Preparation and Characterization of Carbon Quantum Dots from *Salvia rosmarinus* (Rosemary) Plant via Hydrothermal Synthesis Method

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Abstract

In this study, Carbon Quantum Dots (CQDs) were synthesized via hydrothermal synthesis (CQDs) from the rosemary (*Salvia Rosmarinus*) plant as a natural carbon source. Dried and ground rosemary extracts were subjected to hydrothermal reaction at 200 °C for 18 h. CQD solutions were obtained by two purification methods involving high-speed centrifugation and 22 µm injector filter methods. The obtained CQDs were characterized by UV–Vis spectroscopy (UV-Vis) and Fourier transform infrared (FTIR) spectroscopy to analyze their optical and surface chemical properties. UV-Vis analyses revealed that CQDs produced by both purification methods exhibited absorption in the range of 260–320 nm. The lower absorbance values observed after the 22 µm injector filtration process compared to the centrifuged samples showed that the purification method affected the particle size and optical properties. FTIR results proved the presence of hydroxyl (-OH), carboxyl (-COOH), carbonyl (C=O), double bond (C=C) and epoxy (C–O–C) groups on the surface of CQDs. The sharper and more distinct IR bands observed in the CQD samples purified by high-speed centrifugation indicate a more homogeneous and pure structure. As a result of the findings obtained, it is seen that the rosemary plant can be used as an environmentally friendly carbon source by the hydrothermal method, and synthesized CQDs have the potential to be used in biosensors, imaging, and other biomedical applications.

Keywords: Quantum dots, CQDs, rosemary, green synthesis, hydrothermal synthesis

Introduction

Carbon Quantum Dots (CQDs) are a class of nanoparticles with sizes smaller than 10 nm. CQDs were first reported in 2004 during the purification of single-walled carbon nanotubes. CQDs have powerful and tunable fluorescence properties that enable their applications in biomedicine, optronics, sensors, and catalysis. These materials, which have excellent photostability, small size, highly tunable photoluminescence, biocompatibility, electrochemiluminescence, and multiphoton excitation (up conversion), can be functionalized with biomolecules, are less toxic, and chemically inert, and are used as effective nanocarriers for drug delivery and imaging. CQDs also have promising applications in sensors, optronics, and electrochemiluminescence [1]. Since the accidental discovery of CQDs in 2004, numerous synthesis techniques have been used to produce carbon dots: laser ablation, arc discharge, hydrothermal/solvothermal combustion/heating, and microwave irradiation are some methods to synthesize water-soluble carbon dots. However, some factors limit the production of carbon dots and the expansion of current applications. These are energy consumption, the complexity of carbon dot production, and the synthetic conditions. However, hydrothermal methods with biomass carbon sources to synthesize carbon dots have attracted great interest due to their compatibility with green chemistry processes. There is a growing interest in the hydrothermal synthesis of carbon dots from different biomass as carbon sources [2]. Hydrothermal synthesis stands out with its many advantages, such as relatively mild working conditions (reaction temperatures <300°C), single-step synthetic procedure, environmental friendliness, and good dispersion properties in solution [3]. Rosemary (Rosmarinus officinalis L.), which will be used as a carbon source in this study, is an aromatic plant grown in the Mediterranean region of Turkey, which has been used for medicinal, culinary, fragrance, and ornamental purposes since ancient times [2]. Rosemary has attracted attention by containing secondary metabolites with therapeutic potential such as carnosol and carnosic, rosmarinic, ursolic, oleanolic, and micromeric acids [4]. In addition, rosemary plants have antifungal, antiviral, antibacterial, anti-inflammatory, antitumor, antioxidant [5], neuroprotective [6] and dermal protective [7] activities. Current studies have also revealed the anticancer activity and therapeutic potential of rosemary [8]. Rosemary has been used

in traditional medicine to relieve various diseases, including headache, dysmenorrhea, abdominal pain, epilepsy, rheumatic pain, spasms, nervous agitation, improvement of memory, hysteria, depression, as well as physical and mental fatigue [9,10]. In this study, CQDs were synthesized by the hydrothermal synthesis method using rosemary plant extracts, and the characterization of the obtained CQDs was carried out.

