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Natural and Green Protocol for Synthesizing Novel Ag@CQDs Nanoparticles, Their Characteristics, and Anticancer Activity against Oral Cancer Cell Lines

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Abstract: Oral cancer remains a major global health burden influenced by genetic, environmental, and lifestyle factors. Although conventional chemotherapies are available, their toxicity and limited selectivity have driven an increase in the use of nanomaterials and natural-mediated alternatives. In this study, clove, a well-known traditional medicinal plant, was utilized as a green precursor for the synthesis of silver-doped carbon quantum dots. An aqueous extract is prepared from the plant, followed by mixing with a silver salt to afford the targeted products through the hydrothermal protocol. The products were fully characterized, and their production is confirmed via physicochemical techniques. The results showed that the synthesized silver-doped carbon dot nanoparticles are spherical in shape, having a size of 63.25 nm with an amorphous carbon structure, demonstrating a broader peak centered at $2\theta = 27°$. Additionally, their chemical constituents were analyzed by gas chromatography mass spectroscopy. The cytotoxic activity of the three samples (clove extract, carbon dots, and silver-doped carbon dots) was assessed against oral squamous cancer cells (OSCC) and oral squamous normal cells (OSNC). The results showed effective dose-dependent cytotoxic potency for all the products; their IC_{50} is recorded as 346.8, 353.4, and 286.7 µg/mL for clove extract, carbon dots, and silver-doped carbon dots, respectively. In contrast, their activity for normal cells was observed as IC50= 705.4, 1292, and 939.3 µg/mL for clove extract, carbon dots, and silver-doped carbon dots, respectively. These findings revealed that the synthesized silver-doped carbon dots are efficient compounds as anticancer agents against OSCC with comparable low toxicity.

Keywords: *Ag@CQD; Clove;, Cytotoxicity; HRTEM; Hydrothermal; Oral Cancer; Oral Squamous Cell Carcinoma; X-Ray Diffraction.*

1. Introduction

Cancer is a threatening concept in the world, since it's a second major cause of death [1]. Cancer is defined as non-controlled proliferation of cells [2]. Change in genetic and continuous cell proliferation around the mouth, head, and neck causes oral cancer (OC) [3]. Several environmental factors may contribute to the occurrence and development of oral cancer, such as irradiation, lack of nutrients, microbial infections, etc. Oral cancer can be prevented by good-quality nutrients, such as fruits and vegetables, as well as vitamins [4], while smoking and drinking alcohol promote oral cancer [5].

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer, which involves the skin of the neck and head, in addition to the lip and oral cavity. This type of oral cancer can occur in the floor of the mouth, upper and lower gums, palate, and tongue [6]. Oral cancer is asymptomatic; hence, detection at early stages is challenging. Survival rate of 5 years for OSCC is only 41%; in contrast, if detected in early stages, OSCC survival rate increases to more than 85% [7]. In 2020, the occurrence of oral cancer recorded 377,713 new cases and 177,757 deaths [8]. Although OSSC is recording high incidences, fortunately, it can be treated by radiotherapy, surgery, and chemotherapy. Therefore, scientists are trying to innovate new chemical therapies and tumor markers [6, 9].

Recently, researchers have been interested in nanoparticles (NPs); due to their outstanding properties, such as stability, electrical and optical properties, physiochemical properties. The NPs can be synthesized from metals and/or nonmetals. In general, the size of NPs varies from 1 nm to 100 nm, but when their size is less than 10 nm, they are called carbon quantum dots [10]. They have amazing properties, such as biocompatibility, electrical conductivity, low toxicity, and good water solubility; therefore, they are implemented in several applications, including sensors, optical purposes, diodes, and biomedicine [10, 11]. Silver Nanoparticles (Ag NPs) are interested by the researchers due to their remarkable properties, such as compatibility, electrical conductivity, and optical properties. These characteristics make Ag NPs compatible for several activities, including biological activities, pharmaceuticals, photocatalytic, catalysis, and biosensors [12, 13].

The best method to prepare nanoparticles with high safety and biocompatibility is using biological sources such as plants (using their secondary metabolites), bacteria, or fungi (microorganism enzymes) [14, 15]. Medicinal plants are still in great demand in almost all nations as a source for health care due to high bioactivity, low costs, and safety issues [16-19]. Clove with botanical name (*Syzygium aromaticum*)[20] and has a synonym of *Eugenia caryophyllus* [21], has been used as a traditional medicine for centuries [22]. It contains several bioactive constituents such as flavonoids, phenolics, acids, and essential oils [20, 23]. The presence of these bioactive phytochemicals makes *S. aromaticum* an efficient antioxidant, antimicrobial, antiviral, anti-inflammatory, analgesic, and anticancer agent [20, 22-26]. Besides the medicinal uses, clove is used in other industries and as an aroma for soaps, detergents, perfumes, and aroma therapies [27].

Several studies indicated the synthesis of solver-doped carbon dots, including the fluorescence technique to detect the uric acid [28]. Another study mentioned the synthesis of Ag@CQDs from lignin and utilized them to determine the valsartan [29]. A nanocomposite of Ag-doped CQDs was synthesized for effective charge extraction [30]. Novel silver-doped carbon dots were synthesized from L-citrulline, phthalimide, and silver nitrate and utilized as emission enables fluorescence and tetrazine detection [31]. Furthermore, Ag-CQDs have also been employed in chemical castration against rat models, resulting in which resulted in reduction in testosterone levels, motility, and sperm concentration [32].

