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Anticancer Activity of *Senna* Leaf and *Fenugreek* Seed against Lung and Prostate Cancer Cell Lines

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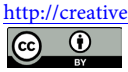

Abstract

Cancer is one of the leading causes of death worldwide. The United States is anticipated to experience 1,958,310 new cases of cancer and 609,820 cancer-related deaths in 2023. The most common cases of cancer are breast cancer, prostate cancer, leukemia, thyroid cancer, bladder cancer, and liver cancer. Cancer treatment represents a major challenge for governments and the health and pharmaceutical fields, due to the high cost in addition to the presence of side effects. Plants are a rich source of secondary metabolites, which contain therapeutic properties for many diseases such as microbial diseases, infections, hyperglycemia, cholesterol, in addition to cancers. The sole purpose of the study is to determine the advantages of using natural cancer treatments such as the use of Senna leaf and fenugreek seed. Different concentrations of extracts of *Senna* leaf and *fenugreek* seed were exposed to cancer cell lines taken from lung and prostate. Cell viability was measured using crystal violet, along with a Caspase-3 antagonist to detect apoptosis. Peroxidation of lipid assays was evaluated to determine antioxidant activity. It was found that the cell viability decreased in general with increasing concentration of extracts in two types of cancer cells (PC3 and A549). MDA (Malondialdehyde) level activities were reduced in cancer cell lines demonstrating reduction of lipid peroxidation in the cancer cell, and the tested samples indicated that the inhibition was dose dependent. With concentrations ranging from 0.46 mg/ml - 0.9 mg/ml for *Senna* leaf extracts on A549 and PC3 respectively, and a concentration of 1.1 mg/ml for *fenugreek* seed extracts on the two types of cancer cell lines. The study showed an increase in the production of the Caspase-3 enzyme in PC3 and A549 when the concentration of the extracts increased. Also, showed that *Senna* leaf extract and *fenugreek* seed had high efficacy against cancerous cells, which could be an ef-

fective treatment or prevention of cancers caused by PC-3 and A549 cell strains.

Subject Areas

Biological Chemistry

Keywords

Senna Leaf, *fenugreek* Seed, Lung Cancer, Prostate Cancer, Lipid Peroxidation, Caspase-3

1. Introduction

For many decades cancer has been considered a global health problematic issue that faces human life due to its involvement in many deaths in humans. The World Health Organization (WHO) in 2019 was estimated cancer is the first or second primary cause of death under the age of 70 years in more than 120 out of 185 countries [1]. In 2020, 19.3 million new human cancer cases and 10 million cancer deaths occurred worldwide. In both males and females combined, more than 58% of cases of deaths have occurred in Asia, where 22.8% of total cancer cases and more than 19% of deaths in Europe. In the Americas, about 20.9% of cancer cases have been reported and 14.2% of worldwide deaths. In Africa, 7.2% of total cancer cases are estimated in 2020, with more than 49% incidence and 5.7% of worldwide mortality. Prostate cancer was diagnosed at the highest frequency in men worldwide, followed by pulmonary, colorectal, and liver cancer. Concerning fatality, pulmonary cancer is the highest one, followed by prostate, and liver in men. On the other hand, the most frequent for women cancer is breast cancer, followed by cervical cancer. Moreover, regarding mortality, breast cancer is the highest one, and cervical cancer comes in the second class, followed by pulmonary cancer [2]. In the United States, the estimation of new cancer cases was about 1,958,310 in 2023 with more than 609,820 deaths. Regarding mortality rate in the US, the higher rate has been recorded in men in comparison of women, while according to ethnicity, the highest mortality rate has been observed in African-American men (239 per 100,000), whereas the lowest rate has been found among the Asian women (88.3/100,000). The most common types of cancer in the US were breast, prostate, liver, leukemia, thyroid, and bladder [3] [4]. Cancer is characterized by the uncontrollable multiplication of cells which leads to the forming of tumor cells, and then metastatic throughout the human body via lymph nodes [5]. Cancer, in general, is classified according to the site at which the abnormal cell started or the tissue where the tumor was initiated. The distinct characteristics of neoplastic tissue comprise; continued fission signaling, inhibition of the growth suppressors, struggle the cell apoptosis, metastasis, invasion, controlling the energy of metabolism, and evasion of the immune system. The reasons for the high incidence of cancer are unknown. However, it is

known that an increased risk of cancer is associated with tobacco custom, certain infections, radiation, physical inactivity, environmental contaminants, poor feed, obesity, and age. These agents may directly destroy genes or combine with genetic defects within cells to cause disease [6]. Cancer treatment has become an entirely new field of research. There are traditional in addition to ultra-modern techniques in place against cancers. A variety of techniques such as chemotherapy, radiotherapy, or surgery are used to treat cancer. However, all of them have some drawbacks. The use of traditional chemicals has side effects and is toxic to other normal tissues. Moreover, as the problem continues, new ways of disease control are needed, particularly because of the failure of traditional chemotherapy methods [7].

Recently, the study of the curative effects of plants has increased due to their comprehensive medicinal and economic properties and the effective use of some of these plants in the treatment of human diseases. The use of plants for treatment is not new, plants have already been considered a valuable source of bioactive compounds to treat many causes, including cancer, in nearly all cultures and societies for thousands of years [8]. In several industrialized countries, medicinal plants are gaining attractiveness as a substitute for or complement to treatment [9]. Some types of plants are used as food or drugs. These types of plants exhibit a wide variety of bioactive and medicinal compounds. Fenugreek (*Trigonella Foenumgracium*), also known as "Helba" in Arab countries, is a legume plant that belongs to the Leguminosae family. It's usually used as a spice in cooking, and it is recognized as a safe food by the US Food and Drug Administration [10]. Fenugreek seeds were used for a long time as an ancient method of medicine for abundant conditions due to their comprising several bioactive compounds, including flavonoids, alkaloids, terpenoids, and glycosides, which are known to have antimicrobial activities. Fenugreek seed also has other compounds such as galactomannan and steroid sapogenin, which are known to have antioxidant substances. Out of these, fenugreek seed has some amino acids like 4-hydroxyisoleucine, which have anti-diabetes and hypocholesterolemia properties that give a strong potential to treat many types of diseases such as leukemia, fertility, obesity, and cancer [11]. Senna is an herb. The leaves and fruits of the plant are used in the medical industry. Senna is an over-the-counter laxative approved by the Food and Drug Administration. It is used to treat constipation as well as to clean the intestines before diagnostic tests such as a colonoscopy. Senna is also used for irritable bowel syndrome (IBS), hemorrhoids, and weight loss. Studies have shown that ethanolic extract from *Senna alata* led to an expansion of cytotoxicity in malignant growth of human breast, prostate, and colorectal cell lines [12]. Some studies demonstrated that the flavonoid derivatives of the antioxidants, anthraquinones, and piceatannol extracted from Senna enhance B16F10-Nex2 melanoma cell death by apoptosis induced by caspase-3 activation, elevated intracellular calcium and ROS levels, and cell death. Cycle stop in G0/G1 phase. Moreover, the extract showed antitumor activity *in vivo* in models of initial tumor size progression and pulmonary nodule formation. These prom-

ising results open the door to further studies, with both the crude extract and fractions isolated from the root of *Senna velutina*, exploring their potential for use in the treatment of melanoma and other cancers [13] [14].

This study was designed and aimed to test the anti-cancer efficacy of methanol extracts of *Senna* leaf and *fenugreek* seed by treating these extracts at different concentrations with cancer cell lines taken from lung cancer (A549) and prostate cancer (PC-3), by measuring cell vitality, lipid peroxidation, and caspase 3 expressions.

2. Materials and Methods

2.1. Samples Processes

Senna leaf preparation:

The leaf of Senna was collected and prepared to extract. The samples were washed using sterile distilled water three times to remove any depress. The washed leaf was dried in an oven at 45˚C with air circulation, then collected into separate sterile, chemical resistance 50-millimeter plastic centrifuge tubes, which then were frozen at −80˚C for 3 days. The dry-frozen leaf was ground using mortar and pestle.

Fenugreek seed preparation:

Fenugreek seeds were purchased from a commercial market. The seeds were washed and dried as mentioned in section 3.1, and then ground multiple times with an electric grinder to make it in fine powder form.

