Original Article

Carbon Quantum Dots Extracted from Natural Lemon Juice: Efficient Material for Fluorescence and Antibacterial Applications

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Received: 11 Dec 2021 Accepted: 21 Jan 2022

Abstract

Background & Objective: In recent years, the proliferation of microbial organisms has increased alarmingly, and the overuse of various antibiotics against microorganisms has increased drug resistance. On the other hand, the need to reduce health costs, the production of antimicrobials with low costs, and the basic needs of today's human society have become. This led to a large-scale study of new drugs against microorganisms and the use of nanoparticles as antibacterial agents were considered. This study aimed to use biocompatible carbon quantum dots (CQDs) nanoparticles instead of antibiotics resistant to gram-positive and gram-negative microorganisms.
Materials & Methods: Fluorescent carbon quantum dots were extracted from natural lemon juice using the hydrothermal approach. Analyzes of X-ray diffraction (XRD), Fourier transform infrared (FT-IR), ultraviolet-visible (UV-Vis), photoluminescence (PL), transmission electron microscope (TEM), and energy-dispersive spectrometer (EDS). CQDs were investigated on ten types of microorganisms by the microwell dilution method. In this study, the minimal inhibition concentrations (MIC) and the minimum bactericidal concentration (MBC) was determined.
Results: Fluorescent CQDs less than 5 nm in size were fabricated and confirmed by structural and microscopic analysis. This test showed that four microorganisms *B. subtilis, E. coli, P. aeruginosa, S. pyogenes* and *C. albicans* were resistant to the antibiotic nystatin and showed the highest sensitivity to CQDs, the lowest MIC and MBC for CQDs are 250 µg/ml and 1000 µg/ml.
Conclusions: In general, the results obtained from this study can claim that CQDs have antibacterial properties and can be introduced after further studies as candidates are used to treat or prevent a variety of infections caused by microorganisms.

Keywords: Antibacterial activity, hydrothermal, carbon quantum dots, lemon juice

Introduction

The increasing prevalence of microbial infections and the rapid emergence of drug resistance to antibiotics created a critical health menace worldwide. An alarming increase in mortality and morbidity in conventional therapeutics created an increased demand for an elective alternative agent for treating the infections. Therefore, finding elective alternatives with antibacterial activity is now a research hotspot in biomedicine. The emergence of nanotechnology has presented a new chance to remove and remedy these infections (1- 3). Nowadays, with the expansion of using synthetic nanoparticles from chemicals, the concern for environmental pollution, by the nanoparticles that have been produced by this method and the

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production of hazardous by-products has been dramatically increased; hence, the need for synthesis methods that are clean, non-toxic and eco-friendly is much greater than ever. Nowadays, green synthesis is one of the new and practical methods in the world. In this method, natural resources are used as raw materials, which leads to a reduction in the consumption of chemicals and the reduction of pollution caused by them (4, 5). In 2006, Sun et al. increased the intensity of fluorescence by increasing the surface area of nanoparticles, which they called carbon quantum dots (6). CQDs are generally synthesized by two major methods: top-down and down-top. The ways we know consist the arc discharging, chemical oxidation, electrochemistry method, hydrothermal, microwave, thermal decomposition. Many of these methods are high cost, complex, and very difficult, so the green synthesis method, which is less complex and biocompatible, has been considered in recent decades (6- 9). Compared to semiconductor quantum dots and organic pigments, carbon dots are increasingly superior in terms of high water solubility, outstanding light luminosity, biocompatibility properties, optimal bioavailability, chemical ineffectiveness, ease of surface functionalization, and low toxicity (4, 6, 10- 13). The CQDs have attracted considerable interest due to their photoluminescent property since they have photochemical and photo-physical. Carbon quantum dots have many applications in cell imaging, bio-imaging, solar cell, electrocatalyst, elimination, and detection of ions in sensors (11, 14- 16). The biomasses studied are orange juice, banana juice, soybeans, grass, corn stalk, glucose, chitosan, chitin, agricultural biomass, egg and manure (17, 18). One of the activities that have been done due to the properties of carbon quantum dots is the imaging of bacterial and fungal cells. Also, CQDs were synthesized from garlic by Zhao et al in 2015 (19), Kumar and his colleagues from cotton. In the same year, it was synthesized from saffron by Insafi and his colleagues and was used in cell imaging and free radical detection (20), materials at the nanoscale show a distinct function and in recent seals, their antibacterial properties have attracted much attention. The process of killing bacteria causes physical or chemical damage to the microbes. This process occurs on their surface or inside, a somewhat antibacterial mechanism similar to antibiotics (21). In this study, lemon juice was used to synthesize CQDs and its antibacterial properties on gram-positive and gram-negative microorganisms were investigated. The nanoparticles attach to the bacterial membrane by electrostatic reaction and destroy it by disrupting it (22, 23). One of the antimicrobial mechanisms of nanoparticles is the reaction with amines and carboxyl groups of the peptidoglycan layer of the bacterial wall, which causes damage to their cell wall. The mechanism of induction of the antibacterial activity of ion release and subsequent intracellular production of reactive oxygen species depends. CQDs can act on a variety of gram-positive and gram-negative bacteria, targeting bacteria based on the electrostatic interaction between anionic and cation microbial membranes on the surface of carbon dots. Accordingly, carbon dots should be used as a suitable alternative to conventional antibiotics in antibacterial testing (24- 26). However, there remains a challenge regarding identifying and sourcing appropriate and cheap bio-based precursors (27). Disadvantages of CQDs: The metabolism and destruction of quantum dots in the body are still largely unknown, and several studies have shown that quantum dots accumulate in the kidney, spleen, and liver. It is also unclear whether quantum dots can be removed from the body (17, 28).