The present study is the first investigation reporting the synthesis of silver-doped carbon quantum dots (Ag@CQDs) using clove, a traditional medicinal plant, and evaluating their anticancer activity against oral cancer cell lines. The products were afforded by a simple, efficient, eco-friendly, and fast protocol. The extract, carbon quantum dots (CQDs), and Ag@CQDs were tested for their cell viability through measuring the MTT cell proliferation assay.

2. Methods

2.1 Materials and Instrumentations

The cloves were purchased from a local market in Erbil City (Cloves Whole, Afia Company, Jeddah, Saudi Arabia). Silver nitrate was purchased from Sigma-Aldrich (Merck, Germany). FTIR (Shimadzu IR Affinity-1), UV-Vis (UV-Visible Spectrophotometer AE-S60; 1.0 cm quartz cells), Centrifuge (Changsha Yingtai, Ltf), and Ultrasonic Bath (LabQuest, Borosil. 40 kHz, 1 – 60 min., 150W) were performed at the Advance Nanotechnology Lab, at the College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq. HRTEM (TECNAI G2 20S-TWIN, 200 kV), XRD (Bruker D2 Phaser, 380 eV), EDS (Bruker QUANTAX EDS, silicon drift detector), Inverted Microscope (ACCU Scope, EXI-350, f=180 mm, LED Epi-fluorescence), and GC-MS (Agilent 5977C, Thermoquest Finning equipped with fused silica capillary DB-5 column (60m90.25 mm; film thickness 0.25 μm) were performed at the University of Tehran, Tehran, Iran.

2.2 Preparation of clove extract

The dry clove seeds (25 g) were grinder to fine powder, followed by dissolving in water (250 mL). The mixture was set up with an ultrasound technique for 2 h at room temperature, and then further stirred for an additional 4 h at room temperature. The mixture was settled, decanted, and then filtered to have a clear solution (brown color). The solution was kept in a safe place for the tests and preparation of targeted nanoparticles [33].

2.3 Green synthesis of Ag-doped carbon quantum dots

The Ag-CQDs were synthesized through a one-step hydrothermal method. Clove's aqueous extract and silver nitrate were utilized as precursors. In detail, 20 mL of clove's aqueous extract and 0.01 g of silver nitrate were mixed under continuous stirring to ensure homogeneity. The resulting mixture was transferred into a Teflon-lined autoclave, which was then sealed and heated at 180 °C for 6 hours. After the completion of the reaction, the solution was cooled down to room temperature. The compounds were filtered through a 0.22 μm membrane to remove impurities, followed by centrifugation at 12,000 rpm for 20 minutes. The supernatant containing Ag-CQDs was collected and further purified using a dialysis bag (Sigma-Aldrich, Merck, Germany) with a molecular weight cutoff (MWCO) of 1000 for 24 hours to ensure the removal of residual impurities. The purified Ag-CQDs solution was stored at 4 °C until further use. For comparison, bare CDs were synthesized using clove's aqueous plant extract as the sole precursor under identical hydrothermal conditions.[34]

2.4 MTT assay evaluation

The *in vitro* anticancer activity against oral squamous cancer carcinoma (OSCC) was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The OSCC cell lines (IBRC C10995) were obtained from the Cell Culture Laboratory, Tehran University for Medical Sciences, Tehran, Iran.

Cells were seeded in 96-well plates at a density ranging from 1,000 to 10,000 cells per well and incubated for 24 hours under standard conditions (37 °C and 5% CO_2). Subsequently, 10 μL of MTT reagent (5 mg/mL in sterile phosphate buffer saline) was added to each well and incubated for an additional 2 - 4 hours until formation of purple formazan crystals was observed. After that, 100 μL of the solubilization solution (dimethyl sulfoxide, DMSO) was added to dissolve the crystals, and the plates were kept in the dark at room temperature for 2 h. Absorbance was recorded at 570 nm using a microplate reader.

The suspended cells were collected by centrifugation and resuspension at 1×10^6 cells/mL. Serial dilutions were prepared to obtain cell concentrations ranging from 1×10^6 to 1×10^3 cells/mL. Aliquots of 100 μL from each dilute solution were placed into microtiter wells, with three control medium wells serving as blanks.

After incubation for 6 - 48 h (12 h is sufficient for most cells), 10 μL of MTT solution was added, and the cells were further incubated for 4 h. An inverted microscope was used to monitor the formazan formation. After visible purple precipitate, 100 μL of DMSO was added to each well, gently swirled, and incubated in the dark for 2 – 4 h at room temperature. Finally, the absorbance was recorded at 570 nm. Results were expressed as mean ± standard error of mean from three independent replicates (n=3).

