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Endogenous Carbon Dots in Traditional Korean Beverages: Structural Characterization, Antioxidant Activity, and Biocompatibility

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Abstract

Carbon dots (CDs) are emerging nanomaterials with promising applications in food science. Recent reports have shown that carbon dots are inherently present in heat-treated foods and beverages. However, the occurrence of carbon dots in traditional Korean beverages has not yet been investigated. In this study, carbon dots were isolated from three Korean beverages, Nurungji tea, barley tea, and green tea, and characterized using TEM, DLS, ζ-potential, UV-absorbance, photoluminescence (PL), FTIR and XPS analyses. All three beverages contained quasi-spherical (<10 nm) CDs, consistent with quantum dots whose optical properties depend on their size. DLS and ζ-potential measurements (−40 mV for cereal tea and −14 mV for green tea) confirmed their colloidal stability without aggregation in the beverages. PL spectroscopy exhibited excitation-dependent emission with a bathochromic shift, peaking at 412–438 nm, and FTIR spectra revealed abundant O-H, N-H, C=O, and C-N functional groups, reflecting oxygen and nitrogen doping that modulate redox reactions. Due to these surface functional groups, CDs demonstrated excellent antioxidant activity, with green tea CDs achieving 100% scavenging activity at 12.5 μg/mL, while barley and Nurungji CDs reached 100% and 78% scavenging activities at 100 μg/mL, respectively. In the cytotoxicity test using L929 fibroblast cells, grain tea CDs showed a survival rate of over 90% at concentrations of 6.25–100 μg/mL, and green tea CDs showed a survival rate of over 90% at concentrations up to 25 μg/mL, which is consistent with the literature on the biocompatibility of CDs. These results confirm that beverage-derived CDs are non-toxic and powerful antioxidants, reaffirming the safety and functionality of traditional Korean food.

Keywords: endogenous; carbon dots; Nurungji tea; barley tea; green tea; functionality

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Academic Editors: Ogueri Nwaiwu and Helen Onyeaka

Received: 31 March 2026
Revised: 8 May 2026
Accepted: 18 May 2026
Published: 28 May 2026

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1. Introduction

Carbon dots (CDs) are a relatively recent class of fluorescent carbon-based nanomaterials that consist of a graphitic carbon core with a passivated surface and are typically less than 10 nm in diameter [1,2]. CDs can be made from inexpensive and abundant biomaterials, such as food processing by-products or agri-processing waste, using a facile

hydrothermal process [2–5]. CDs are non-toxic and possess unique optical, biocompatibility, water-soluble, and functional properties such as antimicrobial, antioxidant, and UV barrier properties [1]. As such, CDs are most often used not only in biosensing, drug delivery, and bioimaging but also in food packaging and preservation [2].

Interestingly, CDs are known to be present in various foods, including heat-treated foods such as baked meat [6], roasted or grilled fish [7–9], roasted chicken [10], bread and caramels [11,12], hamburger patties [13], and pizza [14], and beverages such as coffee [15], cola [16], and beer [17,18] and honey [19]. Endogenous CDs are not artificially created but are created within the food matrix during cooking or processing due to complex interactions between food components such as proteins, carbohydrates, and fats [20]. These findings indicate that CDs form unintentionally during heat processing methods such as baking, boiling, roasting, frying, grilling, steaming, cooking, and heat sterilization, especially when food is subjected to high temperatures.

Across these different systems, the reported food- and beverage-derived CDs typically exhibit quasi-spherical morphologies with sizes below 10 nm, excitation-dependent emission in the blue–green region, and negatively charged surfaces that contribute to colloidal stability in aqueous media, although the precise zeta potential and quantum yield values vary with the matrix and processing conditions [21]. The exact mechanisms of formation, properties, and potential health risks associated with CDs in food are not yet well understood; however, people have been consuming them for centuries without being aware of their existence [20]. However, public perception of foods containing nanoparticles, such as carbon dots (CDs), is largely negative, and understanding of the occurrence, fate, and toxicity of these nanoparticles in foods remains limited. Therefore, scientific research on the safety of carbon dots can provide valuable insights into assessing the risks and benefits of carbon dot-containing foods and their use in food preservation. In particular, most existing studies on endogenous CDs in foods have focused on Western-style thermally processed products and common beverages (e.g., coffee, cola, beer), and have largely concluded that such CDs are strongly fluorescent and often exhibit appreciable antioxidant activity with low in vitro cytotoxicity, thereby supporting their potential functional roles in food systems [20,21].

In Korea, green tea and various roasted grain teas have traditionally been enjoyed as beverages. Notable examples include Nurungji tea (*Sungnyung*), which is made by steeping scorched rice (Nurungji) in hot water, and barley tea, which is made by steeping roasted barley in hot water. Recently, these grain teas have been developed into bottled beverages and commercial products and are widely consumed. The functional properties and efficacy of green tea are well known, mainly due to the antioxidant effects of polyphenol compounds such as catechins [22,23]. On the other hand, while Nurungji tea and barley tea have been enjoyed for a long time, their efficacy has been little studied. In particular, Nurungji tea has a long history of use in oriental medicine due to its effectiveness for indigestion, gastric ulcers, and reflux esophagitis. The antioxidant effect of Nurungji tea is already known [24,25], but the exact cause has not yet been identified. However, it is presumed that CDs generated during grain roasting may contribute to the antioxidant activity of Nurungji tea, together with other known antioxidant components such as phenolic compounds and Maillard reaction products. Compared with previously investigated food- and beverage-derived CDs [20], CDs in roasted grain-based Korean beverages are particularly relevant because they may originate from cereal-derived carbohydrates, proteins, and Maillard reaction products formed during roasting, whereas green tea-derived CDs may coexist with abundant polyphenolic compounds. Thus, comparison among Nurungji tea, barley tea, and green tea can help determine whether their CD-like properties simply follow

established trends or show source-dependent differences in surface chemistry, colloidal stability, antioxidant activity, and cytocompatibility.

