**Supplementary Material**

***Piper longum* catkin extract mediated synthesis of Ag, Cu, and Ni nanoparticles and their applications as biological and environmental remediation agents**

**Running Title:** *P. longum* catkin mediated nanoparticles

Nargis Jamilaa, \*, Naeem Khanb, \*\*, Amina Bibia, Adnan Haiderc, Sadiq Noor Khand, Amir Atlasb, Sajjad Haidere, Aaliya Minhaza, Fatima Javeda, Faheem Ullahf

aDepartment of Chemistry, Shaheed Benazir Bhutto Women University, Peshawar 25000, Khyber Pakhtunkhwa, Pakistan

bDepartment of Chemistry, Kohat University of Science and Technology, Kohat 26000, Khyber

cDepartment of Biological Sciences, National University of Medical Sciences, Rawalpindi, Punjab Pakistan

dDepartment of Medical Lab Technology, University of Haripur, Haripur 22060, Khyber Pakhtunkhwa, Pakistan

eDepartment of Chemical Engineering, King Saud University, Riyadh, Saudi Arabia

fSchool of Materials and Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, Nibong Tebal, Pulau Pinang, 14300, Malaysia

**To whom the correspondence should be addressed:**

Dr. Nargis Jamila\*

Department of Chemistry, Shaheed Benazir Bhutto Women University

Peshawar, Khyber Pakhtunkhwa, Pakistan

Email: [njk985@gmail.com](mailto:njk985@gmail.com), [nargisjamila@sbbwu.edu.pk](mailto:nargisjamila@sbbwu.edu.pk)

Dr. Naeem Khan\*\*

Department of Chemistry, Kohat University of Science and Technology

Kohat, Khyber Pakhtunkhwa, Pakistan

Email: [nkhan812@gmail.com](mailto:nkhan812@gmail.com)

**Abstract**

*Piper longum* (long pepper) in dried form is used in several traditional medicine and as a spice. The present study highlights nutritional and toxic elements content, synthesis, and characterization of silver, copper oxide, and nickel nanoparticles using *P. longum* catkin extract. The study also determined anticancer, antioxidant, antimicrobial, and redox catalytic activities of the synthesized NPs. The *P. longum* extract mediated nanoparticles (PLNPs) synthesized at different pH and ratios were characterized by UV-Vis (ultra-violet-visible), FT-IR (Fourier-Transform infrared), and scanning electron and atomic force microscopic (SEM, AFM) techniques. Elemental content of *P. longum* catkin determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and ICP-mass spectrometry (ICP-MS) indicatd appreciable concentrations of nutritional elements, and well below permissible ranges of toxic elements. Well-defined and stable silver nanoparticles (PLAgNPs) were formed in 1:4 to 1:6 ratios, while copper oxide and nickel NPs (PLCuONPs and PLNiNPs) were found prominent in 1:6 ratio. In determining the effect of pH on synthesized PLNPs, sharp intense absorption peaks were obtained under slightly neutral to highly basic conditions (pH 6 to 13) for PLAgNPs, whereas for PLCuONPs and PLNiNPs, pH 7–8 was optimum. In biological activities, PLNPs exhibited significant anticancer efficacy against DU-145 (prostate cancer) cell line up to the range of 92.7% (PLCuONPs) to 100% (PLAgNPs, PLNiNPs). Fuurthermore, the sythesized NPs exhibited signifcant antioxidant, antimicrobial, and redox catalytic properties. This study concluded the promising nutritional, biological and environmental remediation applications, and hence, further exploration of the synthesized NPs in biological and clinical applications is currently under investigation.

