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Cytotoxicity and cell cycle arrest induction by acetone banana peels extract on human skin cancer A375 and human leukemia HL-60 cell lines

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Abstract: Today, modern medicine has started to rely on phytoremedies for obtaining safe drugs, particularly for targeting cancer cells. Banana peels are packed with many pharmacologically interesting phytochemical compounds, and research is needed to investigate their anti-cancer activity. Banana peel was extracted using 50% acetone and then analyzed for its antioxidant activity using the DPPH assay, as well as for the presence of some anticancer compounds through HPLC analysis. The cytotoxicity of the banana peel extract on human skin cancer A375 and human leukemia HL60 cell lines was studied via the MTT assay and cell cycle arrest analysis. The results revealed a strong antioxidant potency of the banana peel extract, with an IC50 of 20.85 ppm in the DPPH assay. HPLC analysis revealed the presence of dopamine, quinic acid, and chlorogenic acid in concentrations of 42.65, 74.59, and 42.65 µg/ml, respectively. The MTT assay showed that mesenchymal cells had an IC50 of 746.8 µg/ml and remained viable, while A375 cells had an IC50 of 269.27 µg/ml, and HL60 cells had an IC50 of 317.68 µg/ml, both showing significant declines in viability. The findings of the cell cycle arrest demonstrated that the cell cycle of A375 cells was halted in the G1 and S phases at a concentration of 400 g/ml of banana peels. The extract exhibits strong antioxidant potency and high cytotoxicity for human skin cancer A375 and human leukemia HL60 cell lines, but A375 cells were more sensitive to the extract, while normal mesenchymal cells were not affected.

Keywords: *Banana peels, Cytotoxicity, Leukemia HL-60, Skin Cancer A375.*

1. Introduction

Cancer was a uncontrolled proliferative disorder that involve transformation , deregulation of program cell death [1]. Skin cancer is a significant source of morbidity and mortality in patients with leukemia, which initiate by the leukemia itself [2]. It takes place when neoplastic (growing out of control) white blood cells get away from their bone marrow and intrusion the skin (TSIMBERIDOU et al, 2009). For getting save drug and more specific for cancer cells ,modern medicine started to rely on medicinal plants to treat cancer diseases [3]. Medicinal plants are rich source for many phytochemicals that getting more importance therapeutic activeties [4]. Many phytochemicals apear anti-carcinogenic features by interference with cancer beginning and modifying different routes, that includes cell multiplication, the angiogenesis,distinction, programed cell death, incursion, and metastasis [5].

Banana peels are natural source for many effective compounds like phenolics ,tannins, nutrients, vitamins and minerals [6]. Many studies have indicated that banana peels contain many antioxidants, anti-inflammatory and anti-cancer agents [7]. Thus our present study aimed to study the toxicity of acetone extract of banana peels on human skin Cancer A375 and human leukemia HL-60 cell lines.

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2. Materials and Methods

2.1. Preparation Raw Powder of Banana Pell

Unripe banana *Musa acuminate L.* pells were used for extraction.Banana obtained from local market .The banana pells were cut into small parts for about 2x2cm.These parts were dried in oven under 45 C° . Then the dried pieces of banana pells were milled by electric miller to fine powder.This powder stored in sterile dark glass container untile uses for extraction [8].

2.2. Preparation acetone Extract Of banana pells

The raw powder of banana pells was extracted with acetone 50%. Firstly Mixture was prepared by mix one gram of raw powder of banana pells: 10 ml of solvent. Firstly the mixture was shaked in a high speed for 1 hour and secondly it was put in water path under 40 C° for 2 hour. filter paper was used for filtering the mixture .Then liquid was dried to get a solid texture by oven under 45 C°. Then by electric miller, the solid extract was milled to fine powder . Ultimately, the last powder was sterilized by UV light for 20 minutes and it was saved in sterile, dark and close cup to uses then [9].

2.3. Evaluation Antioxidant Activity by Dpph Assay

To evaluate the antioxidant activity for banana peels extract scavenging activity assay by DPPH (1-diphenyl-2-picrylhydrazyl) was performed. Two milliliters of banana peels extract with the following quantities were added: 200, 150, 100, 50, 25, 12.5, 6.25, and 3.125 g/ml. After half hour, absorbance was estimated at 517 nm.

The extract was tested three times at every concentration. The formula below was used to compute the percentage reduction of DPPH (Q) .

$q = 100 \times (a0 - aC) / a0$

Where: a0= absorbance of control

ac=absorbance of the two samples after 30 minute of incubation.

The IC50 value means the concentration of extract by which 50% of DPPH particles was scavenged .It was calculated from the displayed activity graph of scavenger against plotted graph of scavenging activity against the various doses of the extract [10].