Relative cell viability% = [(Absorbance of treated cells)/(Absorbance of control cells) × 100]

2.5 Gas Chromatography Mass Spectroscopy (GC/MS) Analysis

The volatile chemical constituents from the extract, carbon dots, and silver-doped carbon dots were analyzed by using gas chromatography mass spectroscopy (GC/MS) utilizing a Thermoquest-Finnigan equipped with a fused silica capillary DB-5 column (film thickness 0.25 µm, 60m 90.25mm) with a trace MS detector. The instrument's speed rate was set-up on 5 °C/minute, with the variable temperature ranging between 60 – 250 °C, which is finally fixed at 250 °C for 10 minutes. The measurements were documented after three minutes of running. Ionization voltage was maintained at 70 eV, and nitrogen gas was employed as carrier gas with a flow rate of 1 mL min^{1}. Ion source and interface temperatures were set on 200 and 250 °C, respectively; while the injector's temperature was set on 250 oC and the detector's temperature on 300 °C. Finally, the mass range was scanned from 43 to 456 m/z (Anwar GCMS).

2.6 Statistical analysis

The data of the present study were analyzed using Graph-pad Prism (Version 8) program. Data were compared using t-test, ANOVA, and descriptive statistics. The level of significance is assigned as p-value < 0.05.

3. Results and Discussion

3.1 Chemical composition of the crude extract

Gas chromatography mass spectroscopy (GC-MS) analysis revealed that the extract underwent a chemical change upon conversion to nanosized particles. The GC-MS results of the aqueous extract, synthesized carbon dot, and silver-doped carbon dots are presented in Table 1.

The chemical structure of the main volatile phytochemicals in the aqueous extract of E. caryophyllus is presented in Figure 1. Structures were drawn by ChemDraw (Version 16.0) Software based on the molecular data obtained from the NIST mass spectral library.

The findings revealed that the clove crude extract contains the highest amount of Eugenol (61.25%). Whereas the eugenol has been reduced to about 9% in its corresponding carbon quantum dots, and was absent in Ag@CQDs. This transformation may be due to overheating of the chemical constituents in the hydrothermal techniques, in which the phytochemicals are changed to a carbon core and surrounded by functional groups. In CQDs, 4-Pyridinol (25.2%) recorded the highest amount. Ag@CQDs mainly contain "Phenol, 4-(ethoxymethyl)-2-methoxy-" (69.1%).

The clove is used as a traditional medicine because of containing several phytochemicals, which are effective in curing various illnesses. Additionally, silver (Ag) plays a crucial role in medicinal chemistry because of its broad-spectrum anticancer, anti-inflammatory, and antimicrobial activity, through reactive oxygen species (ROS) generation and DNA interaction. When free radicals are activated in the cells, they participate in gene expression and induce cellular transformation signaling, apoptosis suppression, invasion, proliferation, and metastasis to other cells [35].

Eugenol, presented in clove at more than 70%, has antioxidant properties, resisting the modulation of damaging hydrogen peroxide to produce radicals on the DNA of various strains in individuals. Eugenol has radical scavenging activity and genotoxic effects on several cancer cells with different efficiency [20, 36, 37].

Table 1: Percentage of phytochemicals presented in clove (aqueous extract, CQDs, and Ag-CQDs)

#	Retention Time/ minutes	Phytochemicals % in the extract	Phytochemicals % in the CQDs	Phytochemicals % in the Ag-CQDs
1	2.6	---	---	Quinoline, 2-chloro-6-methoxy-4-methyl- (0.8%)
2	2.8	---	---	Butanoic acid, methyl ester (1.0%)
3	3.0	2,2-Dimethoxybutane (6%)	2,2-Dimethoxybutane (21.2%)	2,2-Dimethoxybutane (5.6%)
4	3.8	Furfural (1.36%)	---	---
5	4.0	Undecane (1.3%)	---	Benzoic acid, 2-amino-4-methyl- (4.5%)
6	4.8	2-Chloro-4-ethoxyquinoline (2.1%)	---	---
7	5.0	---	Decane (7.4%)	Decane (1.5%)
8	6.0	---	4-Pyridinol (25.2%)	Quinoline, 2-chloro-6-methoxy-4-methyl- (1.4%)
9	7.1	5-Hydroxymethylfurfural (4.3%)	---	---
10	7.3	Phenol, 4-(2-propenyl)- (2.0%)	---	---
11	7.4	---	---	Hydroquinone (1.35%)
12	7.6	---	---	Ethyl 5-(furan-2-yl)-1,2-oxazole-3-carboxylate (1.65%)
13	8.0	Eugenol (61.25%)	Eugenol (9.3%)	---
14	8.2	---	---	1,2,3-Benzenetriol (2.4%)
15	8.4	---	---	Benzeneethanol, 4-hydroxy- (1.75%)
16	9.0	---	Methyl-(2-hydoxy-3-ethoxy-benzyl) ether (21.7%)	Phenol, 4-(ethoxymethyl)-2-methoxy- (69.1%)
17	9.1	Phenol, 2-methoxy-4-(2-propenyl)-, acetate/ (3.7%)	---	---
18	9.6	---	---	2,4'-Dihydroxy-3'-methoxyacetophenone (5.7%)
19	9.7	---	Pyrido[2,3-d] pyrimidine, 4-phenyl- (15.2%)	---
20	10.0	---	---	Isoelemicin (3.0%)
21	10.5	2-Ethylacridine (3.0%)	---	---

