE to synthesize and stabilize the bio-reduced CuNPs. The synthesis was rapid and completed within 5 h which is in agreement with our earlier reports.^[17,18,20,22] *P. orientalis* leaf extract has shown similar potential to synthesize AgNPs which was considered to be faster and environmentally benign.^[32] The biogenic AgNPs formed by *P. orientalis* were found to be between 15 to 500 nm which is in agreement with the size distribution observed in our study. *L. chinensis* is also reported to synthesize antimicrobial AgNPs which are with a size 20 to 50 nm.^[33] Earlier, *Calotropis procera* L. is reported to synthesize CuNPs. Cysteine proteases present in the latex of *C. procera* L. played the key role in fabrication of CuNPs with average size of 15 ± 1.7 nm. It is already proved that phyto-genic CuNPs are non-cytotoxic when checked against HeLa, A549 and BHK21 cell lines by MTT dye reduction assay.^[34] *P. orientalis* leaf contains flavonoids, pentacyclic triterpenoids, tannins and caffeic acid in addition to various acylated flavonol glycosides and kaempferol derivatives.^[35,29] Similarly, *L. chinensis* is also rich in flavonoids, isoflavone daidzein, cyanidin-3-glucoside and malvidin-3-glucoside, cyanidin-3-rutinoside, quercetin-3-rutinoside, and quercetin glucoside.^[36,37] Similarly, *B. prionitis* is rich in various phytochemicals like balarenone, pipataline prionisides, barlerinoside, verbascoside, shanzhiside methyl ester, barlerin, acetylbarlerin, lupulinoside, scutellarein.^[38] These phytochemical compositions rationalize the potential of the extracts to reduce metal ions to nanoparticles and its stabilization. The bio-reduced CuNPs showed anti-diabetic activity by inhibition of α -amylase and α -glucosidase which is supported by our earlier report on CuNPs synthesized by *D. bulbifera*.^[14] Elevated concentrations of Cu^{2+} ions are reported to inhibit the digestive enzyme, amylase in giant freshwater prawn *Macrobrachium rosenbergii*.^[40] Likewise, Cu (II) ion and its complexes were reported to exhibit strong α -glucosidase inhibitory activity even greater than clinically used acarbose in *in vitro* studies.^[41-45] This report provides the first hand evidence that CuNPs synthesized by medicinal plants demonstrate excellent inhibitory potential against α -amylase and α -glucosidase which are considered to be significant pharmacological targets for treatment of T2DM. This intensely rationalizes the promises of phyto-genic CuNPs in emerging therapy and management of T2DM.

CONCLUSION

This is the first report on synthesis of CuNPs using *Barleria prionitis*, *Litchi chinensis* and

Platanus orientalis. The bioreduction process was rapid and efficient yielding monodispersed, extremely small and stable CuNPs which is of utmost significance for a candidate nanomedicine. The CuNPs exhibited potent antidiabetic activity by inhibition of α -amylase and α -glucosidase. Hence phytogetic CuNPs can be considered as promising therapeutic nanomaterial for treatment of T2DM.

ACKNOWLEDGMENT

The authors acknowledge the help extended for the use of TEM and HRTEM facilities in Chemical Engineering and CRNTS funded by the DST through Nanomission and IRPHA schemes. The authors thank Dr. M. Jayakannan, Indian Institute of Science Education and Research (IISER), Pune for DLS facility.

DISCLOSURE

The authors report no conflicts of interest in this work.

REFERENCE

1. Ghosh S, Harke AN, Chacko MJ, Gurav SP, Joshi KA, Dhepe A, Dewle A, Tomar GB, Kitture R, Parihar VS, Banerjee K, Kamble N, Bellare J, Chopade BA. *Gloriosa superba* mediated synthesis of silver and gold nanoparticles for anticancer applications. J Nanomed Nanotechnol, 2016; 7: 390.
2. Ghosh S, Patil S, Chopade NB, Luikham S, Kitture R, Gurav DD, Patil AB, Phadatare SD, Sontakke V, Kale S, Shinde V, Bellare J, Chopade BA. *Gnidia glauca* leaf and stem extract mediated synthesis of gold nanocatalysts with free radical scavenging potential. J Nanomed Nanotechnol, 2016; 7: 2.
3. Camacho-Flores BA, Martínez-Álvarez O, Arenas-Arrocena MC, Garcia-Contreras R, Argueta-Figueroa L, de la Fuente-Hernández J, Acosta-Torres LS. Copper: synthesis techniques in nanoscale and powerful application as an antimicrobial agent. J Nanomater, 2015; 2015: Article ID 415238.
4. Adersh A, Kulkarni AR, Ghosh S, More P, Chopade BA, Gandhi MN. Surface defect rich ZnO quantum dots as antioxidant inhibiting α -amylase and α -glucosidase: A potential anti-diabetic nanomedicine. J Mater Chem B, 2015; 3(22): 4597-4606.
5. Ghosh S, Ahire M, Patil S, Jabgunde A, Dusane MB, Joshi BN, Pardesi K, Jachak S, Dhavale DD, Chopade BA. Antidiabetic activity of *Gnidia glauca* and *Dioscorea bulbifera*: potent amylase and glucosidase inhibitors. Evid Based Complement Alternat Med, 2012; 2012: 929051.