**Keywords:** *P. longum*; elemental content; nickel nanoparticles; inductively coupled plasma-optical emission spectroscopy; atomic force microscopy; anticancer

C:\Users\lg\Desktop\SBBWUP- Tasks\SBBWU Research Activities\Research Students 2017-2019\Thesis\Amina Bibi Thesis\Amina 1.tif

Figure S1.*P. longum* aqueous extract preparation and synthesis of PLNPs under different conditions

C:\Users\lg\Desktop\Amina Photos.pptx1.tif

Figure S2. Synthesis of AgNPs at different pH using *P. longum* catkin

Table S1. Analytical methods validation parameters for ICP-OES and ICP-MS in the macro, micro, and trace elements analysis

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Element | Correlation coefficient  (*R2*) | Limits of detection  (ng/g) | Limits of quantification  (ng/g) | Coefficient  of variance  (CV%) | Spike recovery† (%) |
| Macro elements | |  |  |  |  |
| Al | 0.99994 | 8.502 | 28.086 | 1.212 | 103.5 |
| Ca | 0.99743 | 9.120 | 30.105 | 1.423 | 93.7 |
| Fe | 0.99979 | 0.447 | 1.4813 | 1.145 | 94.0 |
| K | 0.99773 | 12.228 | 40.337 | 1.191 | 94.1 |
| Mg | 0.99792 | 8.110 | 26.766 | 1.138 | 95.0 |
| Na | 0.99850 | 12.983 | 42.852 | 2.195 | 94.9 |
| P | 0.99987 | 7.564 | 24.977 | 1.193 | 97.9 |
| S | 0.99990 | 11.110 | 36.676 | 2.155 | 93.8 |
| Micro elements | |  |  |  |  |
| Cu | 0.99958 | 0.053 | 0.1685 | 1.154 | 99.4 |
| Ni | 0.99978 | 0.251 | 0.8345 | 1.029 | 102.9 |
| Rb | 0.99993 | 0.056 | 0.1911 | 1.137 | 98.8 |
| Sr | 0.99985 | 0.476 | 1.5641 | 1.160 | 96.9 |
| Zn | 0.99987 | 0.213 | 0.7091 | 1.361 | 97.2 |
| Trace essential elements | | | | | |
| Co | 0.99989 | 0.046 | 0.1452 | 0.891 | 93.7 |
| Cr | 0.99993 | 0.097 | 0.3264 | 0.989 | 97.2 |
| Se | 0.99994 | 0.242 | 0.7954 | 1.151 | 103.0 |
| V | 0.99995 | 0.034 | 0.1053 | 1.650 | 97.4 |
| Trace non-toxic element | | | | | |
| Ba | 0.99998 | 0.078 | 0.2503 | 0.928 | 98.2 |
| Be | 0.99998 | 0.070 | 0.2215 | 0.553 | 96.1 |
| Ga | 0.99993 | 0.045 | 0.1584 | 0.525 | 94.9 |
| Li | 0.99997 | 0.076 | 0.2341 | 0.975 | 98.5 |
| Trace toxic elements | |  |  |  |  |
| As | 0.99998 | 0.065 | 0.2211 | 1.403 | 98.8 |
| Cd | 0.99996 | 0.083 | 0.2736 | 1.092 | 104.0 |
| In | 0.99998 | 0.036 | 0.1252 | 0.932 | 93.6 |
| Pb | 0.99996 | 0.054 | 0.1714 | 0.880 | 105.7 |
| Tl | 0.99988 | 0.033 | 0.1224 | 1.112 | 95.2 |
| U | 0.99996 | 0.008 | 0.012 | 1.530 | 102.7 |

†Macro elements were spiked at 3,000 ug/kg, while micro and trace elements were spiked at 50 µg/kg

Table S2. Accuracy determination of ICP-OES and ICP-MS techniques by analyzing standard reference material (NIST SRM-1573a), tomato leaves

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Instrument | Element | Certified value  (mg/kg) | Measured value (mg/kg) | Recovery (%) |
| ICP-MS | Cr | 1.99 ± 0.05 | 2.05 ± 0.06 | 103.0 |
| Co | 0.59 ± 0.01 | 0.58 ± 0.02 | 98.3 |
| Cu | 4.30 ± 0.14 | 4.38 ± 0.43 | 101.8 |
| Se | 0.055 ± 0.002 | 0.058 ± 0.001 | 105.4 |
| Zn | 30.5 ± 0.6 | 30.9 ± 1.01 | 101.3 |
| Cd | 1.51 ± 0.04 | 1.38 ± 0.02 | 91.3 |
| As | 0.114 ± 0.004 | 0.120 ± 0.012 | 105.2 |
| ICP-OES | Al | 593.0 ± 10.0 | 546.0 ± 49.0 | 92.1 |

