# Laetrile and Methotrexate: A Dual-Drug Approach to Inhibiting Cervical Cancer Cell Proliferation

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**Abstract:** *Objective*: This study was done to evaluate the efficacy of laetrile methotrexate combination in inhibiting cervical cancer proliferation and identify the synergistic effects between them

*Method*: During three incubation periods, 24, 48, and 72 hours, the antiproliferative impact of the laetrile and methotrexate combination was assessed by employing the Hela cell line, a human cervical cancer cell line. The concentration range of the tested reagent varied from 0.1 to 1000 µg/ml for each laetrile and methotrexate. At the same time, the concentration of the mixture varied from 0.05 to 500 µg/ml for both laetrile and methotrexate. The combination index value was used to identify whether there was a synergistic impact between laetrile and methotrexate in the combination. The dose reduction index was employed to ascertain the extent of the reduction in the concentration of the constituent mix, which resulted in a notable cytotoxic impact.

*Result*: The study results revealed that the combination of laetrile and methotrexate successfully inhibited the proliferation of cervical cancer cells in a manner that depended on the concentration and duration of treatment. The combination index value exhibited a significant synergistic impact between laetrile and methotrexate in all concentrations except 1000 µg/ml. Furthermore, the dose reduction index outcomes showed that the combination minimized the required concentration of laetrile and methotrexate.

*Conclusion*: Concerning the study findings and their defined pharmacokinetic and safety characteristics of mixture drugs. The laetrile-methotrexate mixture offers an attractive and safer option for the treatment of cervical cancer.

**Keywords:** Cervical cancer, Hela cancer cell line, combination index, dose reduction index, cytotoxicity, laetrile, methotrexate.

## 1. INTRODUCTION

Throughout history, humans have identified and utilized various therapeutic plants. Plants can yield chemical molecules that play a vital role in several biological activities. At least 12,000 unique chemicals have been identified and separated [1-4].

In recent years, a significant amount of promising medical research has focused on the utilization of plants for the management and prevention of cancer. The main disadvantages of synthetic drugs are the potential harmful effects they may cause. Nevertheless, alternative therapies utilizing botanical substances or naturally occurring plant-derived compounds have exhibited promising outcomes in eliminating cancerous cells. In the 1950s, researchers initiated an inquiry into plant-derived anti-cancer medications after the identification and synthesis of vinca alkaloids (vinblastine and vincristine) and the detection of hazardous podophyllotoxins [1, 5-7].

Although Prunus armeniaca kernels have demonstrated potential anticancer properties in certain studies, Prunus americana has also exhibited promising effects in combating cancer [8-10]. The significant concentration of laetrile found in apricot kernels is thought to be the main factor contributing to their cancer-preventive effects. Studies have demonstrated that laetrile has analgesic properties and the additional advantage of selectively inducing apoptosis in cancer cells with reduced adverse effects [11]. Phytochemicals, in addition to laetrile, are plant-derived substances that have both anticarcinogenic and antioxidant characteristics [12, 13].

Laetrile and its derivative, often known as vitamin B17, are both promoted as therapies for cancer [14-17], Laetrile is a glycoside found in the seeds of the bitter almond tree (Prunus dulcis). Laetrile is found in different species of the Prunus genus, including the apricot (Prunus armeniaca) and the black cherry (Prunus serotina) [18-21].

Laetrile has been extensively advocated as an alternative anticancer drug, yet its effectiveness

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remains contentious. Advocates assert that laetrile specifically targets cancer cells by producing cyanide, which causes cytotoxicity while preserving healthy cells owing to their elevated quantities of the detoxifying enzyme rhodanese [22]. However, clinical studies have not substantiated these claims. A systematic review by Milazzo *et al*. (2011) found no reliable evidence supporting laetrile's anticancer effects, with some trials reporting cyanide toxicity in patients. Additionally, the U.S. Food and Drug Administration (FDA) has not approved laetrile due to insufficient scientific validation and potential health risks [23].

