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Synthesis of Carbon Dots (C-Dots) From Active Extracts of Mangrove Fruit (*Sonneratia alba*) and Their Effectiveness as a Cytotoxic Agent Against Lung Cancer Cells

Cynthia Aulia Novariyanti[1], Fahri Ahmad Median Wicaksono[1], Aushofin Hamidah[1*]

[1] MAN 1 JEPARA, Bawu, Batealit, Jepara 59461, Indonesia

*Corresponding author email: aushofin@gmail.com

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Abstract. Cancer is still a chronic disease in Indonesia, based on Globocan the number of new cancer cases reached 396,914 total cancers in Indonesia, with lung cancer reaching 30,843 people (13.2%). One of the potential materials to be developed as an anticancer agent is the C-Dots compound from mangrove fruit extract, this study aims to extract, synthesize, and assess the effectiveness of C-Dots in inhibiting A-549 cancer cells, the stages in this study are the extraction of active substances from mangrove fruit, synthesis of C-Dots with the agitation method, there are three variations of C-Dots samples, including C-Dots A (50 grams of powder + 50 mL of extract), C-Dots B (50 grams of powder + 50 mL of distilled water), and C-Dots C (25 grams of powder + 50 mL of extract), the three samples were characterized through fluorescence tests, UV-Vis, FTIR, PSA, and A-549 cytotoxic tests with MTT-Assay, the characterization results showed the formation of a green color under UV light, in the UV-Vis test the three samples showed two absorbance peaks of 280 nm and 380 nm, the results of the FTIR test of C-Dots A and C have the same functional groups, namely O-H, C-H, C=C, and C-O, while C-Dots B has a typical functional group C=O, PSA test shows the most dominant particle size of 75 nm with a percentage of C-Dots A 22.01%, B 15.2%, and C 16.93%. Based on the characterization results, it shows that C-Dots have been formed from mangrove fruit extract, then the results of MTT Assay, C-Dots A, B, and C produce IC_{50} values of 335.80 µg/mL, 365.70 µg/mL, and 154.50 µg/mL, respectively, then C-Dots C with an IC_{50} value of 154.50 µg/mL is the most effective variation because the IC50 value is the smallest and is included in the toxic category.

Keywords: A-549 Cells, C-Dots, IC_{50}, Mangrove Extract, MTT-Assay.

Abstrak. Penyakit kanker masih menjadi penyakit kronis di Indonesia, berdasarkan *Globocan* jumlah kasus baru kanker mencapai 396.914 total kanker di Indonesia, dengan kanker paru-paru mencapai 30.843 orang (13,2%). Salah satu bahan berpotensi untuk dikembangkan sebagai zat antikanker adalah senyawa C-Dots dari ekstrak buah mangrove, penelitian ini bertujuan untuk mengekstraksi, mensintesis, dan mengkaji efektivitas C-Dots dalam inhibisi sel kanker A-549, tahapan dalam penelitian ini adalah ekstraksi zat aktif buah mangrove, sintesis C-Dots dengan metode *agitasi,* terdapat tiga variasi sampel C-Dots, antara lain C-Dots A (50 gram serbuk + 50mL ekstrak), C-Dots B (50 gram serbuk + 50 mL aquades), dan C-Dots C (25 gram serbuk + 50 mL ekstrak), ketiga sampel dikarakterisasi melalui uji fluoresensi, UV-Vis, FTIR, PSA, dan uji sitotoksik A-549 dengan MTT-*Assay* , hasil karakterisasi menunjukkan terbentuknya warna hijau di bawah sinar UV, pada uji UV-Vis ketiga sampel menunjukkan adanya dua puncak absorbansi 280 nm dan 380 nm, hasil uji FTIR C-Dots A dan C memiliki gugus fungsi yang sama, yakni O-H, C-H, C=C, dan C-O, sementara C-Dots B terdapat gugus fungsi khas C=O, uji PSA menunjukkan adanya ukuran partikel paling dominan 75 nm dengan persentase pada C-Dots A 22,01%, B 15,2%, dan C 16,93%. Berdasarkan hasil karakterisasi menunjukkan telah terbentuknya C-Dots dari ekstrak buah mangrove, selanjutnya hasil MTT *Assay*, C-Dots A, B, dan C menghasilkan nilai IC_{50} berturut-turut 335.80 µg/mL, 365.70 µg/mL, dan 154,50 µg/mL, maka C-Dots C dengan nilai IC_{50} 154,50 µg/mL merupakan variasi paling efektif karena nilai IC_{50} paling kecil dan termasuk dalam kategori toksik.

