Bull. Chem. Soc. Ethiop. **2025**, 39(5), 907-920.
DOI: https://dx.doi.org/10.4314/bcse.v39i5.8

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

MICROWAVE SYNTHESIS OF CARBON DOT FROM ASPARAGUS RACEMOSUS FOR Ag^+ ION SENSING, ANTI-OXIDANT AND CYTOTOXICITY STUDIES

Priya Bijesh[1,2], V. Andal[2] and V. Selvaraj[1*]

[1]Department of Chemistry, University College of Engineering-Villupuram, Kakuppam Villupuram 605 13, India
[2]Department of Chemistry, KCG College of Technology, Chennai 600 097, India

(Received September 30, 2024; Revised December 26, 2024; Accepted January 26, 2025)

ABSTRACT. In this study, we provide a single-step, environmentally friendly microwave method for producing water-soluble, monodisperse carbon dots (CDs) using the natural herb *Asparagus racemosus* root as a carbon precursor. These CDs were characterised structurally and morphologically using TEM, XRD, and FTIR techniques. TEM measurements of the newly created CDs showed that they were ranged in size of 2 to 10 nm. By using FTIR spectroscopy, the functional groups -OH and COOH on the exterior of the CDs was proved. The potential of the synthesized carbon dots was used to sense Ag^+ ion in an aqueous media. Here, we provide a colorimetric carbon dot (CD) probe for detecting the Ag^+ ion in water visually. Moreover, antioxidant characteristics were assessed using the DPPH method, and their cytotoxicity was studied upon A549 cell-lines. Results from the DPPH assay revealed that CDs at higher concentrations had greater antioxidant capacity than the standard L-ascorbic acid and the MTT assay reveals the cytotoxic properties by increasing the rate of cell death as the concentration of CDs increases. There is a rise in cytotoxic activity, or 95% cell inhibition, at a dose of 500 µg/mL anti-cancerous and antioxidant effects may be favourably correlated.

KEY WORDS: Antioxidant activity, Free radical scavenging, Lung cancer cell line

INTRODUCTION

The nanospheres known as carbon dots have a graphitic core and a surface layer, which has experienced sp^2 hybridization [1]. C-dots are among the fascinating nanomaterials because of their high-water solubility, low toxicity, distinctive luminous characteristics, excellent biocompatibility, high chemical stability, and simplicity of functionalization [2]. By changing the synthesis parameters, such as the source, temperature, reagents, etc., it is possible to tailor the properties of C-dots [3]. Due to their remarkable characteristics, C-dots are an excellent option for a wide range of applications in technology and medical science, including bioimaging [4], photocatalysis [5], sensing [6], and security fluorescent coding [7]. Top down and bottom-up methods are used to prepare C-dots. Bottom-up methods includes microwave irradiation, ultrasonication, hydrothermal, laser ablation [8, 9] etc. Among other techniques, the microwave method produces CDs with high yields, little impurities, and precise size control. Additionally, microwave heating is the contactless heat transmission to the reactants, allowing the opportunity for the reactions to occur in a short period of time. Microwave synthesis of carbon dots (CDs) from *Asparagus racemosus* offers several significant advantages compared to traditional synthesis methods. Here are the key benefits: rapid synthesis (microwave-assisted synthesis significantly reduces the reaction time required to produce carbon dots.), higher yield (the microwave method tends to produce a greater yield of carbon dots compared to conventional heating methods), uniform heating (microwave irradiation provides volumetric heating, meaning that heat is distributed uniformly throughout the material), eco-friendly process, cost effectiveness [10-11].

*Corresponding authors. E-mail: vaithilingamselvaraj@gmail.com

Nowadays, plant and other green biomasses are used as precursors in the production of CDs. Due to their easy availability and growing environmental concerns, a variety of biomass precursors, such as pineapple [12], pears [13], musk melons [14], pumpkin seeds [15], milk protein [16], and watermelon [17] have been employed as the raw materials. However, very little research on the synthesis of CDs utilising the root of the *Asparagus racemosus* plant has been documented. *A. racemosus*, often known as "Shatavari," is a perennial herb that is a member of the Lamiaceae family. Due to its numerous applications (including those for neurological diseases, dyspepsia, stomach ulcers, and galactagogues), demand is always increasing [18].

The overuse of heavy metals in several industrial and domestic applications poses a major hazard to the environment [19]. Carbon dot was synthesized by Das and his colleagues from jute waste, and it was used as a nano button for the detection of inorganic pollutant Cr ions [20]. Tan and his team utilised L-arginine to make carbon dots, which they then used as fluorescent sensors to detect the metal ions Cd and Hg in food samples [21]. Some recent research described the use of CDs as fluorescent agents to detect the ions Hg^{2+}, Pb^{2+}, Cu^{2+}, Fe^{3+}, Cd^{2+}, and Cr^{6+} [22, 23].

