Evaluation of Antineoplastic Activity of Aloe vera latex extract on Human Hepatic Cancer Cell Line

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Abstract

Liver cancer is one of the most common fatal types of cancer over the world. Conventional cancer treatments such as surgery, chemotherapy and radiation therapy are costly and requires long-term commitment to be effective, which eventually leads to financial burden and suffering of patients and their families. Natural extracts originated from plants have been studied and exploited as pharmaceutical products or functional products supporting cancer treatments for years. The purpose of this study is to evaluate the antineoplastic activity of *Aloe vera* on Huh-7 cell line. The results showed that dimethyl sulfoxide extract from *Aloe vera* latex had cytotoxicity on Huh-7 cell line after (24-48) h of treatment with IC_{50} value at (160.72 ± 2.95) µg/mL and (156.41 ± 1.30) µg/mL respectively. Besides, the extract also inhibited the migration of cancer cells in (24-48) h treatment at minimum concentration of 100 µg/mL. Hence, *Aloe vera* latex extract is considered a potential anticancer agent against the proliferation of hepatic cancer cell.

Received 30/09/2022
Accepted 03/03/2023
Published 30/03/2023

Keywords
natural extracts, anticancer agent, Huh-7 cell line, *Aloe vera*, liver cancer

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1 Introduction

Aloe vera is a succulent plant species which widely distributed in many regions around the world [1]. In Viet Nam, *Aloe vera* is considered one of the typical agricultural products of Ninh Thuan and Binh Thuan provinces with high economic value. For years, *Aloe vera* extracts containing bioactive compounds have been studied and commercialized into cosmetics, function foods and pharmaceutical products[2]. The latex, also known as exudate, is a yellow-brownish liquid that exists between the rind and the pulp of *Aloe vera*. It has a bitter taste and serves as a component of defense response of the plant. Approximately 80 compounds have been found in the latex and most of them are anthraquinone C- glycosides, anthrones, and free anthraquinones [3-4].

The major components of Aloe vera latex are aloin A, also known as barbaloin A, aloin B (isobarbaloin), aloesin (aloeresin B), and aloeresin A [5-6]. These chemical constituents have been proven to have cytotoxicity and anticancer potential against several cancer cell lines [7]. Until now, in Viet Nam there is no research focusing on antineoplastic potential of *Aloe vera* extracts against hepatocellular carcinoma cell (HCC) lines and Huh-7 cell line in particular. Therefore, this study aims to investigate and evaluate the antineoplastic activity of *Aloe vera* latex extract, specifically its potential to induce apoptosis in cancer cell.

Different types of *Aloe vera* extracts have been studied and their antiproliferation activities against several types of cancer cells, including hepatocellular carcinoma cell lines such as HepG2 and Hep3B, have been assessed thoroughly worldwide. However, Huh-7 cell line has never been the target of the previous research. This cell line is one of the most important and

beneficial *in vitro* models for hepatitis B, hepatitis C, hepatoma and liver cancer research at molecular level. Furthermore, since liver cancer cell has a strong correlation with hepatitis B and hepatitis C (more than 85 % HCC cases caused by chronic viral hepatitis infection), a wide range of research focus on several types of liver cancer relies on Huh-7 model [8]. Consequently, this study aim to evaluate antineoplastic effects of *Aloe vera* latex extract on Huh-7 cell line and investigating the IC_{50} value of *Aloe vera* latex extract against this model cell line. Besides, the latex of this plant must be removed in the food and cosmetic production. Therefore, another goal of this study is to provide the basic information serving for exploiting low-cost raw materials and develop them into functional products supporting the cancer treatments for human.

2 Materials and method

2.1 Materials

Aloe vera plants were purchased from Minh Phuc *Aloe vera* garden in Binh Chanh district - Ho Chi Minh City. All chemicals were purchased from Sigma-Aldrich Company, USA. Huh-7 cell line was purchased from Thermo Fisher Scientific Company.

2.2 Method

2.2.1 *Aloe vera* latex extract preparation

Aloe vera leaves were cut at the nearest position to the leaf root, dust and soil were wiped off. Then, the leaves were vertically placed in clean containers in 60 minutes to collect the latex. The latex was filtered through Whatman filter paper No. 1 under pressure in shaded condition to ensure the activity of the substances contained in the latex. The liquid obtained after filtration had an amber color and a slight yellow fluorescence. Then, it was evenly divided into 50 mL falcon tubes and centrifuged at high speed (13,000 rpm) at 4 ^{0}C for 15 minutes to completely remove the residue. The supernatant was freeze-dried at −80 ^{0}C, 0.1333 Pa pressure until obtaining dry powder with the moisture that met the standards specified in Vietnames Pharmacopoeia (8.53 ± 0.02) %. Extraction efficiency (0.09 ± 0.01) %, was identified by calculating the ratio between the mass of *Aloe vera* leaves collected and the mass of the extract in dry powder form obtained after freeze-dry process. The extract powder was dried until there was no change in its mass. The difference between the mass of the extract before and after heat-drying process at 65 ^{0}C was determined as its moisture. *Aloe vera* latex extract in powder form was dissolved in dimethyl sulfoxide (DMSO) to make a set of extract solutions with different concentrations, served for further experiments.