22	10.6	Methyltris(trimethylsiloxy)silane (12.3%)	---	---
23	11.2	Thieno[2,3-c] furan-3-carbonitrile,2-amino-4,6-dihydro-4,4,6,6-tetramethyl (2.6%)	---	---

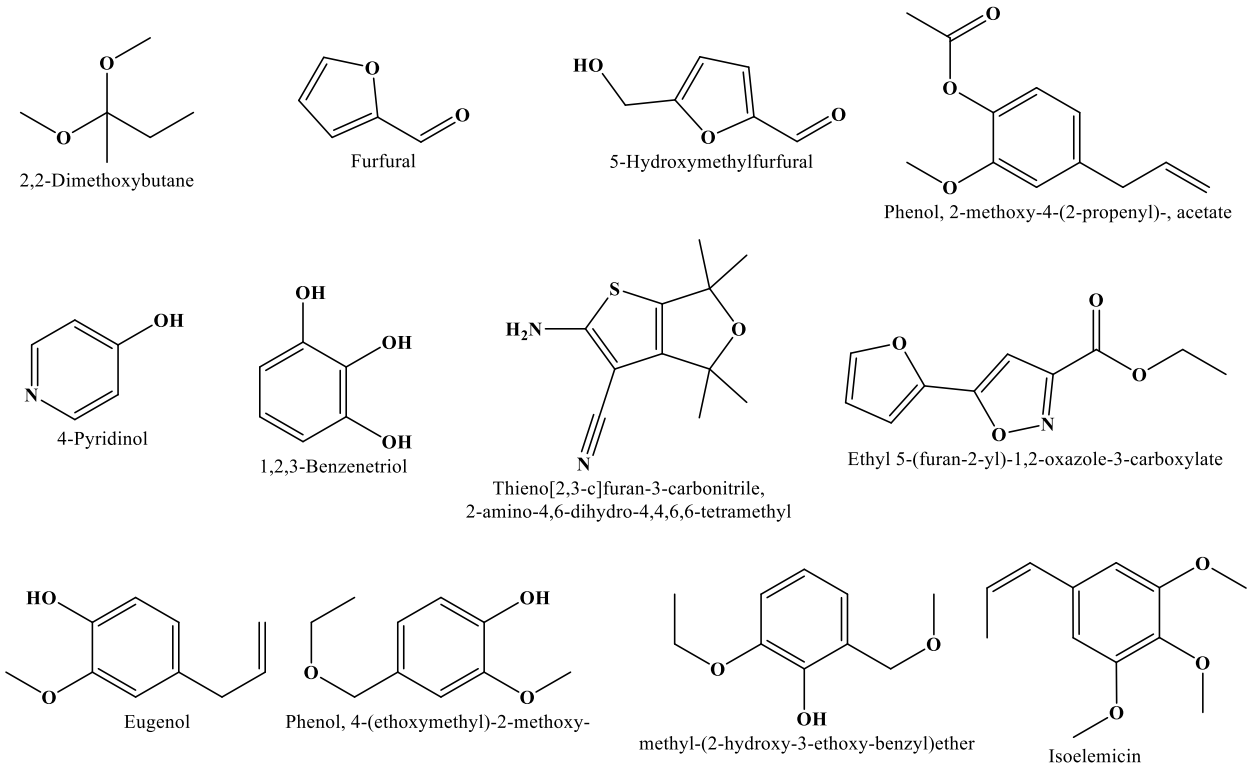

Figure 1: Chemical structure of main volatile constituents found in aqueous extract of clove; measured and identified by GCMS.

3.2 Characterization of Ag-doped CQDs

All the chemical constituents in clove are carbon-based compounds. They are considered a good source for the synthesis of carbon dot nanoparticles. The morphology of the synthesized silver-doped carbon quantum dots (Ag-CQDs) was studied using high-resolution transmission electron microscopy (HRTEM) and field emission scanning electron microscopy (FE-SEM). The obtained result recorded spherical shape and well-dispersed Ag-CQDs, as shown in Figures 2a and 2b, which are aligned with previously published articles [28]. The size distribution and green-synthesized Ag-CQDs are calculated by using ImageJ software. The obtained size of Ag-CQDs was 63.25 nm, as shown in Figure 2c. The Fluorescence and Ultraviolet-Visible (UV-Vis) absorption was selected to analysis the optical properties of green synthesized Ag-CQDs. The difference in fluorescence intensity between CDs that are prepared alone from plant extract and Ag-doped CDs, as explained in Figure 2d emphasized that Ag doping of carbon quantum dots enhances the fluorescence intensity, which ensures the improvement in its optical property after doping. The green synthesized Ag-CQDs were size-dependent when the fluorescence was studied under different excitation wavelengths. The green synthesized Ag-CQDs have green fluorescence intensity at ʎ equal 500 nm. Figure 2e is the UV-Vis absorption spectra of the extract alone, the extract converted to CDs, and Ag-CQDs. The absorption spectra of Ag-CQDs exhibited a distinct absorption peak at 390 nm, 325 nm, and 233 nm. When CDs were analyzed by UV-Vis, the first peak did not appear; this could be an indication of the presence of silver in the structure of the nanoparticles after doping.