Ag^{+}, a noble metal ion, is employed in a variety of industrial applications, including the pharmaceutical, electronic, photographic, and mirror sectors. A significant amount of silver ion is released into the environment as industrial waste, particularly through water channels and sludge. But extremely high levels of silver ions in natural environments can be harmful to aquatic life and to people's health [24, 25]. As a result, finding out how much silver ion is present in water samples becomes crucial.

To ascertain the presence of the silver ion and its concentration, modern analytical methods based on ICP-AES, AAS, and EC techniques, such as ion selective electrodes, etc. can be employed. However, the widespread use of such techniques for regular analysis is constrained by issues like costs, complicated apparatus, etc. Therefore, a straightforward, less expensive, and speedy analytical device is needed for such analysis. Carbon dots (CDs) have emerged as a promising material for use in colorimetric sensors due to their unique properties. Few advantages of using carbon dots for this application includes high sensitivity and selectivity [26], low toxicity and biocompatibility, tunable optical properties [27], cost effectiveness, easy integration into devices [28], etc.

Cancer is the leading global cause of death, which affects both developed and developing countries. Chemotherapy is a widely used cancer treatment, but it has a number of drawbacks, including the toxicity it causes to normal cells because the chemical medications can't tell them apart from diseased ones [29]. Only a few numbers of studies have been published on the intracellular activity of carbon dots and the mechanisms behind their potential anticancer effects. Li and his team synthesised carbon dot using fresh ginger extract, and they examined its anticancer properties in human hepatocellular carcinoma cells, healthy liver cells, and healthy mammary epithelial cells. Due to the induced intracellular formation of reactive oxygen species, they identified a good inhibitory effect for human hepatocellular than others [30]. Similarly, Mathew *et al*. group has prepared carbon dot from the green source using hydrothermal method and studied its anticancer activity against MCF-7 breast cancer cells [31]. Fahmi and his team recently developed chalcone-loaded carbon dots and tested their anticancer efficiency on He La cells [32]. Researchers also synthesized carbon dot from agro waste. Surendran and his group successfully synthesized carbon quantum dots (CQDs) from the agro-waste of *Ananas comosus* (pineapple) and cassava (*Manihot esculenta*) using a facile hydrothermal method. They also studied its NLO property, antibacterial and antioxidant properties [33]. Researchers have focused on the development of anticancer drugs that precisely cause cell death and are less hazardous to normal cells. Additionally, numerous studies have been done on the anticancer properties of CDs.

The present work focuses on the synthesis of carbon dots (CDs) using the root of the *Asparagus racemosus* plant through a microwave method. This innovative approach leverages the natural compounds present in the plant's root for the production of CDs, which are nanometer-sized carbon materials with unique optical properties. Various methodologies were used to

characterise the physicochemical characteristics of CDs, including their elemental composition, surface functional groups, and particle size. Evaluation of the CDs' cytotoxic and antioxidant effects on a lung cancer cell line is another goal of the current investigation. The standard DPPH assay was used to investigate the antioxidant activity, and the MTT assay was used to investigate the cytotoxic activity.

EXPERIMENTAL

Chemicals

Without any additional purification, all of the chemicals used were of analytical quality. We bought various metal chlorides and nitrates ($FeCl_3$, $BaCl_2$, $MnCl_2$, $PbCl_2$, $CuCl_2$, $BiNO_3$, $ZnCl_2$, $AgNO_3$ and $NiCl_2$) from Hi Media in India. The roots of *Asparagus racemosus* were collected from the shop. The collected roots were thoroughly cleaned before being dried and pulverised for usage. All of the tests were conducted using deionized water (DI).

Synthesis of CDs from Asparagus racemosus root by microwave method

The preparation of yellowish green, carbon dots from natural biodegradable carbon source. *Asparagus racemosus* root by microwave reaction, which includes the following steps; (i) Preparing a solution by mixing *Asparagus racemosus* root powder (carbon source) with deionized water. The obtained solution was heated for 10 min at 900 watts in a microwave oven. (ii) After the microwave reaction, the solution was cooled. (iii) A Whatman Grade 1 Qualitative filter paper was used to filter the resultant yellow solution after centrifuging it at 10,000 rpm for 15 min to get rid of any big particles. Figure. 1 shows a pictorial illustration of the CDs preparation and colorimetric sensing of Ag ions.

