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Article

Amino Acid Analysis and Cytotoxicity Study of Iraqi *Ocimum Basilicum* Plant

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Abstract

Context: Medicinal plants have long been a cornerstone of traditional medicine and modern pharmacology, offering a rich source of biologically active compounds with diverse therapeutic applications**. Objective:** Detection and identification of amino acid composition of Iraqi *Ocimum basilicum* (basil) leaves and evaluation the cytotoxic effects of the plant leaf extract on human colorectal cancer cells (HRT-18). **Methods:** Leaves *of Ocimum basilicum* were collected from Tikrit in November 2024. After drying and powdering, the plant material goes through cold methanol extraction. Initial phytochemical screening was conducted to identify the presence of alkaloids, flavonoids, coumarins, and terpenoids. Amino acids analysis was completed by amino acid analyzer with fluorescence detection. Cytotoxic effect was evaluated via the MTT assay on HRT-18 cell lines exposed to various concentrations of the plant extract. Morphological changes were further tested using dual Propidium Iodide/Acridine Orange assay fluorescent staining. **Results:** Seventeen amino acids were detected in the plant extract. The extract showed dose-dependent cytotoxic effects on HRT-18 cells, with significant reduction in cell viability at concentrations of more than 25 μg/mL. Morphological alterations of membrane blebbing and cell shrinkage were observed suggesting apoptotic activity. The IC_{50} value confirmed strong cytotoxic potential. **Conclusion:** The extract of *Ocimum basilicum* leaf cultivated in Iraq shows a rich amino acid profile and significant cytotoxic activity against colorectal cancer cells that highlights its potential effect as a natural source of anticancer compounds and permit further analysis into its active constituents and the mechanisms of action.

Keywords: amino acids; cytotoxicity; HRT-18 cell line; leaf extract

1. Introduction

Medicinal plants have long been a cornerstone of traditional medicine and modern pharmacology, offering a rich source of biologically active compounds with diverse therapeutic applications. Among these, *Ocimum basilicum* Linn., a member of the Lamiaceae family, is widely cultivated in Asia, the Middle East, and the Mediterranean for culinary, aromatic, and medicinal uses [1]. "Sweet basil" is the popular name of *Ocimum basilicum* that is used in both Ayurvedic and Unani medicine system [2]. There were more than 150 species of the genus Ocimum present. Basil is the main crop of essential oil that is cultivated commercially in many countries [3]. Its popularity is due to its rich and spicy, mildly peppery flavor with a trace amounts of clove and mint and has been widely used as a food ingredient for flavoring enhancement, meat products and baked foods [4].

The plant is used for its stomachic, antipyretic and alexipharmic effects. It also has emmenagogue and diuretic properties. An infusion of the plant extract is considered to be anthelminthic, antiemetic, diaphoretic and anti diarrheaic in Annam. Diuretic, anti-dysenteric and aphrodisiac effects have also been attributed to this plant seeds. The plant juice exhibits stimulant, carminative and antibacterial effects; its essential oil has antifungal, antibacterial and insecticidal

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properties [5]. The plant flowers have diuretic, demulcent and stimulant properties. These flowers are also considered to have anti-spasmodic, carminative and digestive stimulant effects [6].

High concentration of vitamins, minerals and oils are present in the green plant leaves [7]. The phytochemical screening reports of *O.basilicum* showed the presence of the following I it: proteins, amino acids, glycoside, mucilage, gums, tannins, triterpenoids steroids, phenolic compound, saponins, sterols, flavones and flavonoids. A total of 29 compounds representing 98.0–99.7% of the oils are identified in this plant which are contributed to its pharmacological potential [8,9]. However, limited studies have explored the amino acid composition of basil, particularly in local varieties grown in Iraq. Amino acids are crucial components of plant metabolism and human nutrition; they play central roles in protein synthesis, neurotransmission, metabolic regulation, and immune function [10]. Profiling amino acids in medicinal plants like *O. basilicum* can provide insights into their nutritional value and enhance their use in dietary supplements and therapeutic formulations [11].

In recent years, increasing attention has been paid to the anticancer properties of medicinal plants. Plant products are promising alternative or adjunct to conventional chemotherapy owing to their potential for high efficacy and lower toxicity [12]. One of the most common malignancies worldwide is the colorectal cancer, and the study of plant-derived compounds with selective cytotoxic effects is of critical importance [13]. The HRT-18 cell line that is derived from human colorectal adenocarcinoma can serve as a reliable in vitro model for screening cytotoxicity and evaluating apoptosis-inducing activity [14].