Kata Kunci: C-Dots, Ekstrak Mangrove, IC_{50}, Sel A-549, MTT-Assay

1. Introduction

Cancer is one of the diseases that causes high morbidity and mortality rates in Indonesia and worldwide. The Global Burden of Cancer (GLOBOCAN) reported that there were 396,914 cancer cases and 234,511 cancer-related deaths in Indonesia. One type of cancer that urgently requires further research is lung cancer. According to data from the World Health Organization (2020), lung cancer ranks third in prevalence (8.8%) and is the leading cause of cancer-related deaths (13.2%) in Indonesia. The primary factor contributing to lung cancer is smoking, as cigarettes contain harmful chemical substances such as tar, which is carcinogenic. Repeated exposure to these carcinogenic substances may lead to abnormal cell growth in the lung epithelium and affect protein synthesis, thereby disrupting the cell cycle and increasing carcinogenesis within the body (Siddiqui et al., 2022).

Indonesia, as a megadiverse country, offers enormous potential and opportunities for researchers in the discovery of new natural product-based drugs. One natural resource that is interesting and has strong research potential from the waters of Jepara is mangrove fruit (*Sonneratia alba*). Until now, mangrove fruit has not been optimally utilized by local communities, despite its chemical composition containing secondary metabolites such as alkaloids, steroids, terpenoids, and flavonoids. Based on the study conducted by Haryoto and Putri (2019), mangrove plant extract demonstrated cytotoxic effectiveness against myeloma cancer cells, with an IC_{50} value of 508.19 µg/mL. Research by Bruguiera and Giyanirfani (2020) also reported that mangrove fruit contains squalene compounds, which showed anticancer activity against MCF-7 cancer cells, with IC_{50} values of 644.008 and 595.164 µg/mL, respectively. Therefore, mangrove fruit has the potential to be further developed as an active compound for inhibiting cancer cells, including lung cancer cells (A-549), which has not yet been explored in previous studies. To improve the effectiveness of substances in inhibiting cell growth, an innovation is needed to increase the surface area of substances by reducing particle size. One approach that can be applied through recent scientific developments is nanotechnology.

Carbon Nanodots (C-Dots) are carbon-based nanomaterials with sizes below 10 nm and unique fluorescence properties. To date, the development of carbon-based fluorescent materials continues to advance because C-Dots possess significant potential for broad applications, such as sensors, chemosensors, and bioimaging (Li et al., 2012; Liu et al., 2016). One of the requirements for a material to be used as a source for C-Dots synthesis is the presence of high carbon (C) content, such as mangrove fruit, which is known to contain chemical compounds with high carbon composition, including flavonoids, terpenoids, and alkaloids. Furthermore, in the field of microbiology, particularly in anticancer applications, C-Dots are used as active agents with high biocompatibility because of their extremely small particle size, allowing more optimal cell inhibition. In addition, C-Dots can also function as carriers for chemotherapy drugs, thereby enhancing drug performance and minimizing side effects.

Based on the explanation above, we propose an innovation and objective to synthesize C-Dots derived from mangrove fruit to inhibit the growth of lung cancer cells (A-549 cells) through in-vitro testing. This innovation also represents our contribution as the younger generation in advancing science and technology, particularly in the field of healthcare in Indonesia.

2. Method and Experimental

2.1 Research Time and Location

This research was conducted from May to September 2025 at the Integrated Science Laboratory of MAN 1 Jepara. Meanwhile, the characterization testing of C-Dots was carried out at the Integrated Laboratory of Diponegoro University, and the cytotoxicity testing was conducted at the Integrated Laboratory of Padjadjaran University. The stages of this research included: extraction of active compounds from mangrove fruit, synthesis of mangrove fruit-based carbon dots (C-Dots), characterization of C-Dots derived from mangrove fruit extract, and in-vitro cytotoxicity testing on lung cancer cells (A-549).