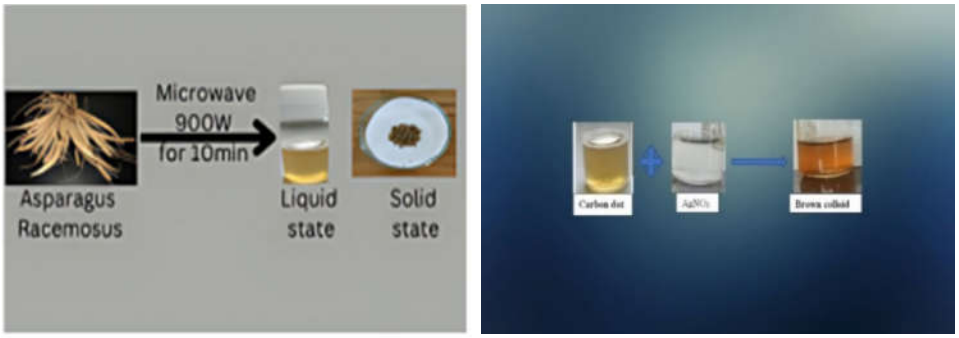


Figure 1. Schematic representation of carbon dot synthesis and colorimetric sensing of Ag ions.

Characterization

The CDs were examined using UV–Vis spectroscopy (Hitachi Double beam spectrophotometer Model U2800), powder X-ray diffraction (CuK, PANalytical), FT-IR spectroscopy (ThermoNicolet-330 spectrometer) and TEM (JEOL 3010).

Ag^+ sensing analysis

Lead nitrate**,** calcium nitrate, potassium nitrate, sodium nitrate, silver nitrate, cadmium nitrate, sodium hydrogen arsenate, zinc acetate, magnesium nitrate, and mercury nitrate have all been

prepared as bulk solutions ($2x10^{-3}$ M). In order to determine each metal ion's response, 10 mL of the metal ion solution were first mixed to 1 mL of CDs colloid. Within a few seconds of the CDs colloid being added, the mixture at room temperature underwent a slight shake, changing the colour. In order to evaluate the colloid's selectivity in sensing the metal ions, 1 mL of CDs colloid was added to a homogenised solution containing 1 mL of each metal ion. Using a UV-Vis spectrophotometer, the resulting solutions were examined.

MTT assay and antioxidant studies

Antioxidant assay

DPPH assay

The free radical scavenging activity was used to determine the antioxidant assay's percentage (2, 2-diphenyl-1-picryl-hydrazyl-hydrate). A total of 1.0 mL of different CD concentrations (10–50 g) were combined with 0.1 mL of DPPH-methanol solution (0.135 mM). After complete vortexing, the reaction mixture was kept at ambient temperature in the dark for half an hour. Spectrophotometric analysis was used to determine the mixture's absorbance at 517 nm. Vitamin C was used as standard drugs [34]. The following equation was used to determine the percentage of free radical scavenging:

$$\% \text{ Scavenging} = 100–(\text{Abs sample}–\text{Abs blank})/\text{Abs Control} \times 100 \quad (1)$$

Cell culture maintenance

The National Centre for Cell Sciences (NCCS), located in Pune, India, provided the lung cancer A549 cell lines that were used in this study. The cell line was maintained in Dulbecco's Modified Eagle Media (DMEM), which was augmented with 10% Fetal Bovine Serum (FBS). In order to avoid bacterial contamination, penicillin (100 U/mL) and streptomycin (100 g/mL) were added to the medium. The cell line medium was kept at 37 °C in a humid atmosphere with 5% CO_2.

MTT assay

The cytotoxicity of CDs on A549 cells was determined by the method of Mosmann [35]. In 10 mL of PBS was MTT(3-4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazoliumbromide) (50 mg) dye was dissolved. After vortexing for 1 min, it was filtered through 0.45 micro filters. Since MTT was light-sensitive, the bottle was wrapped in aluminium foil to block off light. At 4 °C, the preparation was kept.

Cell viability assay

A549 viable cells were collected and counted using a hemocytometer. They were then diluted in DMEM media to a density of 1 x 10^4 cells/mL, planted in 96-well plates for each well, and incubated for 24 hours to promote adhesion. A549 cells were treated with a control, and then varied amounts of CDs (50 to 300 g) were added to each well. For 24 hours, A549 cells were incubated at 37 °C in an incubator with humidified 95% air and 5% CO_2. The MTT (5 mg/mL in PBS) dye was added to each well after the drug-containing cells had been incubated, and they were then washed with fresh culture media and left to continue incubating for an additional 4 h at 37 °C. The cell viability was evaluated by absorbance at 540 nm using a multi-well plate reader, and the purple precipitated formazan was dissolved in 100 mL of DMSO. The percentage of stable

cells compared to the control was used to express the results. Calculating the half maximal inhibitory concentrations (IC_{50}) values and analysing the best doses at various times were done.

$$\text{Inhibitory of cell proliferation (\%)} = \frac{\text{Mean absorbence of the control} - \text{Mean absorbence of the sample}}{\text{Mean absorbence of the control}} \text{ X } 100 \quad (2)$$

The sample CDs dose response curve, which showed a 50% reduction in cytotoxicity when compared to vehicle control cells, served as the basis for calculating the IC_{50} values. At least three replicates of each experiment were run in each study.