Materials and Methods

Materials

The rosemary plant, which will be used as the main carbon source in the project, was supplied from the rural area of Ağaçbeyli village in Sivaslı district of Uşak province, and its woody parts were separated and dried in sunlight. The deionized water used as solvent was supplied by the Biosensor and Nano Drug Laboratory of the Central Research Laboratories Application and Research Center of İzmir Kâtip Çelebi University of the Republic of Türkiye.

Method

In the experimental phase of this project, it was aimed to produce CQDs from the rosemary plant by following the procedure established by considering the carbon dot synthesis method of Eskalen et al. [5], and certain analyses were carried out to characterize these dots.

To achieve the goals, firstly, the woody parts of the dried rosemary plant were ground and powdered. The extract was prepared by mixing 1 mg of powdered rosemary and 50% deionized water in a magnetic stirrer for 4 hours. The amounts were changed in direct proportion to the amount to be used in the characterization phase.

The prepared extract was filtered twice with filter paper to remove large rosemary pieces. The obtained liquid was transferred to a constant-pressure steel autoclave reactor at 200°C for 18 hours to thoroughly dissolve the remaining rosemary particles in water and to ensure a homogeneous distribution with water. After the hydrothermal treatment, the steel autoclave reactor was allowed to cool to room temperature and then filtered again with filter paper. The liquid obtained after filtration was first centrifuged 3 times at 7500 rpm for 10 minutes each. The purer liquid obtained after centrifugation was separated into two and purified in two separate processes. The liquid, initially 50 ml, decreased to 40 ml due to absorption by the filter paper and remaining in the pellet part at the bottom of the falcon tubes. 20 ml of this 40 ml liquid was centrifuged 2 times at 10000 rpm for 10 minutes each with a high-speed centrifuge, and the remaining 20 ml was passed through a 22-micrometer syringe tip filter.