Nevertheless, although it is expected that these grain teas contain CDs, manufacturers are often unaware of their existence or reluctant to disclose them to the public, even if they are aware. This avoidance phenomenon stems from concerns that many consumers may feel uneasy about the presence of nanomaterials in their beverages.

The main objective of this study was to confirm the presence of CDs in traditional Korean beverages such as Nurungji tea, barley tea, and green tea and to evaluate the safety of these beverages by analyzing their functional characteristics and cytotoxicity. To this end, CDs were extracted from commercially available Nurungji, barley, and green tea, analyzed for their characteristics, and evaluated for their antioxidant and cytotoxic effects.

2. Materials and Methods

2.1. Materials

Commercial bottled barley tea, Nurungji tea, and green tea beverages produced by three different manufacturers were purchased from a local supermarket in Seoul, Republic of Korea. All other analytical grade reagents were procured from Daejung Chemicals and Metals Co., Ltd. (Siheung, Gyeonggi-do, Republic of Korea) and used as received.

2.2. Carbon Dots Extraction and Purification

Each beverage was centrifuged at 10,000 rpm for 15 min to remove particulates. The supernatant was passed through a Whatman syringe filter with a 0.22 µm pore size to obtain a clear, purified original solution (Figure 1). This filtered original solution was used as the starting material for all subsequent experiments.

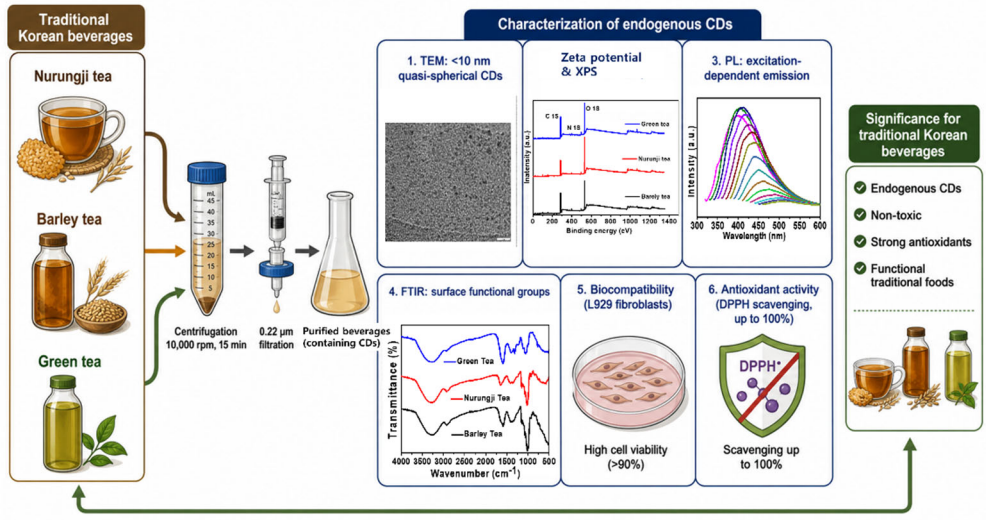


Figure 1. Schematic illustration of the extraction, purification, characterization, antioxidant activity, and biocompatibility evaluation of endogenous carbon dots isolated from traditional Korean beverages.

2.3. Physicochemical Characterization of Carbon Dots

The morphology and average size of the carbon dots were examined on a JEM-2100F field-emission transmission electron microscope (TEM, Jeol Ltd., Tokyo, Japan). Samples were drop-cast onto copper grids and air-dried, and the images were acquired. Particle size distributions and mean diameters were calculated from at least 50 particles per sample using ImageJ software (ImageJ 1.53 k Wayne Rasband and Contributors, National Institute of Health, Bethesda, MD, USA).

The UV-visible optical absorption spectra were recorded on a Mecasys Optizen POP UV–Vis spectrophotometer (Seoul, Republic of Korea) over the wavelength range 200–800 nm. Photoluminescence measurements were carried out on a Horiba Fluoromax fluorescence spectrophotometer (Kyoto, Japan) by exciting samples at various wavelengths (310–420 nm) and collecting emission spectra from 350–600 nm. Surface functional groups were identified by Fourier-Transform Infrared Spectroscopy (ATR-FTIR; TENSOR™ 37, Bruker Optics Inc., Billerica, MA, USA) over the range of 4000–500 cm^{-1}. The photoluminescence quantum yield (PLQY) of CDs extracted from barley tea, Nurungji tea, and green tea was measured by connecting an integrating sphere (model ILF-385 Jasco, Tokyo, Japan) to the same spectrofluorometer as reported by Rayappa Naveen et al. [26].