According to the mentioned articles, in addition to being used to identify bacteria and identify cancer cells, CQDs can also be used in treatment. In this study, the effect of CQDs on ten types of gram-positive and negative bacteria was measured and good results were obtained. These nanoparticles can be a perfect alternative to antibiotics due to their low side effects, cost-effectiveness, and biocompatibility with the environment.

Experimental details

Synthesis of CQDs

Lemon juice was used for the green synthesis of carbon quantum dots by the hydrothermal method. First, 10 ml of Shirazi lemon juice was added to 20 ml of Deionized Water. It was then placed on a magnetic stirrer for one hour and dissolved. The prepared solution was placed in an autoclave and placed in an oven at 180 °C for 24 hours. After the reaction, the obtaining solution was separated using filter paper to remove excess material, then examined. Figure 1 shows a diagram of the synthesis of CQDs using hydrothermal methods.

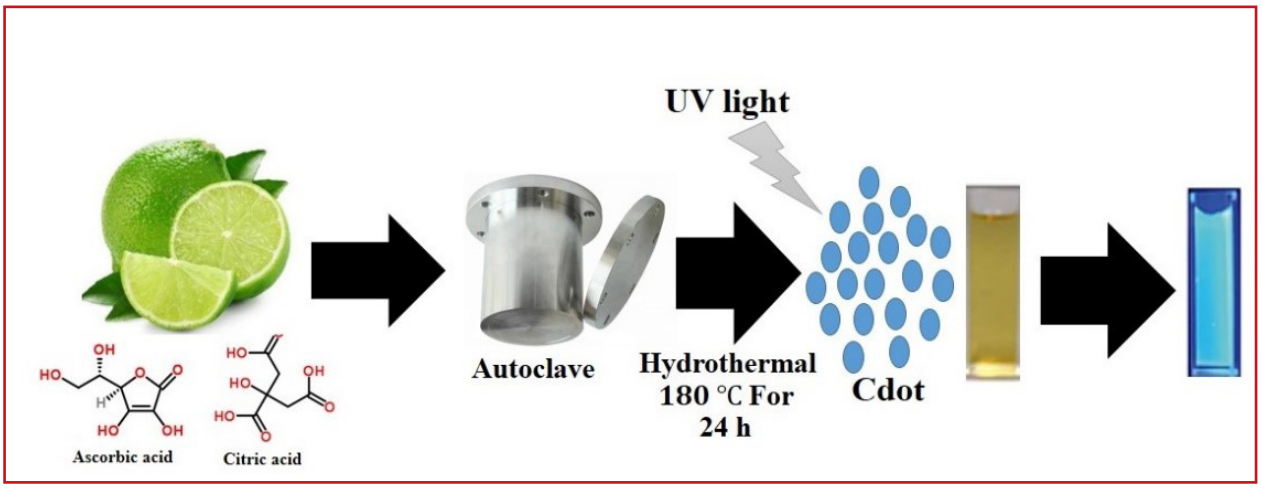


Figure 1. The diagram of the synthesis of CQDs using hydrothermal methods

Characterizations

The crystal structure was investigated using analysis of X-ray diffraction (XRD; model: Philips X'pert Pro MPP with Cu Kα radiation filtered by Ni, and λ= 0.1540 nm). In this regard, the average crystallite size (D) of CQDs was estimated based on the Scherrer formula as given below:

$$D = \frac{K\lambda}{\beta \cos\theta} \qquad (1)$$

where k is the shape factor (k= 0.9), λ is the wavelength of the incident beam, ß is the full width at half maximum, and θ is the incident beam angle.

A scanning electron microscope determined morphology, composition and particle size of the NPs (SEM; model: TESCAN Mira 3–XMU) equipped with an energy-dispersive spectrometer (EDS), and a transmission electron microscope (TEM; model: Zeiss EM900). A Fourier transform infrared (FT-IR; model: Magna–IR550) spectrometer was used to investigate the presence of functional groups. Photoluminescence spectroscopy of the PerkinElmer (LS55 model) spectrometer was used in the range of 200−900 nm to investigate optical properties at room temperature. An ultraviolet-visible (UV-Vis; UVS-2500, PHYSTEC/Iran) spectrometer was used in the range of 200−800 nm to investigate optical properties at room temperature.

Antimicrobial activity

Antibacterial activity assay