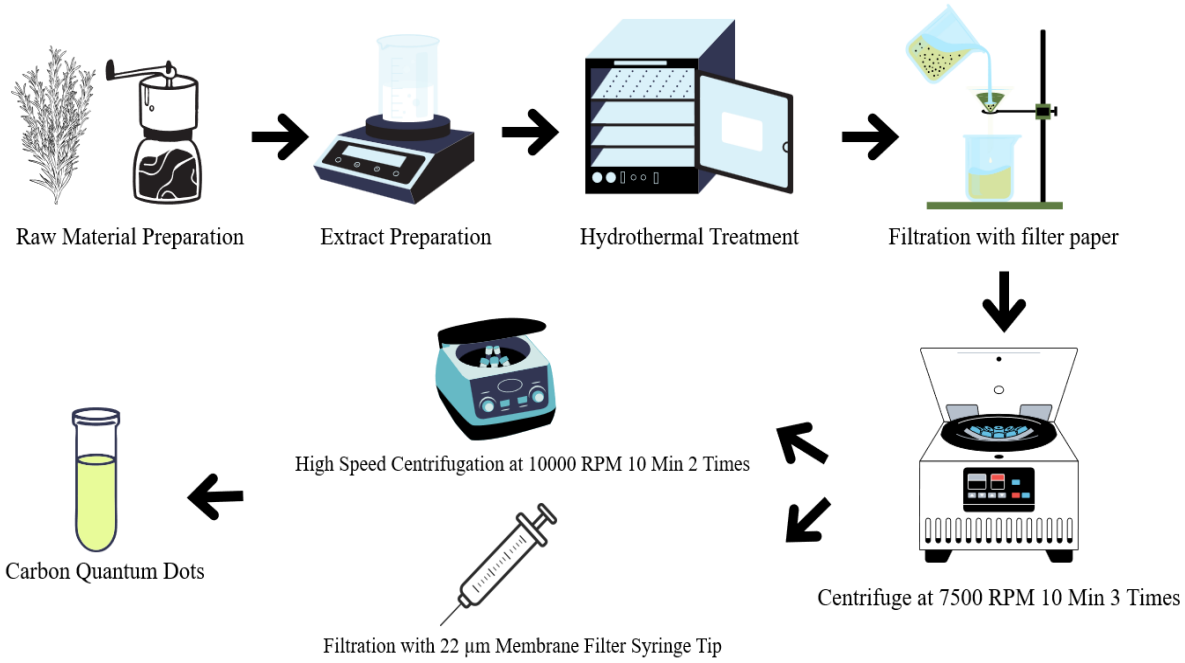

Figure 1. Synthesis of CQD via the hydrothermal method

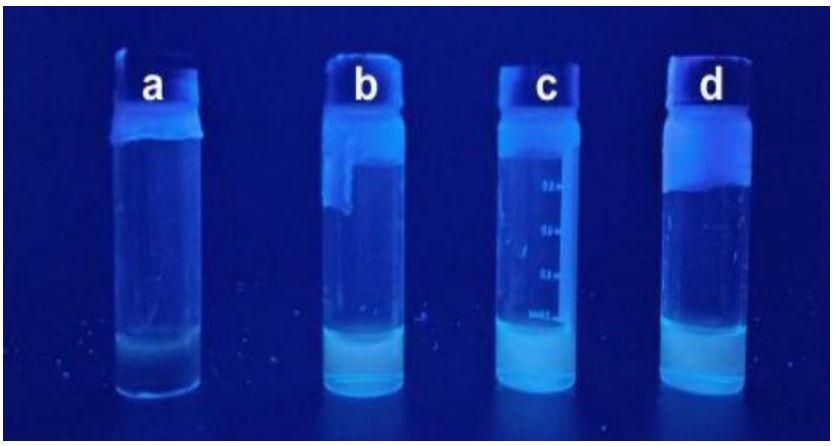

Figure 2. CQDs under 365nm UV light (a. After hydrothermal synthesis; b. After 3 re-centrifuges at 7500 rpm 10 minutes; c. After 3 re-centrifuges at 7500 rpm 10 minutes + 2 re-centrifuges at 1000 rpm 10 minutes; d. After 3 re-centrifuges at 7500 rpm 10 minutes + 22 micrometer syringe filter)

Characterization Studies

In this project, during the characterization phase of CQDs, the following was performed

- Ultraviolet and visible light (UV-Vis) absorption spectroscopy,
- Fourier transform infrared spectroscopy (FTIR).

Ultraviolet and Visible Light (UV-Vis) Absorption Spectroscopy

UV-VIS spectroscopy is a useful analysis for the characterization of CQDs. This spectroscopic study provides information about the surface of the synthesized CQDs. In addition, by imaging the UV-VIS spectrum, it can be determined whether a ground state complex formation occurs between CQDs and a foreign substrate, and if complex formation occurs, it always leads to a change in the spectrum [11]. During the UV spectroscopy analysis of the produced dots, 100 microliters of quantum dots uniformly distributed with distilled water were taken and deposited into a quartz cuvette. Then, 1900 microliters of deionized water were added to the crystal cuvette, and the analysis was carried out in the measuring device between the wavelengths of 200 nm and 500 nm.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is an advanced spectroscopic technique used to characterize different functions on the surface of synthesized CQDs. These characterizations are used to understand the field where CQDs can be applied. This spectroscopic technique is also used to monitor the synthesis during post-synthesis surface modification of CQDs [12].

While performing FTIR measurement of our CQDs, 2 microliters of our samples were taken with a micropipette and dropped onto the ATR crystal, and some of the water was allowed to evaporate. Then the measurement was performed.

Results

Ultraviolet and visible light absorption spectroscopy

The peaks of the UV-visible region absorption calibration curves of the CQDs we produced in our project are seen to be between 260-320 nm, consistent with previous reports [5, 13- 17].

The maximum absorption of the CQDs we produced with both purification methods suggest at a wavelength of 283 nm, as seen in Figure 4. It can be thought that this absorption is due to the pi-pi bond transition, C=C or C=O bond. The fact that the absorbance of the CQDs filtered with a syringe filter is lower than that of the ones purified using a high-speed centrifuge suggests that filtration method influences particle size and, therefore, the optical properties. The rapid decrease in absorption after approximately 300 nm indicates that CQDs have a strong absorption in the ultraviolet (UV) region. However, there is also a low absorbance after 400 nm, that is, in the visible region.

