Section A-Research paper

ANTIOXIDANT AND CYTOTOXICITY EVALUATION OF CARBON NANOPARTICLES SYNTHESIZED FROM *EICHHORNIA CRASSIPES*

K. Sindhu Priya and N.K. Udaya Prakash*

Department of Biotechnology, School of Life Sciences,
Vels Institute of Science, Technology and Advanced Studies (VISTAS),
Pallavaram, Chennai, India

nkudayaprakash@gmail.com

ABSTRACT

The studies on nanotechnology are mostly oriented towards synthesis and applications and not widely studied for their antioxidant ability and toxicity. In this study, the carbon nanoparticles synthesized from *Eichhornia crassipes* were evaluated for their antioxidant ability using DPPH, metal chelating ability using $FeCl_2$, Phosphomolybdenum reduction and cytotoxicity using A549 cell lines. The IC_{50} value recorded for DPPH scavenging was more than 500µL and 300µL and 600µL concentration of CNPs for Phosphomolybdenum reducing activity. The IC_{50} value for metal chelating activity was recorded nearly at 1000µL. The cell growth inhibition was recorded less than 50% against the cell line A549 even at the concentration of 1000µL. The study proves that the carbon nanoparticles synthesized from *Eichhornia crassipes* was less toxic and safer to use as a biocompatible material.

Keywords: Carbon nanoparticles, *Eichhornia crassipes*, Antioxidant activity, DPPH, Cytotoxicity, A549, Biocompatibility

Corresponding Author:

Dr. N.K. Udaya Prakash
Professor,
Department of Biotechnology,
School of Life Sciences,
Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, India
Email: nkudayaprakash@gmail.com
Mob: +91 9444896061

1.INTRODUCTION

The research pertaining to nanotechnology are manifold and mostly oriented towards synthesis and applications. Thus, exposure to these nanoparticles is unavoidable and the impact depends upon their nature and toxicity. However, studies to characterise their effects after exposure and to address their potential toxicity are scarce in comparison to the studies on the synthetic methods and applications.Nanoparticles are being used in diagnostic and therapeutic instruments, particularly in the medical industry, to better understand, detect, and cure human diseases. They are also used as a carrier component for medication. Understanding the characteristics of nanoparticles and how they interact with the body is essential before clinical use since exposure to nanoparticles for medicinal purpose involves intentional encounter or medication. Hence, the research towards nanotoxicology is important and now receiving more attention.

Carbon nanoparticles are used in many scientific domains due to their characters, including fluorescence, conductivity, absorbance and their biocompatibility. Carbon nanoparticles may become one of the most common materials in biomedicine as a result of these characteristics. For a very long time, CNPs were thought to be 10 nm-sized crystalline or amorphous carbon-based nanoparticles. Recently, several publications depicted CNPs as a combination of structures with similar or overlapping emission [20]. CNPs are used mostly in sensing and bioimaging, chemical analysis, optoelectronic devices, catalyst design, agriculture and optoelectronic devices [5]. CNPs are potential agent in theronastics, distributing the drugs and as diagnostic agent by integrating imaging and therapy. The toxicity of CNPs mostly depends upon the source of preparation. In this study, the CNPs synthesized in the form of Biosoot from the leaves of *Eichhornia crassipes* were evaluated for its antioxidant potency and toxicity.

A free-floating aquatic plant *Eichhornia crassipes*, also known as water hyacinth, is a member of the family Pontederiaceae and it is Monocotyledonous [22]. The plant has a high rate of growth, spreads quickly and widely and has a high tolerance for changes in temperature, pH and nutrient levels. Therefore, it has been recognized as one of the 10 most severe weed plants in the world and listed as one of the 100 most invasive and aggressive species by the International Union for Conservation of Nature [14]. *Eichhornia crassipes*, however, has a number of negative effects on the environment and the economy [21]. Due to its capacity to take up heavy metals and grow in contaminated water, it has been utilised as a

phytoremediation agent for wastewater treatments [12,13]. Additionally, it has been speculated to be a source of bioenergy and fertilisers [10,14].