This study was carried out to consider two key features of the Iraqi *Ocimum basilicum* plant: (1) to determine its amino acid profile using a high-performance amino acid analyzer, and (2) to evaluate cytotoxic effects of the plant on HRT-18 colorectal cancer cells by using the MTT assay and Propidium Iodide/Acridine Orange assay fluorescent staining (AO/PI) fluorescent staining.

2. Material and Methods

2.1. Plant Material

Ocimum basilicum leaves were collected from Tikrit in November 2024. Leaves were washed thoroughly, dried under shade, and ground in a mechanical grinder to a fine powder.

2.2. Experimental Work

2.2.1. Extraction Method (Cold Method)

A Hundred grams of the powdered plant material was soaked in 1500ml methanol, with occasional shaking, at room temperature. After 3 days, the methanol-soluble materials were filtered off. The filtrate was evaporated to dryness under a vacuum using a rotary evaporator. A dark greenish residue was obtained. The residue evaporated to dryness and take to the laboratory for detection of amino acid.

2.2.2. Preliminary Phytochemical Examination of Crude Extracts

Phytochemical analysis for the screening and identification of bioactive chemical constituents in the medicinal plants under study was carried out on crude extracts, and fractions as well as powder specimens using the standard procedures as described [15,16].

Alkaloid test: approximately 0.5 to 0.6 g of each of plant extract and fractions were mixed in 8 ml of 1% HCl, warmed, and filtered. Two ml of the filtrate was treated separately with both reagents (Mayer's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.

Coumarins test: 0.5 g of each of plant extract and fractions were mixed in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. The test tube was placed

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for a few minutes in a boiling water, and then the filter paper was removed and examined under the UV light for yellow fluorescence indicating the presence of coumarins.

Terpenoids test (Salkowski test): 5 ml of each of plant extract and fractions were mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H_2SO_4). A layer of reddish-brown coloration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Flavonoids test: 0.5 g of each of plant extract and fractions were shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests:

(a) Three ml of the filtrate was mixed with 4 ml of 1% aluminum chloride in methanol in a test tube, and the color was observed. The formation of yellow color indicated the presence of flavonoids.

(b) Three ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube, and the color was observed. A dark yellow color indicated the presence of flavonoids.

2.3. Amino Acids Determination

One ml was taken from the extracted sample and 200 microliters of orthophthalein aldehyde (5%) was added to it and the sample was shaken for two minutes after which 100 microliters of the last mixture were taken and injected into the amino acid analyzer [17].

The test was conducted in the laboratories of the Scientific Research Authority / Environment and Water Research Center using the amino acid analyzer (Korean made). The method provided by the scientist (Scriver CR, 2001) was used, where the carrier phase consisting of (methanol: acetonitrile: 5% formic acid) was used in proportions (20: 60: 20) at a flow rate of (1 ml / minute). A separation column (C18–NH2 (250mm * 4.6 mm) was used to separate the amino acids, while a fluorescence detector was used to detect the amino acids at wavelengths (Ex = 445 nm, Em = 465 nm). The (clarity 2015) program was used to analyze the amino acids [18].

2.4. Cytotoxicity Assay

2.4.1. Cell Lines Used

HCT-8 [HRT-18] cells were isolated from the large intestine of a 67-year-old, male, adenocarcinoma patient. HCT-8 [HRT-18] is used for cancer and toxicology research.

2.4.2. MTT Cytotoxicity Assay

Cell Culture Conditions

The cell lines were cultured in cultured in MEM (US Biological, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Capricorn-Scientific, Germany), and 100 IU penicillin, and 100µg streptomycin (Capricorn-Scientific, Germany) and incubated in a humidified atmosphere at 37 °C. Exponentially growing cells were used for experiments [19].

MTT Procedure

Cells were seeded at a density of 10000 cells in a 96-well microplate (NEST Biotech, China) and incubated at 37 °C for 72 h until monolayer confluence was achieved. Cytotoxicity was investigated through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Elabscience, China). The cells were exposed to a range of concentrations (**1000, 500, 250, 125, 62, 31 ug**). After 72h of infection, MTT dye solution 28 µL of (2 mg/ml) was added to each well. The incubation continued for three hours. A total of 100 µl of DMSO was added to each well and incubated for 15 min. The optical density was measured at 492 nm using a microplate reader [19]. Cytotoxicity % was calculated using the following equation:

Cytotoxicity % = (OD Control − OD sample)/OD Control × 100,

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were OD control being the mean optical density of untreated wells, and OD Sample is the optical density of treated wells [20].