HPLC analysis for qualitatively and quantitatively investigating about:

2.4. Dopamine

The HPLC analysis was carried out by using C18-ODS (25.0cm X 4.60 mm X 5 µm) column. The stage of mobility ascertained by following the method of Akowuah, et al. [11]. Which was consist of acetone (C) and Distilled water(D) . The linear gradient was starting with C:D (90:10) V/V to four minute, changing to C:D (85:15) for three minute, C:D (80:20) for three minute, A:B (70:30) to 10 minute, C:D (60:40) for eight minute, C :D (50:50) to four minute. The flow rate was 0.5 ml/ minute. The Injection volume was 100 µL with flow rate 0.5 ml/ minute, and the column temperature was 25 °C. The detection was performed under 340 nm wavelength [11].

2.5. QUINIC ACID

The HPLC analysis was investigated by column C18-ODS (25cm X 4.6 mm X 5 µm). O-phosphoric acid 25% (A) was used in a linear gradient as the mobile phase: acetonitrile (B) began with A:B (95:5) for 2 minutes then altering to A:B (90:10) for 5 minutes A:B (85:15) for 3 minutes, A:B (80:20) for 13 minutes, A:B (70:30) for 5 minutes, A:B (50:50) for 4 minutes. The rate of flowing was 1 ml/minutes.For all samples and standard solusions the Injection volume was 100 µL. The temperature of column was 25 °C. and the detection wavelength was 360 nm [12].

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2.6. Chloroginic Acid

The HPLC technique was carried out by using C18-ODS (25cm X 4.6 mm X 5 µm) column. According to Zubillaga and Maerker [13] method the mobile phase was founded which was consist of two sort of solutions (E) acetone: acetic acid: distilled water (10:2:88) and (F) methanol: acetic acid: distilled water (90: 3: 7). The gradient began at 40 % of E, 60% of F for four minute, 50% of E, 50% of F In a bid to five-eight minute and 60 % of E,40% of F for eight -ten minutes. 100 µL was the injection volume for standard solutions and all samples, the column temperature was 25 ˚C. the detection wavelength 280 nm [12].

The concentration of each phytochemical (Dopamine acid, Quinic acid, Chloroginic acid) was calculated by using the following equation which depending on the area under the peak [12].

$$\text{Conc. of sample}(\mu g/ml) = \frac{\text{Conc. of standard} \times \text{A of sample}}{\text{A of Standard}} \times \text{DF}$$

Where Conc = Concentration
DF= Delusion factor , A = Area

2.7. Preparation of Sample for HPLC

A 100 µl of banana peel extract was added to 1 ml methanol grade for HPLC , 100 µl of sample injected to HPLC device after 3 minutes of vortex [11].

2.8. Preparation of HPLC Standard

Phytochemical stander solution was prepared by melting 1 milligram in 100 milliletter of methanol evaluating for HPLC [12].

2.9. Cell Line Culture

Human skin cancer A375 and human leukemia HL-60 cell lines and type of normal cell HDFn were storage in vapor phase of liquid nitrogen at temperature below -130 C° in frozen vial, The frozen cell line vials was storage in Tissue Culture Laboratory in the university of Malaya Kuala Lampur [14].

3. Estimation Cytotoxicity of Extract by MTT Assay

A 96 flat-well plate was used to grow cells (1×10^4 to 1×10^6 cells ml^{-1}) with A finale volume of 200 mL $well^{-1}$. The dishware were insured with sterilized parafilm , and then incubated for 24 hrs. at 37° C and 5% CO_2. The medium was removed after incubation period ends, 200 mL of the one-fold serial dilution of acetone extract of banana peels (25.0, 50.0, 100.0, 200.0 , 400.0) µg ml^{-1} were introduced to the wells at each dose and controls in triplicates. Plates were incubated at 37°C and 5% carbon dioxide (gas) for one day. The cells were washed twice in the wells with the solution of phosphate buffer. The solution of of MTT staining (20 µL) was added to each well and then the plate was incubated at 37 C. After 4h, 100 µL of dimethyl sulfoxide (DMSO) was put to dissolved the formazan crystals for each well, then recorded absorbance with a 575 nm . Viability percentage of cells exposed to the extract was estimated by the following equation [14].

$$\text{Cell viability \%} = \frac{\text{absorbance of treated cells}}{\text{absorbance of control}} \times 100$$

4. Cell Cycle Tests

4.1. Preparation of Cells

Cells were initially established with a confluence of 5 x 105 cells in well in 12- well plates. Theses plates were cultivated at 37˚C with 5% CO for 24 h. After incubation and removal of the culture medium , the cells were treated with banana peels extract at a concentration of 400 µg/ ml for 24h. to perform cell cycle analysis. To obtain a homogeneous cell suspension , the following steps were followed: removed growth medium and the cell flask was rinsed with PBS. Then added (2 – 3) ml of

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atrypsin/EDTA solution to the cells and the vessel was gently shaken to ensure complete coverage of the monolayer. The vessel was then incubated at 37˚C for 1-2 min until the cell detached. Afterward , the cells were distributed by pipetting from the surface after the complete RPMI fresh medium (15 – 20 ml) has been added. The resulting suspension of cells was transferred to tube wcich labeled 17x 100 mm. The cells were rotated at room temperature for 5 minute at 300 g, then the supernatant was removed. The cells were gently vortexed at low speed to resuspend them after adding (1ml) of buffer solution, and. After another round of centrifugation under the same conditions, removed the supernatant, and resuspention the pellet in 1ml of buffer solusion .Counted the cells by hemocytometer and the cell concentration 1.0 x 106 cells/ml with buffer solution was used for immediate staining and flow cytometry examination [15, 16].