2.2 Research Instruments and Materials

The instruments used in this research included: 250 mL beakers, 250 mL Erlenmeyer flasks, a blender, an oven, Whatman filter paper, a magnetic stirrer, a glass stirring rod, an analytical balance, 100 mL vial bottles, a centrifuge, universal pH indicator paper, and a UV light source. Meanwhile, the materials used in this study were mangrove fruit (*Sonneratia alba*) obtained from Jepara, distilled water (aquades), and A-549 cells.

2.3 Reseacrh Design and Producers

A. Extaction of Active Compounds from Mangrove Fruit

The extraction of active compounds from mangrove fruit was carried out using the decoction method. Initially, 125 grams of mangrove fruit were prepared by washing and drying them, followed by grinding with a blender until a fine powder (simplicia) was obtained. The powder was then extracted at a temperature of 50°C using distilled water for 1 hour. Subsequently, the mixture was filtered to separate the solid residue from the active extract. The remaining solid residue was then dried at 105°C for 2 hours until active powder was obtained.

B. Synthesis, Characterization, and Cytotoxicity Testing of C-Dots Against A-549 Cells

The fluorescence testing of synthesized C-Dots was carried out using three variations, namely: sample A variation (50 grams of active powder + 50 mL of active extract), sample B (50 grams of active powder + 50 mL of distilled water), and sample C (25 grams of active powder + 50 mL of active extract). Each sample was stirred for 24 hours until homogeneous, then filtered and centrifuged at 4000 rpm for 5 minutes to separate the filtrate from the precipitate. Furthermore, all samples (C-Dots A, C-Dots B, and C-Dots C) were characterized using fluorescence, UV-Visible, FTIR, and PSA analyses.

2.4 Data Processing and Analysis

The fluorescence test is a qualitative analysis used to determine the emission of UV light through the absorption of light and its re-emission at a longer wavelength, which can be observed through the emitted fluorescence color.

A. Wavelength Analysis Using UV-Visible Spectroscopy

The UV-Vis test was conducted to determine the absorbance peak. The synthesized samples were dissolved in distilled water to facilitate the readability of absorbance and wavelength measurements in the samples. The data obtained from the UV-Vis characterization were presented in the form of UV-Vis absorbance graphs.

B. Functional Group Analysis Using FTIR

Functional group analysis is an important parameter used to identify the active functional groups within the chemical structures formed from each variation of C-Dots. Therefore, the results obtained from FTIR characterization consisted of wavenumber and transmittance data. The resulting spectral peaks indicated the presence of functional groups in the synthesized C-Dots.

C. Particle Size Analysis Using PSA

The particle size analysis of C-Dots was conducted using specific volume ranges. The results of the PSA test provided particle size data in nanometer (nm) or micrometer (μm) units, along with particle distribution data indicating the size distribution of the particles. Particle size analysis was carried out using a Particle Size Analyzer (PSA), with the objective of determining the particle size within the synthesized C-Dots samples.

Meanwhile, the effectiveness of the cytotoxic (anticancer) activity was determined using the MTT Assay test, with the test results expressed as the IC_{50} value (Inhibitory Concentration 50%) calculated using a logarithmic equation. The effectiveness test data were analyzed using probit regression analysis to determine the Inhibitory Concentration (IC_{50}) value.

3. Result and Disscussion

3.1 Mangrove Fruit Extraction

Extraction is a process of separating substances from their mixtures through chemical processes. The extraction of mangrove fruit was carried out using the decoction method, which is a hot extraction method applied to simplicia using water as the solvent (1:10, w/v) with heating at a temperature of 90°C to obtain the active extract from mangrove fruit. The decoction method was selected because mangrove fruit is considered a hard fruit tissue of mangrove plants. In addition, the decoction method utilizes water as a solvent, which is polar, non-toxic, and supportive of green chemistry principles in chemical processes.