2.5. DNA Damage

Apoptosis Estimation (Propidium Iodide/Acridine Orange Assay)

The apoptotic attentions in cell lines (infected and control) were measured using (AO/PI). 5000 cells/well were seeded in plate, next infected with (***Ocimum Basilicum***) for 24 hours in a 37 °C incubator. The tested wells received exactly 50µl of the AO/PI stain mixture (at room temperature) for 30 seconds. After then, the stain was removed. The images were taken using a Leica fluorescent microscope [21]. Fluorescent intensity measured by fluorescent microscopy and via using image j software.

Statistical analysis:

The obtained data were statically analyzed using an unpaired t-test and Tukey's ANOVA multiple comparisons test with GraphPad Prism 8. The values were presented as the mean ± SD of triplicate measurements [20].

3. Results

3.1. Amino Acid Detection

The chromatographic analysis clearly (Figure 1) demonstrates multiple peaks corresponding to different amino acids present in the extract. The sharp and well-separated peaks indicate good resolution and efficient separation by the amino acid analyzer. The presence of numerous peaks suggests a diverse amino acid profile, which may contribute to the biological activity of the plant. While, Table 1 illustrates the specific retention times for the 17 amino acids identified in the sample. These times were consistent with standard references, confirming the identity of the compounds. The reproducibility of retention times across multiple runs confirms the reliability and sensitivity of the analytical method. Therefore, *Ocimum basilicum* leaves contained 17 amino acid detected by amino acid analyzer.

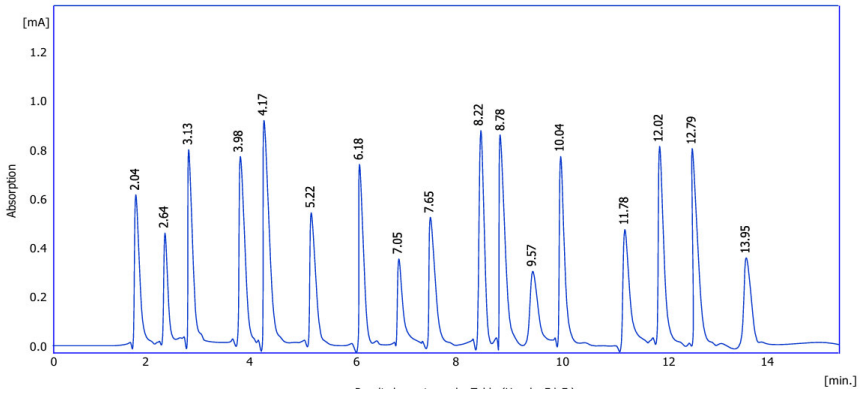

Figure 1. Chromatogram of *Ocimum basilicum* leaves extract.

Table 1. Retention times of amino acid detect of *Ocimum basilicum* Leaves.

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Amount (g/100 g)	Calculation	Peak type	Compound Name
1	2.04	4562.9	604.7	17.45	Calibration carve	Order	Aspartic acid
2	2.64	4152.6	643.6	26.09	Calibration carve	Order	Glycine
3	3.13	6325.9	794.9	24.12	Calibration carve	Order	Lysine
4	3.98	5854.0	789.8	27.88	Calibration carve	Order	Serine