RESULTS AND DISCUSSION

CD's morphological, optical, and structural characteristics

With the use of UV-Vis absorption spectroscopy, the generation of carbon dots from the powdered *Asparagus racemosus* root is demonstrated. The UV-Vis spectra of CDs generated after a 10-minute microwave exposure are shown in Figure 2. Two prominent absorption peaks at 211 nm and 370 nm are visible, and they correspond to the transitions of the functional groups n-π* of C––O and π-π* of C—C. We have not seen any evidence of the second shoulder peak that Naik saw at 465 nm. The emission data, however, are consistent with their reports [18]. The CDs are excited at several wavelengths, extending from 216 nm to 290 nm, and photoluminescence spectroscopy is used to examine the fluorescence characteristics.

Figure 2b displays the PL emission spectra of the CDs generated from *Asparagus racemosus* root at different excitation wavelengths. Two emission peaks at 447 nm and 569 nm were found, for the excitation wavelength of 216 nm. However, because of the n–π* transition, the highest intensity was achieved at the low 216 nm excitation wavelength. From 216 to 290 nm, the excitation wavelength is progressively increased, while the emission wavelength is shortened. The surface states of C—O bonds, polydispersity, and surface heterogeneity, are the major determinants of this excitation dependent emission property [36]. However, the multi-emissions disappear with the excitation wavelength over 290 nm. Similarly, Zhu *et al.* observed multi-emission luminescent peaks for carbon dots obtained from 2,4-diaminotoluene EDA and phosphoric acid by one pot hydrothermal method [37]. Ren *et al.* also observed multi-emission luminescent peaks for micropores carbon quantum dots [38].

The zeta potential measurement was carried out to determine what charge is present on CDs' surface. The zeta potential of CDs is depicted in the Figure 2c, with the largest peak being at -48.4 mV and a wide peak on the negative side. These negative surface charges demonstrated extremely high stability for CDs, leading to excellent dispersion in aqueous conditions and making them a strong option for the adsorption of positively charged substances like metal ions. As a result of electrostatic interactions, synthesised CDs are capable of adsorbing or detecting metal ions [39].

Figure 3a shows the X-ray diffraction pattern of microwave synthesized CDs heated at 900 W for 10 min. A large peak with a central angle of about $2\theta = 27.9°$ was visible in the prepared CDs' XRD pattern (Figure 4), indicating that they are of a highly disordered graphitic nature. Since there are lesser number of peaks compared to graphite the crystal structure of CDs is not well defined [40]. The obtained results are well-matched with the CD synthesized from Mexican mint by Archita *et al*. [41].