Extraction of bioactive compounds:

The prepared leaf and seed were soaked separately in methanol with interval reflux for 24 hours at room temperature to extract the bioactive compounds. Then the mixture was distilled in a Soxhlet for 6 hours, and the resulting supernatant of each mixture was evaporated using a rotary evaporator to remove methanol residuals. Finally, the yielding crude was weighed and dissolved in an appropriate amount of dimethyl sulfoxide (DMSO) (Sigma, USA) as concentrated stock. (**Figure 1**)

Crude extraction dilution:

The resulting crude extracted from both Senna and Fenugreek was serially diluted by 0.5 ml in DMSO for crude, or phosphate buffer saline (PBS) for aqueous extraction to obtain the appropriate concentration. The range of dilution was 1 to 1/64.

Cancer cell line:

To evaluate the cytotoxicity and antiproliferation activity of extracted crude, two types of human cancer derivative cell lines were used, which are represented in (**Table 1**). A549 (lung cancer) and PC3 (prostate cancer) cell lines were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). These cell lines were examined and verified to be free of contaminated bacteria (*Mycoplasma*). The cell lines were incubated in 5% CO_2 at 37˚C using RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Atlanta Biologicals), 2 mM L-glutamine, and 1% penicillin-streptomycin. A549

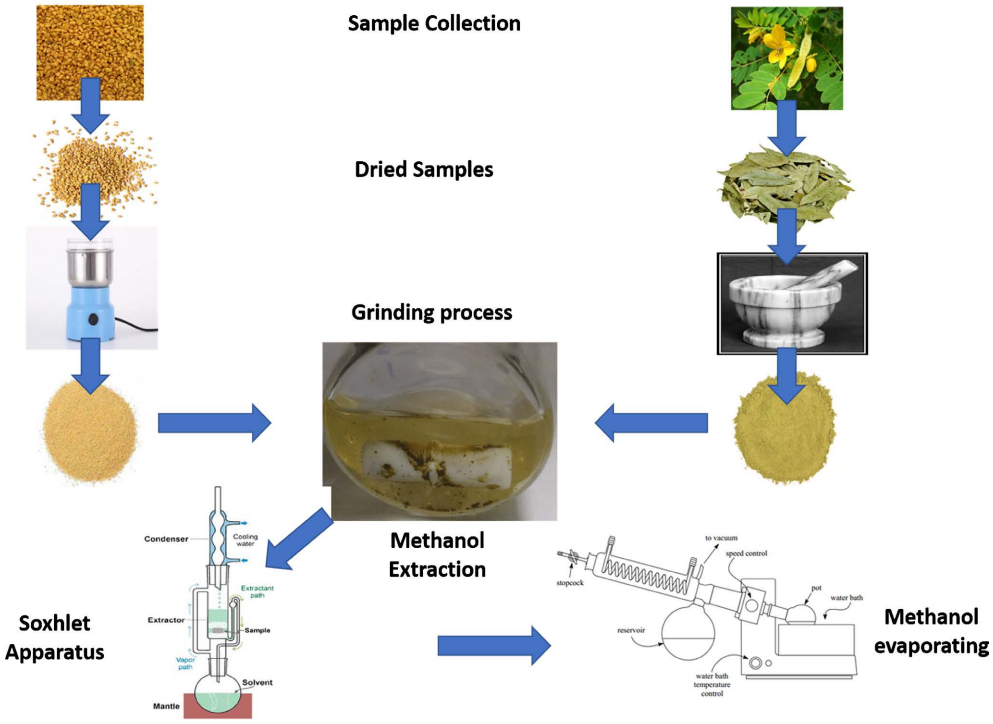

Figure 1. Samples preparation and crude extraction processes.

Table 1. Description of the cell line that used in the study.

Cancer cell line	ATCC No#	Cell Origin	Site of Tissue	Culture Medium
PC3	CRL-1435	Epithelial	Prostate; derived from metastatic site: bone	DMEM-F12
A549	CCL-185	Epithelial	Lung	DMEM-F12

(lung cancer) and PC3 (prostate cancer) cell lines were cultivated into DMEM medium, a low glucose variant (Gibco), encompassing 2 mM L-alanyl- L- glutamine, non-essential amino acids, penicillin-streptomycin, 5% fetal calf serum (Atlanta Biologicals) and also supplemented with 0.01 mg/mL insulin and 1 mM sodium pyruvate. Cells were incubated in 5% CO_2 at 37˚C and passaged once a fortnight after attainment of 80% confluency in 1:2/1:4 ratios for no more than forty passages.

2.2. Crystal Violet Cell Cytotoxicity Assay

Crystal violet cell cytotoxicity assay was used to detect cell viability. Crystal violet is a triarylmethane stain that can attach to ribose-type molecules such as nucleic acid in the nucleus. Generally, dead cells will detach from cell culture plates and will be removed during washing steps. Crystal violet staining can be used to measure the total DNA of the remaining viable cells and thus determine cell viability. The Crystal violet dyeing is right proportional to the viable cell biomass and can be measured at 550 nm. Crystal violet dyeing is a rapid and

useful assay for the detection of cell viability under various enhancement or suppression conditions.

Two types of cell lines (A549 and PC3) were cultivated into DMEM-F12 medium and incubated at 37˚C overnight at a 5% CO_2 incubator. When the cells reached the 60% - 80% confluence, trypsin was added and consequently by centrifugation to remove it. 5 ml of DMEM-F12 medium was added to disperse the cells. 200 µl of cell suspension ((5 - 10) × 10^4 cells/ml) was added to a 96-well clear flat bottom microtiter plate, and the plate was allowed overnight to let the cells settle down and adhere to the plate. Meanwhile, the compounds were diluted in stock solution in DMSO appropriately, and the final DMSO concentration in each well should be 0.5% or less. The compounds were added to wells in triplicate to avoid processing errors, while the mixture of growth medium with DMSO (without compounds) was used as background control.

The plated cells were incubated at the optimal temperature (37˚C) and for optimal time (18 - 24 hours). Thereafter, the culture media were removed, and the cells were washed gently to avoid the upset of the cells layer, using 1X Phosphate Buffer Saline (PBS). The PBS solution was removed by pipetting, then 50 µl of 0.5% Crystal Violet Staining Solution with methanol was added to each well for 20 minutes at room temperature. Finally, the cells were washed using a stream of running water four times, and the fourth washing solution was decantated as much as possible by pipetting. Each well optical density (OD) was mustered at 550 nm using a microplate reader.

The percentage of cytotoxicity was obtained using the formula below (**Figure 2**).

2.3. Lipid Peroxidation Assay

Lipid peroxidation is the destruction of lipids resulting from oxidative damage and is a good indicator for oxidative stress. Polyunsaturated lipids are sensitive to an oxidative attack, naturally by reactive oxygen species, subsequent in a distinct chain reaction with the making of end products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). Lipid peroxidation may be associated with the occurrence of many diseases including atherosclerosis, diabetes, cancer, and Alzheimer's. The principle of lipid peroxidation assay is based on the direct reaction between hydroperoxide with ferrous ions to reduce the latter into ferric ions. The ferric ions are exposed by thiocyanate ions as the colored compound (**Figure 3**).

The standard curve of Aliquot the Lipid Hydroperoxide Standard (HP) and the chloroform-methanol mixture was prepared as described in **Table 2** and must be run at the same time with each set of samples.

$$\%\ \text{Cytotoxicity} = \frac{OD_{DMSO} - OD_{Sample}}{OD_{DMSO}} \times 100\%$$

Where: OD_{DMSO} is the OD of the DMSO control after background correction
OD_{sample} is the OD of the sample after background correction

Figure 2. Percentage of cytotoxicity formula.

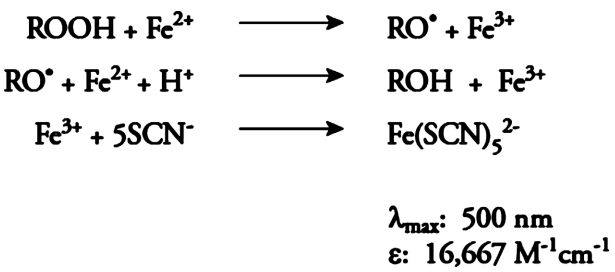

Figure 3. Lipid peroxidation assay reaction (oxidation/reduction reaction).