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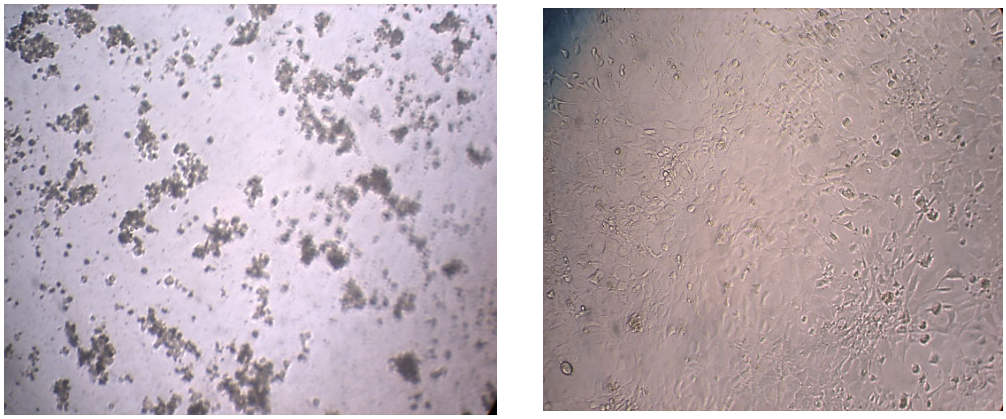
5	4.17	6214.5	835.7	23.65	Calibration carve	Order	Threonine
6	5.22	5062.6	594.4	23.54	Calibration carve	Order	Isoleucine
7	6.18	6541.8	781.1	29.08	Calibration carve	Order	Alanine
8	7.05	4562.6	386.5	35.65	Calibration carve	Order	Valine
9	7.65	4369.0	549.6	18.99	Calibration carve	Order	Tyrosine
10	8.22	7125.8	827.4	26.14	Calibration carve	Order	Arginine
11	8.78	10325.6	812.7	24.15	Calibration carve	Order	Cysteine
12	9.57	6521.4	284.8	19.08	Calibration carve	Order	Methionine
13	10.04	10568.9	741.9	21.65	Calibration carve	Order	Proline
14	11.78	8542.6	418.5	28.9	Calibration carve	Order	Histidine
15	12.02	6985.8	719.4	30.65	Calibration carve	Order	Lucien
16	12.79	11256.6	712.1	27.44	Calibration carve	Order	Glutamic acid
17	13.95	3565.0	362.3	38.0	Calibration carve	Order	Phenylalanine

3.2. Cytotoxic Effects Evaluation

Figure 2A shows HRT-18 cells treated with *Ocimum basilicum* extract with noticeable morphological changes are observed, such as: cell shrinkage, membrane blebbing, detachment from the surface and reduced overall cell density. These features are typical signs of cytotoxicity and apoptosis**.** In contrast, Figure 2B shows untreated cells that appear: well-spread, with normal polygonal shape, forming a dense and uniform monolayer and with no signs of damage. These visual differences support the conclusion that the basil extract induces cell death in cancer cells.



(**a**) (**b**)

Figure 2. Effect of *Ocimum basilicum* Leaves on HRT-18 Cells. A- Represent treated HRT-18 Cells. B- Represent untreated HRT-18.

Figure 3 indicates the inhibitory curve which shows the cytotoxic effect of different concentrations of *Ocimum basilicum* extract on HRT-18 cancer cells. As the concentration of the extract increases, there is a clear increase in the percentage of cell death, indicating a dose-dependent inhibitory effect. Higher doses of the extract can cause greater cytotoxicity, suggesting that the extract contains active compounds capable of killing cancer cells.

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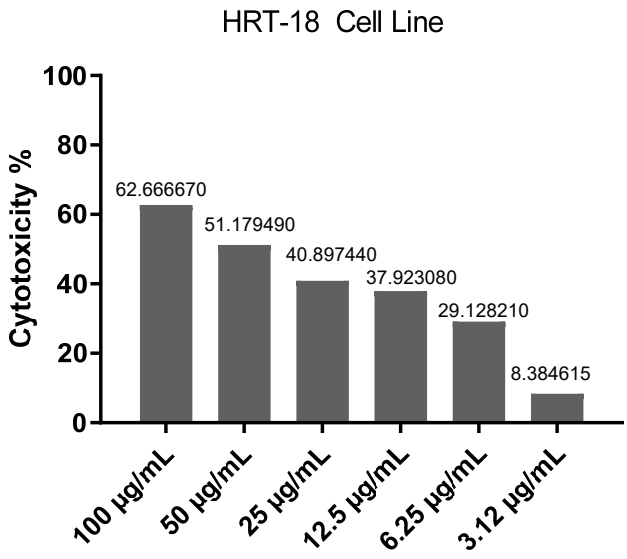

Figure 3. Inhibitory curve showing the effect of *Ocimum basilicum* on cell viability. The x-axis represents the concentration of the extract (µg/mL), while the y-axis shows the percentage of cell death (cytotoxicity).