Hydrodynamic diameter and colloidal stability were assessed using a Malvern Zetasizer Nano instrument (Zetasizer Nano ZS-ZEN 3600, Malvern Instruments Ltd., Malvern, UK). The purified CD suspensions were measured in aqueous medium at 25 °C using disposable cuvettes. Dynamic light scattering (DLS) was used to determine the hydrodynamic size distribution, while zeta potential analysis was performed to evaluate the surface charge and colloidal stability of the CD suspensions.

2.4. Antioxidant Activity of Carbon Dots

The antioxidant activity of the extracted carbon dots was determined using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) oxidative free radical-scavenging assay [27]. For this, the ABTS solution was prepared by mixing ABTS stock and potassium persulfate, incubating in the dark at room temperature for 16 h, then diluting with deionized water to an absorbance of 1.00 at 734 nm. Sample solutions of CDs (12.5, 25, 50, 75, and 100 µg/mL) were mixed with 10 mL of ABTS solution and incubated for 15 min at room temperature. Absorbance at 734 nm was measured using a UV–vis spectrophotometer (Mecasys Optizen POP Series, Seoul, Republic of Korea), and scavenging activity was calculated as:

$$\text{Antioxidant activity}(\%) = \frac{A_o - A_T}{A_o} \times 100 \quad (1)$$

where A_o and A_T represent the absorbance of the control and the sample, respectively.

2.5. Cytotoxicity Assay

The L929 mouse fibroblast cells were used to evaluate CD cytotoxicity at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. The cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay to estimate the cytotoxicity of the CDs towards L929 cells [28]. The MTT assay is an indirect cell viability measurement that uses the reaction of mitochondrial dehydrogenase to reduce tetrazolium salts to insoluble formazan (purple compound) in metabolically active cells. For the MTT test, L929 cells (7×10^3 cells/well) were seeded in 96-well plates and incubated overnight. Then, the cells were treated with different concentrations of CDs, as mentioned above, for 24 h. After the treatment, 20 µL of 2 mg/mL MTT solution was added to each well and incubated at 37 °C for three h. After dissolving formazan crystals with DMSO, the absorbance was monitored at 570 nm using a microplate reader (Molecular Devices, San Jose, CA, USA). The experiments were performed three times independently, and the measurements were repeated three times. Cell viability was determined using a standard colorimetric assay, and results are reported as mean ± SEM.

3. Results and Discussion

3.1. Physicochemical Characterization

The mean size and morphology of carbon dots (CDs) extracted from barley tea, Nurungji tea and green tea were determined using transmission electron microscopy (TEM)

analysis (Figure 2a–c) and size distribution histograms (Figure 2d–f). The average particle sizes of Nurungji tea, barley tea, and green tea were 2.1 nm, 2.6 nm, and 2.4 nm, respectively, all of which fell within the typical 2–10 nm range of CDs [29]. Since these sizes are much smaller than the exciton Bohr radius of graphite, they indicate significant quantum confinement effects that lead to the size-dependent optical behavior observed in CDs [30]. The quasi-spherical morphology of CDs present in these beverages is consistent with numerous reports on CDs synthesized via thermal methods such as hydrothermal carbonization, which produces nearly spherical nanoparticles through isotropic growth [30]. Interestingly, the average size of CDs derived from beverages is somewhat smaller than that often reported for CDs derived from other organic precursors, possibly reflecting the unique molecular composition and heat treatment parameters of these beverages [1,20]. TEM micrographs also show that there is nearly no aggregation of CDs in these beverages, indicating effective dispersion and surface stabilization of CDs during the separation process. This observation aligns with the literature emphasizing the role of surface functional groups in preventing nanoparticle aggregation [27].