In the present study, the antibacterial activity of the CQDs nanoparticles was evaluated against *Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), Bacillus subtilis (ATCC6633), Staphylococcus aureus (ATCC 29737), Streptococcus pyogenes (ATCC 19615), Staphylococcus epidermidis (CIP 81.55), Shigella dysenteriae (PTCC 1188), Salmonella paratyphi-A serotype (ATCC 5702),*

Salmonella paratyphi-A serotype (ATCC 5702), Candida albicans (ATCC 10231) and Aspergillus niger (ATCC 9029).

Determination of the minimum inhibitory concentration (MIC)

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, the minimum inhibitory concentration determines by the broth micro-dilution method. In brief, 100 µL sterile Mueller Hinton broths were added to each well of sterile 96-well plates. After, dilution series of SnO_2: Au nanoparticles were prepared and added to the wells. Finally, 10 µL of bacterial suspension containing approximately 5×10^5 CFU was inoculated into each well. The plates were incubated at 37 ◦C for 18–20 h. The lowest concentration of CQDs nanoparticles that inhibited the growth of bacteria was recorded as the minimum inhibitory concentration. Also, MIC determinations were done with Gentamicin, Rifampin, and Nystatin used as positive inhibitor controls for Gram-positive bacteria, Gram-negative bacteria, and fungus.

Minimum bactericidal concentration (MBC)

The minimum inhibitory concentration determines by the broth microdilution method. 10 µL from the wells where no bacterial growth was subcultured to on MHA plates. After 24 h incubation at 37 °C, the colony forming units (CFU) were detected. The lowest concentration of that killing 99.9% of the bacteria was recorded as MBC.

Results and Discussion

Figure 2 (a) shows XRD patterns used to study the structure of CQDs. The resulting spectrum consists of a peak of 2θ in 10-80. That exhibits a single broad (002) peak at 19◦ (2θ) diffraction is seen, according to this, Scherer equation the crystallite size is obtained to be about 5 nm, and no secondary phase is observed in the XRD pattern. On the other hand, a peak in this area is the main characteristic of carbon quantum dots and confirms the synthesis of QCDs. Following previous studies of CQDs (29, 30). Other articles have confirmed these results. The energy-dispersive X-ray spectroscopy is a technique for elemental analysis and chemical composition determination. The method relies on the generation of characteristic X-rays that reveal the identity of the elements present in the sample Figure 2 (b) shows the EDS spectrum of CQDs The presence of the elements C and O with respective atomic percentages of 66.02% and 33.98% is confirmed (31).