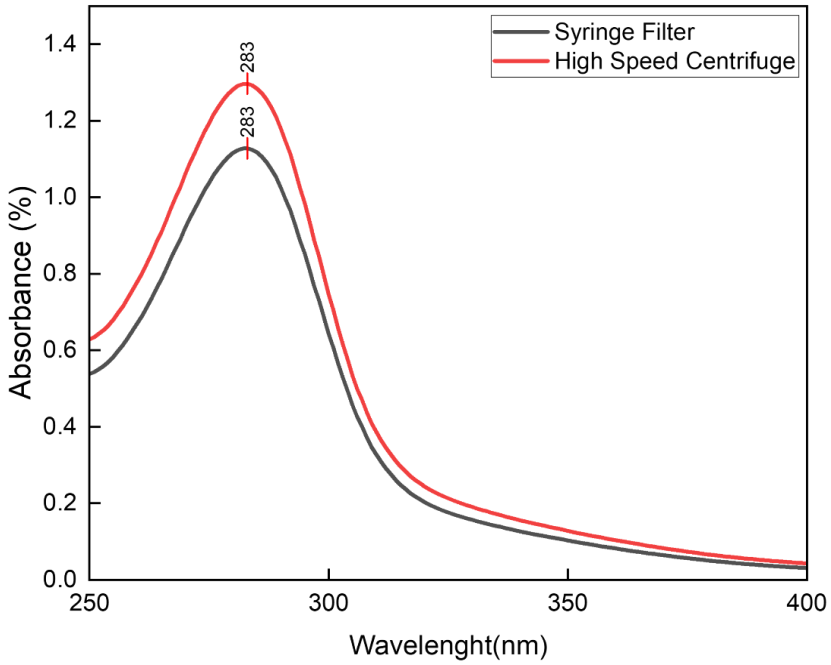

Figure 3. UV Spectroscopy curve of CQDs

Fourier transform infrared spectroscopy

Like this study in the existing literature, Zhang et al. used sweet pepper as a carbon source to create high-quality carbon dots (C dots) with up- and down-conversion fluorescence by low-temperature carbonization. As a result of the characterization of these dots, the hydroxyl group (-OH at 3405 nm-1055 nm) and carboxylic anion (-COO- at 1605 nm- 1405 nm) were detected by FT-IR spectroscopy. The peaks observed at 2940 nm and 860 nm were assigned as C-H tension [18]. Similarly, Feng et al. carried out green synthesis of CQDs from coke in another study. In this study, the hydroxyl group (-OH) was detected at 3415 cm-1, and the C-H bond was detected at 2960 cm-1 by FT-IR spectroscopy. In addition, C=O, C=C, and C-O-C bonds were also detected [19]. This is also the case in other similar studies [20-22].

First, a Fourier transform infrared spectroscopy measurement was made of the carbon source, powdered rosemary that we used in our project. Natural biochemicals present in the plant extract may cause the formation of extra signals in FTIR spectroscopy.

The wide stretching band that we saw between 3200 - 3500 cm^{-1} in the FTIR spectroscopy of dried and powdered woody rosemary (Figure 4) is the -OH stretching band and indicates the presence of plant alcohols. The tension observed around 1700 cm^{-1} is the C=O stretching vibration and indicates the presence of ester or carboxylic groups expressed by compounds such as rosmarinic acid and carnosic acid found in the structure of Rosemary. C=C vibrations between 1600 -1500 cm^{-1} indicate the presence of antioxidant compounds in the rosemary structure.