Table 2. Lipid peroxidation assay standard curve.

Lipid Hydroperoxide Standard (µl)	$CHCl_3$-CH_3OH (µl)	Final HP* (nmol)
0	950	0
10	940	0.5
20	930	1.0
30	920	1.5
40	910	2.0
60	890	3.0
80	870	4.0
100	850	5.0

2.4. Lipid Peroxidation Assay of Tested Samples

The tested cancer cell lines (A549 and PC3) were separately treated with Senna and Fenugreek extracts. The cells were cultivated on 6 flat bottom well plates until reached 70% - 80% confluent. The medium was then removed, DMSO solvent was added as a control into the first two wells. Three concentrations of the extracts were poured into the remaining three wells into a serum-free medium. The medium was removed gently by pipetting after 24 hours of incubation, and the cells were washed with 1 ml of 1× PBS. 600 µl of trypsin was added to the cells and the plate was incubated at room temperature for 3 hours. Thereafter, the cells were harvested and collected in microcentrifuge tubes, then 600 µl of the medium was added and centrifuged at 3000×g for 10 minutes. Finally, the supernatant was discarded, and the cell pellets were kept at −80˚C until used.

The frozen pellets were taken out of and located in an icebox. The cell pellets were destroyed using sonicator apparatus, then centrifuged in a cold centrifuge at 3000×g for 10 minutes, and the cell-free supernatant was utilized to measure the Malondialdehyde (MDA) level according to manufacturer's instruction (MD Biosciencesinc) with some modifications. The procedure in brief; 100 µl of aliquot supernatant was obtained, then R1 solution was added to each sample, then 0.2N of HCl (Chromogen) was added to the mixture and mixed well. The tubes were put at room temperature for 5 minutes, then the mixture and the previous standard were transferred to 96 flat bottom wells microplate. The result was obtained using a Microplate Reader (Bio-Tek Instruments) at wavelength 500 nm.

The MDA levels were calculated corresponding to the standard curve.

2.5. Caspase 3 Activity Assay

Caspase is a protease enzyme that has a highly conserved cysteine amino acid and plays an important role in cell apoptosis. Caspases are cleaved a wide range of cell substrates, such as nuclear proteins, mitochondrial proteins, plasma membrane proteins and resulting in cell death.

The assay is dependent on spectrophotometric detection of the p-nitroaniline (chromophore), after breaking down from the labeled substrate DEVD-p-nitroaniline. The p-nitroaniline light emission can be recognized using a spectrophotometer or a microtiter plate reader at wavelength 400 or 405 nm. The result is obtained by comparing the absorbance of p-nitroaniline released from the apoptotic cell with background control (untreated) Allows identification of a rise in Caspase-3 activity level.

The cancer cell lines (A549 and PC3) were prepared as mentioned above in section 3.5.2. Then the extract of Senna and Fenugreek was added separately in wells of serum-free medium and incubated at room temperature for 6 hours. After the incubation period, the cells were washed with 1× PBS. Thereafter, 500 µl of cold cell lysis buffer was added to the cells to make the suspension, then kept on an icebox or for 10 minutes. After that, the suspension was separated using a centrifuge at 10,000×g for 1 minute. The cell-free supernatant was transferred to a new tube and kept on ice. 100 µl of 2× reaction buffer (10 mM DTT), and 10 µl of 200 mM of DEVD-p-NA substrate were added and mixed very well, then incubated at 37˚C for 2 hours. Eventually, the OD was obtained using a microplate reader at wavelength 400 nm. The steps of the caspase 3 activity were summarized in **Figure 4**.

2.6. Statistical Analysis

The mean of standard deviation was used to calculate the result data. The software StatView 5.0.1 was used to perform a one-way ANOVA study (SAS Institute Inc., Cary, NC). Statistical significance was demarcated as $p < 0.05$.

$$\sigma = \sqrt{\frac{\sum (X - \mu)^2}{N}}$$

- σ = population standard deviation
- Σ = sum of…
- X = each value
- μ = population mean
- N = number of values in the population

3. Results

3.1. Effects of *Senna* Leaf on the Inhibition of A549 and PC3 Cells Proliferation

The two types of tested cell lines lung cancer A549 and prostate cancer PC-3

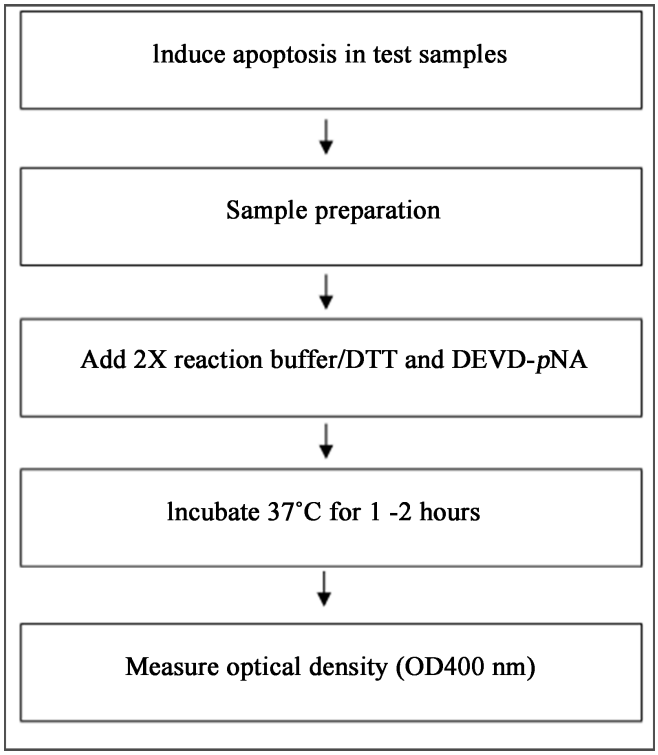

Figure 4. Caspase 3 activity assay steps.

were cultivated under aseptic conditions, in either presence or absence (background control) of different concentrations of *Senna* leaf crude extract. The tested cell lines were plated in 96 clear flat-bottom microplates with a cell density of approximately 10,000 cells/well. Different concentrations of Senna leaf crude extract (1.8, 0.9, 0.23, 0.11, and 0.029 µg/ml) were added separately to wells to assess the effect of the extract. The result was obtained in comparison with the background control.

3.2. Effects of *Senna* Leaf Extract on the Inhibition of A549 Cells Proliferation

Effects of Senna leaf extract on lung cancer cell line (A549) growth were determined by the crystal violet cell cytotoxicity assay after 24 hours of incubation. The result revealed that the high concentration of leaf extracts leads to reducing the proliferation of the A549 cell line. The percentage of cell viability was detected to be the highest in the background control sample (100%), which was not mixed with the tested extract, then the percentage of different concentrations of the extract was calculated against the absorbance of control at 550 nm.

The significant decrease in A549 cells proliferation was clearly shown with 1.8 µg/ml (64.5%) of leaf extract, where the lowest effect was observed with a low concentration of extract (0.029 µg/ml) (18.2%). All results of *Senna* leaf extract on the Inhibition of A549 cells proliferation assay were represented in **Figure 5**, while the effects of Senna leaf extract on A549 cell line under microscopic were illustrated in **Figure 6** ($p < 0.05$, unpaired t-test).