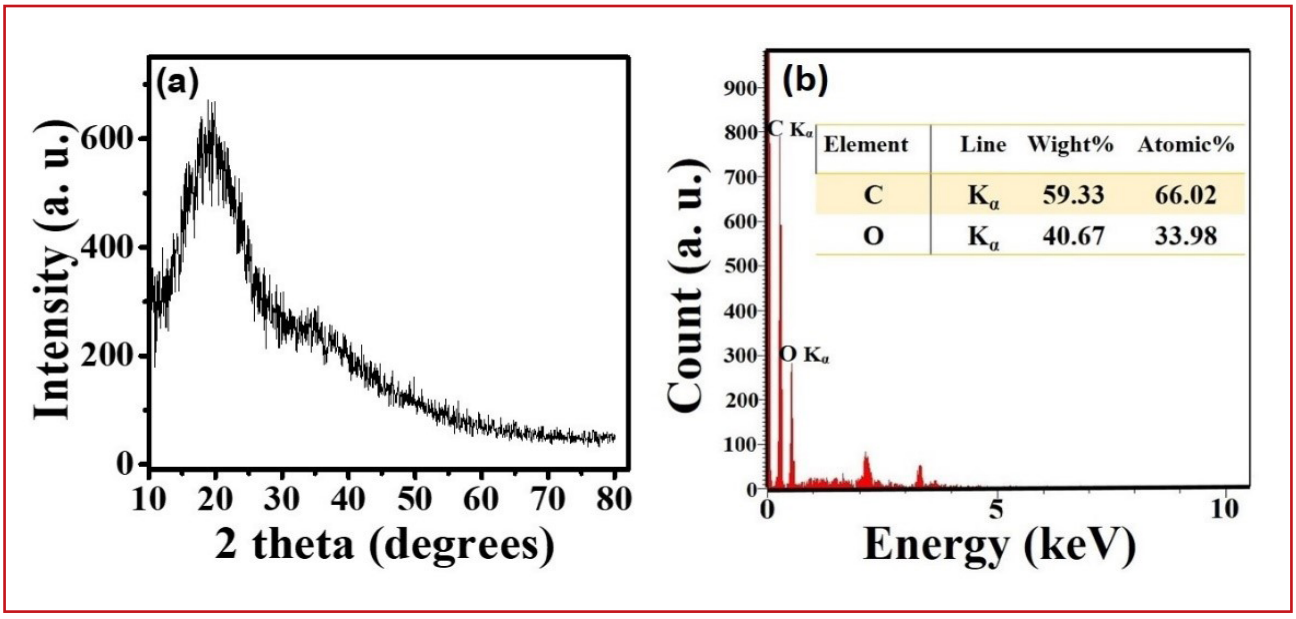


Figure 2. (a) XRD spectrum of CQD. (b) EDS spectrum CQDs

Transmission electron microscope (TEM)

The results of TEM analysis confirmed the fabrication of quantum carbon particles. However, due to the fineness of the particles, it is not possible to accurately estimate the particle size by this analysis. Therefore, TEM analysis was used. The size of the CQDs particles synthesized by this figure is less than 5 nm, and the particles are stacked together, in addition to the TEM image confirming the quantum nanoparticles. The CQDs are spheroidal. Together with the corresponding size distribution histogram [the inset of Figure 3]. As can be seen, the NPs are ultrafine, and uniformly dispersed without considerable agglomeration. Moreover, the size of spherical NPs ranges from 3 to 5 nm, having an average diameter of about 4 nm.

