SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	Asian Journal of Biotechnology and Bioresource Technology
Manuscript Number:	**2017/AJB2T/39126**
Title of the Manuscript:	**GREEN SYNTHESIS OF COPPER NANOPARTICLES USING MANDARIN (Citrus reticulata) PEEL EXTRACT AND ANTIFUNGAL STUDY**
Type of Article:	**Original Research Article**

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	**Authors' response to final evaluator's comments**
My comments about the manuscript entitled as "GREEN SYNTHESIS OF COPPER NANOPARTICLES USING MANDARIN (*Citrus reticulata*) PEEL EXTRACT AND ANTIFUNGAL STUDY" **1. There are many reports on the synthesis of copper nanoparticles (NPs) using different verity of citrus. Hence, what is the unique in this work when compared to the reported one? Moreover, the authors have used PVA during the synthesis so it's an external capping agent. Hence this work is not a biosynthesised one which deals purely with the phytochemicals. Do they experienced any difficulties with the addition of extract and the precursor in order to get CuNPs?** **2. If they want to examine the effect of temperature and pH they have to use the range of values. What would be happened if they increase or decrease the temperature and pH of the medium? There is a controversy in the manuscript regarding the temperature either 60 or 80° C. What is the pH of the extract and the reaction mixture?** **3. What 1000 ppm and mandarin peel/ Cu^{2+} (2:1v/v), Cu^{2+}/PVA (1/10w/w) indicates?** **4. The quality of the language is not good and so may typo errors throughout the manuscript.** **5. "Biologically" term is a wrong one here and UV-Visible absorption spectroscopy is not meant for the morphological studies.** **6. Copper sulfate is not an ideal one to compare the antifungal activity. Author should use the commercial antifungal agent or CuNPs with different size.** **7. There is not much citation of the articles throughout the manuscript.**	**1.** The authors have read and consented to reviewer's comment. All changes are highlighted. 2. Effect of temperature and pH on the synthesis CuNps which are mentioned in the manuscript. 3. The authors have read and consented to reviewer's comment. All changes are highlighted. 4. The authors have read and consented to reviewer's comment. All changes are highlighted. 5. The authors have read and consented to reviewer's comment. All changes are highlighted. 6.We aim to demonstrate that the fungus inhibition efficiency of copper nanoparticles is higher activities than those of copper sulphate. Essa AM, Khallaf MK. Antimicrobial potential of consolidation polymers loaded with biological copper nanoparticles. BMC microbiology. 2016; 16(1): 144. 7. The reference which indicates the presence of ascorbic acid in the extract.