6. Ghosh S, More P, Derle A, Patil AB, Markad P, Asok A, Kumbhar N, Shaikh ML, Ramanamurthy B, Shinde VS, Dhavale DD, Chopade BA. Diosgenin from *Dioscorea bulbifera*: Novel hit for treatment of Type II Diabetes Mellitus with inhibitory activity against α -Amylase and α -Glucosidase. PLoS One, 2014; 9(9): e106039.
7. Ghosh S, Derle A, Ahire M, More P, Jagtap S, Phadatare SD, Patil AB, Jabgunde AM, Sharma GK, Shinde VS, Pardesi K, Dhavale DD, Chopade BA. Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*. PLoS One, 2013; 8(12): e82529.
8. Markad PR, Sonawane DP, Ghosh S, Chopade BA, Kumbhar N, Louat T, Herman J, Waer M, Herdewijn P, Dhavale DD. γ -Hydroxyethyl piperidine iminosugar and *N*-alkylated derivatives: A study of their activity as glycosidase inhibitors and as immunosuppressive agents. Bioorg Med Chem, 2014; 22(21): 5776–5782.
9. Siriwardena A, Sonawane DP, Bande OP, Markad PR, Yonekawa S, Tropak MB, Ghosh S, Chopade BA, Mahuran DJ, Dhavale DD. Synthesis of 1,5-Dideoxy-1,5-Iminoribitol-C-glycosides through a Nitron-olefin Cycloaddition Domino Strategy: Identification of pharmacological chaperones of mutant human lysosomal beta-galactosidase. J Org Chem, 2014; 79(10): 4398–4404.
10. Patil VS, Nandre KP, Ghosh S, Rao VJ, Chopade BA, Sridhar B, Bhosale SV, Bhosale SV. Synthesis, crystal structure and antidiabetic activity of substituted (E)-3- (Benzo [d]thiazol-2-ylamino) phenylprop-2-en-1-one. Eur J Med Chem, 2013; 59: 304- 309.
11. Patil VS, Nandre KP, Ghosh S, Rao VJ, Chopade BA, Sridhar B, Bhosale S V, Bhosale S V. Synthesis and glycosidase inhibitory activity of novel (2-phenyl-4H-benzopyrimido[2,1-b]-thiazol-4-yliden)acetonitrile derivatives. Bioorg Med Chem Lett, 2012; 22: 7011–7014.
12. Mane RS, Ghosh S, Singh S, Chopade BA, Dhavale DD. Synthesis of anomeric 1,5-anhydrosugars as conformationally locked selective α -mannosidase inhibitors. Bioorg Med Chem, 2011; 19: 6720-6725.
13. Sanap SP, Ghosh S, Jabgunde AM, Pinjari RV, Gejji SP, Singh S, Chopade BA Dhavale DD. Synthesis, computational study and glycosidase inhibitory activity of polyhydroxylated conidine alkaloids – a bicyclic iminosugar. Org Biomol Chem, 2010; 8: 3307-3315.
14. Ghosh S, More P, Nitnavare R, Jagtap S, Chippalkatti R, Derle A, Kitture R, Asok A, Kale S, Singh S, Shaikh ML, Ramanamurthy B, Bellare J, Chopade BA. Antidiabetic and antioxidant properties of copper nanoparticles synthesized by medicinal plant *Dioscorea*