Chemotherapy is a conventional method used for treating cancer. The primary concern associated with chemotherapy treatment is its cytotoxic impact on both cancer cells and rapidly proliferating healthy cells [24, 25]. Several therapeutic herbs can be utilized to mitigate the adverse effects of chemotherapy. Apricot kernel's antioxidant properties indicate its potential to alleviate the detrimental effects of methotrexate [26].

Various research has explored the antiproliferative efficacy of combining laetrile with other substances. However, these studies have overlooked the examination of the cytotoxicity of the combination of laetrile and methotrexate on cervical cancer.

The goal of our study is to evaluate the cytotoxic effectiveness of laetrile and methotrexate on a cervical cancer cell line and determine whether they demonstrate any synergistic impact.

## 2. MATERIAL AND METHODS

### 2.1. Methotrexate

Following dilution with the RPMI medium, the methotrexate raw material was employed at diverse concentrations, ranging from 0.1 to 1000 µg/ml.

### 2.2. Laetrile

The laetrile concentration varied from 0.1 to 1000 µg/ml and was acquired from Santa Cruz in Santa Cruz, CA, USA. The concentrations of laetrile were obtained by diluting it with the RPMI medium.

### 2.3. Cell Culture

The HeLa cancer cell line, derived from human malignant cervical cancer, was first established in the tissue culture unit at ICCMGR. Under regulated circumstances, the cells were grown in tissue culture containers with a surface area of 75 cm2, relative humidity of 37 degrees Celsius, and 5% CO2. To culture the cells, they were grown in RPMI-1640 medium (Sigma Chemicals, England) that included 10% fetal calf serum (FBS) and 100 U/mL penicillin-streptomycin (100 µg/mL streptomycin) [27].

### 2.4. Cytotoxicity Assay

Grown cervical cancer cells were exposed to laetrile – methotrexate mixture on a 96-well microtiter plate. During the logarithmic development phase, cancer cell concentration consistently grew, and the toxicity of the studied drugs was estimated at different incubation periods [28].

Each well consists of 10,000 cells. Seeding is the process of employing a medium that contains 10 % of fetal bovine serum. To facilitate cell adhesion, the plates were placed in an incubator set at a temperature of 37°C for 24 hours. Serial dilutions were conducted using free serum RPMI media. Laetrile and methotrexate were diluted in RPMI medium without calf serum to create a range of dilutions from 0.1 to 1000 µg/ml for each constituent. For the mixture of laetrile and methotrexate, the concentration varied from 0.05 to 500 µg/ml for each mixture of ingredients [14].

After 24 hours of cancer cell incubation, the cancer cells were exposed to six replicates with 200 µl for each treatment. For the control wells, 200 µl of maintenance media was added to each well, and the exposure durations ranged from 24 to 72 hours. Afterwards, the plates were hermetically sealed using self-adhesive material and returned to the incubator. The cells were then stained with MTT dye.

The optical density of each well was quantified using a microtiter plate ELISA reader at a transmission wavelength of 550 nm [28, 29].

The inhibition rate is calculated using the following mathematical equation [28].

$$growth\ inhibition\ \% = \frac{optical\ density\ of\ control - optical\ density\ of\ treated\ wells}{optical\ density\ of\ control\ wells} \times 100\%$$

### 2.5. Drug Combination Studies

The study analyzed the combined effects of the drug mixture by measuring concentration-response curves. These curves tracked the percentage of cells with inhibited growth at different drug concentrations after 24, 48, and 72 hours of treatment. To assess whether the interaction was synergistic, additive, or

antagonistic, the Compusyn software (Biosoft, Ferguson, MO, USA) was employed to estimate the combination index (CI) and dose reduction index (DRI) values.

A combination index (CI) value below 1 suggests a synergistic effect between drugs, meaning they work better together. A CI equal to 1 indicates an additive effect, while a CI above 1 signals antagonism—where the drugs interfere with each other's effectiveness.

The dose reduction index (DRI) measures how much each drug's dosage can be lowered in combination therapy while still achieving the same effect as when used alone. A DRI of 1 means no dose reduction is possible. A DRI greater than 1 is beneficial, allowing for lower doses, while a DRI below 1 suggests that reducing the dose would weaken treatment efficacy [30].