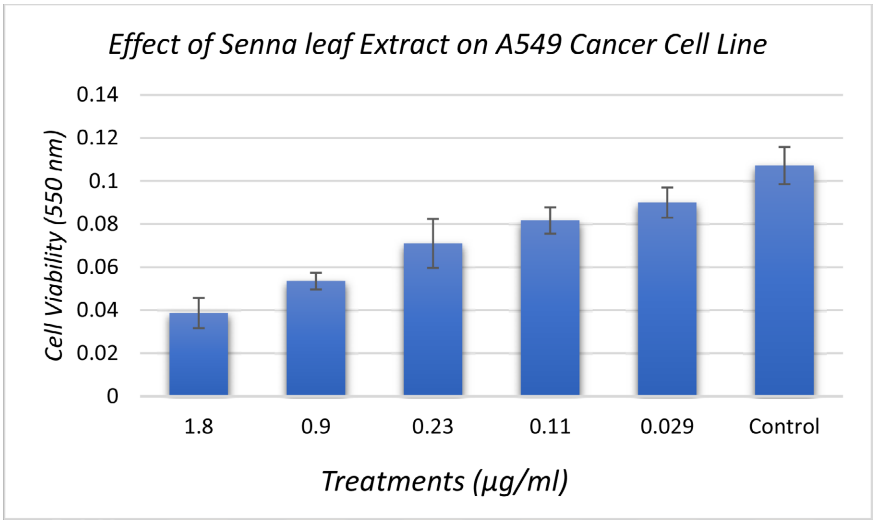

Figure 5. Viability Analysis of Lung cancer cell line A549 After Exposure to *Senna* leaf extract. Cells were plated at 1×10^4 cells per well in a 96-well plate. Values are presented as means (n = 3) ± S.D.*Statistical difference ($p < 0.05$).

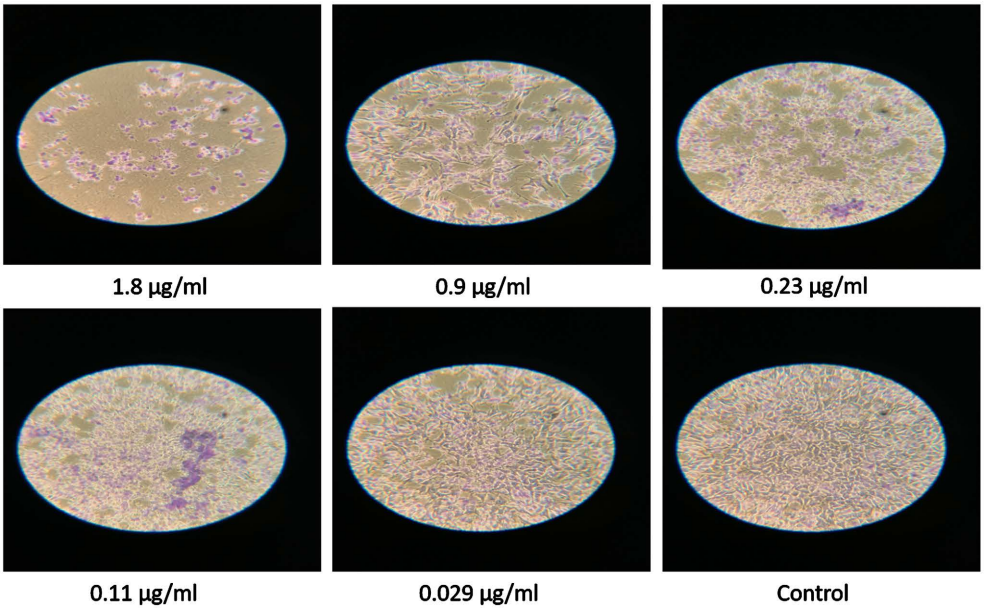

Figure 6. The evaluation of *Senna* leaf extract effects on cell viability in A549 lung cancer cells at different concentrations (1.8, 0.9, 0.23, 0.11, 0.029 µg/ml, and control) under 40× magnification.

3.3. Effects of *Senna* Leaf Extract on the Inhibition of PC-3 Cells Proliferation

The effects of *Senna* leaf extract were also tested on prostate cancer cell lines (PC-3). Our result showed a significant decrease in the viability of the PC-3 cell line about 69.1% at 1.8 µg/ml of leaf extract. Moreover, the lowest concentration of extract (0.029 µg/ml) still gave a reduction in cell line proliferation (18.2%) compared to background control (100%) as shown in **Figure 7**, while the effects of Senna leaf extract on P3 cell line under microscopic were illustrated in **Figure 8** (*P-value* < 0.05).

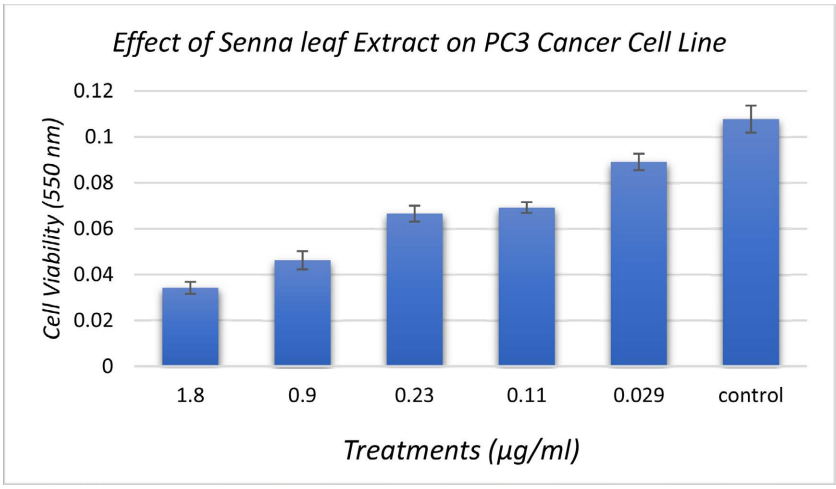

Figure 7. Viability Analysis of Prostate cancer cell line PC3 After Exposure to *Senna* leaf extract. Cells were plated at 1×10^4 cells per well in a 96-well plate. Values are presented as means (n = 3) ± S.D.*Statistical difference ($p < 0.05$).

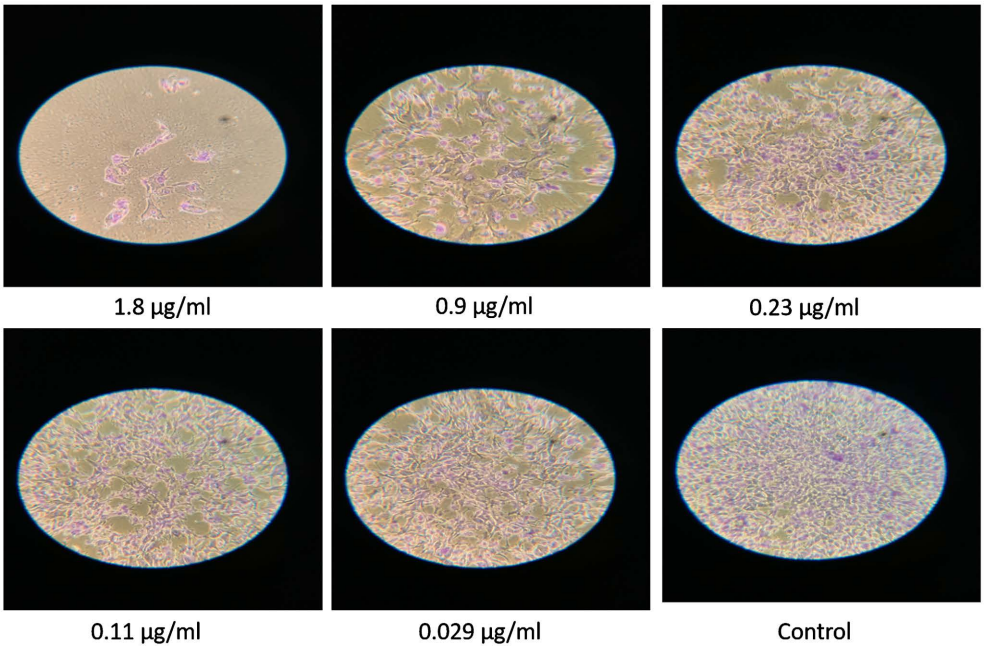

Figure 8. The evaluation of *Senna* leaf extract effects on cell viability in PC-3 prostate cancer cells at different concentrations (1.8, 0.9, 0.23, 0.11, 0.029 µg/ml, and DMSO control) under 40× magnification.

3.4. Effects of Fenugreek Seed on the Inhibition of A549 and PC-3 Cells Proliferation

Also, the effects of Fenugreek seed extracts were investigated against lung cancer A549 and prostate cancer PC-3 cell lines. Different concentrations of Fenugreek seed crude extract (2.2, 1.1, 0.275, 0.138, and 0.0343 µg/ml) were added separately to wells to assess the effect of the extract. The result was obtained in comparison with the background control.