5. Staining Procedure

The suspension of cells was subjected to centrifugation at 400.0 g for five minute at ambient temperature (20 – 2537° C) . After removing the supernatant, 20 µL of trypsin buffer (solusion A)was added to the tube which mixed by hand tapping gently, then incubated at room temperature for 10 min . An aliquot of 200 µL of solusion B was then added to the tube with thorough mixing . The mixture incubated for 10 min. in 25° C . Then , 200 µL of staining solusion (cold solution C) was added to the tube with kind mixing .The mixture was put in adark, cold place in the refrigerator (2 – 8 ° C) for 10 min. At the last, the mixture was filtered by using a 50 µm nylon mesh with a new sterilized tube and analyzed using a flow cytometer [15].

6. Statistical Analyses

One –way analysis of variance (ANOVA) was carried out to evaluated the significance variance of groups. Information were displayed as mean and plus minus Standard Deviation (SD) and statistical significances were performed by utilizing version 6 of GraphPad Prism.

7. Results and Discussion

7.1. Prepare Banana Peel Extract

The raw powder of banana peels was extracted with acetone 50% as clear in Figure 1.

Figure 1.
Raw powder of banana peels.

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Natural banana peel powder. It has a very high level of purity This keeps the final product free of potential contaminants. Highly soluble and easy-to-mix powder activity of the extract by their activity to scavenging DPPH molecules, which was increased as extract concentration increased until reached to 97% in concentration 200 ppm.

Evaluation antioxidant activity of acetone 50% banana peels extract was conducted by DPPH scavenging activity test , and the findings in Table 1 reflect the strength of antioxidant

Table 1.
Scavenging activity test for different concentration of banana peels extract.

Concentration (µg/ml)	Mean of AB under 517 nm	Scavenging percentage
0	0.633	0%
3.125	0.342	46%
6.25	0.304	52%
12.5	0.273	57%
25	0.225	64%
50	0.183	71% Prior and Schaich
100	0.078	88%
200	0.016	97%

The finding of figure (1) show IC50 value of the extract was 20.85 ppm which calculated by the linear regression equations by considering Y is to be 50%. C50 for any phytochemical compound was as an identity for the strength of the antioxidant activity for this compound [17]. And by our results and according to the pharmacopia our extract classify as a strong antioxidant (2005). Researchers such as: Aboul-Enein, et al. [18] and Fatemeh, et al. [19] showed the richness of different types of banana peels extracts with many antioxidant compounds specially phenolic compounds like gallocatechin ,catecholamine , ,dopamine and betanin [20, 21] and that support our results and justified antioxidant strongness of our extract.