### 2.6. Research Ethics

No human subjects were involved during this study.

### 2.7. Statistical Analysis

The research parameters were examined using the Statistical Analysis System (SAS) to evaluate the impact of various factors. The study utilized the mean, standard error, and a Least Significant Difference (LSD) test to compare the means [31].

The study utilized uppercase and lowercase letters in data tables to differentiate between various statistical groupings or significance levels; means (averages) sharing the same letter are not significantly different, whereas means with differing letters are statistically significant. This method facilitates efficient communication of intricate statistical results without necessitating detailed elaboration. Readers can readily discern the similarities or differences among groups based on the assigned letters.

## 3. RESULTS

### 3.1. Cytotoxicity Study

#### *3.1.1. Laetrile Cytotoxicity*

The cytotoxicity of laetrile on cervical cancer cells significantly increased with higher concentrations of laetrile. The maximum growth inhibition of cervical cancer cells was observed at 1000 µg/ml. Additionally, cervical cancer cell growth inhibition increased significantly with longer incubation periods. The maximum growth inhibition was observed after 72 hours of incubation, compared to other incubation periods Table **1**, Figure **1**.

#### *3.1.2. Methotrexate Cytotoxicity*

The outcomes of the cytotoxic impact of methotrexate on cervical cancer exhibited a pattern that depended on the concentration. Nevertheless, as the incubation period advanced, there was a decline in the cytotoxicity of MTX, suggesting that cancer cells acquired significant resistance to the cytotoxic effects of MTX Table **2**, Figure **2**.

#### *3.1.3. The Cytotoxicity of Laetrile-Methotrexate Mixture*

The investigation revealed that the most significant suppression of cancer cell proliferation occurred at a 500 µg/ml concentration for each mixture component. The combination exhibited cytotoxicity that increased over time, resulting in a more significant suppression of cellular growth during the highest incubation period Table **3** Figure **3**.

Moreover, the comparison of methotrexate, laetrile, and mixture cytotoxicity demonstrated the superior

**Table 1: Laetrile's Effect on Hela Cancer Cell Growth Suppression**

| Concentration (µg/ml) | Mean ± SE | | | LSD value |
|---|---|---|---|---|
| | Incubation period | | | |
| | 24 hr. | 48hr. | 72hr. | |
| 0.1 | D 0 ± 0.0 | B 0 ± 0.0 | C 0 ± 0.0 | N. S |
| 1 | CD 6 ± 1.155 b | A 30 ± 2.887 a | B 32 ± 1.155 a | 10.28 * |
| 10 | BC 13 ± 1.155 b | A 33 ± 1.732 a | AB 36 ± 0.577 a | 7.04 * |
| 100 | AB 20 ± 2.887 b | A 35 ± 2.887 a | AB 38 ± 1.732 a | 14.46 * |
| 1000 | A 29 ± 2.309 b | A 37 ± 1.155 ab | A 40 ± 1.155 a | 9.22 * |
| LSD value | 10.16 * | 11.52 * | 6.16 * | --- |

*: Significant at (P≤0.05), N. S: Non- Significant).