(i) (ii)

Fig 1. Results of Mangrove Fruit Extraction Using the Decoction Method (i) Active Powder, (ii) Active Extract

3.2 Synthesis C-Dots From Mangrove Fruit Extract

The synthesis of carbon dots (C-Dots) from mangrove fruit was carried out using the agitation synthesis method. In this study, three variations were prepared to determine the most optimal variation for C-Dots formation, namely: variation A (50 grams of active powder and 50 mL of active extract), variation B (50 grams of active powder + 50 mL of distilled water), and variation C (25 grams of active powder + 50 mL of active extract). The selection of these compositions was based on the study conducted by Emi Kurnia Sari et al. (2020), which successfully synthesized C-Dots from betel leaves. Each variation was synthesized using the agitation method by mechanically stirring the solution to ensure uniform energy distribution throughout all particles in the solution. This process allowed the molecules to break down into more uniform particle sizes, resulting in relatively homogeneous nanoparticles. Furthermore, each synthesized sample was centrifuged at 4000 rpm for 5 minutes to optimally separate the liquid phase from the precipitate. The synthesized results of mangrove fruit extract-based C-Dots A, B, and C are presented in Figure 2.

Fig 2. Results of the Synthesis of C-Dots A, C-Dots B, and C-Dots C Using the Agitation Method

3.3 Characterization of Carbon-Dots

The fluorescence test is a qualitative analysis used to indicate the successful formation of C-Dots by observing the fluorescence color produced under UV light. In this study, the synthesized C-Dots A, C-Dots B, and C-Dots C were each dissolved in distilled water to dilute the concentrated C-Dots solution, thereby preventing interference with fluorescence emission. The fluorescence test results of C-Dots A, C-Dots B, and C-Dots C can be observed in Figure 3.

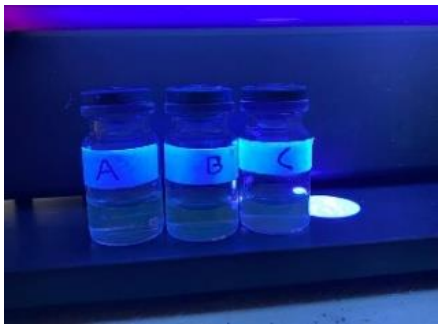


Fig 3. Fluorescence Test Results of C-Dots A, C-Dots B, and C-Dots C

Based on Figure 3, green fluorescence emission was observed in C-Dots A, C-Dots B, and C-Dots C. This result is consistent with the study conducted by Sahu et al. (2012), which reported that C-Dots emit green fluorescence when exposed to UV light. This observation indicates that the particles have successfully formed at the nanometer (nm) scale.

UV-Visible testing was conducted to determine the wavelengths formed in the C-Dots samples. In this study, all samples exhibited two absorbance peaks. The higher the absorbance value, the greater the amount of C-Dots formed. The results of the UV-Visible characterization of C-Dots A, B, and C are presented in Figure 4.