The plant, *Eichhornia crassipes* was used in the synthesis of silver nanoparticles [6,9]. The plant was also used in the synthesis of carbon nanoparticles [18]. However, the antioxidant activity and cytotoxicity of the synthesized carbon nanoparticles was not evaluated so far. Thus, the study is conducted to evaluate antioxidant activity, metal chelating ability, phosphomolybdenum assay and cytotoxicity of Carbon Nanoparticles synthesized using *Eichhornia crassipes.*

2. MATERIAL & METHODS

2.1 Collection of Leaves *(Eichhornia crassipes)*

Theleaves were collected from the Palkeni tank located in Pallavaram, Chennai, India. The leaves were identified and authenticated by Late Prof P. Jayaraman, Plant Anatomy Research Centre (PARC), Tambaram, Chennai, India.

2.2 Preparation of Biosoot

The surface of the collected leaves of *Eichhornia crassipes* were cleaned in tap water, cut into small pieces and kept under shade to air dry. The air dried plants were directly burnt for the formation of soot. The developed soot were collected by deposition of the same on a sterile porcelain tile. The deposited soot were scraped using sterile scalpel and stored in Eppendorf tubes for further analysis.

2.3 Antioxidant activity of CNPs

2.3.1 DPPH radical scavenging activity:

For the evaluation of anti-oxidant activity, CNP's of *Eichhornia crassipes* (methanol and aqueous extracts) of 200, 400, 600, 800 and 1000µLwere taken in a series of test tubes along 1ml of 100µM of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) which is dissolved in methanol. The mixture is made up to 2ml using methanol. This was incubated in dark for the period of 30 minutes to react. After incubation time, the absorption value was measured using UV–vis spectrophotometer (UV-1800, Shimadzu) at 517nm [7]. In this experiment, methanol served as blank, DPPH solution devoid of any test sample as positive control and Ascorbic acid as standard. The experiments was repeated thrice and their mean value for absorbance was recorded. The inhibition percentage was determined with the following equation,

Absorbance of Control – Absorbance of test sample

$$\% \text{ of Radical Scavenging activity} = \frac{\text{Absorbance of Control}}{\text{Absorbance of Control}} \text{X } 100$$

IC_{50} of the value for individual extract (CNPs) with standard deviation is presented.

2.3.2 Phosphomolybdenum assay

The assay was based on the reduction of the CNPs of *Eichhornia crassipes* (methanol and aqueous extract) and subsequent formation of a complex. The CNPs dissolved in water were taken as 200, 400, 600, 800 and 1000µL concentration and allowed to react with 3ml of reagent (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) at 95°C for 30mins. The absorbance was taken at 695nm using a spectrophotometer. The results were calculated in ascorbic acidequivalents.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \text{ X } 100$$

IC_{50} of the value for Individual extract (CNPs) with standard deviation is present

2.3.3 Metal chelating activity

The chelation of ferrous particles by different concentrations of the CNPs was assessed. The chelation of ferrous ions by various concentrations of CNPs of *Eichhornia crassipes* was estimated. The CNPs dispersed in water were taken as 200, 400, 600, 800 and 1000µL and added to 0.1ml of $FeCl_2$. Then the reaction was initiated by adding 0.2ml of Ferrozine and were vortexed and left remaining at room temperature for 10 min. The reaction mixture containing deionized water in place of a sample was considered as the negative control absorbance of the solution was studied for their absorbance using UV-spectrophotometre at 562 nm against the blank (deionized water). EDTA was used as the standard. The chelating ability is calculated as,

$$\text{Inhibition (\%)} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \text{ X } 100$$

Absorbance control

IC_{50} of the value for Individual extract (CNPs) with standard deviation is presented

2.4 Cytotoxicity of CNPs of *Eichhornia crassipes*:

2.4.1 Culturing cell lines

Vero and human lung cancer cells (A549) were cultured in Dulbecco's Minimum Essential Medium (DMEM) amended with Trypsin-Phosphate-Versene glucose (TPVG) solution, 10% New Born Calf Serum (NBCS), 100 U/mL penicillin and 100g/mL streptomycin in a 96-well Tissue Culture (TC) plate. The cells were sub-cultured every 3-4 days in a CO_2 incubator at 37°C in a 95 percent humidified environment enhanced with 5% CO_2.