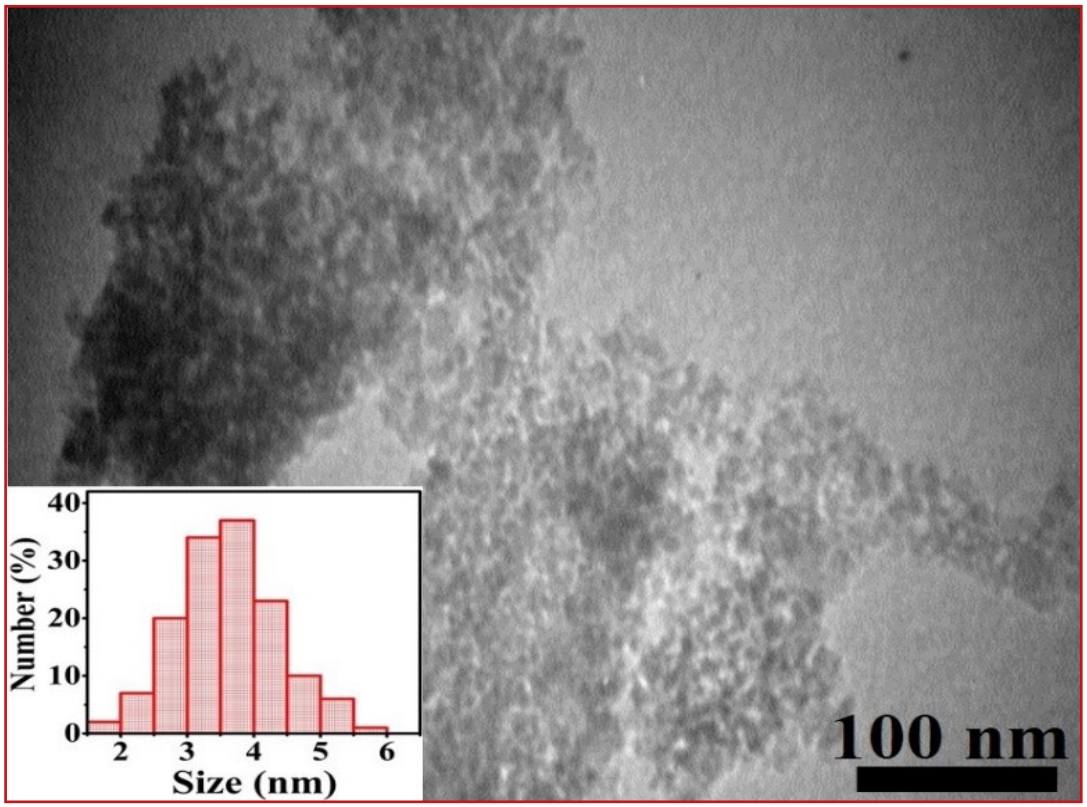
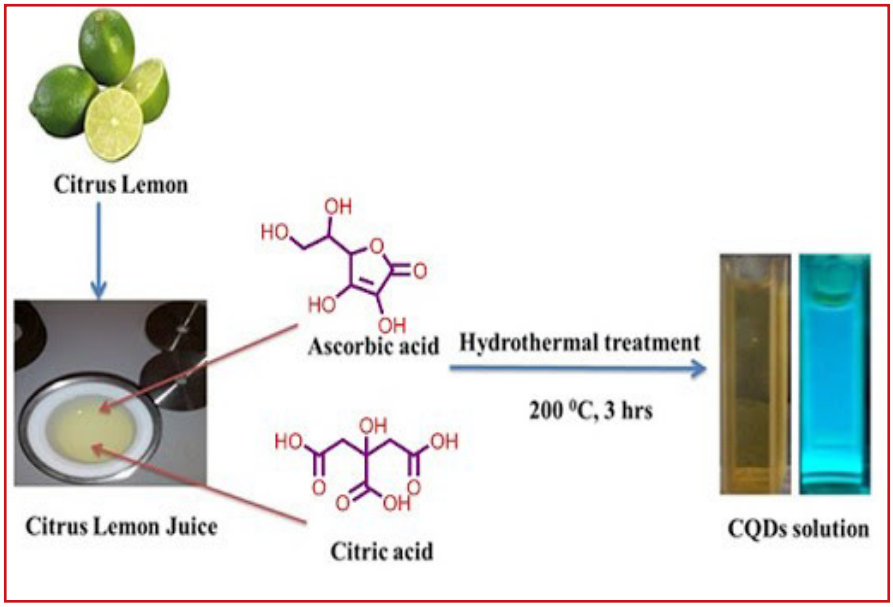


Figure 3. TEM image of green synthesis of CQDs

Photoluminescence spectrum (PL)