Table S3. Food analysis performance assessment scheme (FAPAS) results and Z-scores for tomato paste, milk powder and chili powder analysis

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | Assigned value µg/kg | Result  µg/kg | Z-score\* |
| Tomato paste (2015) | | | |
| Cd | 126.0 | 139.1 | 0.5 |
| Pb | 322 | 302 | 0.3 |
| Milk powder (2014) | | | |
| Cd | 12.2 | 10.7 | -0.6 |
| Hg (total) | 13.4 | 13.7 | 0.1 |
| Pb | 47.5 | 43.85 | -0.4 |
| Chili powder (2013) | | | |
| As (total) | 1006 | 1000.7 | 0.0 |
| Cd | 556 | 574.3 | 0.2 |
| Pb | 620 | 666.8 | 0.4 |

\*A Z-score compares an estimate of the error of a result with a target value for standard deviation

|  |  |
| --- | --- |
|  |  |
| a. 1:1 | b. 1:2 |
|  | |
| c. 1:3 | |

Figure S3. No PLAgNPs formation with ratios (a) 1:1, (b) 1:2, and (c) 1:3

|  |  |
| --- | --- |
|  |  |
| 1. 1:1 | 1. 1:2 |
|  |  |
| 1. 1:3 | 1. 1:4 |

Figure S4. No PLCuONPs formation with ratios (a) 1:1, (b) 1:2, (c) 1:3, and (d) 1:4

|  |  |
| --- | --- |
|  |  |
| 1. 1:5 | 1. 1:6 |
|  |  |
| 1. 1:7 | 1. 1:8 |
|  | |
| 1. 1:9 | |

Figure S5. UV spectra of PLCuONPs with ratios1:5-1:9

|  |  |
| --- | --- |
|  |  |
| 1. 1:1 | 1. 1:2 |
|  | |
| 1. 1:3 | |

Figure S6. No PLNiNPs formation with ratios (a) 1:1, (b) 1:2, and (c) 1:3

|  |  |
| --- | --- |
|  |  |
| 1. 1:4 | 1. 1:5 |
|  |  |
| 1. 1:6 | 1. 1:7 |
|  |  |
| 1. 1:8 | 1. 1:9 |

Figure S7. PLNiNPs formation with ratios 1:4-1:9

|  |  |
| --- | --- |
|  |  |
| 1. PLAgNPs (1:4, heating and stirring) | 1. PLAgNPs (1:5, stirring) |
|  |  |
| c. PLAgNPs (1:6, heating and stirring) | d. PLCuONPs (1:6, heating and stirring) |
|  | |
| e. PLNiNPs (1:6, heating and stirring) | |

Figure S8. UV spectra showing the effect of pH on PLAgNPs, PLCuONPs, and PLNiNPS synthesis

|  |  |
| --- | --- |
| a | C:\Users\lg\Desktop\P- longum paper\PL aq-extract.tif |
| b | C:\Users\lg\Desktop\P- longum paper\PL-AgNPs-IR.tif |
| c | C:\Users\lg\Desktop\P- longum paper\PLCuONPs-IR.tif |
| d | C:\Users\lg\Desktop\P- longum paper\PL-NiNPs-IR.tif |

Figure S9a-d. FT-IR spectra of (a) PLAE, (b) PLAgNPs, (c) PLCuONPs, and (d) PLNiNPs

|  |  |
| --- | --- |
|  |  |
| 1. DG | 1. CR |
|  |  |
| 1. ZY | 1. BR |
|  | |
| E | |

Figure S10. UV-Vis spectra of effect of PLAgNPs on the degradation of dyes and food colour (a) DG, (b) CR, (c) ZY, (d) BR, and (e) MB dyes absorption peak changes by NaBH4