All concentrations of extract obtained a significant reduction in both cells'

viability. The highest cell viability reduction was detected at 2.2 µg/ml in A549 and PC-3 cell lines by 60% and 65.5% respectively. Moreover, the lowest concentration of Fenugreek seed extracts (0.0343 µg/ml) obtained cell viable reduction in both A549 and P3 cell lines by 16% and 11.9% respectively. Noteworthy, at a concentration range (0.275 - 0.138 µg/ml) of Fenugreek seed extract, approximately a similar reduction of cell viability on the A549 cell line was observed (Figure 9), while a clear difference was shown with the PC-3 cell line (Figure 10). Evaluation of morphological changes of A549 lung cancer and PC-3 prostate cell lines were illustrated in Figure 11 and Figure 12 respectively.

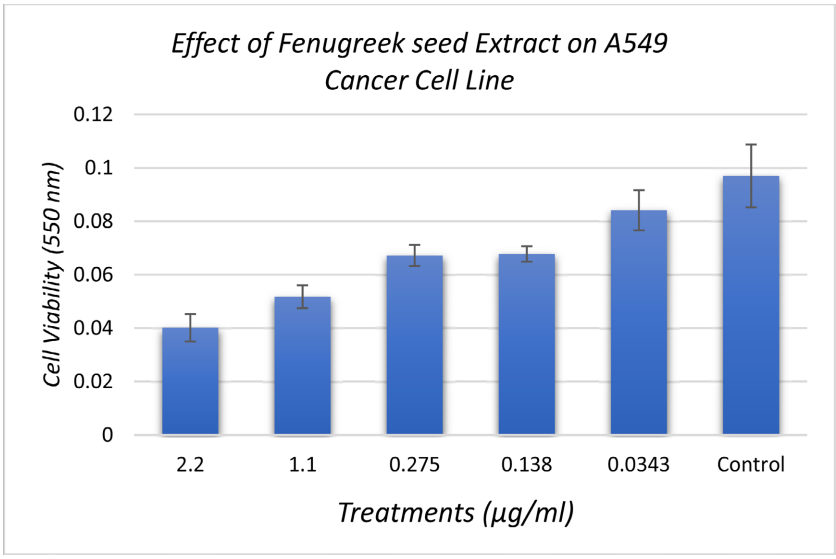

Figure 9. Viability Analysis of Lung cancer cell line A549 after Exposure to Fenugreek seed extract. Cells were plated at 1×10^4 cells per well in a 96-well plate. Values are presented as means (n = 3) ± S.D. *Statistical difference ($p < 0.05$).

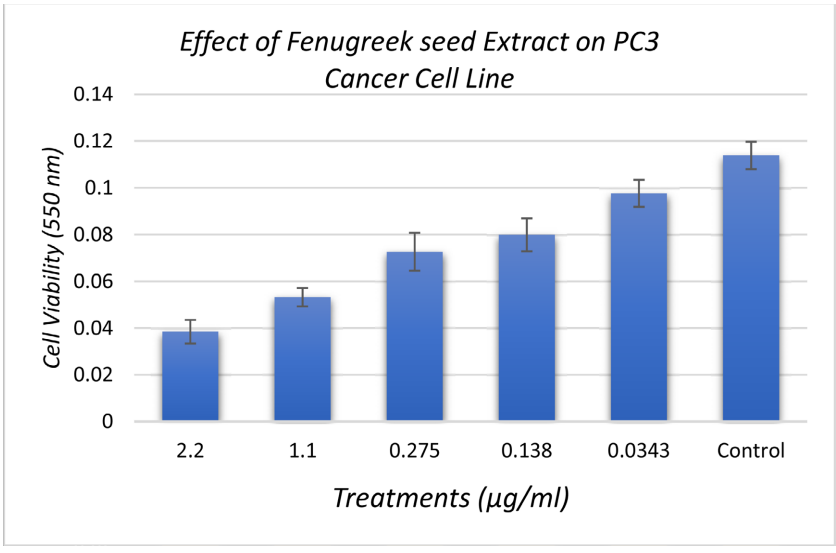

Figure 10. Viability Analysis of Prostate cancer cell line PC-3 After Exposure to Fenugreek seed extract. Cells were plated at 1×10^4 cells per well in a 96-well plate. Values are presented as means (n = 3) ± S.D.*Statistical difference ($p < 0.05$).

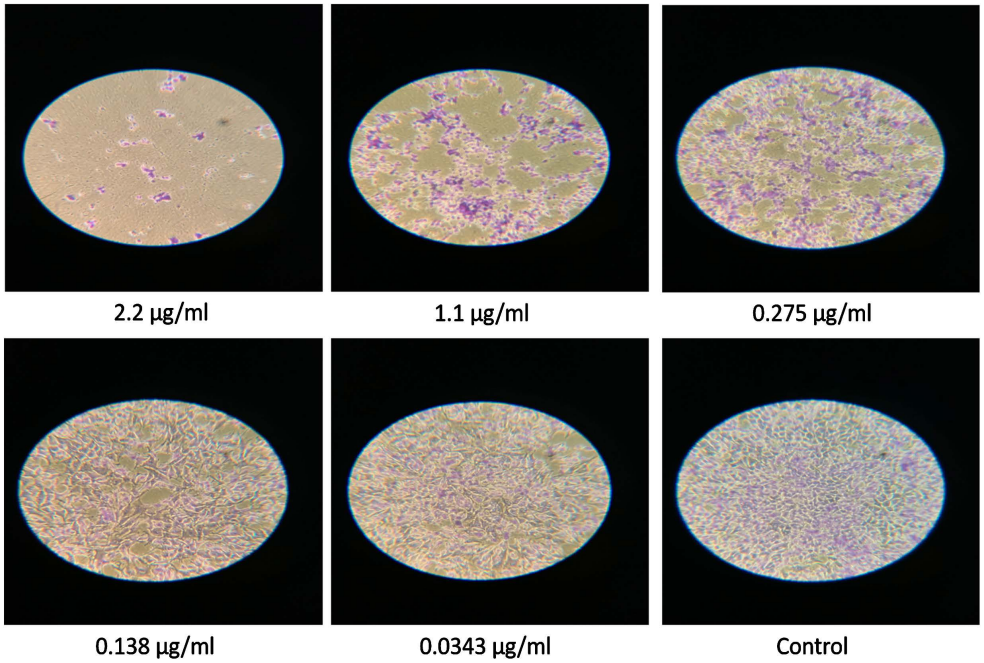

Figure 11. Evaluation of Fenugreek seed extract effects on cell viability in A549 lung cancer cells at different concentrations (2.2, 1.1, 0.275, 0.138, 0.0343 µg/ml, and DMSO control) under 40× magnification.

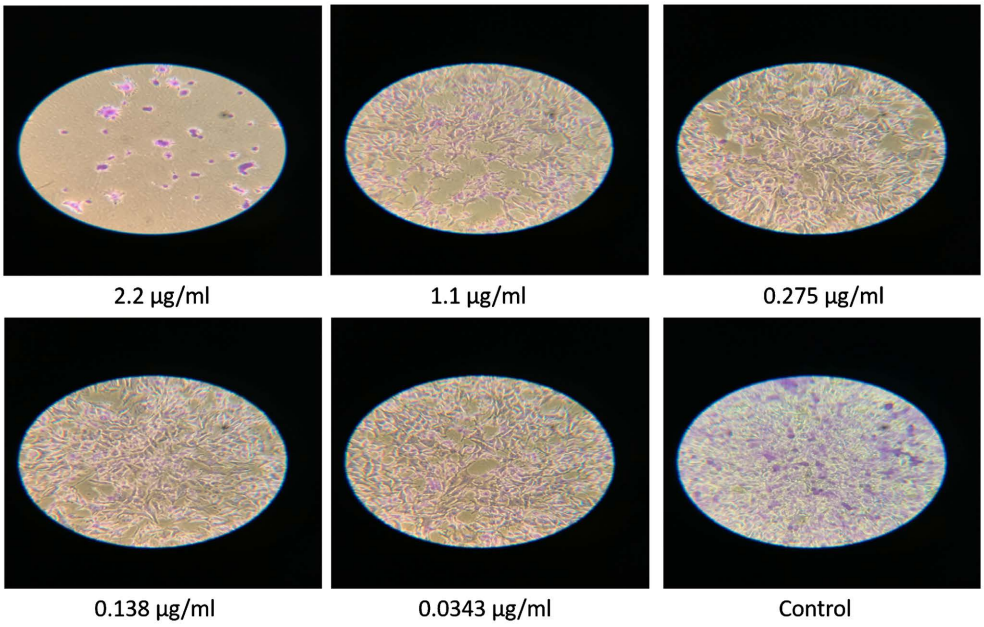

Figure 12. Evaluation of Fenugreek seed extract effects on cell viability in PC-3 prostate cancer cells at different concentrations (2.2, 1.1, 0.275, 0.138, 0.0343 µg/ml, and DMSO control) under 40× magnification.