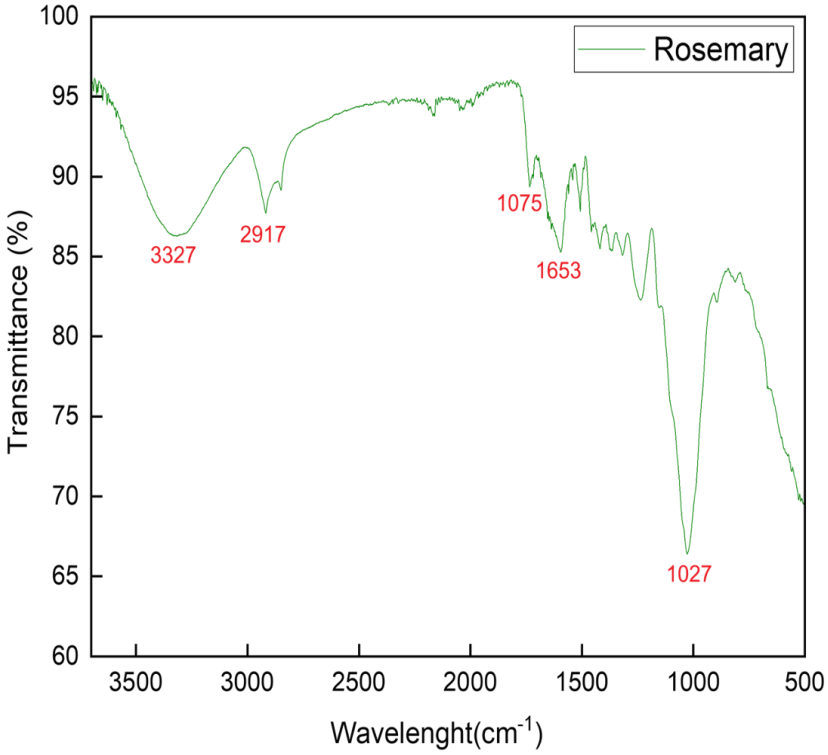

Figure 4. FTIR Spectroscopy of Rosemary extract

As observed in the FTIR spectroscopy of CQDs that were filtered through a syringe filter by Zhang et al. and Feng et al. [23,24], the tension between 3200-3500 cm^{-1} indicates the presence of -OH. This tension may be due to rosemary itself or to water used as a solvent during hydrothermal synthesis. The tension observed between 2800-3000 cm^{-1} may be an aliphatic C-H band, indicating that the carbon source is derived from a herbal extract. Again, Zhang et al. [23] also detected C-H bonds in this range in their study. Tensions between 1700-1750 cm^{-1} indicate the presence of carboxyl groups (-COOH) on the surface of CQDs. The tension observed around 1650 cm^{-1} indicates the presence of C=O, C=C bonds and aromatic systems. Tensions between 1000 – 1300 cm^{-1} indicate the presence of C-O-C and C-O epoxy or hydroxyl groups.

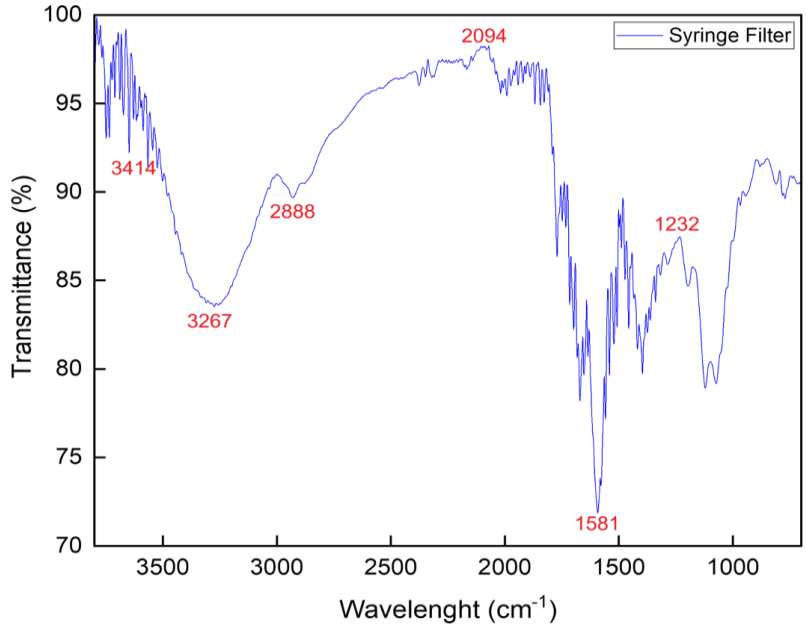

Figure 5. FTIR Spectroscopy of CQDs purified by syringe filter

CQDs purified by a high-speed centrifuge generally have similar spectra to CQDs purified by syringe filters. Similarly, as observed in the studies of Zhang et al. and Feng et al. [23,24] there is a broad -OH band observed between 3200-3500 cm^{-1} and this tension may be due to solvent deionized water. C=O and C=C tensions observed around 1650 nm indicate aromatic systems. Vibrations between 1000-1300 cm^{-1} indicate the presence of C-O and C-O-C groups epoxy.