Figure 4 represents the dose-response curve used to determine the IC50 value of *Ocimum basilicum* on cancer cells. The x-axis represents the concentration of the test compound (in µg), and the y-axis represents the percentage of cell inhibition. The IC50 value, represented by the Log of drug concentration of the test *Ocimum basilicum* that inhibits 50% of cell viability.

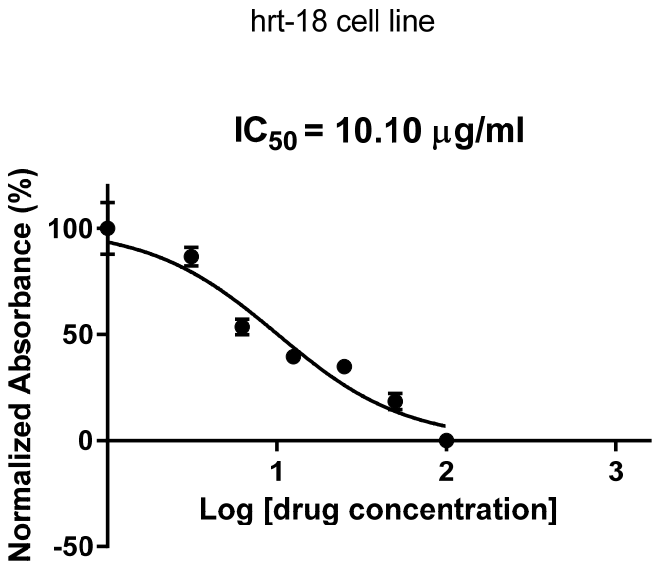

Figure 4. IC50 showing the effect of *Ocimum basilicum* on cell viability.

As shown in Figure 5 the post hoc Tukey's test demonstrates a significant, dose-dependent cytotoxic effect of *Ocimum basilicum* extract on HRT-18 cells. Higher concentrations (particularly 100 µg/mL) show statistically significant reductions in cell viability compared to lower concentrations and the control group ($p<0.0001$). Minimal or non-significant differences at lower doses suggest a threshold concentration is needed to elicit a strong cytotoxic response. These results support the extract's potential as an anticancer agent at higher doses.

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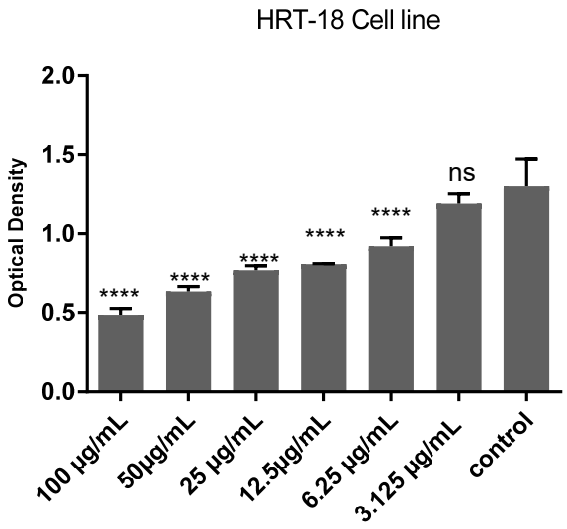

Figure 5. Optical density (OD) reading curve over time for cells treated with *Ocimum basilicum*. The x-axis represents concentrations in ug/ml, and the y-axis represents the OD value at 492 nm. Each data point represents the mean ± standard deviation of triplicates.

3.3. DNA Damage Analysis

Figure 6 illustrates a clear difference in fluorescence intensity between treated and control HRT-18 cells, indicating changes in cell viability. In the treated group, fluorescence from dead cells is significantly higher than viable cells ($p<0.01$), while the control group shows a predominance of viable cells with no significant difference between viability and death signals. This suggests that treatment with *Ocimum basilicum* extract induces cytotoxicity in HRT-18 cells, leading to increased cell death.