3.5. Effects of *Senna* Leaf Extract on Lipid Peroxidation of Lung Cancer A549 and Prostate Cancer PC-3 Cell Lines

The effect of different concentrations of *Senna* leaf extracts in the A549 lung cancer and PC-3 prostate cancer cell line was tested. The cell lines were culti-

vated separately in clear flat bottom 96 microplate wells (approximately 10,000 cells/well), then the cells were treated with several concentrations of *Senna* leaf extracts (0.11, 0.23, 0.46, and 0.9 µg/ml) and incubated for 24 hours. Our results revealed that the MDA level was inversely proportional to the concentrations of extract in both the A549 cell line (**Figure 13**) and PC-3 cell line (**Figure 14**) with some preferred effect in the A549 lung cancer cell line.

3.6. Effects of Fenugreek Seed Extract on Lipid Peroxidation of Lung Cancer A549 and Prostate Cancer PC-3 Cell Lines

The effect of different concentrations of *Fenugreek seed* extracts in the A549 lung cancer and PC-3 prostate cancer cell line was also tested. The cell lines were cultivated separately in clear flat bottom 96 microplate wells (approximately

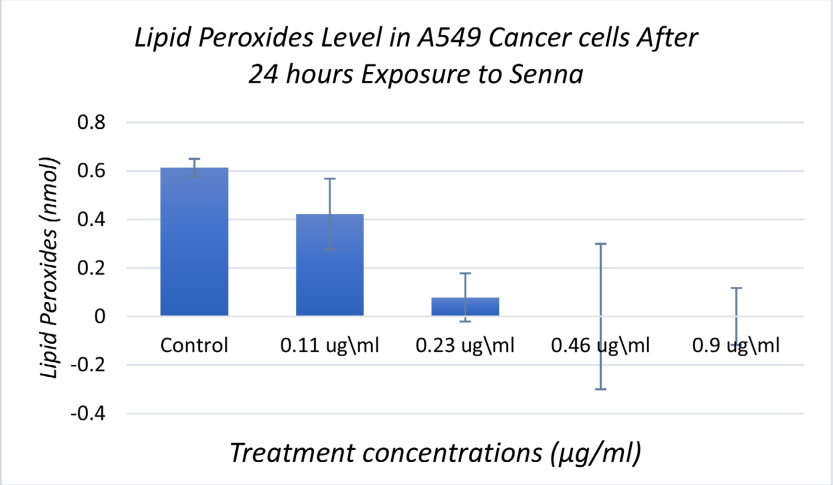

Figure 13. Effect of different concentrations of *Senna* leaf extract on lipid peroxides levels in A549 cancer cell line. ($p < 0.05$).

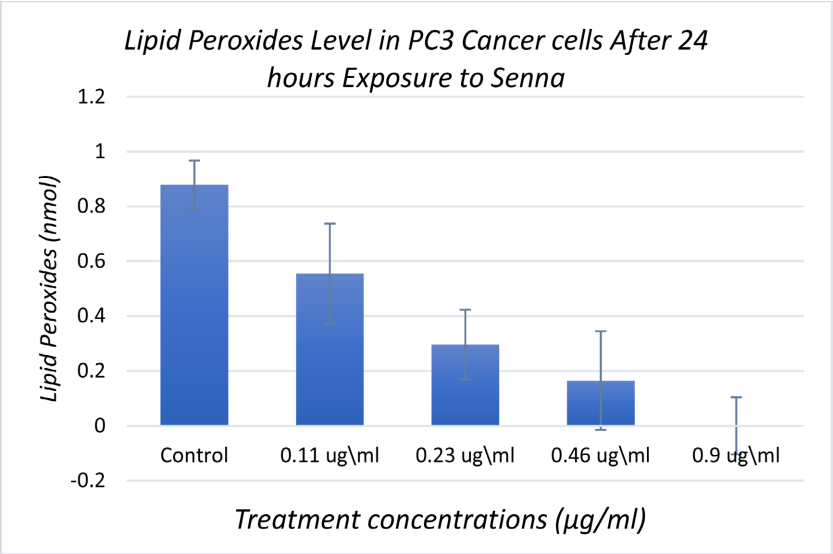

Figure 14. Effect of different concentrations of *Senna* leaf extract on lipid peroxides levels in PC-3 cancer cell line. ($p < 0.05$).

10,000 cells/well), then the cells were treated with several concentrations of *Senna* leaf extracts (0.138, 0.275, 0.55, and 1.1 µg/ml) and incubated for 24 hours. Our results revealed that the MDA level was also inversely proportional to the concentrations of extract in both the A549 cell line (Figure 15) and P3 cell line (Figure 16) with no significant variation in the effectiveness between A549 lung cancer and PC-3 prostate cell lines.

3.7. Effects of *Senna* Leaf Extract on Caspase 3 Expression of Lung Cancer A549 and Prostate Cancer PC-3 Cell Lines

The caspase-3 activity in A549 and PC-3 cancer cell lines was investigated with different concentrations of Senna leaf extracts. The cells were first cultivated in

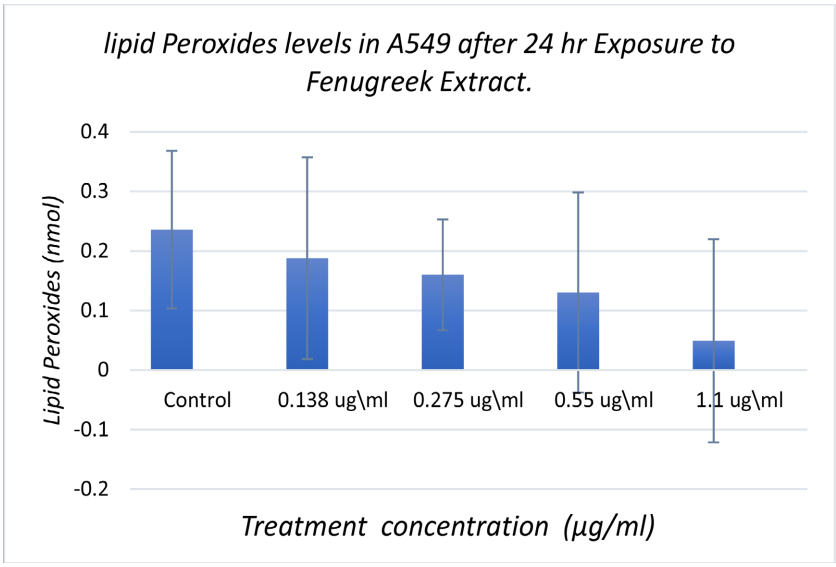

Figure 15. Effect of different concentrations of Fenugreek seed extracts on lipid peroxides levels in A549 cancer cell line. ($p < 0.05$).