- bulbifera*. J Nanomed Nanotechnol, 2015; S6: 007.
15. Ghosh S, Nitnavare R, Dewle A, Tomar GB, Chippalkatti R, More P, Kitture R, Kale S, Bellare J, Chopade BA. Novel platinum–palladium bimetallic nanoparticles synthesized by *Dioscorea bulbifera*: Anticancer and antioxidant activities. Int J Nanomedicine, 2015; 10: 7477–7490.
 16. Ghosh S, Jagtap S, More P, Shete UJ, Maheshwari NO, Rao SJ, Kitture R, Kale S, Bellare J, Patil S, Pal JK, Chopade BA.. *Dioscorea bulbifera* mediated synthesis of novel Au_{core}Ag_{shell} nanoparticles with potent antibiofilm and antileishmanial activity. J Nanomater, 2015; 2015: 562938.
 17. Ghosh S, Patil S, Ahire M, Kitture R, Kale S, Pardesi K, Cameotra SS, Bellare J, Dhavale DD, Jabgunde A, Chopade BA. Synthesis of silver nanoparticles using *Dioscorea bulbifera* tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. Int J Nanomed, 2012; 7: 483–496.
 18. Ghosh S, Patil S, Ahire M, Kitture R, Jabgunde A, Kale S, Pardesi K, Bellare J, Dhavale DD, Chopade BA. Synthesis of gold nanoanisotrops using *Dioscorea bulbifera* tuber extract. J Nanomat, 2011; 2011: 354793-354800.
 19. Ghosh S, Patil S, Ahire M, Kitture R, Gurav DD, Jabgunde AM, Kale S, Pardesi K, Shinde V, Bellare J, Dhavale DD, Chopade BA. *Gnidia glauca* flower extract mediated synthesis of gold nanoparticles and evaluation of its chemocatalytic potential. J Nanobiotechnol, 2012; 10: 17.
 20. Ghosh S, Chacko MJ, Harke AN, Gurav SP, Joshi KA, Dhepe A, Kulkarni AS, Shinde VS, Parihar VS, Asok A, Banerjee K, Kamble N, Bellare J, Chopade BA. *Barleria prionitis* leaf mediated synthesis of silver and gold nanocatalysts. J Nanomed Nanotechnol, 2016; 7: 394.
 21. Sant DG, Gujarathi TR, Harne SR, Ghosh S, Kitture R, Kale S, Chopade BA, Pardesi K R.. *Adiantum philippense* L. frond assisted rapid green synthesis of gold and silver nanoparticles. J Nanopart, 2013; 2013: 1-9.
 22. Ghosh S, Gurav SP, Harke AN, Chacko MJ, Joshi KA, Dhepe A, Charolkar C, Shinde VS, Kitture R, Parihar VS, Banerjee K, Kamble N, Bellare J, Chopade BA. *Dioscorea oppositifolia* mediated synthesis of gold and silver nanoparticles with catalytic activity. J Nanomed Nanotechnol, 2016; 7: 398.
 23. Salunke GR, Ghosh S, Santosh Kumar RJ, Khade S, Vashisth P, Kale T, Chopade S, Pruthi V, Kundu G, Bellare JR, Chopade BA. Rapid efficient synthesis and characterization of silver, gold, and bimetallic nanoparticles from the medicinal plant