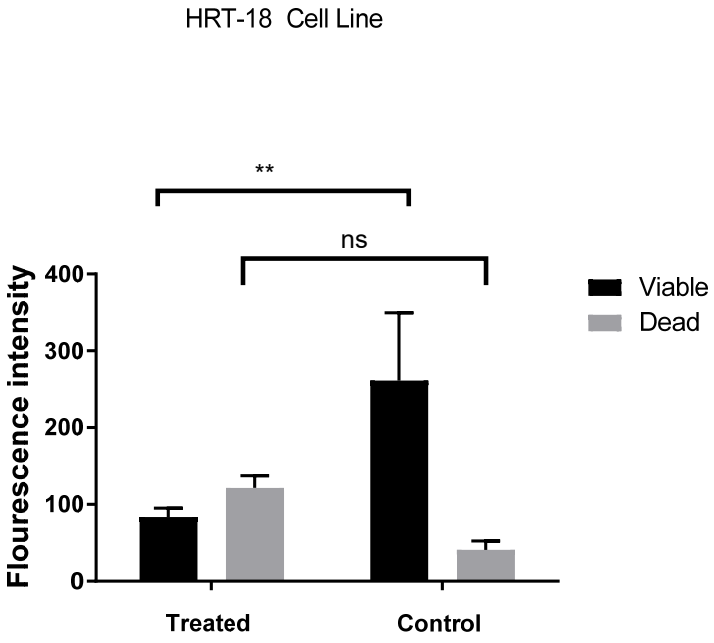

Figure 6. Effect of *Ocimum basilicum* extract on viability and death of HRT-18 cells assessed by fluorescence intensity.

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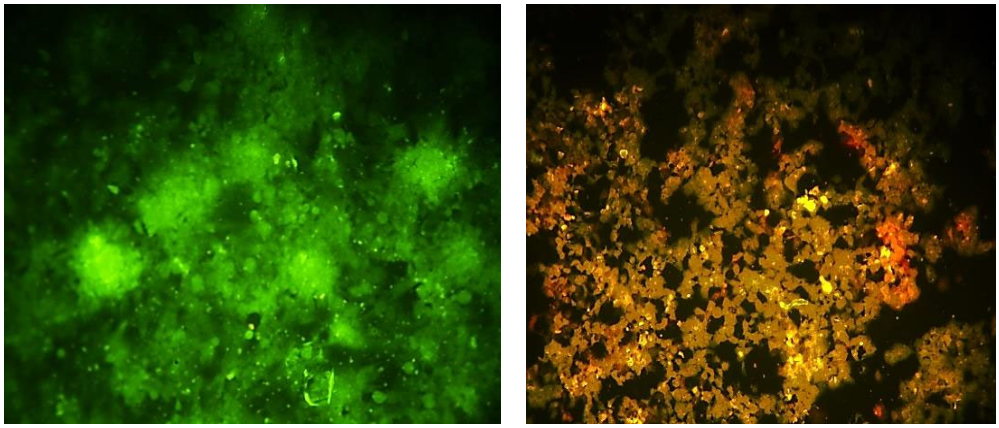
Figure 7 compares HRT-18 colorectal cancer cells before and after treatment with *Ocimum basilicum* extract. Figure 7A, the untreated (control) cells show normal morphology, with a flat, polygonal shape, intact membranes, and a dense, healthy monolayer covering the surface. While, Figure 7B, the treated cells display clear signs of cytotoxicity, including Cell shrinkage, membrane blebbing, loss of adherence, reduced cell density and rounded or fragmented cells, indicating possible apoptosis. These visual differences support the extract's antiproliferative and cytotoxic effects on cancer cells.



(**a**) (**b**)

Figure 7. Effect of *Ocimum basilicum* Leaves on HRT-18 Cells. A- Represent untreated HRT-18 Cells (control). B-Represent treated HRT-18.

4. Discussion

The amino acid analysis of *Ocimum basilicum* leaves reveals a rich and diverse profile of both essential and non-essential amino acids. A total of 17 amino acids were identified, each with varying concentrations, indicating the nutritional and potentially therapeutic value of this plant. Among the detected amino acids, Phenylalanine exhibited the highest concentration (38.00 g/100 g), followed by Alanine (29.08 g/100 g), Histidine (28.90 g/100 g), and Serine (27.88 g/100 g). The high levels of these amino acids are significant meanwhile phenylalanine is an essential amino acid that is involved in protein biosynthesis and to be neurotransmitters precursor like dopamine and norepinephrine [22]. Alanine exhibits a vital role in glucose metabolism and production of energy, especially under stress conditions [23]. Other essential amino acids like Valine (35.65 g/100 g), Leucine (30.65 g/100 g), Isoleucine (23.54 g/100 g), and Methionine (19.08 g/100 g) were also present in considerable amounts. These branched-chain amino acids are principally important for immune function, muscle repair, and neurotransmitter synthesis, making *Ocimum basilicum* potentially beneficial for physical endurance and metabolic health.