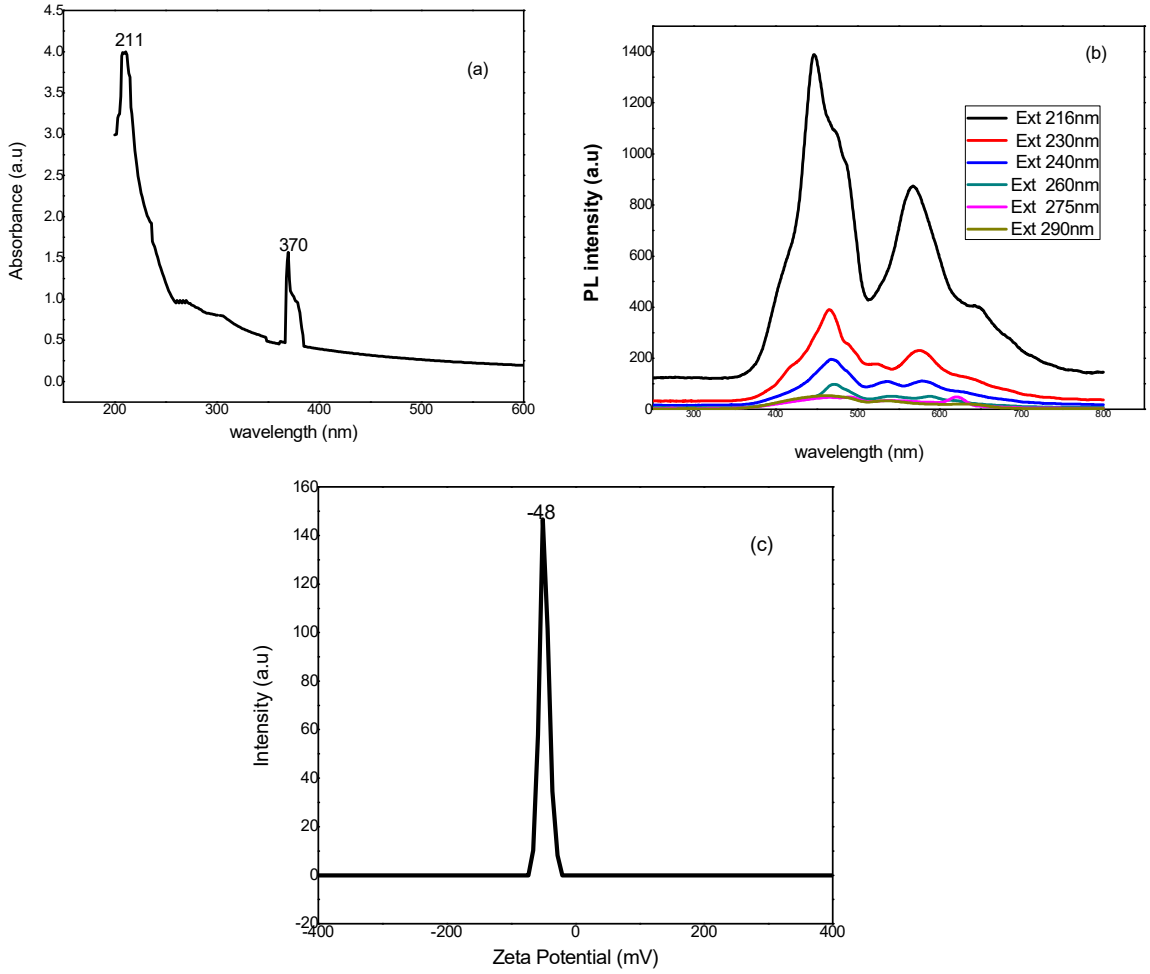


Figure 2. (a) UV-Vis spectra of carbon dot resulted from *Asparagus racemosus* root, (b) photoluminescence spectra of carbon dot excited from 216-290 nm, and (c) zeta potential graph of CDs resulted from *Asparagus racemosus* root.

The broad band seen in the CDs' FTIR spectra (Figure 3b) at 3200–3500 cm^{-1} belongs to C–OH and N–H stretching vibrations. The presence of the C=O and C-H stretching vibrations were revealed, respectively, by peaks emerging at 1600 and 682 cm^{-1}. Similar results were observed for the CDs obtained from fenugreek leaves, watermelon juice, etc. [42].

TEM was used to investigate the shape and size of CDs, as shown in Figure 3c. According to the size distribution histogram, the corresponding average sizes are 8±1 nm. TEM image confirms that the size of the particle is not very uniform.

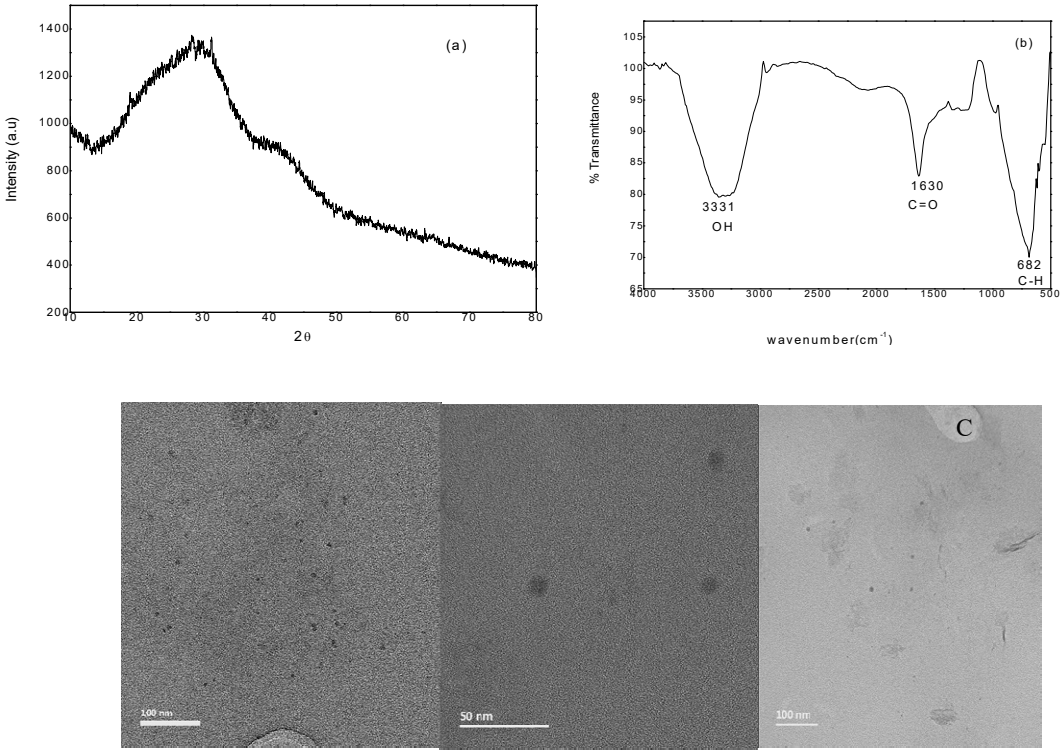


Figure 3. (a) XRD pattern of CDs resulted from *Asparagus racemosus* root, (b) FT-IR spectra of CDs resulted from *Asparagus racemosus* root, and (c) TEM images of CDs resulted from *Asparagus racemosus* root.