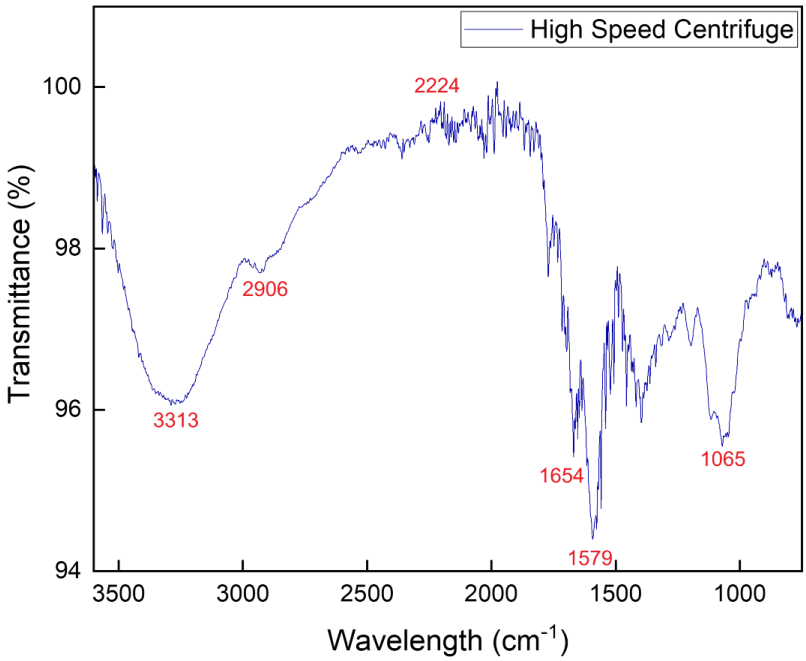

Figure 6. FTIR Spectroscopy of CQDs purified by high-speed centrifugation

Sharper and more distinct tensions are observed in the spectrum of CQDs purified by high-speed centrifugation. This shows that a more homogeneous and pure structure is obtained.

CQDs contain oxygen-containing functional groups compared to plant extracts due to hydrothermal synthesis. The sharper, distinct peaks that CQDs show in the -OH band between 3200-3500 cm-1 indicate the presence of oxygen-containing functional groups. The reason for this band being wide and intense in plant extracts is the compounds and water contained in the plant extract. C=O and C=C vibrations are observed around 1650 cm^{-1} in CQDs, and the presence of carboxyl and amine groups can be considered. The carbonyl group (C=O) is distinct in plant extracts but may be different due to plant proteins and alcohols.

The reason for the additional peaks uvseen in FTIR is that the analysis is performed on a liquid sample, and the FTIR Spectroscopy results we obtained generally coincide with the literature, especially with the study of Zhang et al. [18] and Feng et al. [19].

Conclusions

In this study, CQDs were successfully synthesized by the hydrothermal synthesis method using rosemary plants as plant carbon sources. Optical properties and functional properties of synthesized CQDs were analyzed by UV-visible region absorbance spectroscopy and FTIR. In UV-visible region absorbance analyses, it was observed that CQDs obtained by both purification methods showed maximum absorption at 283 nm wavelength. This absorption is related to π-π* transitions and is thought to be caused by C=C and/or C=O bonds. In the literature, maximum absorption values in the range of 260-320 nm were reported for CQDs obtained by similar syntheses [5, 13-17]. The CQDs purified with syringe filters have lower absorbance values compared to those purified with high-speed centrifuges, indicating that filtration may affect particle size and therefore optical properties. The rapid decrease in absorbance observed after 300 nm indicates that CQDs have strong absorption in the UV region, and absorption continues in the visible region, albeit at a low level, at 400 nm and beyond.