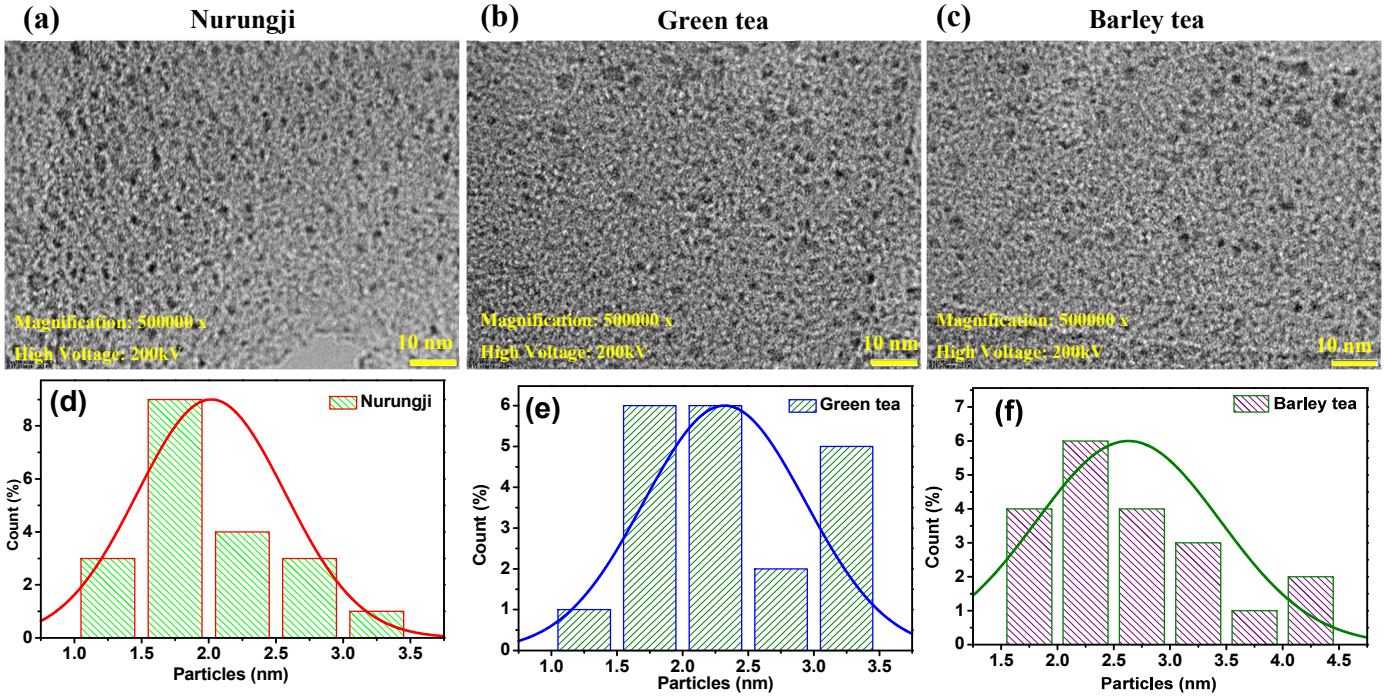


Figure 2. TEM images (**a**–**c**) and size distribution histograms (**d**–**f**) of CDs extracted from barley tea, Nurungji tea, and green tea, respectively.

Dynamic light scattering (DLS) and zeta potential measurements were conducted to evaluate the colloidal stability and hydrodynamic dimensions of beverage-derived carbon dots (CDs). Zeta potential analysis results indicated that both barley tea and Nurungji tea CDs exhibited very high negative charges of approximately −40 mV, while green tea CDs showed −14 mV, suggesting excellent colloidal stability due to strong electrostatic repulsion and minimal aggregation [31]. Particles with zeta potential values lower than −30 mV generally exhibit very stable nanoparticle dispersions due to dominant repulsive forces [32]. The somewhat lower negative zeta potential observed for green tea CDs remains within a reasonably stable range, indicating slightly weaker interparticle repulsions and a marginally higher tendency for aggregation under challenging conditions. Complementary DLS measurements indicated that the hydrodynamic diameters of Nurungji tea CDs were approximately 16 nm, barley tea CDs about 37 nm, and green tea CDs around 20 nm, all of which were larger than the core sizes of 2–3 nm measured by TEM. This discrepancy

arises because DLS measures both the effective diameter of the nanoparticles as well as the solvation shell and surface functional groups, which significantly increase the apparent size in aqueous solution [33].

UV–vis analysis was used to evaluate the optical absorption behavior of CDs isolated from barley tea, Nurungji tea, and green tea. As shown in Figure 3a–c, all samples displayed a distinct absorption band around 290 nm. This absorption can be related to n → π* transitions of carbonyl-containing groups and π → π* transitions within conjugated sp^2 carbon structures [34]. The presence of this band suggests the formation of aromatic carbon domains, which are typical features of biomass-derived CDs. The optical appearance of the CD dispersions further supported this assignment. Under daylight, the dispersions showed a pale-yellow color, whereas irradiation with 365 nm UV light produced visible light-blue fluorescence (insets of Figure 3a–c). This UV-responsive blue emission is a characteristic photoluminescent feature of CDs and supports the presence of endogenous CDs in the tested beverages [27].

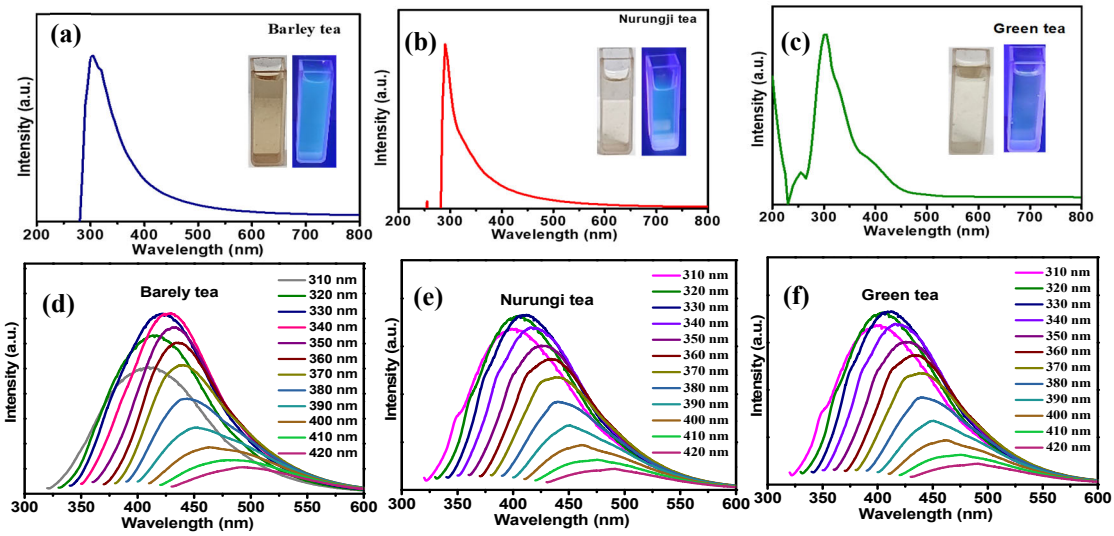


Figure 3. UV–vis absorption spectra of CDs extracted from (**a**) barley tea, (**b**) Nurungji tea, and (**c**) green tea, with inset images under daylight and 365 nm UV light. Fluorescence emission spectra of CDs from (**d**) barley tea, (**e**) Nurungji tea, and (**f**) green tea recorded at excitation wavelengths from 310 to 420 nm, showing excitation-dependent emission behavior.