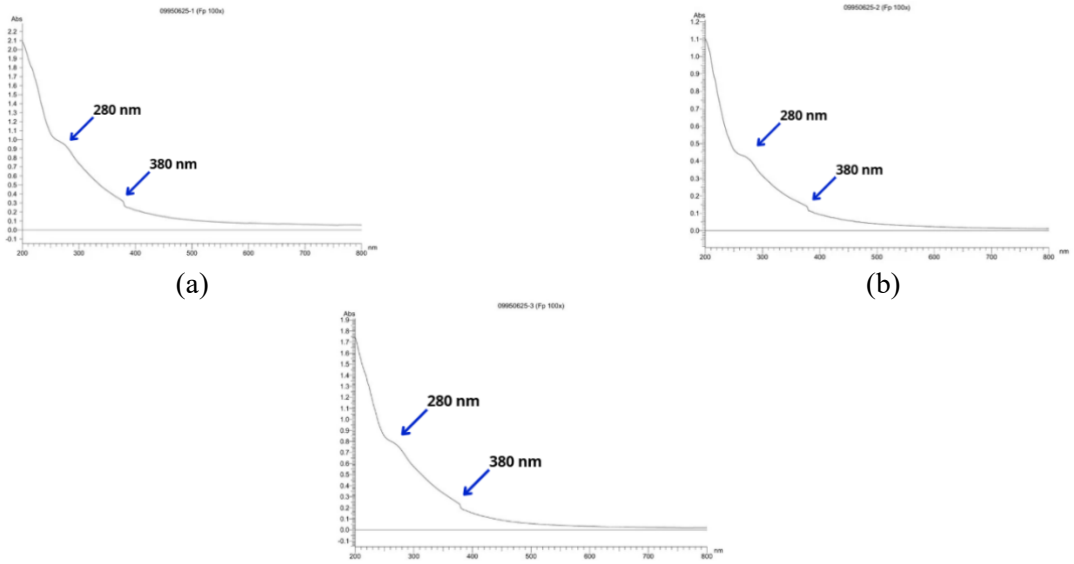


Fig 4. UV-Visible Test Results of (a) C-Dots A, (b) C-Dots B, and (c) C-Dots C

Based on Figure 4, C-Dots A, C-Dots B, and C-Dots C each exhibited two wavelength peaks at 280 nm and 380 nm. This result is consistent with the study conducted by Gultom Erdiana (2024), which successfully synthesized C-Dots from watermelon peel and also reported two absorbance peaks at 294.00 nm and 407.50 nm. The study further explained that C-Dots chemical compounds exhibit strong absorbance in the UV region within the wavelength range of 200–400 nm. Therefore, these findings indicate the successful synthesis of C-Dots A, C-Dots B, and C-Dots C derived from mangrove fruit extract.

FTIR testing is an analysis used to identify the characteristic functional groups that compose chemical compounds, including the characteristic functional groups of C-Dots. Each sample was subjected to FTIR analysis to obtain wavenumber and transmittance data. The FTIR test results of C-Dots A, C-Dots B, and C-Dots C are presented in Table 1, Table 2, and Table 3, respectively, as follows.

Table 1. FTIR Test Results of C-Dots A

C-Dots A	
Wavenumber	**Functional Group**
3200-3400	O-H
2800-2900	C-H
1500-1600	C=C
1000-1200	C-O

Table 2. FTIR Test Results of C-Dots B

C-Dots B	
Wavenumber	**Functional Group**
3200-3400	O-H
2800-2900	C-H
1700-1800	C=O
1500-1600	C=C
1050-1200	C-O

Table 3. FTIR Test Results of C-Dots C

C-Dots C	
Wavenumber	**Functional Group**
3200-3400	O-H
2800-2900	C-H
1500-1600	C=C
1000-1200	C-O

Based on the identification of functional groups using FTIR, it was found that each sample—C-Dots A, C-Dots B, and C-Dots C—contained O–H functional groups (3200–3400 cm^{-1}), C–H bonds (2800–2900 cm^{-1}), C=C bonds (1500–1600 cm^{-1}), and C–O bonds (1000–1200 cm^{-1}). This study is consistent with the findings of Sari et al. (2020), who successfully synthesized C-Dots with functional groups identified as O–H (3438–3441 cm^{-1}), C–O (2063–2083 cm^{-1}), and C=C (1633–1635 cm^{-1}). Based on Table 5 and Table 6, C-Dots A and C-Dots C exhibited similar functional groups, namely active O–H functional groups, C–H bonds, C=C bonds, and C–O bonds. These functional groups are characteristic of flavonoid structures belonging to the flavanol group. Meanwhile, C-Dots B showed the presence of an active C=O functional group, indicating that C-Dots B belongs to the flavonoid compound group classified as flavonols.

PSA (Particle Size Analyzer) is a primary parameter used to determine the success of nanoparticle (C-Dots) synthesis, with the results providing information on the size and distribution of the formed nanoparticles. The PSA test results of C-Dots A, C-Dots B, and C-Dots C are presented in Figure 5.