The Fourier Transform Infrared (FT-IR) spectrum of green synthesized Ag-CQDs was measured to verify the functional groups of Ag-CQDs Figure 2f. The peaks at 3550 to 3159 cm^{1} are identified as a stretching vibration of the O-H and N-H bands. The peak at 1691 cm^{-1} can be explained as the C=O bond. The C=C bending vibration peak appears at 1428 nm. The peak at 1280 cm^{-1} is related to the band of the C-N, and 988 cm-1 is in accordance with the vibrations of C-OH bonds. The result implies the existence of abundant oxygen-containing functional groups on the surface of Ag-CDs, which may provide binding sites for attracting the carcinoma cell line in further sensing tests and support the Ag-CDs' great water-solubility.

The typical X-Ray Diffraction (XRD) patterns of both CDs alone and Ag-CDs are depicted in Figure 2g. Green synthesized CDs alone exhibited a broader peak centered at 2θ = 27°, which indicates that the structures of CDs are amorphous carbon structures. The XRD pattern of green synthesized Ag-CDs has the same broad peak with a little shift to a greater 2theta nearly 30°, also appearing an extra peak at 2θ equal to 43° and 65 °. This supports that Ag interred into a crystalline structure and makes the doping process successful. The Energy Dispersive Spectroscopy (EDS) analysis for the elemental composition of Ag-CDs is represented in Figure 2h. The result indicates that green Ag-CDs mostly contain four main elements, which include C, O, N, and Ag. These results are consistent with those obtained from the other characterization techniques and further confirm the successful green synthesis of silver-doped carbon dots. The relatively high oxygen content supports the FTIR findings, indicating the presence of abundant oxygen-containing functional groups on the nanoparticles' surface [38].

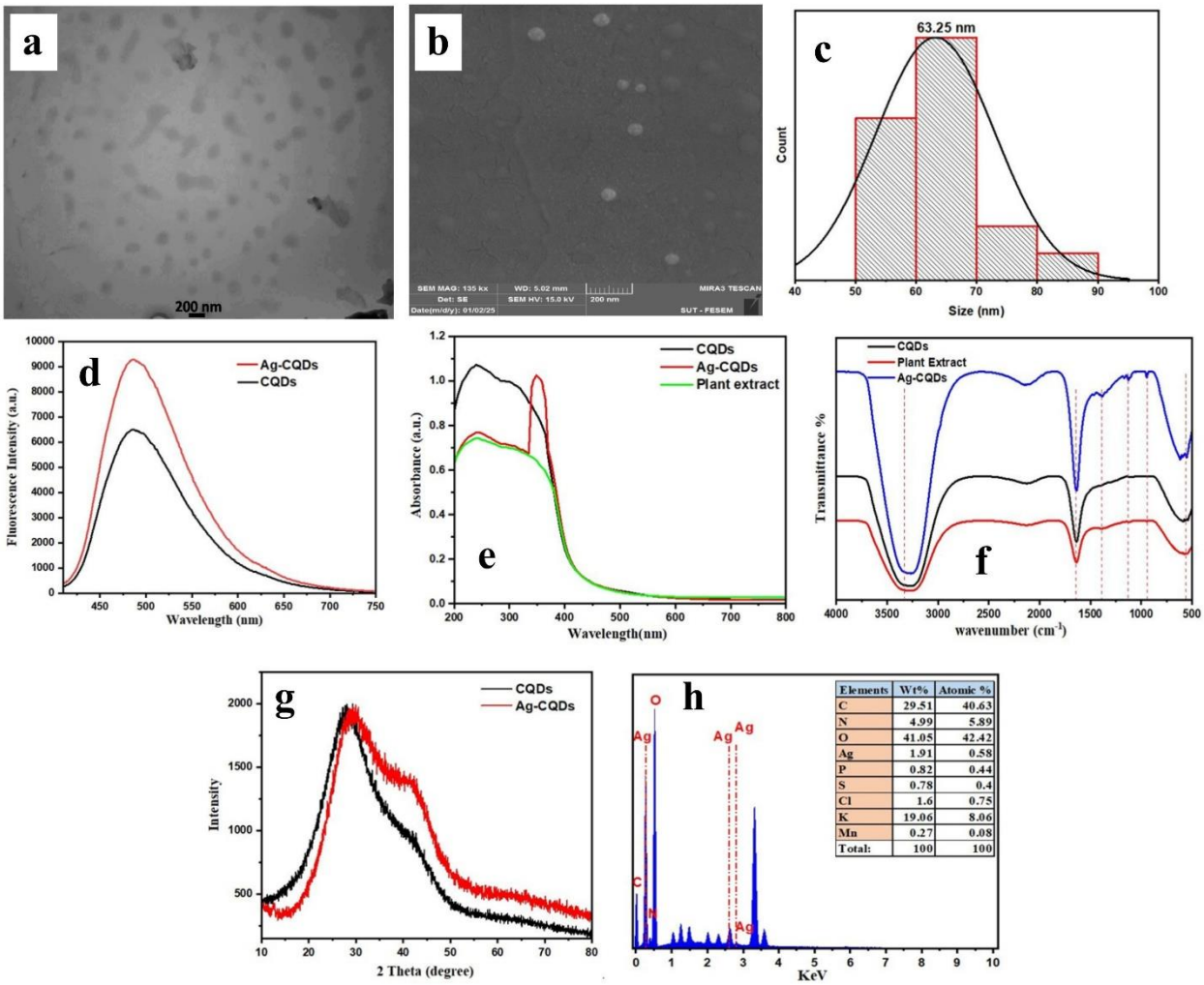

Figure 2: Characterization of the synthesized NPs by different techniques, including TEM and FE-SEM image (a and b), Size distribution of Ag-CDs (c), Fluorescence intensity study for both CDs

and Ag-CDs (d), UV-Vis spectra for Ag-CDs (e), FT-IR study of functional groups (f), XRD pattern for both CDs and Ag-CDs (g), and EDS analysis for elemental composition of Ag-CDs (h).