2.4.2 MTT cell viability assay

The MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) experiment was performed by trypsinizing a monolayer of cell culture and adjusting the cell count to $1.0x10^5$ cells/ml using growth media [7]. 0.1ml of the diluted cell suspension (about 10,000 cells per well) was added to each well of the 96 well microtitre plate. The supernatant was flicked off after 24 hours, biosoot sample with various concentrations (250, 500,750 and 1000µg/ml) was added to each well.

The plates were then incubated at 37°C for 3 days in a 5% CO_2 environment and the results were microscopically evaluated after 24 hours. After 24 hours, the test solutions in the wells were withdrawn and MTT in HBSS-PR was added to each well. The plates were lightly shaken before being incubated at 37°C for 3 hours in a 5% CO_2 environment. After removing the supernatant and adding 50µl of propanol, the plates were gently shaken to solubilize the produced formazan. At a wavelength of 540 nm, the absorbance was measured using a microplate reader. The growth was calculated as [(Test-Blank) / (Control-Blank)] and Percentage growth as:

$$\text{Percentage growth} = [(\text{Test-Blank})/(\text{Control-Blank})] \times 100$$

3. RESULTS

3.1 Antioxidant activity of CNPs of soot from *Eichhornia crassipes*

3.1.1 DPPH radical scavenging activity:

The antioxidant properties of methanol and aqueous extract using CNPs of *Eichhornia crassipes* were evaluated using DPPH. The methanol and aqueous extract of the CNPs of *Eichhornia crassipes* at the concentration of 200µl, 400µl, 600µl, 800µl and 1000µl of individual extract were evaluated against the DPPH and their IC_{50} value were estimated. The IC_{50} value of 502.22µl for methanol extract and IC_{50} value of 581.33µl for Aqueous extract were recorded. The DPPH inhibitory percent for individual concentration of methanol and aqueous extract is presented in Table 1 & Figure 1 and 2.

Table 1. Anti-oxidant activity of CNPs of *Eichhornia crassipes* against DPPH

Sample	200µL	400µL	600µL	800µL	1000µL	IC_{50} (µl)
Methanol extract	32.43	44.9	56.12	71	82.42	502.22
Aqueous extract	22.56	40.39	51.48	65.16	75.38	581.13

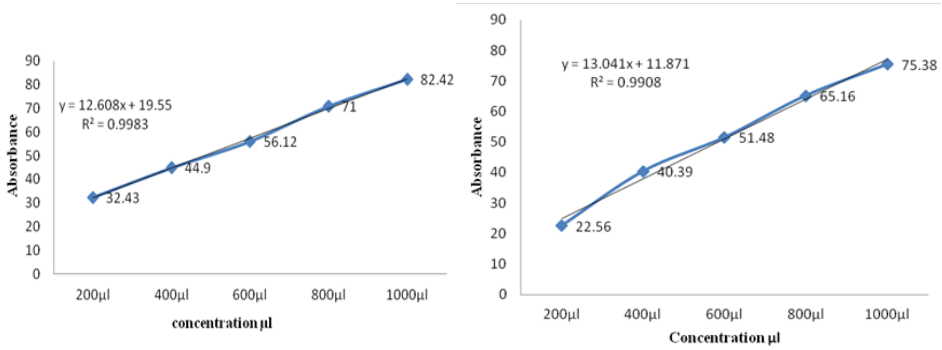

Figure 1. DPPH scavenging activity of methanolic and aqueous extracts of the CNPs of *Eichhornia crassipes*

3.1.2 Phosphomolybdenum activity

The methanol and aqueous extract of the CNPs of *E. crassipes* at the concentration of 200µl,400µl, 600µl,800µl and 1000µl were evaluated against the phosphomolybdenum activity and their IC_{50} value were estimated.The IC_{50} value of 303.61µl for methanol extract and IC_{50} value of 611.43µl for Aqueous extract were recorded. The inhibition percent of Phosphomolybdenum of methanol and aqueous extract of the CNPs of *E. crassipes* is presented in Table 2 & Figure 3 and 4.