Photoluminescence (PL) spectroscopy of carbon dots (CDs) extracted from barley tea, Nurungji tea, and green tea clearly revealed excitation-dependent luminescence (Figure 3d–f), which is characteristic of quantum dots [27]. All samples were measured under the same instrumental conditions while varying the excitation wavelength from 310 to 420 nm. When excited in the wavelength range of 310–420 nm, each sample exhibited a bathochromic shift in the emission maximum, indicating changes in optical transitions due to differences in surface states and size distributions [35]. Specifically, the Nurungji tea CDs exhibited an emission peak at 412 nm when excited at 330 nm, the barley tea CDs displayed an emission peak at 428 nm when excited at 340 nm, and the green tea CDs demonstrated an emission peak at 438 nm when excited at 320 nm. The observed excitation-dependent luminescence and the red shift in luminescence with increasing excitation wavelength are generally attributed to the sequential excitation of non-uniformly distributed surface functional groups, such as C=O, C-OH, and amino functional groups, which occur during the heat treatment of tea biomolecules [36].

In comparison, the emission maxima of CDs derived from beverages prepared in this study fall within the blue-green region frequently reported for CDs prepared by

hydrothermal methods, reflecting similar core–shell structures and surface passivation profiles [37]. The slight differences in the positions of the emission peaks among the three teas are likely due to variations in their phytochemical compositions and processing conditions, which influence the abundance and energy distribution of the surface emission sites. In addition, the PLQY values of CDs from barley tea, Nurungji tea, and green tea were calculated to be 3.3%, 2.9%, and 3.2%, respectively. Overall, these PL characteristics confirm the quantum dot nature of the isolated particles from these beverages.

To further compare the extracted beverage-derived CDs, their particle size and optical properties were evaluated alongside previously reported CDs synthesized from different carbon precursors (Table 1). The comparable nanoscale size, UV–vis absorption features, blue fluorescence, and excitation-dependent emission behavior support the presence of endogenous CDs in barley tea, Nurungji tea, and green tea.

Table 1. Comparison of extracted endogenous CDs with previously reported CDs synthesized from different carbon precursors.

Cds Source	Particle Diameter	Emission Response Under UV Light	UV	PL	Ref
Galla chinensis waste	4.09 nm and 5.02 nm	Emitted bright blue fluorescence	280 nm (n-π* transition of the C=C bonds and π-π* transition of the C=O)	Excitation wavelength-dependent fluorescence response	[38]
Oolong tea	2.04 nm	Emitted bright blue fluorescence	270 nm (n-π* conjugated transitions of C=O)	Excitation wavelength-dependent fluorescence response	[39]
Purple cabbage residue	10 nm	Emitted bright green fluorescence	290 nm (π-π* transition of the C=C)	emission peak redshifted with increasing excitation wavelength	[40]
Charcoal	1 to 8 nm	Emitted bright green fluorescence	290 nm (n-π* transition of C=O groups)	Emission wavelength shifted with increasing excitation wavelength	[41]
Glucose	2.4 to 6.3 nm	Emitted blue fluorescence	297 nm n-π* (C–OH or C = O bonds)	Red shift in emission wavelength with increasing excitation wavelength	[42]
Endogenous CDs from Nurungji tea, barley tea, and green tea	2–10 nm range	Emitted blue fluorescence	290–300 nm (n-π* transition of C=O groups)	Red shift in emission wavelength with increasing excitation wavelength	This work

The Fourier transform infrared (FTIR) spectra of carbon dots (CDs) isolated from barley tea, Nurungji tea, and green tea, as shown in Figure 4a, exhibit characteristic absorption bands of oxygen- and nitrogen-containing surface functional groups typically generated during the heat treatment of organic precursors. The broad band centered at 3270 cm^{-1} corresponds to the O-H and N-H stretching vibrations, indicating the presence of abundant hydroxyl and amine groups on the CD surface. In contrast, the peak at ~2930 cm^{-1} results from aliphatic C-H stretching [42]. The mid-region bands further confirm the rich chemical heterogeneity. The absorption at 1645 cm^{-1} is assigned to the C=C stretching within the sp^2-hybridized graphitic domains, while the nearby band at 1590 cm^{-1} corresponds to the N-H bending of primary amines [27]. The peak near 1400 cm^{-1} can be attributed to O-H bending or C=C stretching of polycyclic aromatics, consistent with the formation of a conjugated carbon core [16,17]. The feature at 1350 cm^{-1} is characteristic of C-H bending, while the region of 1110–1150 cm^{-1} reflects C-N stretching, highlighting nitrogen

incorporation during the secondary heat treatment. Finally, the band at 1020–1060 cm^{-1} is identified as aromatic C-O-C or alkoxy bonds, suggesting surface passivation by polyphenol components [43,44].