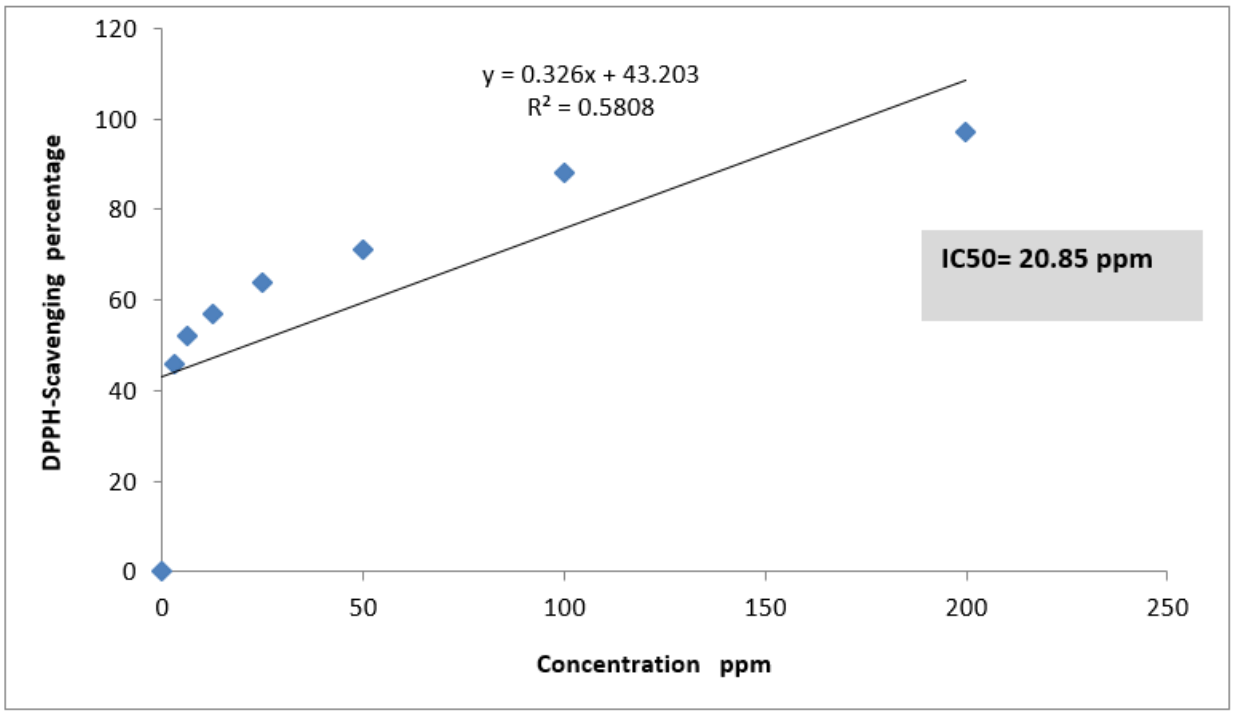

Figure 2.
Scavenging activity test for different concentration of banana peels extract and evaluated (IC50) by linear regression equations.

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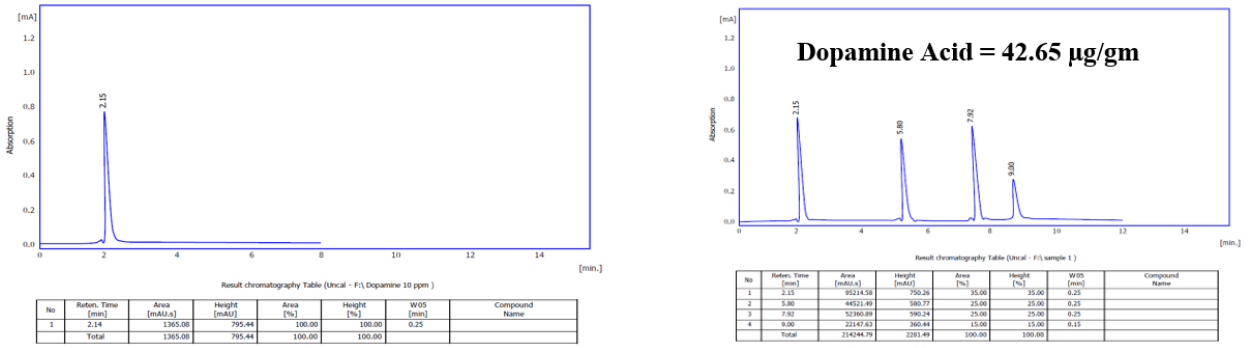

Figure 2.
Detection for dopamine in banana peels extract by HPLC.

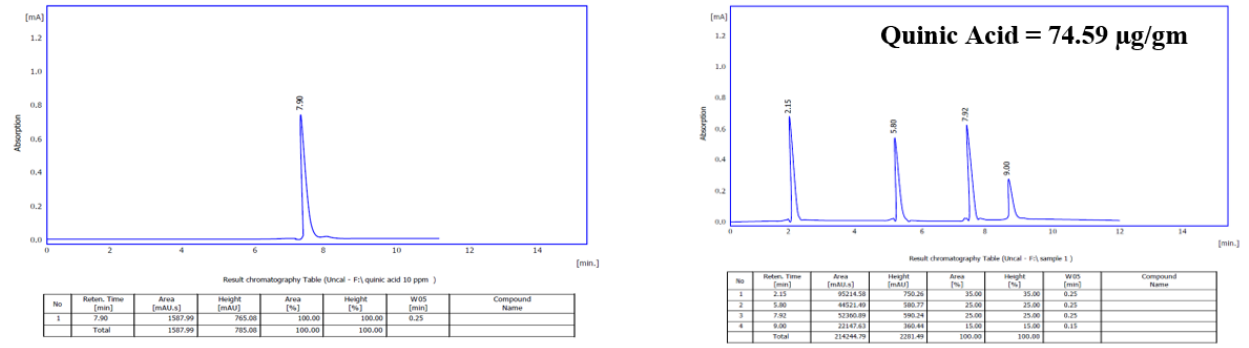

Figure 3.
Quinic acid detection in banana peels extract by HPLC.

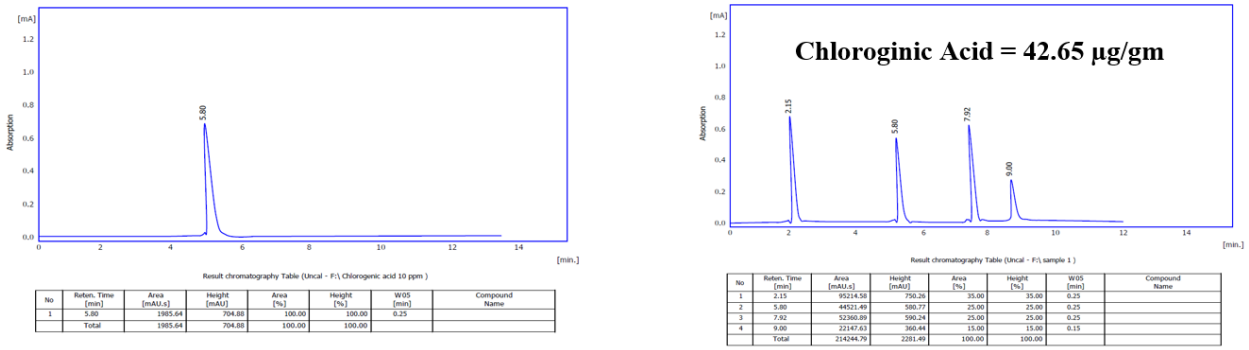

Figure 4.
Detection the Chloroginic acid in the extract of banana peels by HPLC.