3.3 Anticancer activity

In this study, the assay was conducted on Oral Squamous Cancer Cells (OSCC) and Oral Squamous Normal Cells (OSNC) cell lines. The MTT assay is a well-established and reliable method for evaluating the cytotoxicity of various compounds by measuring cell metabolic activity. The cells were compared to determine the effects of the tested compounds on cancerous and non-cancerous oral epithelial cells. The OSCC cells are widely used as a model for oral cancer, while the OSNC cells serve as a non-tumorigenic control, allowing for an assessment of selective toxicity. The principle of the MTT assay relies on the ability of mitochondrial dehydrogenase enzymes in viable cells to reduce the yellow tetrazolium salt (MTT) into insoluble purple formazan crystals. After a specific incubation period, the formazan crystals were solubilized using dimethyl sulfoxide (DMSO) to facilitate optical density (OD) measurements at 570 nm using a microplate reader. The intensity of the absorbance is directly proportional to the number of metabolically active cells, providing a quantitative measure of cell viability. In this study, a dose-dependent reduction in OSCC cell viability was observed following treatment with increasing concentrations of the test compounds. This suggests a moderate to weak cytotoxic effect, potentially mediated through apoptosis or inhibition of cellular proliferation. Conversely, OSNC cells exhibited significantly higher resistance, with minimal reduction in viability across comparable concentrations, indicating that the compounds selectively target cancerous cells while sparing normal epithelial cells. This selectivity is crucial for the development of effective anticancer agents with reduced side effects. Table 2 summarizes the statistical ANOVA results of each cell line for the tested compounds.

Table 2: ANOVA summary for the tested compounds against OSCC and OSNC cell lines

Cell line	Assessed compound	F	p-value	R Square	IC_{50} µg/mL
Oral Squamous Cancer Cell (OSCC)	Clove extract	209.4	<0.0001	0.9892	346.8
	CQDs	735.1	<0.0001	0.9969	353.4
	Ag-CQDs	382.8	<0.0001	0.9941	286.7
Oral Squamous Normal Cell (OSNC)	Clove extract	546.5	<0.0001	0.9958	705.4
	CQDs	722.2	<0.0001	0.9968	1292
	Ag-CQDs	272.5	<0.0001	0.9917	939.3

3.4 Investigation of the clove extract, CDs, and Ag-CDs on OSCC cancer cell line

Figure 3 displays dose-dependent effects of the products on OSCC. The MTT assay results for clove extract on OSCC cells after 48 hours of treatment reveal a dose-dependent cytotoxic effect, as evidenced by the gradual decline in cell viability with increasing concentrations. At lower concentrations (10–50 µg/mL), cell viability remains relatively high (>80%), indicating minimal cytotoxic effects. However, beyond 100 µg/mL, a significant reduction in viability is observed, with substantial cytotoxicity at 500–1000 µg/mL. The IC_{50} value of 346.8 µg/mL suggests that the clove extract exhibits moderate cytotoxicity against OSCC cells, with potency comparable to synthesized carbon dots (IC_{50} = 353.4 µg/mL) but lower than silver-doped carbon dots (IC_{50} = 286.7 µg/mL). This implies that while crude extract effectively reduces cancer cell viability, it is slightly less potent than silver-doped CDs. The variations in cytotoxicity could be attributed to differences in structural composition, cellular uptake, or specific molecular interactions.

The cell viability gradually decreases with increasing concentrations of carbon quantum dots, indicating its potential anticancer activity. At lower concentrations (10–50 µg/mL), cell viability

remains above 80%, suggesting minimal cytotoxicity. However, a significant reduction in viability is observed at higher doses (100–1000 µg/mL), with the lowest viability recorded at 1000 µg/mL. The IC_{50} value of 353.4 µg/mL represents the concentration at which 50% of the OSCC cells lose viability, serving as a key parameter for evaluating cytotoxic potency. This suggests that CDs exhibit moderate cytotoxicity against OSCC cells.