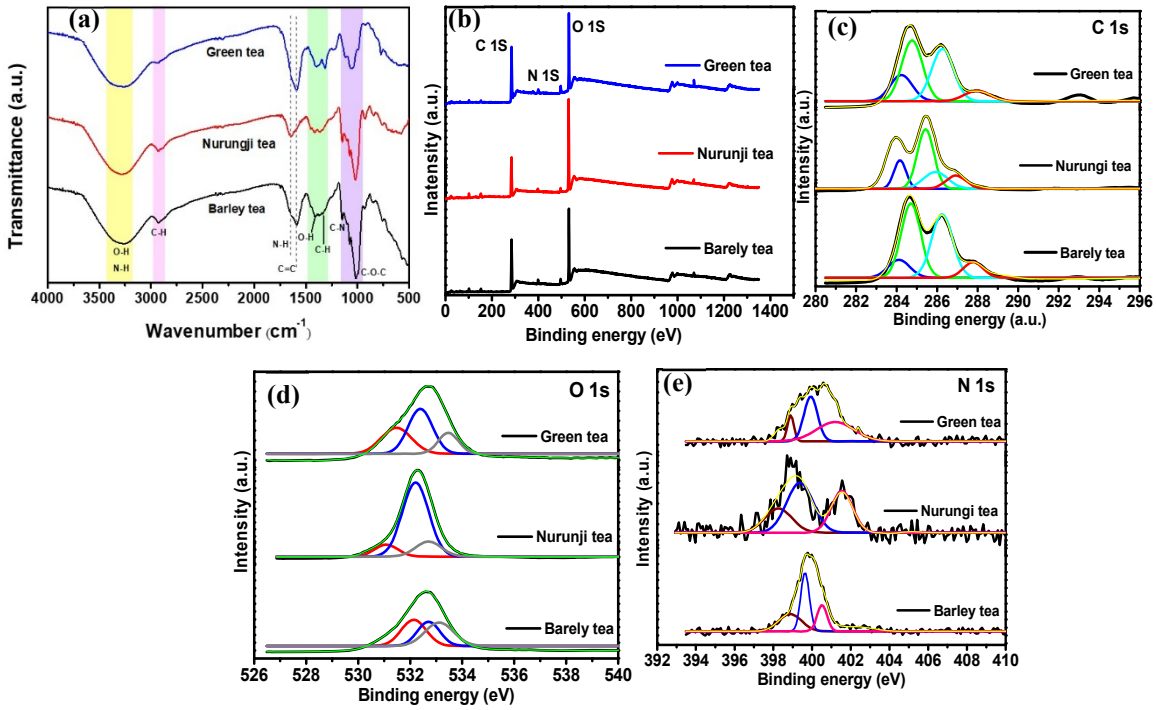


Figure 4. (**a**) FTIR spectra, (**b**) XPS survey spectra, and high-resolution XPS spectra of (**c**) C 1s, (**d**) O 1s, and (**e**) N 1s for CDs obtained from barley tea, Nurungji tea, and green tea.

Distinct variations in band intensities among the three tea-derived CDs reveal differences in surface chemistry likely stemming from their unique phytochemical compositions and roasting histories. Nurungji tea CDs display a pronounced C=C stretching band at 1645 cm^{-1}, whereas their N-H bending signal at 1590 cm^{-1} is comparatively weak; this suggests a higher relative abundance of sp^2 carbon networks versus amine groups. In contrast, green tea CDs exhibit the most intense 1590 cm^{-1} band, reflecting a greater presence of primary amine groups derived from amino acids and catechin-based nitrogenous compounds, in line with reports on nitrogen-functionalized CDs prepared from tea leaves [45]. Barley tea CDs, like Nurungji CDs, show relatively stronger absorption around 1020 cm^{-1} (C-O-C stretching) than at 1590 cm^{-1}, indicating that aromatic ether functionalities predominate over amine groups in these grain-based beverages. The rich surface chemistry, combining hydroxyl, carboxyl, amine, and polyaromatic groups, provides CDs with both hydrophilicity and electron-donating capabilities, which have been correlated with strong radical scavenging activity in similar systems [27].

XPS analysis was conducted to examine further the surface elemental composition and chemical bonding states of the CDs isolated from barley tea, Nurungji tea, and green tea. The XPS survey spectra (Figure 4b) showed three dominant signals at approximately 284.8, 400.1, and 531.1 eV, corresponding to C 1s, N 1s, and O 1s, respectively, confirming that carbon, nitrogen, and oxygen were the main surface elements of the beverage-derived CDs [46]. The high-resolution C 1s spectra (Figure 3c) were fitted into several components, including peaks near 284.2 eV assigned to C-C/C-H bonds, around 285.1–286.3 eV related to C-N and C-O bonds, and approximately 287.8 eV associated with carbonyl-related O–C-O or C=O groups [45]. The O 1s spectra (Figure 4d) were resolved into peaks centered at about 531.8, 532.7, and 533.2 eV, which can be attributed to C-O/N-O, C=O, and C–O–H groups,

respectively [39,41]. In addition, the N 1s spectra (Figure 3e) contained three fitted peaks at approximately 398.8, 399.6, and 400.5 eV, corresponding to C-N-C, graphitic/tertiary N [N-$(C)_3$], and N-H bonding configurations, respectively [45]. These results confirm the presence of oxygen- and nitrogen-containing surface functionalities, which are consistent with the FTIR findings and may contribute to the optical and antioxidant behavior of the extracted CDs.

Furthermore, the relative abundance of nitrogen-containing moieties in green tea CDs may enhance their electron transfer antioxidant mechanisms compared to grain-derived CDs. In contrast, the high aromatic contents in Nurungji and barley tea CDs may contribute to the π-electron stabilization of radicals, indicating complementary pathways for their antioxidant functions. Thus, these FTIR analysis results not only confirm the successful extraction of chemically diverse CDs from traditional Korean beverages but also provide a molecular basis for interpreting their subsequent antioxidant and cytotoxicity profiles.