The presence and concentration of Dopamine acid, Quinic acid and Chloroginic acid were identified by carried out HPLC analysis of stationary phase in a proper condition and a phase of polarity gradient mobile phase system as shown in figures (1 , 2 and 3) , where the retention times of 50% acetone banana peels extract was respectively (2.15 ,7.92 and 5.80) min and areas was (95214.58 , 52360.89 and 44521.49) and when compared with Dopamine acid, Quinic acid and Chloroginic acid standard retention time and area we find the presence of Dopamine acid, Quinic acid and Chloroginic acid in concentrations (42.65 , 74.59 and 42.65) µg/ml respectively. Many other studies like Kanazawa and Sakakibara [22] and Danijela, et al. [23] refer to predominant bioactive contents of many

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phytochemical compounds in banana peels extract among of them Dopamine acid, Quinic acid and Chloroginic acid and they have attributed many of therapeutic properties of this extract , such as antioxidant and anticancer activity , to the presence of of these active compounds in it [22, 23].

Table 2.
Viability of A375, HL-60 and HDFn cell lines at different concentrations of banana peels extract after incubation for 24 hour.

Banana peels extract concentration (µg/ml)	Cell viability % (mean ± SD)		
	A375	HL-60	HdFn
25	87.07± 3.06	88.54± 0.70	94.29 ± 0.43
50	75.77± 4.17	71.79± 1.23	93.55± 1.18
100	65.35± 0.86	61.84± 0.96	83.06± 1.79
200	51.23± 5.18	55.55± 0.53	79.59± 5.03
400	38.69± 5.42	46.68± 2.25	72.02± 4.97

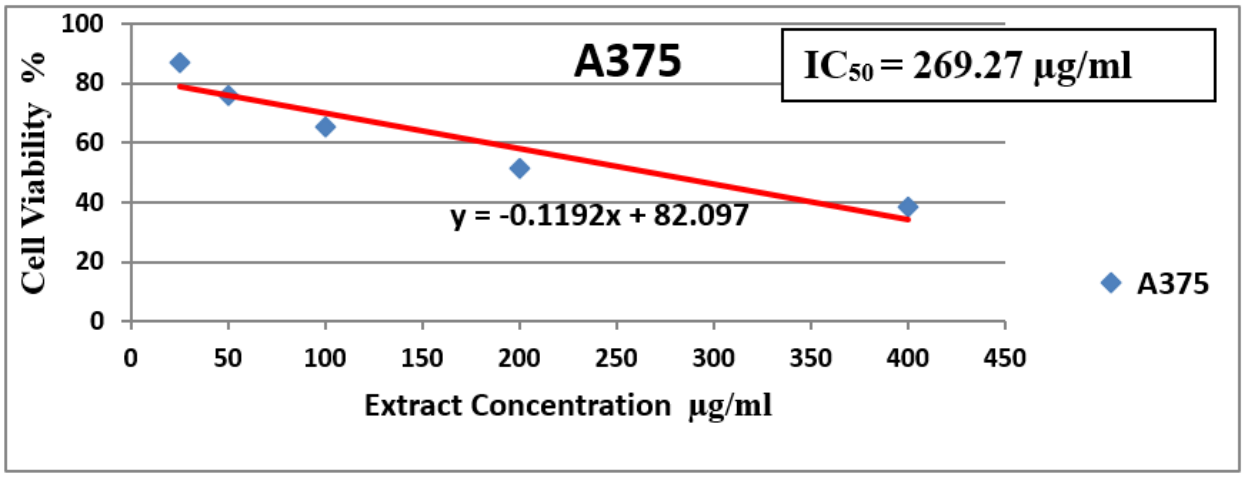

Figure 5.
Cytotoxicity effect and IC50 of banana peels extract on A375 cells.