The MTT assay results for silver-doped carbon dots on OSCC cells after 48 hours of exposure indicate a dose-dependent cytotoxic effect, with cell viability decreasing as the concentration of Ag-CDs increases. At lower doses (10–50 µg/mL), cell viability remains above 80%, suggesting limited cytotoxicity. However, as the concentration increases beyond 100 µg/mL, a marked decline in viability is observed, with higher cell death occurring at 500–1000 µg/mL. The calculated IC_{50} value of 286.7 µg/mL indicates that Ag-CDs exhibit moderate cytotoxicity against OSCC cells compared to CQDs (IC_{50} = 353.4 µg/mL). This suggests that silver-doped carbon dots are more effective in reducing cancer cell viability. The differences in cytotoxicity may be attributed to variations in chemical composition, uptake efficiency, or interaction with cellular components. [39-41]

Further mechanistic studies, such as apoptosis assays, oxidative stress evaluation, and gene expression analysis, are necessary to elucidate the mode of action of *S. aromaticum* extract, synthesized CDs, and Ag-CDs. Additionally, assessing their effects on non-cancerous OSNC cells would provide critical insights into their selectivity and potential therapeutic application in Oral cancer treatment.

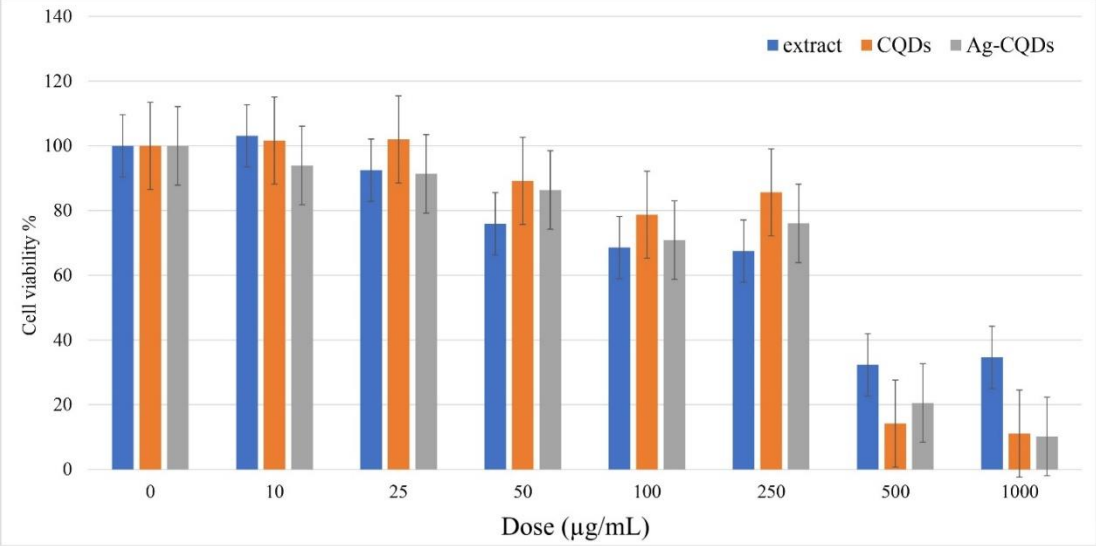

Figure 3: Dose-dependent cell viability % of the samples (clove extract, CQDs, and Ag-CQDs) against OSCC cell lines. The effect is directly proportional; increasing the concentration of the sample increases activity, and more cells are diminished. Cell viability % was normalized to untreated control cells (100%); values more than 100% in indicating improved metabolic activity relevant to the control. All the data were performed in triplicate. The data represent mean ± SEM of three independent experiments (n=3).

3.5 Investigation of the clove extract, CDs, and Ag-CDs on the OSNC normal cell line

Figure 4 illustrates the cytotoxic effects of clove extract on OSNC normal epithelial cells after 48 hours of exposure, as evaluated using the MTT assay. The y-axis represents the percentage of viable cells relative to the untreated control, while the x-axis denotes the concentration of the products (µg/mL). When plant extract is utilized, the results exhibit a biphasic response, where cell viability increases at lower concentrations (10–50 µg/mL), peaking at 50 µg/mL, suggesting a possible stimulatory effect on metabolic activity. However, at higher concentrations (100–1000 µg/mL), a dose-dependent decline

in cell viability is observed, with significant cytotoxic effects at 250 µg/mL and beyond. The IC_{50} value of 705.4 µg/mL indicates that a 50% reduction in viability occurs at a relatively high concentration, suggesting moderate cytotoxicity against normal epithelial cells.

Interestingly, carbon dots at lower concentrations (10–250 µg/mL) showed an unexpected increase in cell viability, which may be attributed to a hormetic response, experimental variability, or potential metabolic stimulation at sub-toxic doses. However, a significant decline in cell viability is evident at higher concentrations (500 and 1000 µg/mL), indicating a dose-dependent cytotoxic effect. The reported IC_{50} value of 1292 µg/mL suggests that a 50% reduction in viability occurs at a concentration exceeding the highest tested dose (1000 µg/mL), implying that the cytotoxic potential of synthesized carbon dots against OSNC cells is relatively low. This high IC_{50} value indicates a degree of biocompatibility with normal epithelial cells, which is a favorable characteristic for potential biomedical applications.