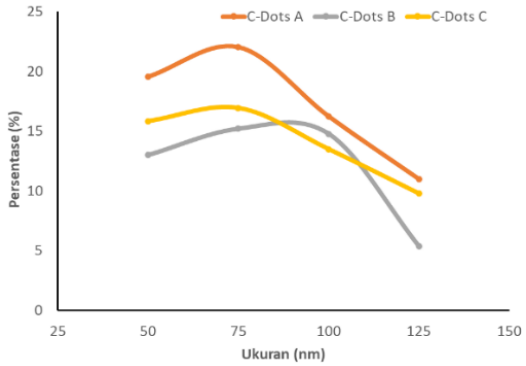


Fig 5. PSA Test Results of C-Dots A, C-Dots B, and C-Dots C

Based on Figure 5, C-Dots A exhibited a better particle size distribution compared to C-Dots B and C-Dots C. This can be observed from the fact that C-Dots A showed the highest percentage distribution at a particle size of 75 nm, reaching 22.01%, followed by C-Dots C at 16.93%, and C-Dots B at 15.2%. Based on the identification results, all three samples demonstrated homogeneous properties because they shared the same dominant particle size, namely 75 nm. According to the study conducted by Jaynis et al. (2024), the particle size range of C-Dots derived from natural extracts generally falls between 1–200 nm. Therefore, the C-Dots synthesized in this study are consistent with those findings, as they exhibited a particle size of 75 nm.

3.4 Cytotoxicity Test on Lung Cancer Cells

The cytotoxicity test was conducted as a preliminary assay to determine the effectiveness of a chemical compound in inhibiting cancer cells. One of the methods used to assess cytotoxic compounds is the in-vitro MTT (Microculture Tetrazolium Technique) Assay. The principle of this test is based on measuring mitochondrial dehydrogenase activity in living cells, which have the ability to convert MTT into formazan (Sirait et al., 2019). This method was chosen because it is fast, requires only a small amount of test compounds, does not require experimental animals, and can directly provide information on its potential effects on target cells. The MTT assay mechanism involves determining the absorbance value of the formed formazan, which is measured using an ELISA reader at wavelengths of 600 nm and 570 nm. The tetrazolium salt in this assay is used to measure mitochondrial metabolic activity and indirectly indicates the number of viable cells. MTT tetrazolium salt is reduced into insoluble purple formazan crystals within metabolically active cells by mitochondrial dehydrogenase enzymes. The total amount of formazan produced is directly proportional to the number of living cells in the culture (Rai et al., 2018). The mechanism of the MTT assay reaction is illustrated in Figure 6.

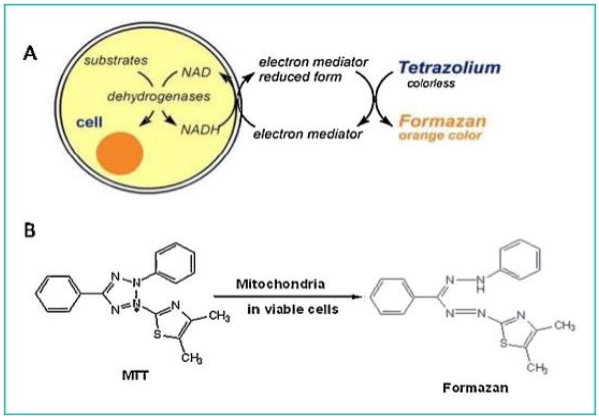
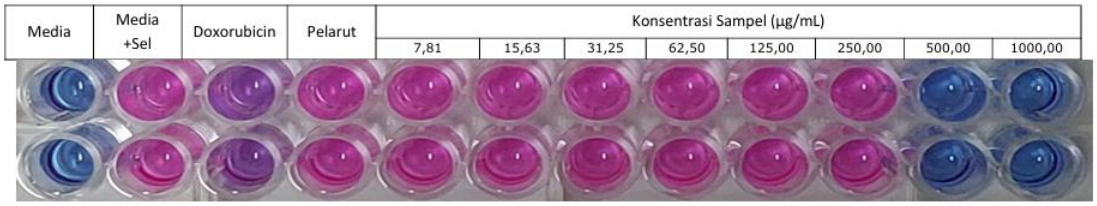
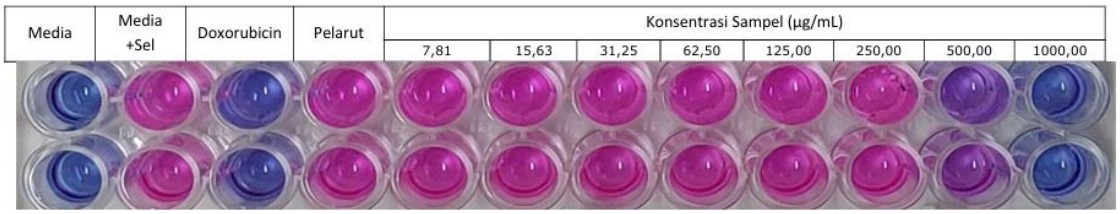