Table 2. Phosphomolybdenum activity of CNPs of *Eichhornia crassipes*

Sample	200µL	400µL	600µL	800µL	1000µL	IC_{50} (µl)
Methanol extract	38.87	61.04	72.57	82.12	91.68	303.61
Aqueous extract	20.6	29.17	49.67	57.68	69.16	611.43

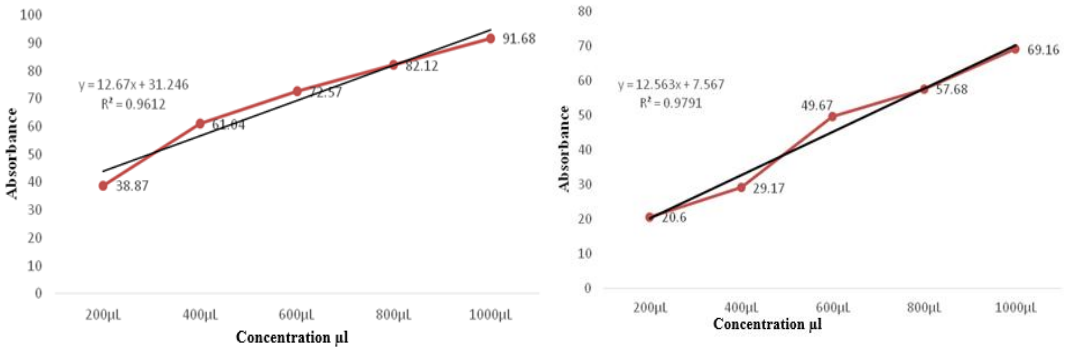

Figure 3. Phosphomolybdenum activity of the methanol and aqueous extracts of CNPs of *Eichhornia crassipes*

3.1.3 Metal chelating activity

The metal ion chelating activity of the CNPs of methanol and aqueous extract from *Eichhornia crassipes* were analyzed and shown in Table 3 & Figure 6 and 7. The methanol and aqueous extract concentration of 200µl, 400µl, 600µl, 800µl and 1000µl of individual extract were evaluated against the metal chelating activity and their IC_{50} valuewere estimated. The IC_{50} value of 964.67µl for methanol extract and IC_{50} value of >1000µl for Aqueous extract were recorded. The metal chelating activity in percent of methanol and aqueous extract of the CNPs of *E. crassipes* is presented in Table 3 & Figure 5 and 6.

Table 3. Metal chelating activity of CNPs of *Eichhornia crassipes*

Sample	200µL	400µL	600µL	800µL	1000µL	IC_{50}(µl)
Methanol Extract	18.59	23.45	31.65	40.45	52.76	964.67
Aqueous water	16.85	21.97	33.58	38.56	47.96	>1000