2.2.2 Evaluation of cancer cell migration inhibition via Wound-healing assay

Huh-7 cells was cultured in Dulbecco's Modified Eagle Medium (DMEM) media (with 10 % FBS and 1 % antibiotics supplement) at 37 ^{0}C/5 % CO_2, sub-cultured when cell concentration reached 80 % area of the flask. Approximately 10^5 cell/mL was transferred into 6-well plate. 1 mm – width “wound space” was created by using sterile 10 µL-tip. Huh-7 cells were incubated with *Aloe vera* latex extract with final concentration at 100 µg/mL and 200 µg/mL in (24 and 48) h. The inhibition of cancer cell migration was observed and recorded using inverted microscope EUROMEX, 10X objective lens.

2.2.3 Evaluation of cytotoxicity by IC_{50} value determination

Approximately $5 \cdot 10^4$ cells/mL were cultured in 96-well plate at 37 ^{0}C, 5 % CO_2 overnight. Huh-7 cells were treated with *Aloe vera* latex extract at different final concentrations in (24-48) h to determine the IC_{50} value by using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Optical absorbance was measured at 570 nm wavelength by ELISA machine.

2.2.4 Evaluation of gene expression of intracellular signaling molecules in the apoptosis pathway

Gene expression of intracellular signaling molecules was evaluated by RT-Realtime PCR. Sequences of the primers (5’ to 3’ end) used for amplification process described below were purchased from PHUSA Biochem Company:

Caspase3-F TGGCACCCATTTACACCTAC
Caspase3-R GATCTCAGGAGTGACAGGG
Caspase8-F GCAACGTGAACATCTTCGAC
Caspase8-R TCCTCAAAGACCGAGTCCAG
Caspase9-F AACTTGTTTCTTGTTGCTGC
Caspase9-R GCCGGTGTAGGTGTAGATAG
Bcl-2-F CAGCGAGGAGTTTCTCATTG
Bcl2-R AGTGTAGGTCTTGGTGAAGC
GAPDH-F ATCATCAGCAATGCCTCCTG
GAPDH-R TGAGTCCTTCCACGATACCA

Huh-7 cells were treated with *Aloe vera* latex extract at (100 and 200) μg/mL in 48 h. Total RNA extraction was performed using QIAzol Lysis agent then total cDNA were synthesized. The concentration of cDNA required for qPCR assay was 50 ng/mL. All procedures were conducted according to the manufacturer's recommendations. Gene expression was calculated based on Livak's $2^{-\Delta\Delta Ct}$ relative quantification method.

2.2.5 Statistical data analysis

All results shown in images or graphs are expressed as mean value ± standard deviation, and statistically significant differences were determined using GraphPad Prism software (version 8.4.3).

3 Results & discussion

3.1 Evaluation of antineoplastic activity of crude extract from *Aloe vera* latex

3.1.1 Changes in morphism, concentration and migration of Huh-7 cancer cell

Cancer cells migration inhibition was evaluated by Wound-healing assay. Huh-7 cells were incubated with *Aloe vera* latex extract with final concentration at 100 μg/mL and 200 μg/mL in (24 and 48) h. Figure 1 and Figure 2 describe the difference in cell concentration and migration between treatments and control samples. After 24 h of treating, the wound space in treatment A (100 μg/mL) and treatment B (200 μg/mL) was larger compared to that of control sample, indicated the inhibition of cell migration and the decrease in cell concentration caused by the extract. After 48 h of treating, the wound space in treatment samples slightly changed, while the concentration and the migration of cancer cells obviously declined, compared to the control. Between treatment A and treatment B, the higher concentration of *Aloe vera* latex extract, the more obvious in cell concentration decrease and cell morphological alterations. Cancer cells detached from each other and from the flask surface, cell death was recognizable. The results also indicated that extract of *Aloe vera* latex had inhibitory effects on the proliferation and migration of Huh-7 cell line.