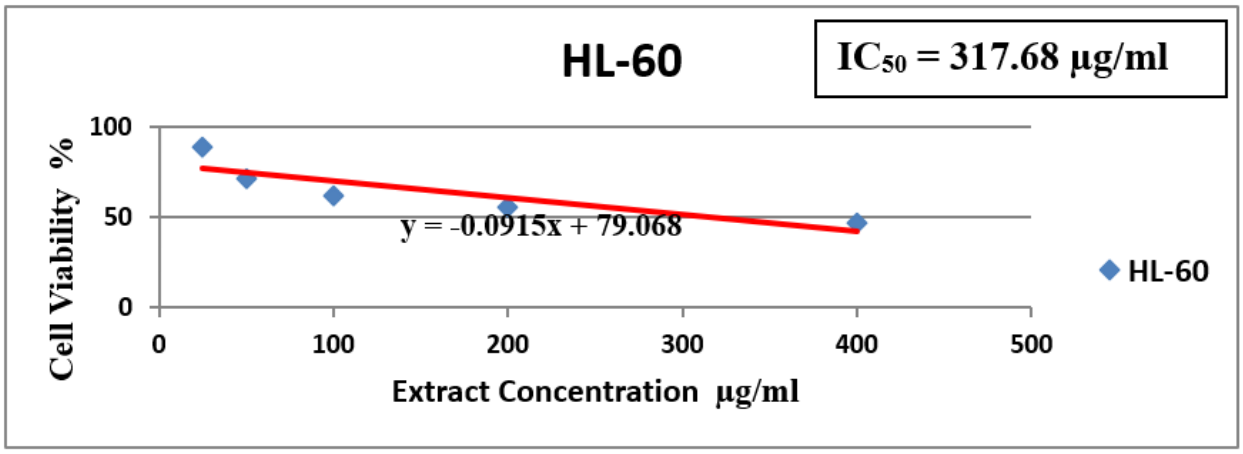

Figure 6.
Cytotoxicity effect and IC50 of banana peels extract on HL-60 cells.

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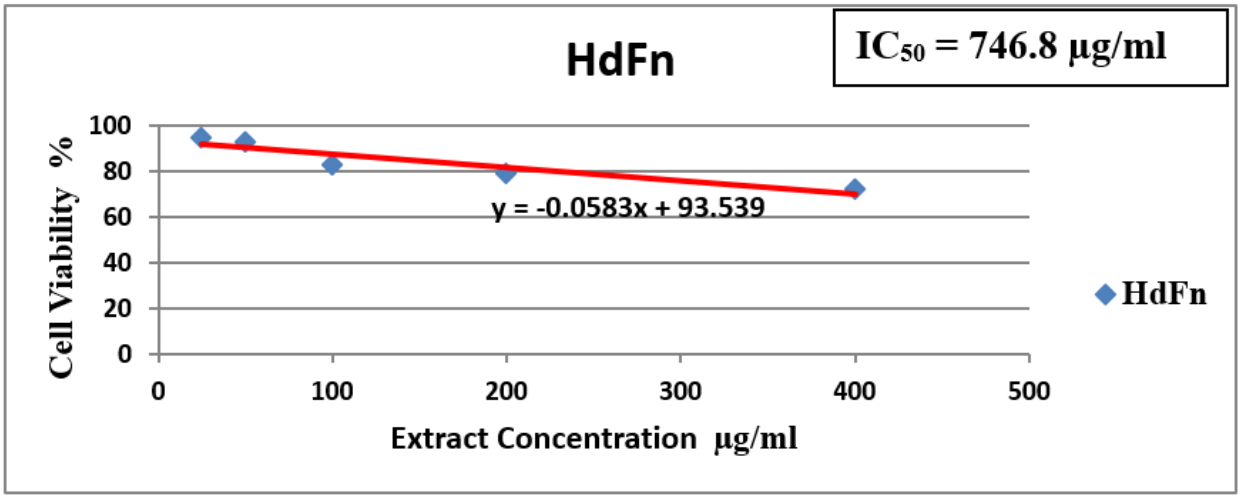

Figure 7.
Cytotoxicity effect and IC50 of banana peels extract on HdFn cells.

Cytotoxic activity of 50% acetone banana peels extract was studied on two type of human cancer cell lines : human skin cancer A375 and human leukemia HL60 cell lines in compere to one type of normal cell HDFn in vitro by using MTT assay .Different concentrations of the extract of banana peels (400, 200, 100, 50 and 25) µg/ml were used and the results in Table 2 showed that incubation of these cells with the extract at various concentrations for 24 hours appeared significantly decrease in cell viability in a manner reliant on dosage. The concentration of 400 µg/ ml was the most affected. The skin cancer cells A375 were more sensitive than leukemia HL60 cells. The viability of A375 cells was (87.07 ,75.77 , 65.35, 51.23 ,38.69) % in concentrations (25 ,50 ,100 ,200,400) µg/ml respectively with IC50 (269.27) µg/ml. The viability of HL60 cells were (88.54 ,71. 79 ,61.84 ,55.55 ,46.68) µg/ml in concentrations (25 ,50 ,100 ,200,400)_ µg/ml respectively with IC50 (317.68) µg/ml. While the viability of HDFn were (94.29 , 93.55 ,83.06 ,79.59 ,72.02) µg/ml in concentrations (25,50 ,100, 200,400)% respectively with IC50 (746.8) µg/ml . The extract was appeared selectively cytotoxic activity on A375cancer cell line and HL60 when comparing with normal cells line HDFn as shown in Table 2 and figures (5 ,6 and 7). The cytotoxic activity of banana peels extract may be contribute to the presence of many active substances for cancer treatment in the extract like: Dopamine acid, Quinic acid and Chloroginic acid as show in figures (2,3 and 4). All these compound have a strong anticancer and antioxidant activity [24]. Study by De, et al. [25] refer to the role of Dopamine as an anti-tumor which provoking apoptosis through activating the cyrochrome-c and caspase dependent apoptotic pathway and catalysed reactive oxygen species creation [25]. And other studies performed by Singh, et al. [26] and Kim, et al. [27] finding that Chloroginic acid and Quinic acid have reduce the expression of the Bcl-2 anti- apoptosis gene and the Bcl-2/Bax ratio, as well as MYCN mRNA levels and induce cell apoptosis through the up-regulation of P53 and P21, as well as , down – regulation of Cyclin D1, Cdk2 and Cdk7 which cause G2 phase cell cycle arrest for cancer cells and apoptosis [26, 27].