The cytotoxic effects of manufactured silver-doped carbon dots on OSNC normal Oral epithelial cells following 48 hours of exposure, as determined by the MTT assay, are also presented in Figure 4. The results reveal a biphasic response, where cell viability initially decreases at lower concentrations (10–50 µg/mL), followed by an unexpected increase at 100 µg/mL, which may be attributed to a hormetic effect or metabolic adaptation. However, at higher concentrations (250–1000 µg/mL), a marked dose-dependent reduction in cell viability is observed, demonstrating the cytotoxic potential of Ag-CDs at elevated doses. The IC_{50} value of 939.3 µg/mL indicates that a 50% reduction in viability occurs within the tested concentration range, suggesting moderate cytotoxicity against normal epithelial cells. [41]

These findings suggest that the tested compounds hold promise for further investigation as potential therapeutic agents against Oral cancer. Future studies should explore the underlying mechanisms of cytotoxicity, such as oxidative stress induction, apoptosis activation, or disruption of cellular signaling pathways, to gain deeper insights into their mode of action.

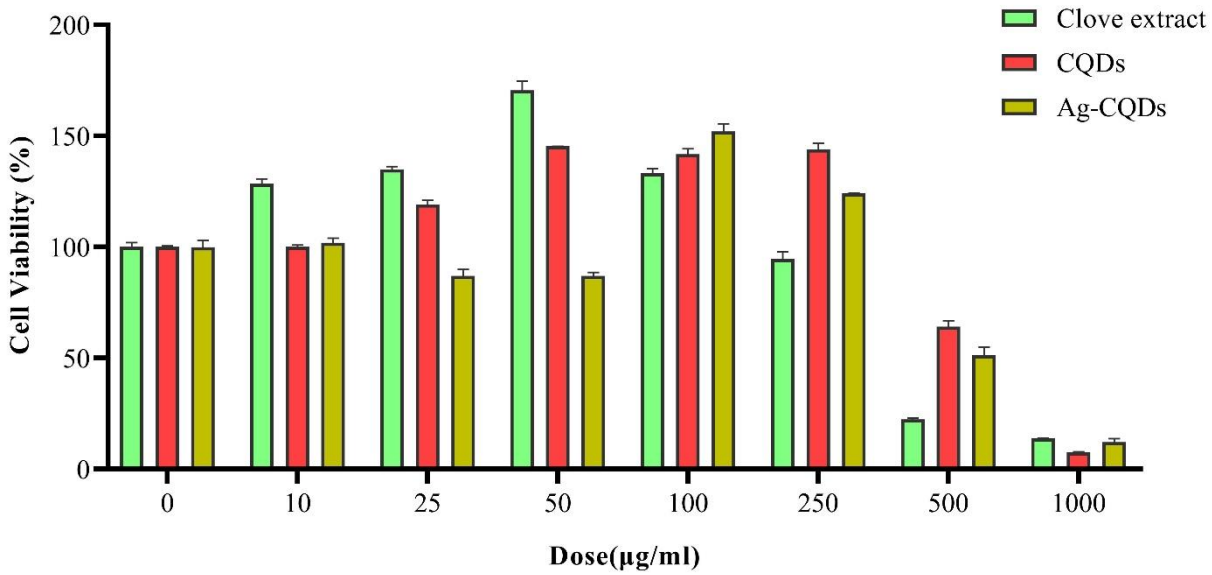

Figure 4: Dose-dependent cell viability % of the samples (clove extract, CQDs, and Ag-CQDs) against OSNC cell lines. The effect is directly proportional; increasing the concentration of the sample increases activity, and more cells are diminished. All the data were performed in triplicate. Cell viability % was normalized to untreated control cells (100%); values more than 100% in indicating improved metabolic activity relevant to the control. The data represent mean ± SEM of three independent experiments (n=3).

4. Conclusion

In this study, silver-doped carbon quantum dots (Ag-CQDs) were synthesized from clove, a traditional medicinal plant. The process of extraction and synthesizing the NPs is simple and efficient within a short period of time. Comprehensive physicochemical characterization confirms the successful synthesis and structural conformation of the nanoparticles. HRTEM and FE-SEM analyses indicated a well-dispersed and spherical shape of the nanoparticles with a size of approximately 63 nm. Optical studies verified enhanced fluorescence intensity after the doping of silver with emission behavior near 500 nm. FTIR spectroscopical analysis demonstrated the presence of abundant nitrogen and oxygen-containing functional groups, while UV-vis spectroscopy verified the distinct presence of band absorption with silver integration. XRD pattern revealed the amorphous structure of the CDs with confirmation of successful doping of the silver through peak shifting and appearance of crystalline silver reflection. EDS further validated elemental analysis (C, N, O, and Ag), supporting the successful incorporation of silver into the carbon quantum dots.

The crude extract, CQDs, and Ag-CQDs were evaluated for their efficacy as anticancer agents against OSCC and OSNC. The results showed moderate to high *in vitro* cell viability of OSCC, whereas less viability of OSNC by the targeted compounds, which means the products are biocompatible since they can kill cancer cells and has low effect on normal cells. Although the products revealed positive activity, further in vivo investigations are required to emphasize the importance and applicability of the novel nanoparticles. Therefore, the products are recommended to be investigated in further experiments, such as cytotoxicity in animal models, immunohistochemistry, and pathological evaluations.

Author's Contribution: "We confirm that all named authors have read and approved the manuscript. We also confirm that each author has the same contribution to the paper. We further confirm that all authors have approved the order of authors listed in the manuscript."

Conflict of Interest: "There is no conflict of interest for this paper."

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Use of AI tool Declaration

The authors declare that no AI tools were used in any part of the preparation of this manuscript.

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