3.2. Antioxidant Activity

The antioxidant activities of CDs obtained from three types of bottled beverages were evaluated based on ABTS oxidative free radical scavenging activity, with the results shown in Figure 5. When assessed at five concentrations (12.5–100 μg/mL), barley tea CD scavenged approximately 20% of ABTS at 12.5 μg/mL, which rapidly increased to 100% at 75 μg/mL. Nurungji tea CD exhibited lower antioxidant activity than CDs from other beverages, with 15% at 12.5 μg/mL and 78% at 100 μg/mL. Conversely, green tea CD demonstrated strong antioxidant activity, achieving 100% ABTS scavenging efficacy even at a low concentration of 12.5 μg/mL. This concentration-dependent behavior of antioxidant activity aligns with previous reports on biomass-derived CDs, which indicated that higher concentrations of CDs increase the active sites in the reaction system, leading to enhanced radical quenching efficiency [47]. The superior antioxidant activity of green tea CDs is attributed to the abundant nitrogen-doped surface moieties, primarily amine and amide functionalities, as confirmed by FTIR analysis. These nitrogen functionalities serve as strong electron donors, reducing ABTS through electron transfer pathways [48]. The enhancement of the antioxidant activity of CDs due to nitrogen functionalities has been widely reported [42]. Collectively, these results indicate that CDs isolated from traditional Korean beverages show notable antioxidant activity. This activity may be partly related to their surface functional groups, particularly nitrogen- and oxygen-containing groups, which can enhance radical-scavenging ability. However, considering the complex composition of beverages, possible residual beverage-derived molecules associated with the CD fraction may also contribute to the observed antioxidant activity.

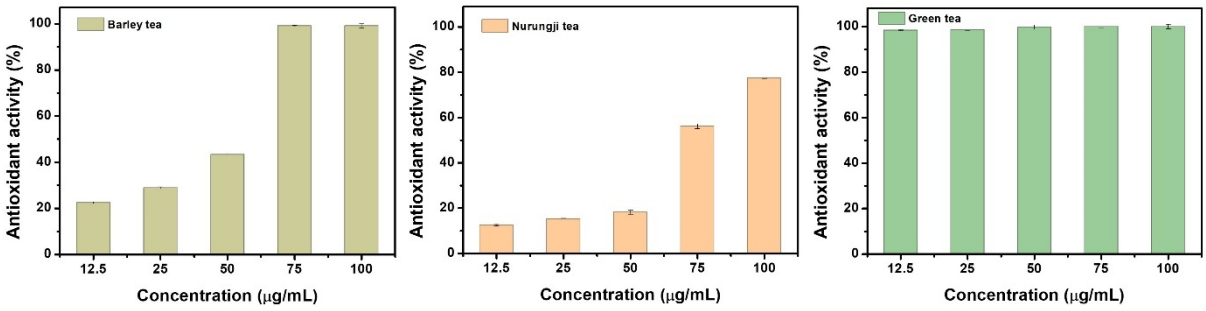


Figure 5. Antioxidant activity of CDs extracted from barley tea, Nurungji tea, and green tea (left to right) assessed using the ABTS free radical scavenging assay.

3.3. Cytotoxicity Analysis

The cytotoxicity of CDs obtained from three beverages was tested against L929 mouse fibroblast cells (Figure 6). At low concentrations, CDs showed no signs of toxicity toward the cell line, but effects on cell proliferation were observed at concentrations below 25 μg/mL. Barley tea and Nurungji tea CDs exhibited minimal cytotoxicity even at high concentrations, maintaining more than 90% cell viability in L929 mouse fibroblast cells. Conversely, green tea CDs showed no toxicity up to 25 μg/mL; however, viability decreased in a dose-dependent manner at 50 μg/mL and 100 μg/mL. The IC_{50} value of CDs obtained from green tea beverage was 64.7 μg/mL.

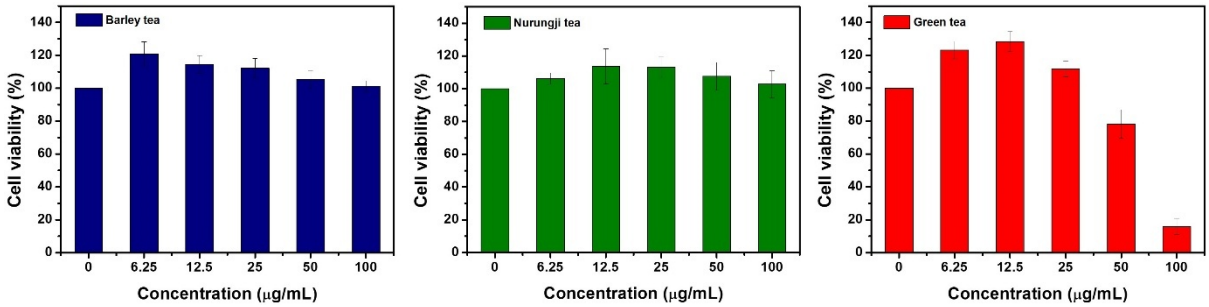


Figure 6. Cytotoxicity assay for CDs extracted from barley tea, Nurungji tea, and green tea (left to right) in terms of L929 mouse fibroblast cell viability at different exposure concentrations.