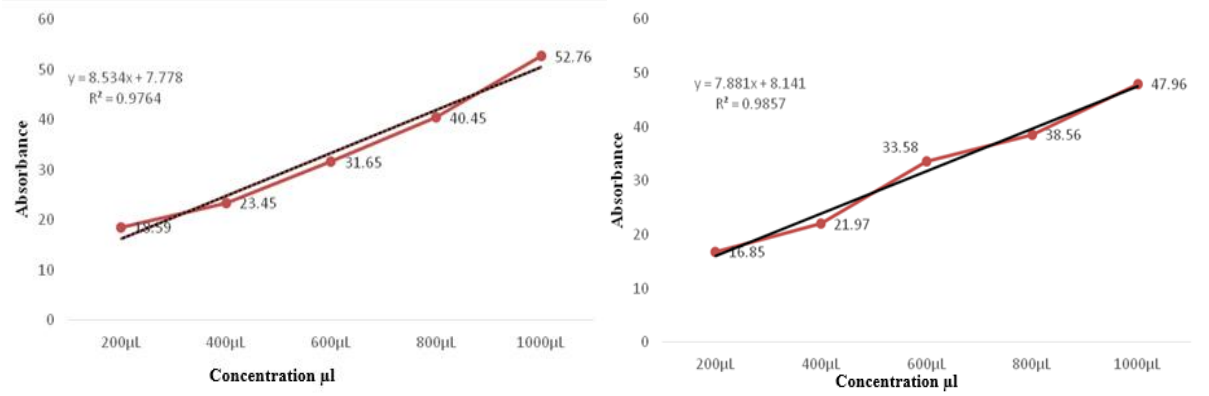

Figure 5. The metal chelating activity of the methanol and aqueous extracts of CNPs of *Eichhornia crassipes*

3.2 Cytotoxicity of *Eichhornia crassipes* against A549 Cell line :

The Cytotoxicity activity of CNPs of *Eichhornia crassipes* soot sample against the A549 cell was treated with different concentrations of CNPs for 24 hours and their growth was examined. The CNPs of *Eichhornia crassipes* demonstrated a minimal inhibition against A549 cells even at the concentration of 1000µg/ml. At a dose of 1000µg/ml, a maximum inhibition was observed (42.79%) which is less than 50% of cell death in current study. The percent inhibition recorded for different concentration of CNPs is presented in Table 4. The photomicrographs showing cell death in Fig. 7.