Table 3.
Follow cytometric investigation the effect of banana peel extract on A375, cells at 400 µg/ml of banana peels extract after incubation for 24.

Phase / Concentration µg/ml	G1	S	G2
Control	46.67 A	39.54 B	14.1 b
400	62.72 B	24.33 A	6.21 a

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The cell cycle phase distribution assay were performed for A375 cells after were treated by 400 µg/ml of 50 % acetone banana peels extract for 24 h in compere with untreated A375 cells (control) . and the results as in Table 3 and figures (8, 9 and 10) appear significantly increasing ($p \leq 0.05$) in the proportion of A375 cells which handled with 400 µg/ml of the extract in G1 phase when compared with control cells , which were respectively (62.72 and 46.67)% . While S Phase results showed that cells percentage of A375 cells which treated with 400 µg/ml of the extract significantly decreasing ($p \leq 0.05$) when compared with control cells , which were respectively (24.33 and 39.54)% . Also we note that G2 phase results show significantly decreasing ($p \leq 0.05$) in the percentage of A375 cells which dealt with 400 µg/ml of the extract in G2 phase when compared with control cells , which were respectively (6.21 and 14.1)% .Induction cell cycle arrest is the most prominent goal in anticancer drug development , when the cancer cell cycle will significantly inhibit cell proliferation and lead to apoptosis [28]. The resulting flow cytometry analysis showed that the acetone – watyery extract of banab peels in concentration 400 µg/ml lead to accumulate A375 cells in G2/M phase ,This result indicate that the extract in concentration400 µg/ml inhibit conversion of A375 cells from G1 phase to S phase.

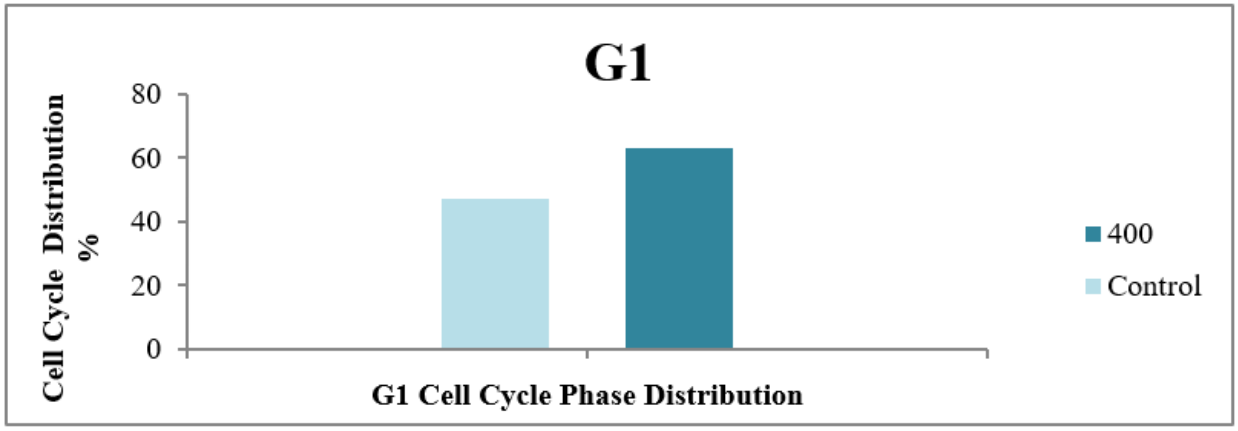

Figure 8.
The effect of 400 µg/ml of banana peels extract on G1 cell cycle phase distribution in A375 cells.