Fig 6. MTT Assay Reaction Mechanism

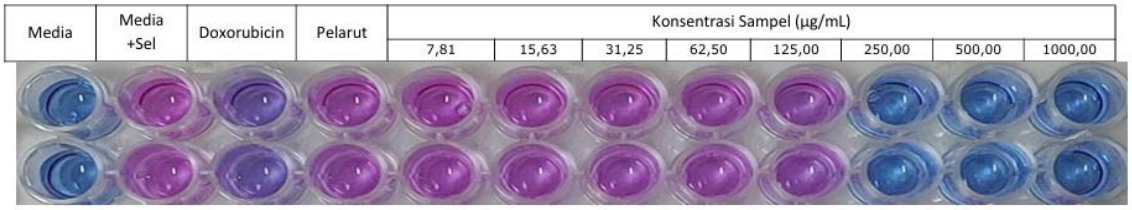
The results of the staining test of C-Dots A, C-Dots B, and C-Dots C for anticancer activity against lung cancer cells are presented in Figure 7.



(i)



(ii)



(iii)

Fig 7. Staining Test Results of Mangrove Fruit Extract for Anticancer Activity Against Lung Cancer Cells

Based on Figure 7, an increase in sample concentration (µg/mL) resulted in a more intense coloration of the medium due to the formation of purple formazan crystals. The intensity of the purple color is proportional to the number of viable cells; thus, a stronger purple intensity indicates a higher number of living cells. From the observed color changes in C-Dots A, C-Dots B, and C-Dots C samples, it can be seen that C-Dots C showed the weakest purple intensity, followed by C-Dots A, while C-Dots B exhibited the strongest intensity. This indicates that C-Dots C had the lowest number of viable cells, suggesting that C-Dots C possesses the highest cytotoxic activity, meaning it has the strongest ability to inhibit lung cancer cell growth. The color changes in the 96-well plate were further quantified by measuring absorbance using an ELISA (Enzyme-Linked Immunosorbent Assay) reader at wavelengths of 600 nm for blue color and 570 nm for pink color. From these results, the IC50 values were determined, as presented in Figure 8 below.