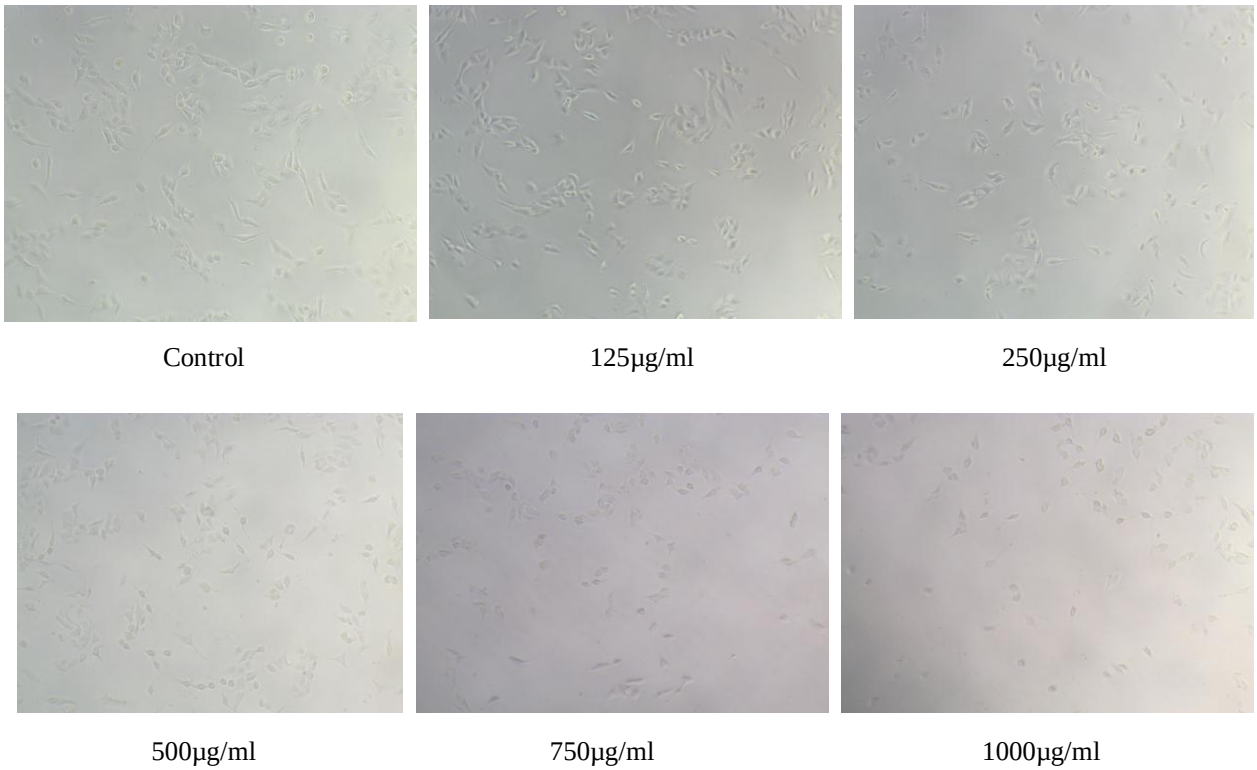

Figure 7. Photomicrograph depicting anti-proliferative activity of the cell line (A549) of CNPs of *Eichhornia crassipes*

Table 4.Cytotoxicity of CNPs of *Eichhornia crassipes*

	OD value			
Concentration	**1**	**2**	**3**	**Percent Inhibition**
Control	1.896	2.831	2.253	100
125µg	2.234	2.711	2.362	0
250µg	1.698	2.559	2.21	5.61
500µg	1.997	2.118	2.276	21.87
750µg	1.715	1.605	1.734	40.80
1000µg	1.662	1.551	2.513	42.79

4. DISCUSSION

Carbon nanomaterials are typically composed of complex mixtures of compounds that differ only slightly in molecular weight, structure, isomerism and so on [3]. It is also known that some of these materials have different properties and structures when acquired from different manufacturers [5] depending upon the mode and source of synthesis. The characterization of Carbon nanoparticles synthesized in the form of biosoot from *E. crassipes* using Dynamic Light Scattering (DLS), Scanning Electron Microscope (SEM) and X-ray crystallography (XRD) as a carbon nanoparticle is already reported [18]. The antioxidant activity for DPPH against *Eichhornia crassipes* has highest free radical scavengingactivity that wasexertedbymethanol extract of soot sample (IC_{50}value – 502.22µl) when compared with Aqueous extract (581.13µl). This shows that the Carbon nanoparticles synthesized using *Eichhornia crassipes* are poor antioxidative agent. The metal chelating activity, of *Eichhornia crassipes* has minimum chelations that were observed for the methanol extract of leaf soot sample (964.67µl) and for aqueous extract the IC_{50} value is >1000µl. This is again proved that the carbon nanoparticles are not having any associated compounds to react with or reduce the metallic salts.

For Phosphomolybdenum activity, *Eichhornia crassipes* showed methanol has higher phosphomolybdenum activity (303.61µl) followed by Aqueous extract (611.43µl). This again shows that the carbon nanoparticles synthesized using *E. crassipes* is not a good reducing agent on its own. So far, there was no study conducted on Phosphomolybdenum activity of any of the carbon nanoparticles synthesized. This is the first report to study the Phosphomolybdenum activity against the CNPs. The poor antioxidant ability, metal chelating activity and phosphomolybdenum reducing capacity of CNPs synthesized using *E. crassipes* is

attributed to lesser compounds associated with the CNPs synthesized. The CNPs prepared using the leaves of *Chromolaena odorata* was found to possess many associated compounds [16]. However, the Fourier Transform Infra-Red spectrophotometer analysis and the Gas Chromatography studies on the CNPs synthesized using the leaves of *E. crassipes* proved the presence of very few compounds associated with CNPs [18].