The presence of Aspartic acid (17.45 g/100 g) and Glutamic acid (27.44 g/100 g) further supports the plant's nutritional value. Glutamic acid is a key neurotransmitter and has a role in memory and learning, while aspartic acid is involved in the citric acid cycle and DNA synthesis [24]. Interestingly, Arginine (26.14 g/100 g) and Cysteine (24.15 g/100 g) were also detected in plant extract. Cysteine contributes to antioxidant defense through glutathione synthesis [25], while arginine supports nitric oxide production, aiding in cardiovascular health and immune modulation [26]. The broad spectrum and high amino acids concentration detected in this analysis support the traditional uses of *Ocimum basilicum* in herbal medicine. The obtained results are consistent with other studies that highlight the nutritional and pharmacological properties of basil species (*Ocimum sanctum* and *Ocimum gratissimum*) for their amino acid content [27,28].

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The cytotoxicity assessment of *Ocimum basilicum* extract on HRT-18 colorectal cancer cells reveals significant dose-dependent antiproliferative activity. Figure 2A demonstrates clear morphological changes in treated cells, including shrinkage, membrane blebbing, and detachment, all of which are hallmark indicators of apoptosis and cell stress. In contrast, Figure 2B shows the untreated control group maintaining normal morphology, indicating that the observed effects are indeed induced by the basil extract. Figure 3 illustrates a dose-dependent inhibitory curve, confirming that increasing concentrations of *O. basilicum* extract result in elevated cytotoxic effects. This supports the hypothesis that the plant contains bioactive compounds—such as flavonoids, terpenoids, and phenolics—that interfere with cancer cell metabolism or induce programmed cell death, as supported by literature indicating the anticancer potential of plant-derived compounds [29,30].

The IC50 curve in Figure 4 further quantifies the extract's potency, showing that the concentration required to inhibit 50% of the cell population is within a practical therapeutic range. This reinforces the potential use of *O. basilicum* in cancer treatment formulations, especially when standardized for high-yielding cytotoxic compounds. The post hoc Tukey's test shown in Figure 5 provides strong statistical evidence for the cytotoxic effect, particularly at 100 µg/mL, which showed highly significant reductions in cell viability ($p<0.0001$). The lack of significant difference at lower doses (e.g., 3.125 µg/mL) suggests a threshold concentration is needed to initiate noticeable cellular damage. These results align with findings from previous studies, which reported similar dose-dependent cytotoxicity of *Ocimum* species on various cancer cell lines [31,32].

The assessment of DNA damage in HRT-18 colorectal cancer cells following treatment with *Ocimum basilicum* extract reveals significant cytotoxic and antiproliferative effects. As illustrated in Figure 6, fluorescence intensity analysis clearly distinguishes between treated and untreated cells. The treated group exhibits markedly increased fluorescence associated with cell death, while the control group maintains high levels of viable cell fluorescence with minimal death signals. The significant difference in viability ($p<0.01$) confirms that the basil extract induces cytotoxicity, likely through mechanisms involving DNA damage and apoptosis. Figure 7 provides visual confirmation of this effect. Untreated cells (Figure 7A) maintain typical epithelial morphology—polygonal, well-adhered, and forming a confluent monolayer. In contrast, treated cells (Figure 7B) display multiple morphological signs of cytotoxicity, including cell shrinkage, membrane blebbing, and detachment from the culture surface. These features are consistent with apoptotic cell death, supporting the fluorescence data and previous cytotoxicity assays.

These findings align with earlier reports that plant-derived compounds, including flavonoids and essential oils found in *O. basilicum*, can cause oxidative stress and DNA fragmentation in cancer cells, leading to apoptosis [33,34].. The observed cell damage and morphological alterations suggest that the extract's active constituents may disrupt cellular homeostasis and promote DNA damage pathways [35].

5. Conclusions

Ocimum basilicum leaf extract exhibits a rich amino acid profile and significant cytotoxic activity against HRT-18 colorectal cancer cells. The extract induced dose-dependent morphological changes, reduced cell viability, and caused DNA damage indicative of apoptosis. These findings highlight its potential as a natural source of anticancer compounds and warrant further investigation into its active constituents and mechanisms of action.

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