In previous studies by other researchers, plant extract CD exhibited dose-dependent cytotoxicity in L929 cells, with ~76% viability at 100 μg/mL ($IC_{50} \approx 314$ μg/mL), highlighting the influence of surface chemistry on biocompatibility [49]. The strongly negative zeta potential (≈−40 mV) measured for barley and Nurungji tea CDs may contribute to their inactivation profile by reducing cellular uptake and membrane disruption [50,51]. In contrast, the abundant nitrogen groups on the surface of green tea CDs may promote enhanced internalization and reactive oxygen species production at higher concentrations, which may explain the toxicity observed above 25 μg/mL. In addition, the tested concentrations refer to the concentration of the filtered CD-containing suspension used in the biological assays, rather than purified CDs alone.

Nevertheless, charge-dependent cellular uptake of nanoparticles has been reported to vary depending on the cell's chemical structure and surface charge. For example, He et al. found that when the absolute values of zeta potentials were similar, positively charged NPs exhibited higher phagocytic uptake than negatively charged NPs, which was reported to be due to the complementary surface charges of cells [50].

4. Limitations

This study was limited to a small number of traditional Korean beverages, such as Nurungji, barley, and green tea samples, so the present findings may not fully represent all commercial or traditionally prepared beverage products. In addition, the analyses were restricted to physicochemical characterization, antioxidant activity, and in vitro cytotoxicity in L929 cells. More comprehensive structural analyses, broader sample coverage, multiple biological models, and in vivo safety evaluations are needed to confirm the generality and biological relevance of these findings.

5. Conclusions

This study provided evidence for the presence of endogenous CDs in traditional Korean beverages prepared through heat processing, including Nurungji tea, barley tea, and green tea. TEM results showed that the carbon dots of Nurungji, barley, and green tea

exhibited a uniformly distributed quasi-spherical shape with average diameters of 2.1, 2.6, and 2.4 nm, respectively. These sizes are significantly smaller than the exciton Bohr radius of graphite, indicating that the quantum confinement effect governs the photophysical behavior. DLS and zeta potential data showed hydrodynamic sizes ranging from 16 to 37 nm and high negative charges (ca. −40 mV for cereal teas and −14 mV for green tea), confirming that the effective solvation shell and surface functional groups provide excellent colloidal stability without aggregation. Photoluminescence measurements at excitation wavelengths of 310–420 nm revealed excitation-dependent emission peaks at 412 nm for Nurungji tea, 412 nm for barley tea, and 438 nm for green tea, consistent with heterogeneous emission traps on the CD surface resulting from various oxygen- and nitrogen-containing molecules. FTIR spectra and XPS analysis highlighted the differences in surface chemical functionalities, confirming the presence of O-H, N-H, C=O, C=C, and C-N groups. Green tea CDs exhibited a higher abundance of amine functionalities, while grain tea CDs displayed more aromatic ether linkages, reflecting their unique precursor compositions and roasting routes. However, because the beverages are chemically complex and the purification procedure was relatively mild, the possible contribution of strongly associated low-molecular-weight beverage components cannot be completely excluded.

Functionally, green tea CDs completely scavenged ABTS radicals even at a concentration of 12.5 μg/mL, whereas barley tea CDs showed a 100% radical scavenging effect at 75 μg/mL, and Nurungji tea CDs showed a 78% radical scavenging effect at 100 μg/mL. These antioxidant effects may be associated with the surface functional groups of the CD fraction, particularly oxygen- and nitrogen-containing groups, but may also be influenced by residual beverage-derived antioxidants such as phenolic compounds and Maillard reaction products.

Cytotoxicity for L929 fibroblast cells showed that barley and Nurungji tea CDs were non-toxic up to 100 μg/mL, and green tea CDs were safe up to 25 μg/mL. These findings provide preliminary in vitro evidence of cytocompatibility under the tested conditions; however, they are not sufficient to conclude consumer safety or long-term health effects. Further purification, compositional analysis, multiple biological models, and in vivo studies are required to clarify the specific contribution of CDs and confirm their biological relevance.

Author Contributions: Conceptualization, G.K., A.K. and J.-W.R.; methodology, G.K., A.K. and S.H.; software, G.K., A.K. and S.H.; validation, A.K., J.-W.R. and S.-G.L.; formal analysis, G.K. and A.K.; investigation, G.K., A.K. and S.H.; resources, J.-W.R.; data curation, J.-W.R., S.-G.L. and J.T.K.; writing—original draft preparation, R.P., A.K., J.-W.R., S.-G.L. and J.T.K.; writing—review and editing, A.K. and J.-W.R.; visualization R.P., J.T.K. and S.-G.L.; supervision, J.-W.R.; project administration, J.-W.R.; funding acquisition, J.-W.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from Kyung Hee University (KHU-20233671) and the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (IPET) through the High Value-added Food Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (RS-2025-02304869).

Institutional Review Board Statement: The L929 mouse fibroblast cell line was obtained from the Korean Cell Line Bank (KCLB), Seoul, Republic of Korea.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be made available upon request.

Acknowledgments: In preparing this paper, the authors used the AI tools QuillBot and Grammarly to enhance linguistic expression and clarity. After utilizing these tools, the authors reviewed and edited the content as necessary and took full responsibility for the material.

Conflicts of Interest: The authors have no competing financial or non-financial interests to declare.

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