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# Anticancer Assessment of Green Synthesized Carbon Nanodots from *Calotropis Procera*

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**Abstract**
Carbon based nanomaterials with a diameter less than 10nm, known as carbon dots, have caught the interest of scientists since they were discovered. Scientists from all fields related to health have uncovered limitless uses for these CDs due to their therapeutic nature. The surface functional groups of CDs dictate their ability to penetrate cells and interact with receptors. Modifying the surface of CDs is therefore an important factor when determining their potency for medical applications. The preparation of these CDs from biologically active precursors is of greater potency. However, the synthesis would be expensive and probably risky to the environment. Therefore, we propose the green synthesis of CDs from the Latex of *C. procera* owing to the high content of potently anticancer Cardenolides present in the latex. Besides its cost-effectiveness and ease of preparation, this approach could also be beneficial due to the possibility of synthesizing cellular-specific CDs, thereby reducing the toxic side effects caused by conventional treatments. In conclusion, at the end of the research we hope to obtain promising Lat-CDs that could serve as substitute to the current noxious cancer treatments.
**Keywords:** Carbon dots, *C. procera,* Cancer, Green synthesis

## Introduction

Carbon based nanomaterials with a diameter less than 10nm, known as carbon dots (CDs), have received huge attention from researchers since they were discovered in 2004 [21]. Realization of their photoluminescence property upon surface passivation [18] unveiled their unlimited applications in different fields. Potential use for bioimaging both in vitro and in vivo have been demonstrated [5,11].

One of two approaches is generally used to synthesize nanomaterials, top down or bottom up. The former involves crushing of a bulk material to nano-sized dimensions while the latter is the reverse of the top-down approach [3,15,17]. Top down approached has been used for the synthesis of CDs. Several techniques including fragmentation using laser [14], selective oxidation of the bulk material using hydrogen peroxide [8], and a mixture of strong acids ($H_2SO_4$ and $HNO_3$) [2] were employed

Synthesis of CDs is regarded as green when less toxic starting materials are used. Basically, both the solvent and the precursor should pose no threat to the human and environment in any way [3]. CDs with synergistic anticancer property were synthesized from dried lemon peel [6], CDs for tumor imaging application were prepared from milk [19], *Trapa bispinosa* derived CDs with exceptional biocompatibility were also prepared [13]. In addition to its cheap precursors, this approach does not require further surface passivating material. As earlier shown [18], detectable photoluminescence behavior of CDs has been directly linked to the addition of surface passivator. Also, CDs properties were shown to be influenced by the nature of surface functional groups on CDs. As such, synthesis of CDs with desired properties could be achieved by engineering the method of synthesis.

*Calotropis procera,* commonly called apple of Sodom, is known locally as “tumfafiya” in Nigeria’s Hausa language. It is a broad-leaved shrub from *Apocynaceae* family usually found in dry habitats and is native to Africa and Asia [7]. Traditionally, *C. procera* have been used for treating various ailments especially skin diseases, diabetes, and tumors [16]. The anticancer activity of the various compounds extracted from this plant is being investigated despite its toxicity [9]. Anti-cancer property of extracts andprotein from different parts of *C. procera* has been widely reported previously [4,16].

According to Yaniv *et al* [24] a compound which shows high anticancer activity was developed from cardenolides. Cardenolides are mostly found in high concentration at the latex of a tree belonging to these families [23]. Therefore, exploring extracts from latex of an Apocynaceae plant could serve as an avenue for the discovery of novel cancer drugs. Herein, we propose the synthesis of CDs from the latex of *C. procera* using green synthesis approach. The technique to be employed is pyrolysis method due to its easier procedure. Since precursors for CDs could directly predict their biological activity [1], Cardenolides rich latex would likely confer anticancer activity to the as-would-be synthesized CDs. Due to the selective interaction shown by some previously reported CDs [10] as well as selective toxicity [12], these CDs would be expected to be selective towards certain cancer cells while having lower toxicity against normal cells. Thus, ultimately leading to highly specific nano-based cancer treatments that are sourced from widely available raw materials thereby providing affordable and more effective treatments.

**Materials and methods**

**Materials:** Latex of *C. procera* would be collected from *C. procera* available Bayero University, Kano Old Campus, Kano Nigeria. Cell Counting Kit-8 (CCK-8), a cytotoxicity kit, was purchased from MedChemExpress (USA). Human breast cancer MCF-7, hepatoblastoma HepG2 cell lines, human cervical adenocarcinoma (HeLa) and prostate cancer PC3 cell lines would be used for anticancer assays. Reagents of analytical grade would be used as received without any further purification.

**Synthesis of CDs:** CDs were prepared using pyrolysis method as described by [11,22]. 50mg of powdered latex would be placed in an isolated reactor so as to limit air flow. Then the powder would be pyrolyzed at 250°C for 2h. The resulting Lat-CDs would be homogenized in 10mL purified water by ultrasound. The Lat-CDs would then be filtered using 0.22μm filtration membrane.

**Characterization of Lat-CDs:** Lat-CDs would be characterized by PL, Fourier transforms infrared (FTIR), Raman analysis, TEM and UV-Visible.

**Cytotoxicity Study:** The cytotoxicity test of Lat-CDs would be performed on HeLa, HepG2, MCF-7 and PC3 cancer cells by a CCK-8 assay [20]. Cell lines would first be cultured at a density of $10^5$ cells per well in a 96-well plate containing DMEM and incubated for 24 h. Then, PBS would be added to wash the cells. The cells would then be incubated further in 100 mL of DMEM with different concentrations of Lat-CDs (10 mL) for different durations. Once the adjusted time was reached, the reaction would be continued by adding 1 mL of CCK-8 reagent (500 mg $mL^{-1}$) followed by incubation for 4 h. The absorbance of formazan on each plate is correlated with the living cell and would then be analyzed using a microplate reader at 450 nm. The cytotoxicity effects of Lat-CDs will be assessed at half the cytotoxic concentration (CC50) and fixed using the Origin software.

**Expected Results**

The Lat-CDs in a similar manner to previously reported CDs from natural sources, the Lat-CDs would be expected to show photoluminescence. Their size would be within the range of nanodots (<10nm) and have surface functional groups present in the secondary metabolites responsible for the CDs formation.

As shown previously [1], CDs can take the biological activity of their precursors with better selectivity [12]. It would be expected that these Lat-CDs would show cytotoxicity towards the cancer cell lines under testwhile selectively showing lower toxicity towards normal cells.

**Conclusion**

Cancer poses a greater threat to both developed and low-income countries because of continuous exposure to many carcinogens. Current treatments are too expensive for low-income countries despite the many side effects shown by these treatments. Thus, we propose the synthesis of CDs using widely available sources for use in potentially affordable treatments with improved specificity.

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