CQDs are a new class of nanomaterials that have attracted much attention in the last decade. Two fluorescence emission mechanisms are considered for CQDs. The first mechanism is related to band transfer π-domains and the second mechanism is related to surface defects in carbon dots. PL method was used to study the optical properties of CQDs (12). PL phenomenon studies the emission of light based on optical excitations. Figure 4 (b) shows the intensity of the fluorescence radiation of CQDs particles with different excitation wavelengths, which has a peak in the range of 400-500 nm, and the fluorescence intensity increases with increasing excitation wavelengths (32). The UV-Vis spectrum of the CQDs recorded two significant absorption peaks at 232 and 283 nm (Figure 4 (a)). These distinct peaks were attributed to the presence of $\pi - \pi^*$ transition of C=C and $n - \pi^*$ transition of the carbonyl group C=O, respectively (33). The diluted CQDs show an intense sky blue color upon illumination by a UV-light source shown in figure 4 (b)(18, 34).



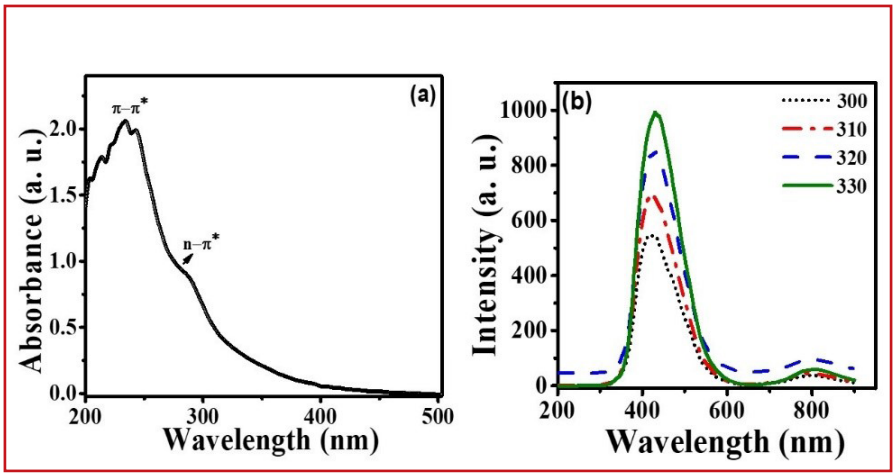


Figure 4. (a) UV-Vis spectrophotometric absorption spectrum of CQDs. (b) Photoluminescence spectrum (PL) of CQDs With different excitation beams

Fourier-transform infrared spectroscopy (FTIR)

In the FT-IR spectroscopy, the radiation of infrared to the NP sample would change the energy of bond vibrations. The absorption amount is identified in terms of the wavenumber for chemical bonds were investigated in the wavenumber interval of 400 −4000 cm^{-1}, and the result is shown in Figure 5. The peak observed in 3439 cm^{-1} can be related to the stretching vibration of the OH group which shows the presence of hydroxyl groups on the sample surface and these functional groups cause water absorption. Peaks in 2985 cm^{-1} correspond to the stretching vibration of C-H, peak at 1733 cm^{-1} is attributed to the presence of vibration absorption C=O group, 1380 cm^{-1} C-O (Carboxy group) and 1206 cm^{-1} C-O (Alkoxy Group) attributed and the results are similar to other articles (35, 36). The results show that the surface of carbon quantum dots has carboxyl and hydroxyl functional groups, which causes the high solubility of carbon quantum dots in the water.