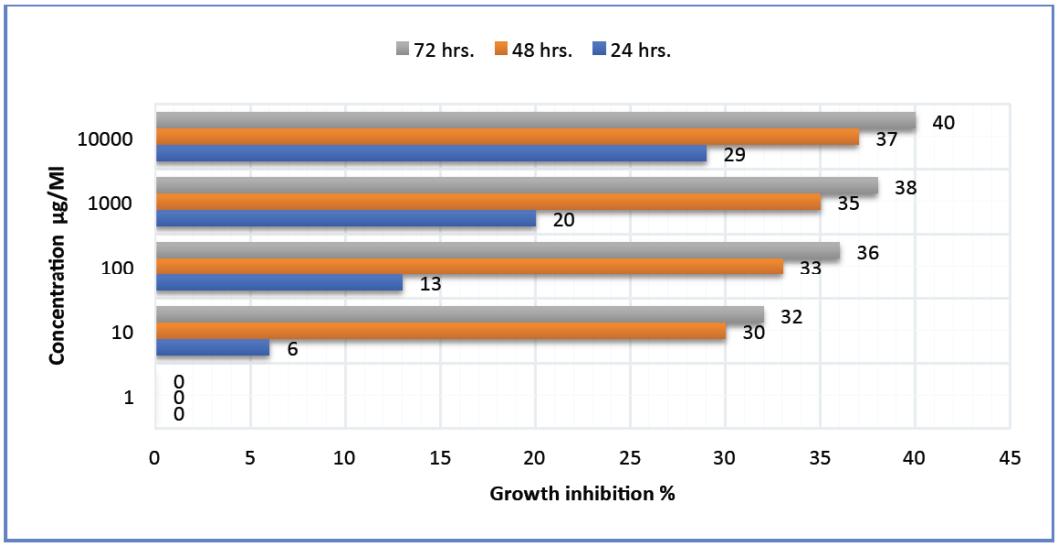


**Figure 1:** Laetrile's effect on Hela cancer cell growth suppression.

**Table 2: Methotrexate's Effect on Hela Cancer Cell Growth Suppression**

| Concentration (µg/ml) | Mean ± SE | | | LSD value |
|---|---|---|---|---|
| | Incubation period | | | |
| | 24 hr. | 48hr. | 72hr. | |
| 0.1 | D 0 ± 0.0 | C 0 ± 0.0 | C 0 ± 0.0 | NS |
| 1 | C 16 ± 0.577 a | BC 3 ± 1.155 b | C 0 ± 0.0 b | 4.22 * |
| 10 | C 17 ± 1.155 a | AB 11 ± 1.732 a | C 0 ± 0.0 b | 6.78 * |
| 100 | A 28 ± 1.732 a | A 13 ± 1.732 b | A 15 ± 1.732 b | 9.78 * |
| 1000 | A 35 ± 2.887 a | A 17 ± 1.155 b | A 25 ± 1.732 ab | 11.6 * |
| LSD value | 10.16 * | 8.3 * | 6.9 * | --- |

*: Significant at (P≤0.05), N. S: Non- Significant).