There supposed to be a reference which indicates the presence of ascorbic acid in the extract. **8. In materials section, the botanical name of the fruit should be written in the bracket. Name of the fungus should be written in a scientific way.** **9. Dose they observe the same color change with the addition of NaOH into Cu Sulfate solution. The author should have to report the blank experiments. They haven't explain the role of PVA in the manuscript. The color of the peel extract is look like stored.** **10. The visual observation of the color change has reported in the experimental part. Hence it should not be repeated in the characterization part. A single title would be fine and no need of subheading in the characterization part.** **11. They have written as "scavenging ability of OH group important for the synthesis" and "The antioxidant property of polyphenolic compounds is mainly due to its redox property which allows them to act as reducing agents" Explain How?** **12. "Pharmocognostic evaluation of synthesized copper nanoparticles", The title doesn't match with the work. The authors have observed the activity of the particles for 3 days. Hence periodic observation should be reported.** **13. There are many unwanted literature reports in the discussion part about the synthesis. It may go to the introduction section.** **14. I couldn't see any dispersed particles in SEM. The size if the particles mentioned inside are not acceptable one, if you have considered about the scale bar in the image. TEM, SEM and DLS data are controversial.** **15. The final color of the nanoparticles are not in a blue color. The table 2 is not at all required.** **16. By seeing UV-Visible absorption spectra, at 0 h it-self there is an absorption peak. What does it mean and where it is from? What is the reason behind the decrease in the absorption intensity and shift in the peak position during the course of the reaction? There is a controversy in the absorption position of CuNPs in the**	Tumbas VT, Ćetković GS, Đilas SM, Čanadanović-Brunet JM, Vulić JJ, Knez Ž, Škerget M. Antioxidant activity of mandarin (Citrus reticulata) peel. Acta periodica technologica. 2010; (41): 195-203. Justin JS, Milton A, Natesan G. Phytochemical evaluation of peel of cittrus reticulata blanco using various solvent extracts. International Journal of Pharmaceutical Sciences and Business Management. 2014; 2(9) : 26-35. de Moraes Barros HR, de Castro Ferreira TAP, Genovese MI. Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. Food Chemistry. 2012; *134*(4): 1892-1898. **8.** The authors have read and consented to reviewer's comment. All changes are highlighted. 9. PVA is frequently used as the stabilizer or capping agent for metal colloids because of its availability, low cost, and non-toxicity (Line 160). The extract obtained was used immediately for the synthesis of copper nanoparticles. 10. The authors have read and consented to reviewer's comment. All changes are highlighted. 11. The authors have read and consented to reviewer's comment. All changes are highlighted. 12. The authors have read and consented to reviewer's comment. All changes are highlighted. 13. The authors have read and consented to reviewer's comment. All changes are highlighted. 14. CuNps was prepared with size of particle form 10 – 40 nm in DLS result. This result is suitable with the SEM and TEM result that CuNps are lower 40 nm. 15. The authors have read and consented to reviewer's comment. All changes are highlighted. 16. 0-h: it means that CuNps were formed after 2 hours and 0 hour-preserved. The result was similar to the previous studies that the peak at 550 – 600 nm of wavelength can be assigned to absorption of CuNps. Xiong J, Wang Y, Xue Q, Wu X. Synthesis of highly stable dispersions of nanosized copper particles using L-ascorbic acid. Green Chemistry. 2011; 13(4): 900-904. Ponmurugan P, Manjukarunambika K, Elango

manuscript (575 nm or 560 nm?). **17. "The effect of ascorbic acid concentration on the UV – Visible absorption spectroscopy of synthesized CuNps is shown in Figure 4" which is not acceptable one. The extract is a mixture of phytochemicals.** **18. There is a difference in the activity of the NPs towards *Corticium salmonicola .Berk* and *Phanerochaete salminicolor.* Explain the reason and mechanism? How effective the antifungal activity of the particle with the reported one? Did they observe the antifungal activity of PVA?** **19. Antimicrobial assay is not clear. The references are not orderly written.**	V, Gnanamangai BM. Antifungal activity of biosynthesised copper nanoparticles evaluated against red root-rot disease in tea plants. Journal of Experimental Nanoscience. 2016; 11(13): 1019-1031. 17. UV-Visible spectra showing stability of copper nanoparticles at room temperature for 30 days 18. Raman et al. has reported that antifungal activity of CuNps solution can be explained on the basis of Overtone's concept and chelation theory. Liposolubility is an important factor that controls the antifungal activity because the lipid membrane that surrounds the cell favors the passage of only lipid soluble matter and this is the Overtone's concept of cell permeability. Due to the overlap of the ligand orbital with the metal orbitals and the slight sharing of positive charge of the metal ion with the donor groups on chelation, the polarity of the metal ion is reduced to a greater extent. Chelation further increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the metal complex. Thus the metal binding sites on the enzymes of microorganisms get blocked because of the increased lipophilicity, which in turn enhances the penetration of the metal complexes into lipid membranes. Copper nanoparticles also disturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organism. Raman N, Joseph J, Velan A, Pothiraj C. Antifungal activities of biorelevant complexes of copper (II) with biosensitive macrocyclic ligands. Mycobiology. 2006; 34(4): 214-218. 19. In vitro, Antifungal activity of CuNps on *Corticium salmonicola* and *Phanerochaete salminicolor* at various concentrations.