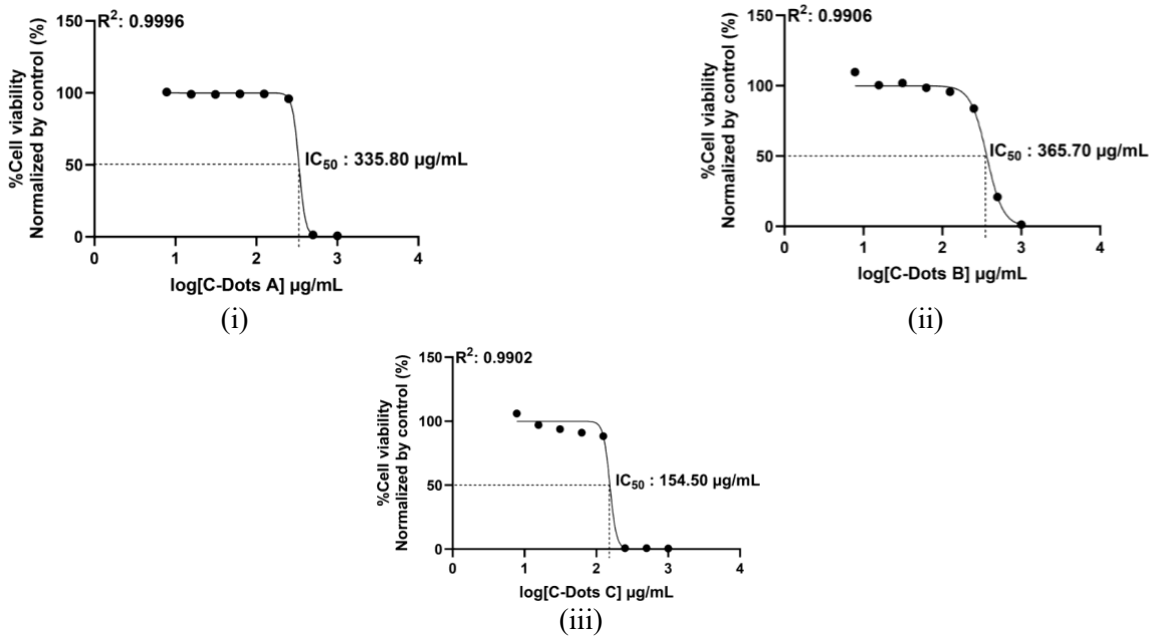


Fig 8. IC50 Results of (i) C-Dots A, (ii) C-Dots B, and (iii) C-Dots C Against A-549 Cells

Based on Figure 8, it is shown that the IC_{50} value of C-Dots C is 154.50 µg/mL. This value indicates that C-Dots C is the most effective variation because it has the lowest IC_{50} value and is classified as toxic. Meanwhile, C-Dots A and C-Dots B produced IC_{50} values of 335.80 µg/mL and 365.70 µg/mL, respectively. Both of these C-Dots are categorized as weakly toxic. The cytotoxicity standards for anticancer activity are presented in Table 4 below.

Table 4. $IC5_0$ Value Categories as Cytotoxic Agents

Category	IC_{50} Value
Very Toxic	<20 ppm
Toxic	21-200 ppm
Weakly Toxic	201-500 ppm
Non-Toxic	>500 ppm

*(Marwati, dkk., 2020)

C-Dots C exhibited a lower IC_{50} value compared to C-Dots A and C-Dots B. When analyzed between C-Dots C and C-Dots B, this difference can be explained through particle size distribution. C-Dots C showed a particle size distribution of 16.93% at 75 nm, while C-Dots B at the same particle size (75 nm) showed a distribution of 15.2%. This indicates that C-Dots C contains a higher proportion of nanoparticles compared to C-Dots B. A higher nanoparticle proportion enhances the effectiveness of cancer cell inhibition, as a greater number of smaller particles allows the compound to penetrate target cells more efficiently. Furthermore, when comparing C-Dots C and C-Dots A, the difference lies in their composition and particle distribution. C-Dots C consists of 50% extract with 25% powder, while C-Dots A contains 50% extract and 50% powder. The difference is in the amount of powder used, where C-Dots C has a lower powder composition, resulting in a more optimal particle distribution compared to C-Dots A. Therefore, C-Dots C is the most effective variation in inhibiting A-549 lung cancer cells, as it produces an IC_{50} value that falls within the toxic category (Marwati et al., 2020).

4. Conclusion

Based on the conducted research, the following conclusions can be drawn:

1. The extraction of active compounds from mangrove fruit (Sonneratia alba) was successfully carried out using the decoction method, resulting in an active extract (decoction) and active powder as starter materials for C-Dots synthesis.
2. C-Dots A, C-Dots B, and C-Dots C were successfully synthesized from mangrove fruit extract using the agitation method. The characterization results confirmed successful synthesis, indicated by green fluorescence under UV light. In the UV-Vis analysis, all three samples showed two absorbance peaks at 280 nm and 380 nm. FTIR analysis revealed that C-Dots A and C shared similar functional groups, namely O–H, C–H, C=C, and C–O, while C-Dots B contained a characteristic C=O functional group. PSA results showed a dominant particle size of 75 nm, with percentages of 22.01% for C-Dots A, 15.2% for C-Dots B, and 16.93% for C-Dots C.
3. C-Dots C produced an IC50 value of 154.50 µg/mL, which is classified as toxic in inhibiting A-549 cells through in-vitro testing (MTT assay). This result can be explained by the fact that C-Dots C has a dominant particle size of 75 nm with a relatively higher proportion compared to C-Dots B, as well as a more homogeneous particle distribution compared to C-Dots A.

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