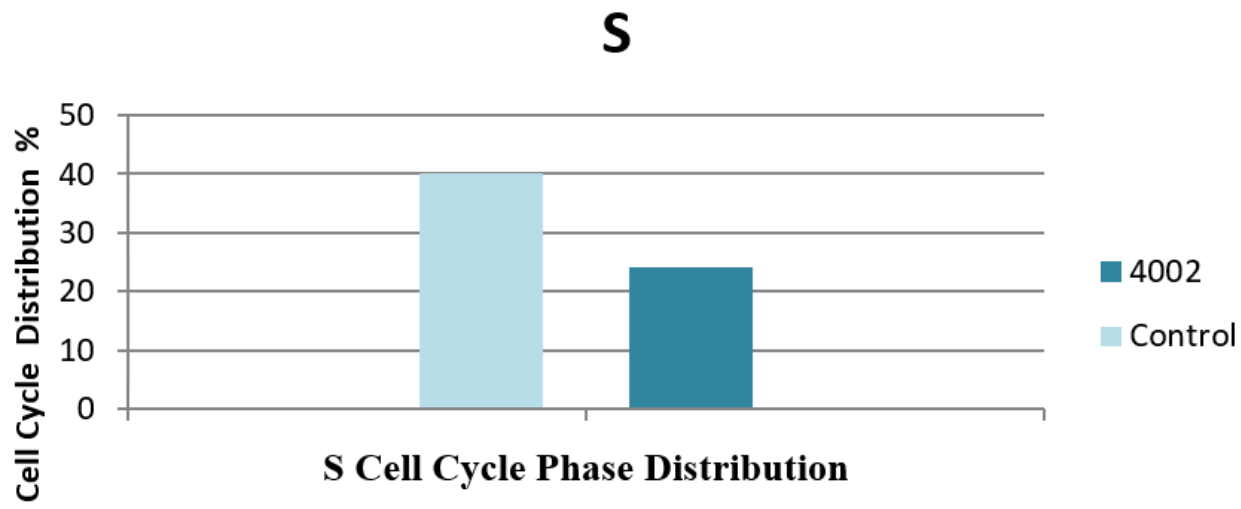

Figure 9.
The effect of 400 µg/ml of banana peels extract on S cell cycle phase distribution in A375 cells.

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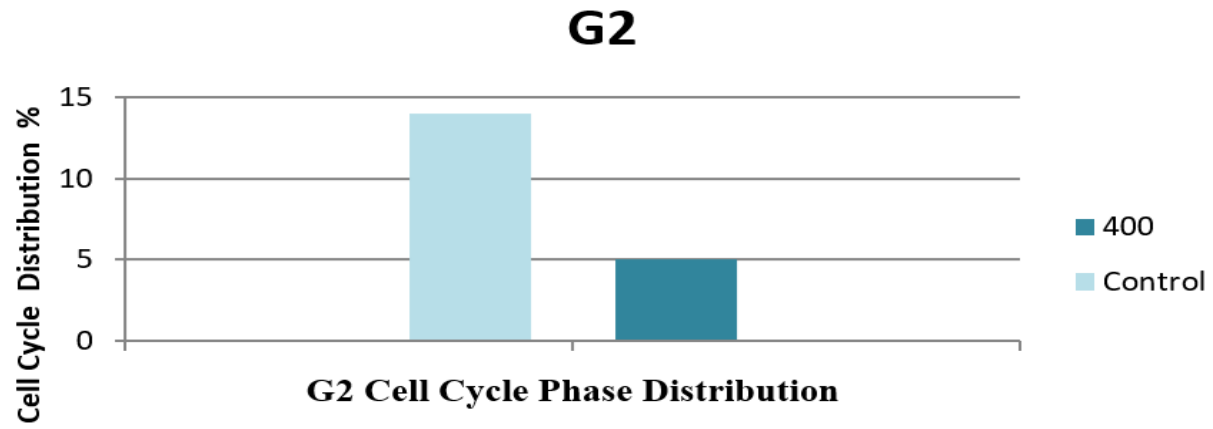

Figure 10.
The effect of 400 µg/ml of banana peels extract on G2 cell cycle phase distribution in A375 cells.

Cell cycle is one of the basic mechanisms to prevent the spread of cancer and its regulated by many genes, the most important of which is P53,which plays a crucial role in regulating cellular proliferation, especially in the G1 and G2 phases of the cell cycle [29, 30].

The richness of the extract in Chloroginic acid as shown in figure (3) may be one of the reasons for this effect . Many studies refered to the activity of Chloroginic acid in inhibition the proliferation of cancers cells in vitro like Shima, et al. [31] refers that Chloroginic acid induce cell cycle arrest in HT-29 by enhancing the expression of P21 and P53 and additionally

refere that induce programmed cell death by lowering Bcl-2 and NF- κB expression and increasing caspase 3 and 9 expresion and Reactive species concentration [31] other study performed by Carolina, et al. [32] refered that Chloroginic acid could induce cell cycle arrest and apoptosis in melanoma cells by up- regulate bax and caspase 3 expression and down –regulate cyclin D1, BCL2 VEGFA and surviving which lead to arrest for cell cycle in G1 and S phase [32]and by these studies , we can justified the role of the extract in induction arrest for cell cycle in G1 and S phase.

8. Conclusions

The extract has a strong antioxidant potency and high cytotoxicity for human skin cancer A375 and human leukemia HL60 cell lines, but (A375) cells was more sensitive for the extract and normal mesenchymal cells were not affected .

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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