- Plumbago zeylanica* and their application in biofilm control. *Int J Nanomedicine*, 2014; 9: 2635–2653.
24. Kitture R, Ghosh S, More PA, Date K, Gaware S, Datar S, Chopade BA, Kale SN. Curcumin-loaded, self-assembled *Aloe vera* template for superior antioxidant activity and trans-membrane drug release. *J Nanosci Nanotechnol*, 2015; 15(6): 4039-4045.
25. Kitture R, Chordiya K, Gaware S, Ghosh S, More PA, Kulkarni P, Chopade BA, Kale SN. ZnO nanoparticles-red sandalwood conjugate: A promising anti-diabetic agent. *J Nanosci Nanotechnol*, 2015; 15(6): 4046-4051.
26. Kitture R, Ghosh S, Kulkarni P, Liu XL, Maity D, Patil SI, Jun D, Dushing Y, Laware SL, Chopade BA, Kale SN. Fe₃O₄-citrate-curcumin: Promising conjugates for superoxide scavenging, tumor suppression and cancer hyperthermia. *J Appl Phys*, 2012; 111: 064702- 064707.
27. Ghosh S, Parihar VS, More P, Dhavale DD, Chopade BA. Phytochemistry and therapeutic potential of medicinal plant: *Dioscorea bulbifera*. *Med Chem*, 2015; 5(4): 154-159.
28. Ghosh S, Parihar VS, Dhavale DD, Chopade BA. Commentary on therapeutic potential of *Gnidia glauca*: A novel medicinal plant. *Med Chem*, 2015; 5(8): 351-353.
29. Tantry MA, Akbar S, Dar JA, Irtiza S, Galal A, Khuroo MA, Ghazanfar K. Acylated flavonol glycoside from *Platanus orientalis*. *Fitoterapia*, 2012; 83: 281–285.
30. Sung Y, Yang WK, Kim HK. Antiplatelet, anticoagulant and fibrinolytic effects of *Litchi chinensis* Sonn. extract. *Mol Med Rep*, 2012; 5: 721-724.
31. Wen L, You L, Yang X, Yang J, Chen F, Jiang Y, Yang B. Identification of phenolics in litchi and evaluation of anticancer cell proliferation activity and intracellular antioxidant activity. *Free Radic Biol Med*, 2015; 84: 171–184.
32. Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess Biosyst Eng*, 2009; 32(1): 79–84.
33. Singh D, Gupta R. Antibacterial properties of silver nanoparticles synthesized from *Litchi chinensis* fruit peel extract. *Int J Biotechnol Biomed Sci*, 2016; 2(1): 6-8.
34. Harne S, Sharma A, Dhaygude M, Joglekar S, Kodam K, Hudlikar M. Novel route for rapid biosynthesis of copper nanoparticles using aqueous extract of *Calotropis procera* L. latex and their cytotoxicity on tumor cells. *Colloids Surf B Biointerfaces*, 2012; 95: 284-288.
35. Irtiza S, Bhat GA, Ahmad M, Ganaie HA, Ganai BA, Kamili AN, Akbar S, Tantry MA. Antioxidant and anti-inflammatory activities of *Platanus orientalis*: An oriental plant

- endemic to Kashmir planes. *Pharmacologia*, 2016; 7: 217-222.
36. Shende S, Joshi KA, Kulkarni AS, Shinde VS, Parihar VS, Kitture R, Banerjee K, Kamble N, Bellare J, Ghosh S. *Litchi chinensis* peel : A novel source for synthesis of gold and silver nanocatalysts. *Glob J Nano*, 2017; 3(1): 555603.
37. Zhao M, Yang B, Wang J, Liu Y, Yu L, Jiang Y. Immunomodulatory and anticancer activities of flavonoids extracted from litchi (*Litchi chinensis* Sonn.) pericarp. *Int Immunopharmacol*, 2007; 7: 162–166.
38. Rokade SS, Joshi KA, Mahajan K, Tomar G, Dubal DS, Parihar VS, Kitture R, Bellare J, Ghosh S. Novel anticancer platinum and palladium nanoparticles from *Barleria prionitis*. *Glob J Nano*, 2017; 2(5): 555600.
39. Zhao M, Yang B, Wang J, Li B, Jiang Y. Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. *Food Chem*, 2006; 98: 539–544.
40. Li N, Zhao Y, Yang J. Effects of water-borne copper on digestive and metabolic enzymes of the giant freshwater prawn *Macrobrachium rosenbergii*. *Arch Environ Contam Toxicol*, 2008; 55: 86–93.
41. Wang Y, Ma L, Li Z, Du Z, Liu Z, Qin J, Wang X, Huang Z, Gu L, Chen AS. Synergetic inhibition of metal ions and genistein on alpha-glucosidase. *FEBS Lett*, 2004; 576: 46-50.
42. Yoshikawa Y, Hirata R, Yasui H, Sakurai H. Alpha-glucosidase inhibitory effect of anti-diabetic metal ions and their complexes. *Biochimie*, 2009; 91: 1339–1341.
43. Vančo J, Marek J, Trávníček Z, Račanská E, Muselík J, Svajlenová O. Synthesis, structural characterization, antiradical and antidiabetic activities of copper(II) and zinc(II) Schiff base complexes derived from salicylaldehyde and β -alanine. *J Inorg Biochem*, 2008; 102: 595–605.
44. Yasumatsu N, Yoshikawa Y, Adachi Y, Sakurai H. Antidiabetic copper(II)-picolinate: Impact of the first transition metal in the metallopicolinate complexes. *Bioorganic Med Chem*, 2007; 15: 4917–4922.
45. Jiao Z, Liu J, Wang S. Antioxidant activities of total pigment extract from blackberries. *Food Technol Biotechnol*, 2005; 43: 97-102.