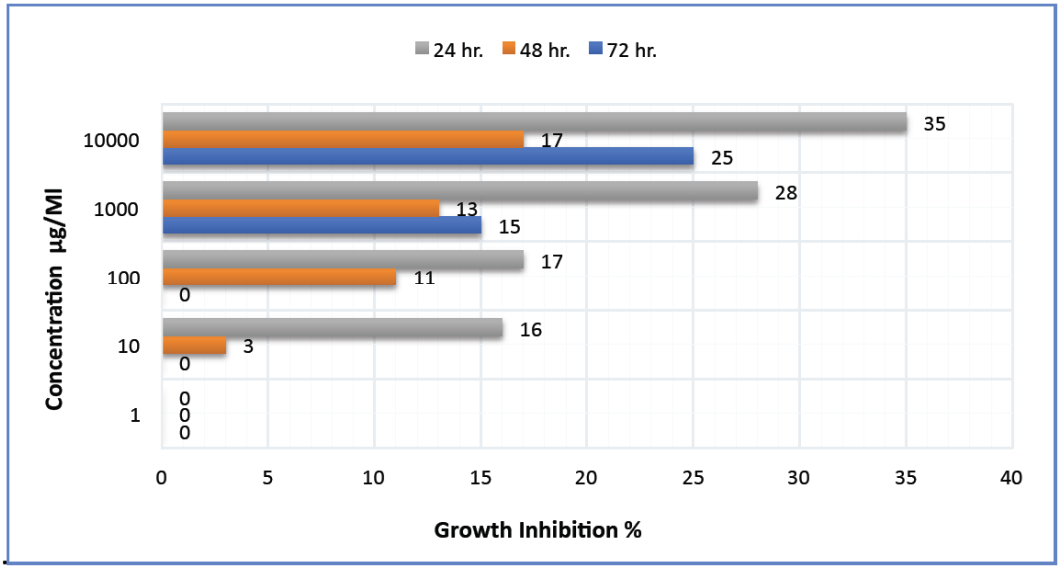


**Figure 2:** Methotrexate's effect on Hela cancer cell growth suppression.

**Table 3: Effect of the Laetrile-Methotrexate Mixture on Hela Cancer Cell Growth Suppression**

| Concentration (µg/ml) | Mean ± SE | | | LSD value |
|---|---|---|---|---|
| | Incubation period | | | |
| | 24 hr. | 48hr. | 72hr. | |
| 0.1 | B 23 ± 1.732 a | B 25 ± 2.887 a | D 30 ± 1.155 a | N. S |
| 1 | B 25 ± 2.887 b | AB 33 ± 1.732 b | C 50 ± 2.887 a | 10.02 * |
| 10 | B 29 ± 0.577 b | A 36 ± 1.732 b | BC 55 ± 2.887 a | 9.72 * |
| 100 | A 45 ± 1.732 b | A 39 ± 0.577 b | BC 58 ± 1.155 a | 9.06 * |
| 1000 | A 50 ± 1.732 b | A 41± 2.309 b | A 62 ± 1.155 a | 10.33 * |
| LSD value | 10.21* | 9.75 NS | 10.35 * | --- |

*: Significant at (P≤0.05), N. S: Non- Significant).

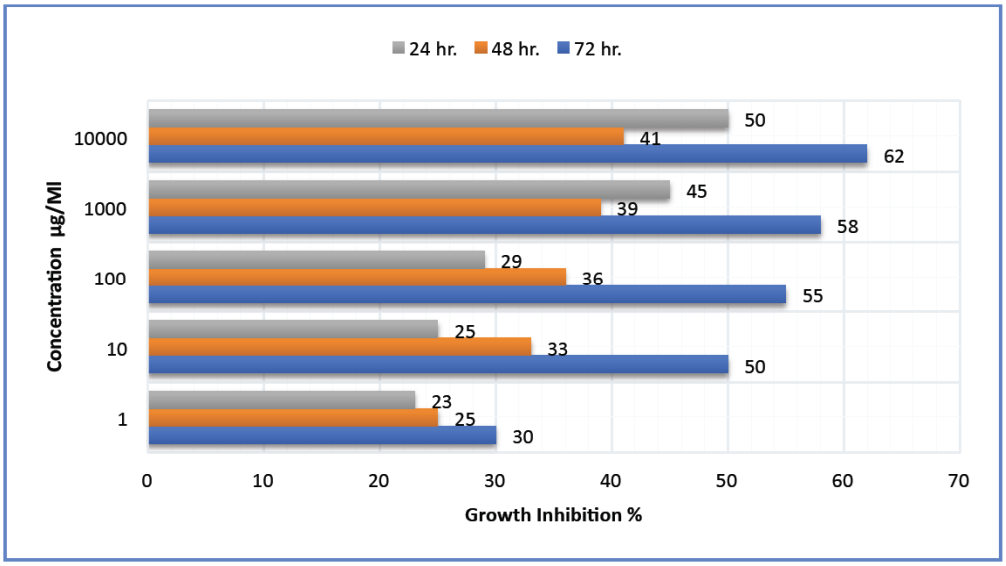


**Figure 3:** Effect of laetrile-methotrexate on Hela cancer cell growth suppression.

**Table 4: Comparing Laetrile, Methotrexate, and Laetrile-Methotrexate Cytotoxicity on Hela Cancer Cells at 24 Hours**

| Concentration (µg/ml) | Mean ± SE | | | LSD value |
|---|---|---|---|---|
| | laetrile | MTX | Mixture | |
| 0.1 | D 0 ± 0.0 b | D 0 ± 0.0 b | B 23 ± 1.732 a | 6.92* |
| 1 | CD 6 ± 1.155 b | C 16 ± 0.577 ab | B 25 ± 2.887 a | 10.32 * |
| 10 | BC 13 ± 1.155 b | C 17 ± 1.155 b | B 29 ± 0.577 a | 5.64 * |
| 100 | AB 20 ± 2.887 b | A 28 ± 1.732 b | A 45 ± 1.732 a | 12.34 * |
| 1000 | A 29 ± 2.309 b | A 35 ± 2.887 ab | A 50 ± 1.732 a | 20.23 * |
| LSD value | 10.16 * | 10.16 * | 10.21* | --- |

*: Significant at (P≤0.05), N. S: Non- Significant).

impact of mixture cytotoxicity over both methotrexate and laetrile when used individually over three incubation periods Tables **4**,**5**,**6**, Figures **4**,**5**,**6**,**7**.