The CNPs of *Eichhornia crassipes* against A549 human lung cancer cell line showed maximum inhibition at 1000µg/ml when compared to 125, 250, 500, 750µg/ml concentrations studied. They are able to control the cancer cells only at higher concentration of CNPs. The results prove that carbon nanoparticles of aquatic plants are less toxic when compared to that of diesel soot which attributes to the interference in the viability of the data [1]. The combustion generated particles that released into atmosphere will have increased toxicity (oxidized flame soot) [1]. The airborne particles by burning wood has induced cytotoxicity against 16HBE cell line [8]. It is reported that the cytotoxicity of Carbon black nanoparticles depends upon their surface modification [9].

The biosoot of common weeds, i.e. *Calotropis gigantea, Lantana camara* and *Parthenium hysterophorus* were evaluated for their antibacterial and antifungal activity which showed that they are better agents of antimicrobial compounds [15]. However, the CNPs of *Eichhornia crassipes* showed poor antimicrobial potency when compared with the previous weeds reported. They were evaluated for their nanocarrier ability using streptomycin to act against bacteria of dental caries was already reported and usage of CNPs was recommended after studies on toxicity evaluation [17] This current study provides the data related to the antioxidant ability, metal chelating activity and the cytotoxicity against the cell line A549. The study aptly demonstrates that the CNPs synthesized in the form of Biosoot from the leaves of *E. crassipes* are safer to use and can be used as a successful nanocarrier.

5. CONCLUSION

The Carbon nanoparticles synthesized in the form of Biosoot from the leaves of *Eichhornia crassipes* were evaluated for their antioxidant, metal chelating and cytotoxicity. The study showed that they are low in their antioxidant activity, metal chelating ability and cytotoxicity. This is attributed to the less reductive oxidative species (ROS) associated compound with that of Carbon nanoparticle. The study aptly demonstrates that the CNPs synthesized using *E. crassipes* are less toxic and much safer in its applications. Thus, the biosoot in the form of carbon nanoparticle can be potentially used in the field of medicine and cosmetics.

6. CONFLICT OF INTEREST

The authors declare that there is no conflict of Interest exist.

7. REEFERENCES

1. Amara L. Holder, Brietta J. Carter, Regine Goth–Goldstein, Donald Lucas, Catherine P. Koshland. Increased cytotoxicity of oxidized flame soot. Atmospheric pollution Research. 2012 Jan;3(1):25-31.
2. Chantiratikul P, Meechai P, Nakbanpote W: Antioxidant activities and phenolic contents of extracts from Salviniamolesta and *Eichorrnia crassipes*. Res J Biol Sci. 2009;4:1113-7
3. Deepthi P D, Raghuraman M, Venkata V K V and Amit N. Green Synthesis of Full Color Fluorescent Carbon Nanoparticles from Eucalyptus Twigs for Sensing the Synthetic Food Colorant and Bioimaging.ACS Publications.2020;5(31):19905–19918.
4. Ibrahim khan, Khalid saeed, Idrees khan. Nanoparticles : properties, applications and toxicities. Arabian journal of chemistry. 2019;12(7):908-931.
5. IlijaRasovic; Water-soluble fullerenes for medical applications. Materials Science and Technology 2016;33:777-794.
6. Juan Carlos Matinez-Espinosa, Maria Antonieta Ramirez- Morales and Raul Carrera Cerritos. Silver Nanoparticles Synthesized using *Eichhornia crassipes* extract from Yuriria Lagoon, and the Perspective for Application as Antimicrobial Agent. Crystals MDPI.2022;12:814.
7. Karina Weinhold, Peter Konig. The cytotoxicity of carbon black nanoparticles depends on their surface modification. European Respiratory Journal. 2014;44:p3856.