Colorimetric sensing property of CDs towards Ag^+ ion

When exposed to solutions containing different metal ions, such as Na^+, K^+, Ca^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Hg^{2+}, Mg^{2+}, and As^{5+}, with the exception of Ag^+, CDs retain their yellow colour. The addition of Ag^+ ion solution causes a noticeable colour change of the CDs from yellow to brown. The homogenised brown solution UV-Vis spectrum is recorded. Figure 4a displays UV-Vis spectra before and after the addition of silver ions. The shift from 370 to 454 nm in the "λ_{max}" value explains the colour change [43]. As reported in the previous reports the concentration of Ag ion as a linear relationship for the detection. Hence experiments were carried out by varying the Ag^+ ion concentrations from 2 x10^{-4} to 2 x10^{-5} M. It is clear that as the concentration of the silver ion solution declines, the absorbance value does as well. When the experiment is run with a solution containing all of the aforementioned cations, no interference with the detection of silver ions is seen. In Figure 4b, the selectivity of CDs in the detection of Ag^+ ion is depicted. The ligand functionalized chemosensors require considerable time to create, and they frequently cause interference with the sensitivity and selectivity of detection. The present CDs-based chemosensor, in contrast, reacts to Ag^+ ions at ambient temperature relatively quickly. Another important benefit of these CDs is that they are non-toxic and environmentally safe.

The mechanism behind the silver ion detection by CDs is analyzed by the characterization of brown solution using XRD. The X-ray diffraction analysis confirms the formation of nano Ag when the CDs is added to the silver nitrate solution. Consequently, during the reaction for the determination of silver ions, the CDs significantly contribute as reductants and stabilisers. Compared to other uses of similar systems that rely on luminescence, the role of carbon dots as a reducing agent is less studied. The potential of carbon dots as a reductant for the detection of Ag ions has been investigated by Raveendran and his colleagues [44]. Similar outcomes are seen when adding carbon dot to an Ag ion solution.

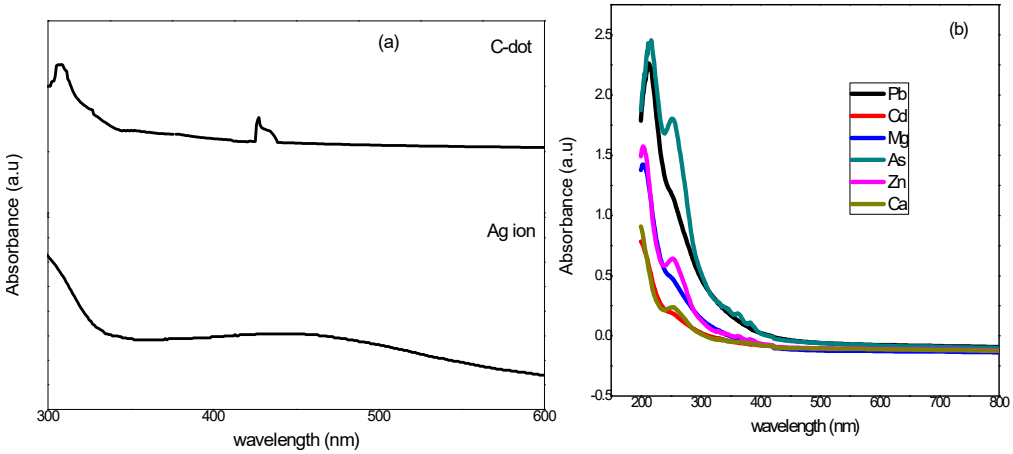


Figure 4. (a) UV-Vis spectra before and after the addition of silver ions and (b) selectivity of C-dots for the detection of metal ions.

The mechanism behind the color change in carbon dot-based colorimetric sensors involves several key interactions and processes that affect the optical properties of the carbon dots (CDs). The specific interactions that cause color change in carbon dot-based sensors are primarily driven by the interactions between the carbon dots (CDs) and target analytes. Because of the specific interactions the carbon dots (CDs) play a crucial role in the reduction of silver nitrate ($AgNO_3$) to form silver nanoparticles (AgNPs) and act as sensors.