FTIR spectroscopy results revealed the presence of oxygen-containing functional groups such as hydroxyl (-OH), carboxyl (-COOH), carbonyl (C=O), alkene (C=C), and epoxy (C-O-C) on the surface of CQDs. These findings are consistent with the results of similar studies reported by Zhang et al. [18] and Feng et al. [19]. The -OH stretching band, observed especially in the range of 3200–3500 cm^{-1}, is present in both plant extracts and CQDs and has become more pronounced due to the effect of water used as a solvent in the hydrothermal process. The C=O and C=C tensions around 1650 cm^{-1} indicate the presence of aromatic structures and functional groups formed after carbonization.

The sharper and clearer FTIR spectra in CQDs purified by high-speed centrifugation indicate that this method provides more homogeneous and pure particles. The results revealed that the structural and chemical properties of CQDs vary depending on both the carbon source used and the purification method applied. In this context, it was concluded that natural and sustainable carbon sources, such as the rosemary plant used in our study, demonstrate potential for the production of highly functional CQDs with environmentally friendly synthesis methods and can be used in areas such as biosensors and biomedical imaging.

These results confirm rosemary as a viable, sustainable carbon source for CQD synthesis with potential for biomedical applications.

References

[1] Das, R., Bandyopadhyay, R., & Pramanik, P. (2018). CQDs from natural resource: A review. *Materials Today Chemistry, 8*, 96–109.

[2] Yan, H., Li, P., Wen, F., Xu, Q., Guo, Q., & Su, W. (2023). Green synthesis of CQDs from plant turmeric holds promise as novel photosensitizer for in vitro photodynamic antimicrobial activity. *Journal of Materials Research and Technology, 22*, 17–34. https://doi.org/10.1016/j.jmrt.2022.11.090

[3] Yang, H.-L., Bai, L.-F., Geng, Z.-R., Chen, H., Xu, L.-T., Xie, Y.-C., Wang, D.-J., Gu, H.-W., & Wang, X.-M. (2023). CQDs: Preparation, optical properties, and biomedical applications. *Materials Today Advances, 18*, 100376. https://doi.org/10.1016/j.mtadv.2023.100376

[4] Öztürk, D., Ömeroğlu, İ., & Durmuş, M. (2023). Quantum dots in photodynamic therapy. In P. Kesharwani (Ed.), *Nanomaterials for photodynamic therapy* (pp. 401–439). Woodhead Publishing. https://doi.org/10.1016/B978-0-323-85595-2.00009-8

[5] Eskalen, H., Çeşme, M., Kerli, S., & Özğan, Ş. (2021). Green synthesis of water-soluble fluorescent carbon dots from rosemary leaves: Applications in food storage capacity, fingerprint detection, and antibacterial activity. *Journal of Chemical Research, 45*(5-6), 428–435.

[6] Duke, J. A. (2000). *Handbook of medicinal herbs* (p. 630–632). CRC Press.

[7] Heinrich, M., Kufer, J., Leonti, M., & Pardo-de-Santayana, M. (2006). Ethnobotany and ethnopharmacology—Interdisciplinary links with the historical sciences. *Journal of Ethnopharmacology, 107*, 157–160.

[8] de Macedo, L. M., Santos, É. M. D., Militão, L., Tundisi, L. L., Ataide, J. A., Souto, E. B., & Mazzola, P. G. (2020). Rosemary (*Rosmarinus officinalis* L., syn *Salvia rosmarinus* Spenn.) and its topical applications: A review. *Plants, 9*(5), 651. https://doi.org/10.3390/plants9050651

[9] Begum, A., Sandhya, S., Vinod, K. R., Reddy, S., & Banji, D. (2013). An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). *Acta Scientiarum Polonorum Technologia Alimentaria, 12*, 61–74.

[10] Hou, C. W., Lin, Y. T., Chen, Y. L., Wang, Y. H., Chou, J. L., Ping, L. Y., & Jeng, K. C. (2012). Neuroprotective effects of carnosic acid on neuronal cells under ischemic and hypoxic stress. *Nutritional Neuroscience, 15*(6), 257–263.