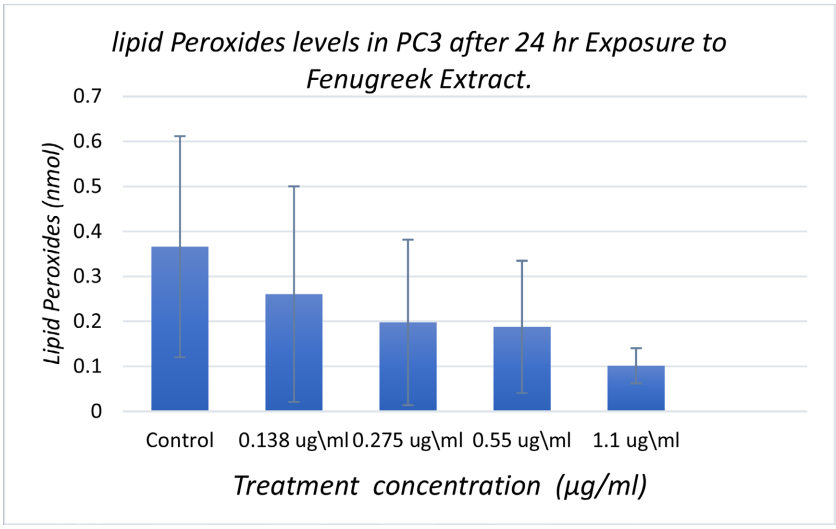

Figure 16. Effect of different concentrations of Fenugreek seed extracts on lipid peroxides levels in PC-3 cancer cell line. ($p < 0.05$).

a clear flat-bottom 6-well plate until reaches 70% - 80% confluent. Therefore, A549 lung cancer and PC-3 prostate cancer cell lines were treated with different two concentrations of extract (0.9 and 0.23 µg/ml). Our results indicated a significant increase in caspase 3 activity in both treated cell lines (A549 and PC-3) if compared with untreated control (cell line + DMSO) as illustrated in Figure 17 and Figure 18 respectively. $p < 0.05$ as compared to the background controls (cell line + DMSO), using the unpaired Student *t*-test.

3.8. Effects of *Fenugreek* Seed Extract on Caspase 3 Expressions of Lung Cancer A549 and Prostate Cancer PC-3 Cell Lines

Also, the caspase-3 activity in A549 and PC-3 cancer cell lines was tested with different concentrations of Fenugreek Seed extracts. The cells were prepared as mentioned in Section 4.5 in the clear flat bottom 6-wells plate until reached 70% - 80% confluent. Therefore, A549 lung cancer and PC-3 prostate cancer cell lines were treated with different two concentrations of extract (1.1 and 0.275 µg/ml).

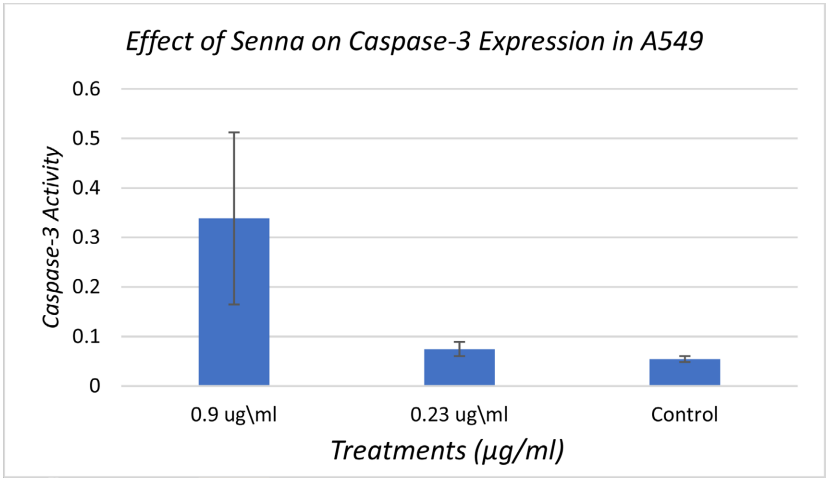

Figure 17. Effect of *Senna* leaf extract on the caspase-3 activity of A549 cells. Cells were treated with or without 0.9 and 0.23 µg/ml. The untreated cell was used as a control.

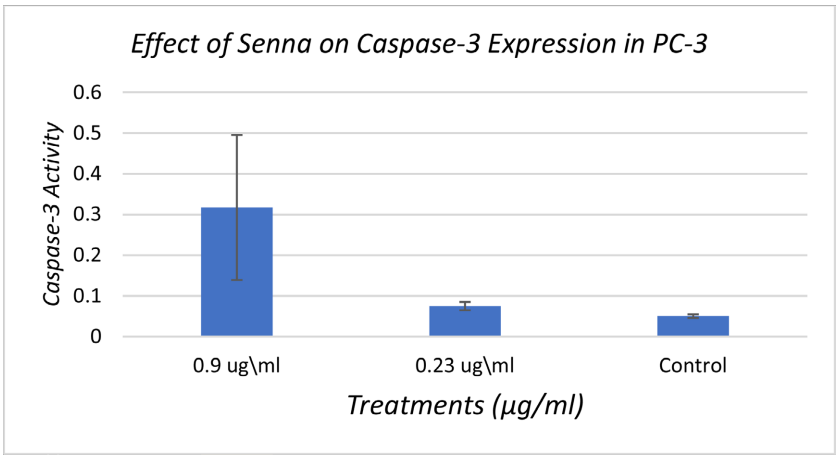

Figure 18. Effect of *Senna* leaf extract on the caspase-3 activity of PC-3 cells. Cells were treated with or without 0.9 and 0.23 µg/ml. The untreated cell was used as a control.

Our results indicated that a significant increase in caspase 3 activity in both treated cell lines (A549 and PC-3) if compared with untreated control (cell line + DMSO) as illustrated in Figure 19 and Figure 20 respectively. $p < 0.05$ as compared to the untreated controls, using the unpaired Student *t*-test.

4. Discussion

4.1. Effects of *Senna* Leaf and *Fenugreek* Seed Extract on the Inhibition of A549 and PC-3 Cell Line Viability

The bioactive compounds in the *Senna* leaf and Fenugreek seed were extracted using the absolute methanol method, then the methanol was evaporated using a rotatory evaporator. The resulting extract was resuspended in the appropriate concentrations of MDSO to investigate their effects on cell line viability. The

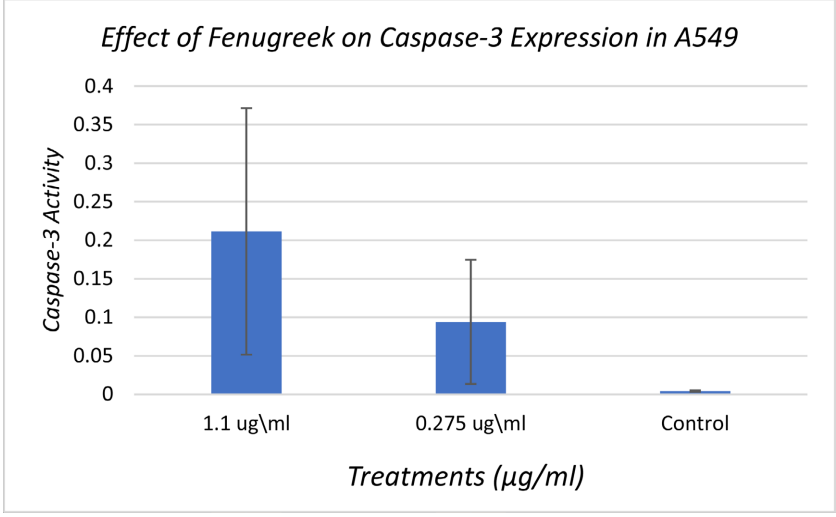

Figure 19. Effect of fenugreek seed extracts on the caspase-3 activity of A549 cells. Cells were treated with or without 1.1 and 0.275 µg/ml. The untreated cell was used as a control.