Carbon dots contain various surface functional groups, such as hydroxyl (-OH), carbonyl (C=O), and carboxyl (-COOH) groups, which can facilitate electron transfer. When $AgNO_3$ is introduced to a solution containing CDs, these functional groups interact with silver ions (Ag^+), allowing the carbon dots to donate electrons to reduce Ag^+ to metallic silver (Ag^0). This reduction process is crucial for the formation of silver nanoparticles. The presence of CDs not only aids in the reduction but also stabilizes the resulting AgNPs. The surface functional groups on the CDs provide steric and electrostatic stabilization, preventing agglomeration of the nanoparticles.

The oxygen-containing functional groups on the surface of CDs are critical for both reducing silver ions and facilitating interactions with analytes. These groups enhance the electron-rich nature of CDs, promoting efficient electron transfer during the reduction process and enabling selective binding with analytes.

The interaction between these functional groups and Ag^+ enhances the sensitivity and selectivity of the sensor. The binding of silver ions can lead to structural changes in the carbon dots, further affecting their optical properties and enabling more precise detection [45].

The advantages of our current research to the previous studies is highlighted in Table.1

Table 1. Highlights of the present work.

Parameter	Present work	Reported literature
Carbon dot source and method of synthesis	*Asparagus racemosus* -root - microwave method for the first time	*Asparagus racemosus* -root- hydrothermal method [46]
Colorimetric sensor	Senses Ag^+ ion colorimetrically without any modification	Senses Ag^+ ion by modification with carrageenan and polyethyleneimine [47]

Biological properties

Cytotoxicity and antioxidant property of C-dots

Chronic diseases including cancer and cardiovascular (CAD) diseases are drastically decreased due to antioxidant properties. Even though there are many reports on the oxidative stress caused by various nanomaterials, carbon nanomaterials were thought to be unusual because of their naturally non-toxic intrinsic carbonaceous nature. A common approach to assess the antioxidant activity of any chemical used in industry is the DPPH assay. The antioxidant activity of any chemical can be quickly and easily assessed with this assay. In this investigation, CDs made from the roots of *Asparagus racemosus* showed excellent potential for combatting free radicals. It is noteworthy that the antioxidant activity rose exponentially with a negligibly altered concentration. The concentration known as the EC_{50} is the point at which a system exhibits 50% of the desired effect. A value of 20 µg/mL was determined as the EC_{50} for CDs based on Figure 5. Due to the numerous carboxyl and hydroxyl groups that are present on the surface of CDs, this significant scavenging activity may be caused. The antioxidant activity of carbon dots (CDs) is influenced by several factors that determine their effectiveness in scavenging free radicals and reducing oxidative stress.

The duration of the synthesis process significantly affects the antioxidant properties of CDs. Studies indicate that shorter synthesis times (e.g., 1 to 2 hours) lead to higher antioxidant activity compared to longer synthesis durations (e.g., 5 to 8 hours). This is likely due to the preservation of reactive functional groups and optimal size distribution in shorter synthesis times, which enhances their reactivity and ability to scavenge radicals [48].

The choice of precursor materials used in the synthesis of carbon dots can also impact their antioxidant capabilities. For instance, CDs derived from sources rich in phenolic compounds (like tea waste or grape pomace) tend to exhibit higher antioxidant activity due to the inherent properties of these compounds [48].

The cytotoxic assay was chosen for the investigation as a next step based on the results of the antioxidant assay [49]. Consequently, lung cancer cell line was chosen for cytotoxic activity based on antioxidant assay. Figure 6 shows the morphological changes that cancer cells have undergone, including cellular shrinkage and blabbing, which are indicative of apoptosis. In standard-treated cells, these morphological alterations are less noticeable, and they appear to be getting worse as the concentration of CDs rises. Figure 7 MTT assay findings demonstrate that the methanolic CDs have high cytotoxic activity against the cancer cell line at a concentration of 50 µg/mL with a rise in CD concentration, the lung cancer cell line's cell viability reduced, reaching its greatest level at 500 µg/mL. The enhanced cytotoxicity of carbon dots is due to the generation of reactive oxygen species. ROS can cause oxidative stress, leading to cellular damage, lipid peroxidation, and ultimately cell death. The anticancer activity mechanism diagram of the prepared carbon dots is provided in the Figure 8. The fact that cell viability decreased as CD concentration increased suggests that CDS may be an effective anti-cancer drug. Rajamani had already found that the CDs weren't hazardous to healthy cells [50]. The CDs' antioxidant and free radical scavenging activity may be the cause of their anti-cancer properties.