[11] Rooj, S., & Ghosh, S. (2023). A review on the characterization of CQDs. *Vibrational Spectroscopy, 124*, 103402. https://doi.org/10.1002/vjch.202300022

[12] Baker, S. N., & Baker, G. A. (2010). Luminescent carbon nanodots: emergent nanolights. *Angewandte Chemie International Edition, 49*(38), 6726–6744. https://doi.org/10.1002/anie.200906623

[13] Fang, X.-W., Chang, H., Wu, T., Yeh, C.-H., Hsiao, F.-L., Ko, T.-S., Hsieh, C.-L., Wu, M.-Y., & Lin, Y.-W. (2024). Green synthesis of CQDs and carbon quantum dot-gold nanoparticles for applications in bacterial imaging and catalytic reduction of aromatic nitro compounds. *ACS Omega, 9*(22), 23573–23583. https://doi.org/10.1021/acsomega.4c00833

[14] Sahu, S., Behera, B., Maiti, T. K., & Mohapatra, S. (2012). Simple one-step synthesis of highly luminescent carbon dots from orange juice. *Chemical Communications, 48*, 8835.

[15] Zhu, C., Zhai, J., & Dong, S. (2012). Carbon dots prepared from glucose for sensitive detection of silver ions. *Chemical Communications, 48*, 9367.

[16] Yin, B., Deng, J., Peng, X., Long, Q., Zhao, J., Lu, Q., Chen, Q., Li, H., Tang, H., Zhang, Y., & Yao, S. (2013). Highly sensitive detection of mercury ions using carbon dots. *Analyst*. https://doi.org/10.1039/C3AN01003A

[17] Feng, X., & Zhang, Y. (2019). A simple and green synthesis of CQDs from coke for white light-emitting devices. *RSC Advances, 9*(58), 33789–33793. https://doi.org/10.1039/C9RA06946A

[18] Patravale, V., Dandekar, P., & Jain, R. (2012). Characterization techniques for nanoparticulate carriers. In V. Patravale, P. Dandekar, & R. Jain (Eds.), *Nanoparticulate drug delivery* (pp. 87–121). Woodhead Publishing. https://doi.org/10.1533/9781908818195.87

[19] Selvamani, V. (2019). Stability studies on nanomaterials used in drugs. In S. S. Mohapatra, S. Ranjan, N. Dasgupta, R. K. Mishra, & S. Thomas (Eds.), *Characterization and biology of nanomaterials for drug delivery* (pp. 425–444). Elsevier. https://doi.org/10.1016/B978-0-12-814031-4.00015-5

[20] Nie, H., Li, M., Li, Q., Liang, S., Tan, Y., Sheng, L., Shi, W., & Zhang, S. X.-A. (2014). Carbon dots with continuously tunable full-color emission and their application in ratiometric pH sensing. *Chemistry of Materials, 26*(10), 3104–3112. https://doi.org/10.1021/cm5003669

[21] Manikandan, V., & Lee, N. Y. (2022). Karbon kuantum noktalarının yeşil sentezi ve çevresel uygulamaları. *Environmental Research, 212*(B), 113283.

[22] Alarfaj, N. A., El-Tohamy, M. F., & Oraby, H. F. (2018). CA 19-9 immobilized CQDs conjugated gold nanocomposite for fluorescence immunodetection of pancreatic tumor marker.

International Journal of Molecular Sciences, 19(4), 1162. https://doi.org/10.3390/ijms19041162

[23] Terracciano, M., Tramontano, C., Moretta, R., Miranda, B., Borbone, N., De Stefano, L., & Rea, I. (2021). Protein-modified porous silicon optical devices for biosensing. In H. A. Santos (Ed.), *Porous silicon for biomedical applications* (2nd ed., pp. 113–148). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-821677-4.00017-3

[24] Azam, N., Ali, M. N., & Khan, T. J. (2021). CQDs for biomedical applications: Review and analysis. *Frontiers in Materials, 8*, 700403. https://doi.org/10.3389/fmats.2021.700403