8. Kumar S, Kumar R, Dwivedi A, Pandey AK: In vitro antioxidant, antibacterial, and cytotoxic activity and in vivo effect of Syngoniumpodophyllum and *Eichhornia crassipes* leaf extracts on isoniazid induced oxidative stress and hepatic markers. Biomed Res Int. 2014;4:452-459.
9. Leena V. Hublikar, Sharanabasava V.ganachari, Narasimha Raghavendra and Veerabhadragouda B Patil. Green synthesis of silver nanoparticles via *Eichhornia crassipes* leaves extract and their applications. Current Research in Green and Sustainable chemistry. 2021;4:100212.
10. Manyuchi M, Mbohwa C, Muzenda E, Mutusva T N, and Mpeta M. Degradation of Water Hyacinth (Eichhornia *crassipes*) to Vermicompost through Application of the Vermicomposting Technology. Proc. Int. Conf. Ind. Eng. Oper. Manag. IEOM 1,2019:79– 88. doi:10.33965/ ste2019_201901l010.
11. Mishra S, and Maiti A. The Efficiency of *Eichhornia crassipes* in the Removal of Organic and Inorganic Pollutants from Wastewater: a Review. Environ. Sci. Pollut. Res. Int. 2017;24(9):7921–7937. doi:10.1007/s11356-016-8357-7.
12. Mustafa H M, and Hayder G. Recent Studies on Applications of Aquatic weed Plants in Phytoremediation of Wastewater: A Review Article. Ain Shams Eng J. 2021;12(1):355– 365. doi:10.1016/j.asej.2020.05.009.
13. Patel S. Threats, Management and Envisaged Utilizations of Aquatic weed *Eichhornia crassipes*: An Overview. Rev. Environ. Sci. Biotechnol. 2012;11(3):249–259. doi:10.1007/s11157-012-9289-4.
14. Rodriguez C. Design and Construction of a Biohydrogen and Bioethanol Production System from the Biomass of the *Eichhornia crassipes*. 2018.doi:10.20944/preprints201805.0393.v1
15. Sanjeev Kumar R, Sampath Kumar B, Hemalatha N, Suganthi V, Bhuvaneswari S, and Udaya prakash N K. Antibacterial and antifungal studies on the biosoot of common weeds. 2022;13(8):851-855.
16. Sanjeev kumar R, Sindhu priya K,Sriraman V, Tharani Kumar L, Ramya R, Sundara Venkata Prasad R, Bhuvaneswari S, Udaya Prakash N K. Synthesis of carbon nanoparticles from Chromolaena ordata as Biosoot and their Characterization. Europeon Chemical Bulletin. 2023;12(8):2547-2557.
17. Sindhu priya K, Shaima S, Sampath kumar B, Bhuvaneswari S, and Udaya prakash N K. Antimicrobial activity of Biosoot of *Eichhornia crassipes* against Bacteria of Dental caries and its application as on alternate for charcoal in Toothpaste. YMER journal.2023;22(2):1107-1118.

18. Sindhu Priya, K., Sampath Kumar, B., S. Bhuvaneswari and N.K. Udaya Prakash. Facile synthesis of carbon nanoparticles using an aquatic weed, *Eicchornia crassipes*, Journal of Pharmaceutical Negative Results.2022;13(8):879-885.
19. Surendraraj A, Farvin KHS, Anandan R: Antioxidant potential of water hyacinth (*Eichornia crassipes*): in vitro antioxidant activity and phenolic composition. J Aquat Food Prod Technol. 2013;22:11-26. 10.1080/10498850.2011.621582.
20. Wang; Rajagopalan Sridhar; Techniques for physicochemical characterization of nanomaterials. Biotechnology Advances.2014;32:711-726
21. Yan S H, Song W, and Guo J Y. Advances in Management and Utilization of Invasive Water Hyacinth (*Eichhornia crassipes*) in Aquatic Ecosystems - a Review. Crit. Rev. Biotechnol. 2017;37(2):218–228.doi:10.3109/07388551.2015.1132406.
22. Zhang Y, Zhang D Y, and Barrett S. C. Genetic Uniformity Characterizes the Invasive Spread of Water Hyacinth (*Eichhornia crassipes*), a Clonal Aquatic Plant. Mol. Ecol. 2010;19(9):1774–1786. doi:10.1111/j.1365-294X.2010.04609.x