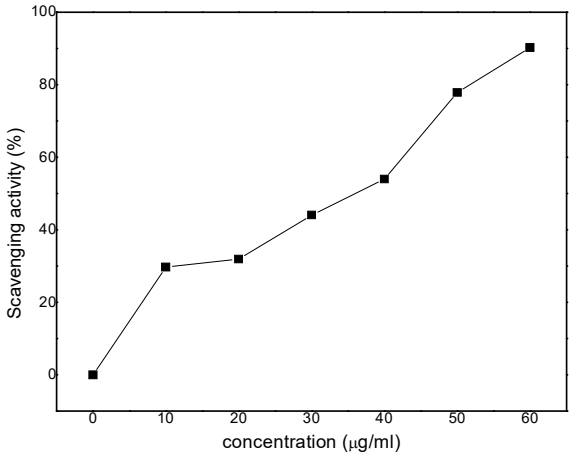
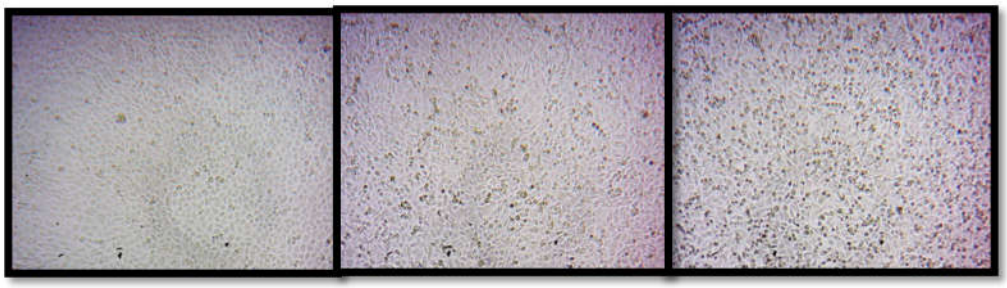


Figure 5. DPPH-free radical scavenging assay of CDs derived from *Asparagus racemosus* root.



Control 150 µg/mL 200 µg/mL

Figure 6. Morphological changes in control and sample carbon dot treated lung cancer A549 cells for 24 h.

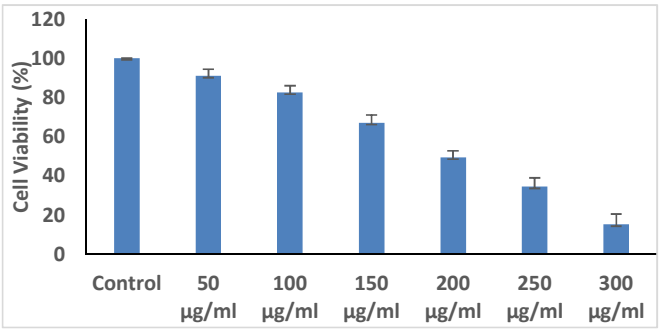


Figure 7. Cell viability graph at various concentrations of CDs.

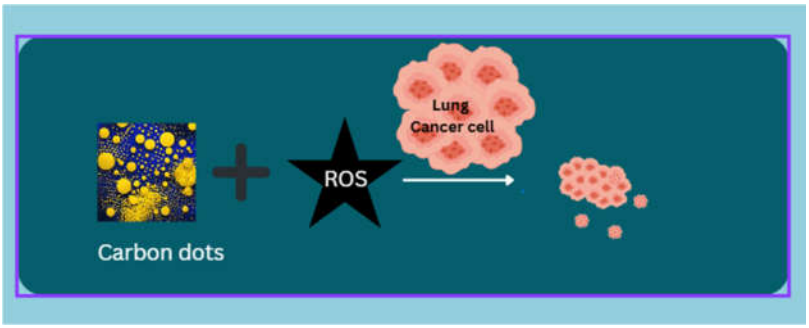


Figure 8. Anticancer activity mechanism.

CONCLUSIONS

In conclusion, the microwave approach was used to successfully synthesize carbon dots from the *Asparagus racemosus* root. The CDs had a spherical shape and an 8 nm average diameter. Additionally, CDs' characterisation revealed that they had functional groups on their surface that include oxygen, which is advantageous for increasing their water solubility. The CDs was used as the colorimetric probe for highly sensitive and selective Ag^+ ion detection. Thus this CDs can be a best tool to detect Ag^+ ions in the environmental samples. Additionally, these carbon dots were used in real-world applications, such as the detection of Ag ions in water samples. The current investigation found that CDs have the potential to function as an alternate medication for lung cancer. In the development of a cancer drug, the CDs may be an effective ingredient. The anti-oxidant potentials of this chemical still need to be validated in vivo, and future research will broaden the study to incorporate additional cancer cell lines. Carbon dots represent a promising avenue for cancer treatment due to their antioxidant properties and biocompatibility. However, extensive research is needed to validate their antioxidant potentials in vivo and expand their application across various cancer types. Future studies should aim to elucidate the mechanisms underlying their action, optimize their formulation, and explore combination therapies to maximize their therapeutic benefits.

ACKNOWLEDGEMENT

The authors thank KCG College of Technology for providing all required facilities to carry out the experiments.

Conflict of interest

The authors declare that they have no conflicts of interest.

Data availability

All data used to support the findings of this study are included within the article.

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