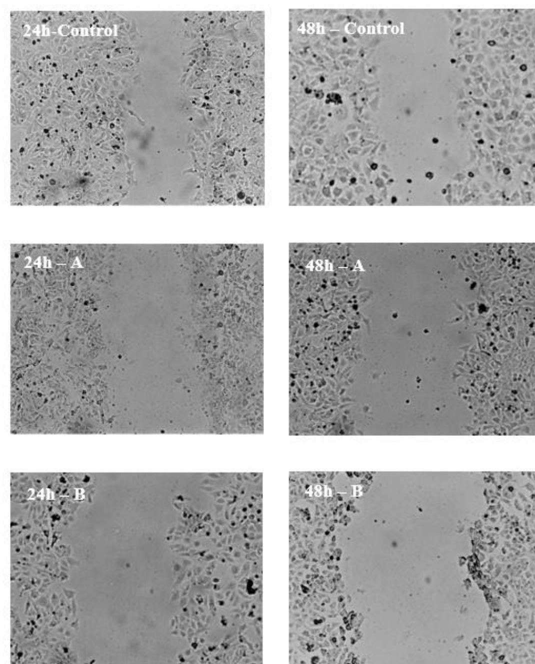

Figure 1 “Wound-healing” assay of Huh-7 hepatic cancer cells. Huh-7 cells were treated with the extract in (24-48) h, Treatment A (100 μg/mL) and Treatment B (200 μg/mL) compared to control sample. Images were taken by EUROMEX inverted microscope, objective lens 10X.

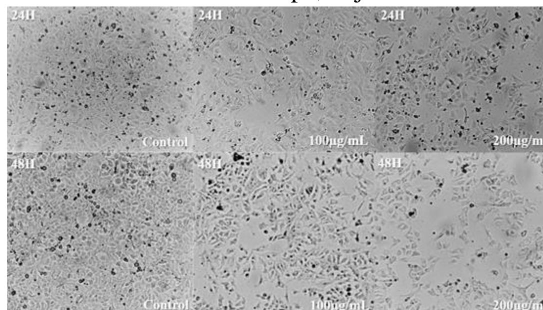

Figure 2 Huh-7 cell concentration drastically decreased in treatment group (final concentration of Aloe vera latex extract at 100 μg/mL and 200 μg/mL, incubated in (24-48) h compared to control group. Images were taken by EUROMEX inverted microscope, objective lens 10X.

3.1.2 Evaluation of *Aloe vera* latex extract’s cytotoxicity on Huh-7 hepatic cancer cells

Huh-7 cells were incubated with *Aloe vera* latex extract with stepwise concentration gradient in (24 to 48) h to determine the half maximal inhibitory concentration (IC_{50} value). IC_{50} value of the extract was (160.72 ± 2.95) μg/mL and (156.41 ± 1.30) μg/mL for each treatment mentioned above, respectively.

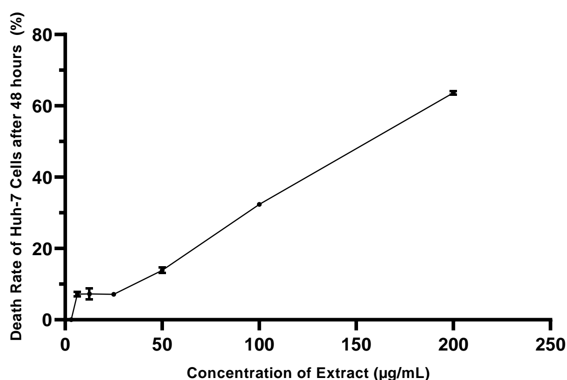

Figure 3 Correlation between Huh-7 cells death rate and the final concentration of Aloe vera latex extract in culture medium. Data was assessed by MTT viability assay and measured in a 96-well ELISA machine at 570 nm wavelength.

In these experiments, crude extract of *Aloe vera* latex was utilized, which may explain the why IC_{50} value was higher than 150 µg/mL. Purified bioactive substances are expected to have more effective antineoplastic activities. Further studies on anticancer reagents isolated from *Aloe vera* latex against Huh-7 cell line in the future are recommended .

3.2 Evaluation of Apoptosis Molecular Signals Gene Expression Rate by RT-Realtime PCR assay

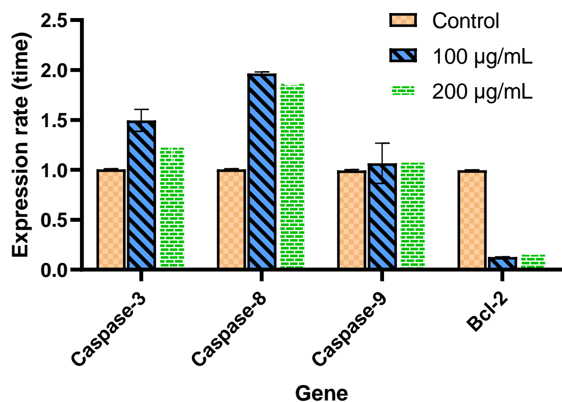

Figure 4 Changes in expression rate of molecular apoptosis markers in Huh-7 cells after being treated with Aloe vera latex extract at 100 µg/mL and 200 µg/mL in 48 h. The graph was constructed by GraphPad Prism 8.4.3 software.