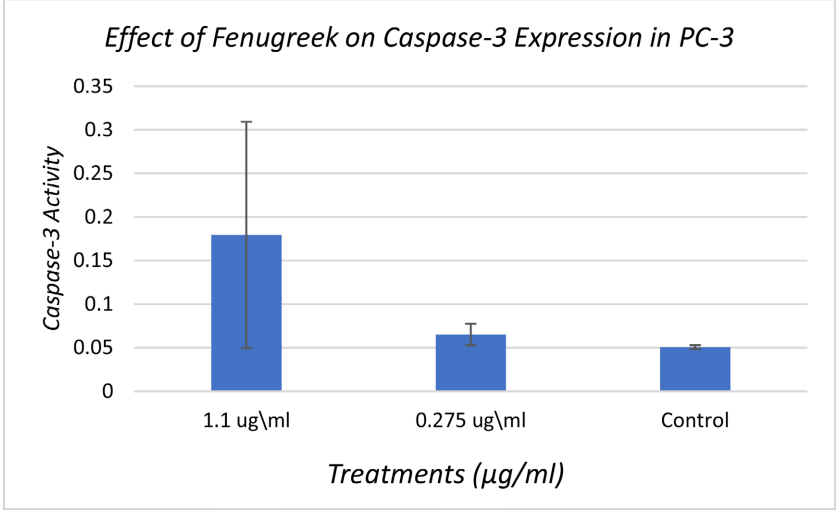

Figure 20. Effect of fenugreek seed extracts on the caspase-3 activity of PC-3 cells. Cells were treated with or without 1.1 and 0.275 µg/ml. The untreated cell was used as a control.

results of this study support the hypothesis that *Senna* plant extracts contain substances with biological characteristics that have a positive effect on cancer cell inhibition in vitro. Our results showed the effectiveness of *Senna* extracts in inhibiting two types of cancer cell derivatives (A549 lung cancer and PC-3 prostate cancer) at all used concentrations in a consistent proportion. If the concentration of the extract increased, the percentage of cell inhibition increased. This effectiveness is attributed to the presence of bioactive compounds, including different types of flavonoids, which were reported in several studies. A study conducted by Xiujuan and his colleagues on experimental animals showed that flavonoids extracted from the *Senna* plant worked to reduce the size and weight of tumors [15]. Another study showed that *Senna* plant extracts inhibit the growth of HT1080 human fibrosarcoma cells through apoptotic cell death [16]. The anticancer activity of flavonoids, attributed to their antioxidant properties, is consistent with their ability to clean out free radicals, defeat radical oxygen species (ROS) and enzymes, and inhibit intracellular and extracellular complex oxidation [17]. Another study found that the hexane fraction of *Senna* has a cytotoxic effect against A549 lung cancer cells and OV2008 ovarian cancer cells [18].

On the other hand, the A549 lung cell line and PC-3 prostate cell line antiproliferation were tested against Fenugreek seed extract with different concentrations (2.2, 1.1, 0.275, 0.138, and 0.0343 μg/ml). Our findings also showed the antiproliferation of both cell lines that were treated with the extracts at all concentrations, and the cell viability decreased gradually with the increase of extract concentrations. Our results support the hypothesis that the anticancer properties of fenugreek seed are due to the presence of many active compounds such as phenolic acids. A study conducted by Mbarki and his colleagues showed *Fenugreek* extract increased free radical clean-up activity against CCl4-induced renal and liver damage in rats, which prevented oxidative damage [19]. Another study performed by Jagadeesan in 2012 observed the anticancer action of diosgenin, which is found in *fenugreek*, in breast cancer-induced rats [20]. Moreover, comparing the effectiveness of *Senna* leaf extracts and *Fenugreek* seed on the two types of cancer cells that were used in the study, the results showed the advantage of *Senna* leaf extracts (64.5% with A549 and 69.1% with PC-3) over *Fenugreek* seed extracts (60.0% with A549 and 65.5% with PC-3), suggesting that the concentrations used for *Senna* leaf extracts in the study were lower than the concentrations of *Fenugreek* seed. Also, the effectiveness of both extracts was obtained with a good result with the PC-3 cell line (prostate cancer) compared to the A549 cell line (lung cancer) antiproliferation.

4.2. The Effects of *Senna* Leaf and *Fenugreek* Seed Extract on Lipid Peroxidation in Lung Cancer A549 and Prostate Cancer PC-3 Cell Lines

Lipid peroxidation is one of the key sources of oxidative damage introduced by reactive oxygen species (ROS) and it has played a main role in altering the membrane structure and enzyme inactivation. In our study, the levels of lipid

peroxidation were measured by the thiobarbituric acid reactive substances assay. Thiobarbituric acid can be used to detect MDA by producing pink-colored products. Our finding showed the presence of an inversely proportional relationship between the concentration of the extracts and MDA levels in tested cell lines. This means that increasing the concentration of extracts leads to a significant decrease in MDA levels. At high concentrations, *Senna* leaf extract at high concentrations, including 0.4 and 0.9 μg/ml decreased the MDA level to zero on the A549 lung cancer cell line, and the decrease was continuous even at low concentrations (**Figure 13**). Unlike the PC-3 prostate cancer cell line, the extracts showed a similar result, with some differences in concentrations. The level of MDA disappeared at a 0.9 g/ml concentration only (**Figure 14**). On the other hand, *Fenugreek* seed extracts showed similar results when used on A549 and PC-3 cell lines, with some variations. The MDA levels were decreased by about 63% on the A549 cell line when treated with a 1.1 μg/ml extract concentration (**Figure 15**), and about 73.3% on the PC-3 cell lines that were treated with the same concentration (**Figure 16**). Notably, *Senna* leaf extracts were found to be slightly superior in reducing MDA levels in the A549 cell line compared to the PC-3 cell line, whereas *Fenugreek* seed extracts had the opposite effect on the cell lines. In a study conducted by Ishak *et al*., who administered an aqueous extract of *Senna* leaf to hyperglycemic rats, they observed a reduction in MDA levels [21]. In another study examining the effects of *Fenugreek* extract on liver lipid peroxidation, antioxidant status, and tumor incidence in colon cancer-induced *in vivo*, it was found that *Fenugreek* extract decreased tumor incidence by 16.6%, increased the antioxidant enzyme levels, and decreased the lipid peroxidation [20].

Finally, it can be said that extracts of *Senna* leaf and *Fenugreek* seed can significantly reduce MDA levels, which is one of the most important products of the process of lipid peroxidation, which positively affects the decrease in ROS levels.

4.3. *Senna* Leaf and *Fenugreek* Seed Extracts' Effects on Lung Cancer A549 and Prostate Cancer PC-3 Cell Lines. Caspase-3 Expression

The caspase-3 enzyme has been involved in apoptotic cell death by activating a cascading of cellular protein degradation enzymes known as caspases. Caspase 3 can degrade numerous cell substrates such as poly-adenosine diphosphate ribose (ADP phosphate), and polymerase (PARP). The degradation of these substrates leads to morphological and biochemical changes that are observed in cell apoptosis. Our findings show that *Senna* leaf extracts and *Fenugreek* seed extracts increase caspase 3 expressions in both A549 and PC-3 cell lines.

As we mentioned above, the anticancer activity of extracts against cancer cell lines was attributed to several factors, including increased caspase 3 expression. In the present study, A549 and PC-3 cell lines were treated with two concentrations of *Senna* leaf (0.9 and 0.23 μg/ml) and *Fenugreek* seed extracts (1.1 and

0.275 μg/ml). The highest expression of caspase 3 was detected at 0.9 and 1.1 μg/ml of *Senna* leaf and *Fenugreek* seed extracts, respectively. Even low concentrations still showed significant induction of enzyme production. Our results confirmed that *Senna* leaf and *Fenugreek* seed extracts induced apoptosis of A549 and PC-3 cells in a caspase-dependent control. A study performed by Chen in 2005 proves that caspase enzymes have cytotoxic properties. Based on Chen study caspase enzymes are in charge of cell death, even that found in the target tumor tissue [22].

5. Conclusion

The results showed that *Senna* leaf extract and *fenugreek* seed had high efficacy against cancerous cells, which could be an effective treatment or prevention of cancers caused by PC-3 and A549 cell strains. Both *Senna* and *Fenugreek* have the potential to treat cancer cells and reduce lipid peroxidase levels as well as induce apoptosis in lung and prostate cancer cell lines A549 and PC3. The current study found that different concentrations of *Senna* leaf and *Fenugreek* extract reduced cell viability and lipid peroxidation in both the A549 lung cancer cell line and the PC-3 prostate cancer cell line. In addition, the extracts also obtained an increase in caspase 3 expression, which makes them a candidate for being a source for the manufacture of new anti-cancer drugs. Our results are consistent with the previous studies and will provide a window for further studies on this issue. Moreover, the traditional use of *Senna* and *fenugreek*, either in food consumption or in medicinal plants, may be useful in decelerating the development and progress of cancer, one of the most important health challenges throughout the world.

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Data Availability Statement

The published article comprises all the data that was generated or analyzed throughout the course of this research.

Conflicts of Interest

The authors declare no conflicts of interest.

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