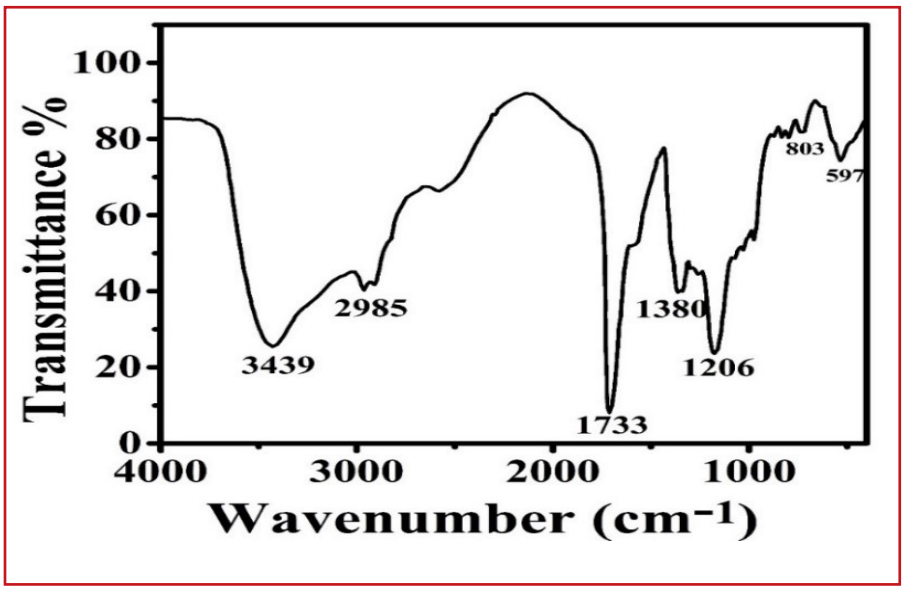


Figure 5. FTIR spectrum of green synthesis of CQDs

Antimicrobial Activity

In the present study, the MICs were evaluated by the broth microdilution method and the results are presented as shown in Table 1. Findings indicated that CQDs NPs considerable antibacterial activity against the tested bacteria. This sensitivity is specifically in the case of, *E.coli*, *B. subtilis*, *C. albicans*, *S.pyogenes*, *P. aeruginosa* were highly regarded and the standard species studied were sensitive to nanoparticles and the MIC was 250 to 500 µg / ml. Also, the results showed that CQDs NPs have no inhibitory effect against *A. niger*. Gentamicin shows a high activity on the tested bacteria than rifampin. However, Nystatin shows a high antifungal activity on *A. niger* and *C. albicans.* Several studies have confirmed the antibacterial activity of CQDs NPs. Usually, GQDs induced membrane stress by free radical formation; then, the ROS damage the cell membrane, resulting in cell content leakage and cell death (31, 37).

Table 1. The antimicrobial activity of the CQDs was evaluated

Test microorganism	CQDs		Antibiotique					
			Rifampin		Gentamicin		Nystatin	
	[b]MIC	[c]MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. subtilis	250	1000<	31.25	31.25	3.90	7.81	NA	NA
S. epidermidis	500	1000<	1.95	3.90	1.95	3.90	NA	NA
S. aureus	1000	1000<	1.95	3.90	1.95	3.90	NA	NA

E. coli	250	500	3.90	3.90	3.90	7.81	NA	NA
S. dysenteriae	500	1000<	15.63	15.63	3.90	7.81	NA	NA
S. paratyphi-A serotype	500	1000<	15.63	15.63	3.90	7.81	NA	NA
P. aeruginosa	250	500	31.25	31.25	7.81	7.81	NA	NA
S. pyogenes	250	500	0.975	1.95	0.975	1.95	NA	NA
A. niger	-	-	-	-	-	-	31.2	31.2
C. albicans	250	1000<	NA	NA	NA	NA	125	125

A dash (–) indicate no antimicrobial activity
[b]MIC minimal inhibition concentrations as µg/ml, NA not applicable
[c]MBC minimum bactericidal concentration µg/ml

Conclusions

Green synthesis of CQDs was performed using Shirazi lemon juice by a hydrothermal method. The resulting CQDs were studied and analyzed using TEM, XRD, UV-Vis, FTIR and PL analyses. The TEM results show that the CQDs are highly homogeneous and are identical in size to the values calculated from the XRD results. According to PL spectra, these CQDs have emission peaks in the wavelength of 400-450 nm and are comparable in terms of light intensity to toxic substances The UV–Vis and fluorescence studies indicate the visible light exposure of the molecules that CQDs may be used for specific blue LED, and UV detector applications. The FTIR spectrum indicates the formation of C and O bonds in the synthesized sample and indicates that the sample surface has hydroxy and alkoxy groups. CQDs showed tenacious antimicrobial activity versus the protected bacterial strains. The above findings detect that biocompatible and easily processed CQDs are useful for nonlinear optical devices and therapeutic applications. The antimicrobial activity of CQDs was evaluated against a set of 10 microorganisms that were assessed qualitatively.

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