Huh-7 cells were treated with *Aloe vera* latex extract at two concentrations of 100 µg/mL and 200 µg/mL in 48 h. Since the IC_{50} value of this extract was determined in between the range of (100-200) µg/mL, changes in gene expression of Huh-7 cells treated with the extract at these concentrations were assessed. RT-Realtime PCR assay was performed to quantify the change in relative expression rate of apoptosis molecular markers including *Bcl-2*, *caspase-3*, *caspase-8*, *caspase-9* genes, and the results of this process were recorded and shown in Figure 4.

There was an obvious decrease in expression rate of *Bcl-2* of both treatment groups (final concentration of extract at 100 µg/mL and 200 µg/mL in culture media). Furthermore, the rate of *Bcl-2* expression in 100 µg/mL treatment was lower than the rate of 200 µg/mL treatment. Meanwhile, expression rate of *caspases* group in both 100 µg/mL and 200 µg/mL treatments markedly increased, particularly, there was a drastically increase of *caspase-8* expression rate in these groups. Overall, the simultaneous appearance of these two phenomena (decrease in *Bcl-2* expression rate & increase in *caspase* group expression rate) in treatment groups confirmed that apoptosis occurred in Huh-7 cells, and it was the cause of cell death in these groups. These results are similar to those of previous studies which successfully demonstrated apoptosis induced by *Aloe vera* latex crude extracts was responsible for inhibitory effects against different types of cancer cells [9-11].

4 Conclusion

Based on the results of *in vitro* experiments, this study successfully proved antineoplastic activity of *Aloe vera* latex extract against Huh-7 hepatic cancer cells and provided basic findings about the mechanism of the cell death cause by this extract. In conclusion, bioactive compounds exist in *Aloe vera* latex extracts are promising anticancer agents which might be developed into pharmaceutical or functional products to support cancer treatment and prevent cancer metastasis.

Acknowledgment This study was funded by the Science and Technology Development Fund of Nguyen Tat Thanh University under the contract code 2021.01.114/HĐ-KHCN.

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Đánh giá tiềm năng ức chế sinh trưởng tế bào ung thư gan của dịch chiết từ nhựa vàng của cây Nha đam

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Tóm tắt Ung thư gan là một trong những loại ung thư phổ biến và có tỉ lệ tử vong cao nhất thế giới. Các phương pháp điều trị ung thư truyền thống như phẫu thuật loại bỏ khối u, hóa trị và xạ trị là rất tốn kém và đòi hỏi quá trình điều trị dài lâu để có thể có hiệu quả, vì vậy tạo ra gánh nặng tài chính lên bệnh nhân và gia đình, gây đau đớn và suy nhược cơ thể của bệnh nhân. Trong nhiều năm trở lại đây, các hợp chất tự nhiên có nguồn gốc thực vật đã được nghiên cứu và khai thác sử dụng dưới dạng dược phẩm hoặc thực phẩm chức năng để hỗ trợ quá trình điều trị ung thư. Nghiên cứu này được thực hiện nhằm đánh giá khả năng ức chế sự sinh trưởng của dịch chiết từ nhựa vàng của cây Nha đam (*Aloe vera*) trên dòng tế bào ung thư gan Huh-7. Kết quả chứng minh dịch chiết có thể tiêu diệt tế bào ung thư gan Huh-7 sau (24-48) giờ xử lí với giá trị IC_{50} lần lượt là (160,72 ± 2,95) µg/mL và (156,41 ± 1,30) µg/mL. Bên cạnh đó, dịch chiết còn có khả năng ức chế sự di chuyển của tế bào ung thư trong (24-48) giờ xử lí ở nồng độ tối thiểu từ 100 µg/mL. Khảo sát các tín hiệu phân tử cho thấy sự tăng biểu hiện của các gene *caspase-3*, *caspase-8*, *caspase-9* và giảm biểu hiện gene *Bcl-2*, điều này chứng minh quá trình apoptosis đã xảy ra ở các tế bào ung thư sau khi xử lí với cao chiết. Từ đó có thể kết luận dịch chiết từ nhựa vàng của Nha đam có tiềm năng lớn trong ức chế sinh trưởng và di chuyển của tế bào ung thư gan.

Từ khóa Hợp chất tự nhiên, chất chống ung thư, dòng tế bào Huh-7, nha đam, ung thư gan