### 3.2. Studying Drug Combinations

The combined study of laetrile and MTX yielded the following results. At all incubation periods, a concentration of (0.1, 1, and 10) µg/ml for the combination demonstrated very strong synergism, while 100 µg/ml exhibited synergism at 24 hrs., 48 hrs., and 72 hrs. showed significant synergism; on the contrary, antagonism was observed at 1000 µg/ml concentration during all incubation times.

The dose reduction index results indicated that the concentrations of the mixture components required to

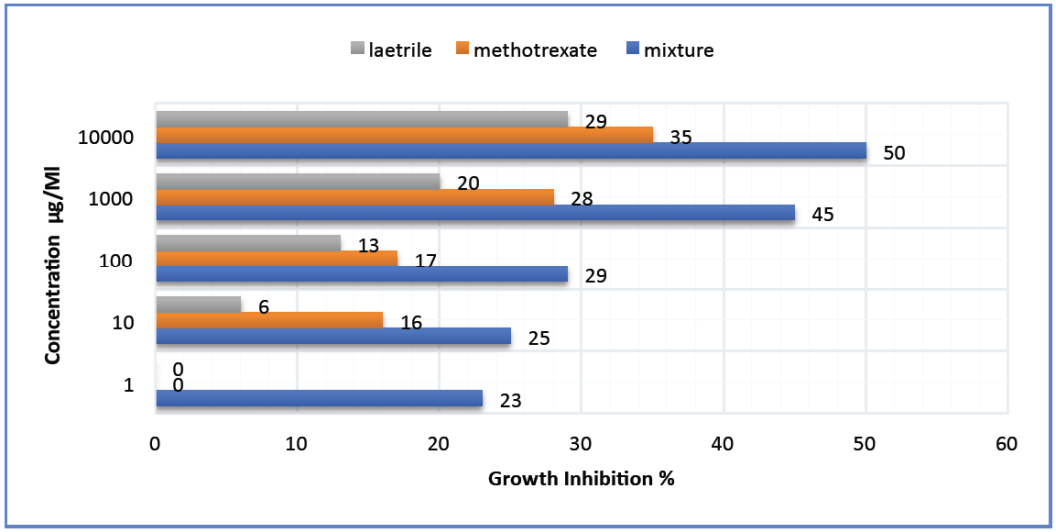


**Figure 4:** Comparing laetrile, methotrexate, and laetrile-methotrexate cytotoxicity on Hela cancer cells at 24 h.

**Table 5: Methotrexate, Laetrile, and Laetrile-Methotrexate Cytotoxicity on Hela Cancer Cells at 48 h**

| Concentration (µg/ml) | Mean ± SE | | | LSD value |
|---|---|---|---|---|
| | laetrile | MTX | Mixture | |
| 0.1 | B 0 ± 0.0 b | C 0 ± 0.0 b | B 25 ± 2.887 a | 3.76 * |
| 1 | A 30 ± 2.887 a | BC 3 ± 1.155 b | AB 33 ± 1.732 a | 13.84 * |
| 10 | A 33 ± 1.732 a | AB 11 ± 1.732 b | A 36 ± 1.732 a | 12.34 * |
| 100 | A 35 ± 2.887 a | A 13 ± 1.732 b | A 39 ± 0.577 a | 11.6 * |
| 1000 | A 37 ± 1.155 a | A 17 ± 1.155 b | A 41± 2.309 a | 6.52 * |
| LSD value | 11.52 * | 8.3 * | 9.75 NS | --- |

*: Significant at (P≤0.05), N. S: Non- Significant).

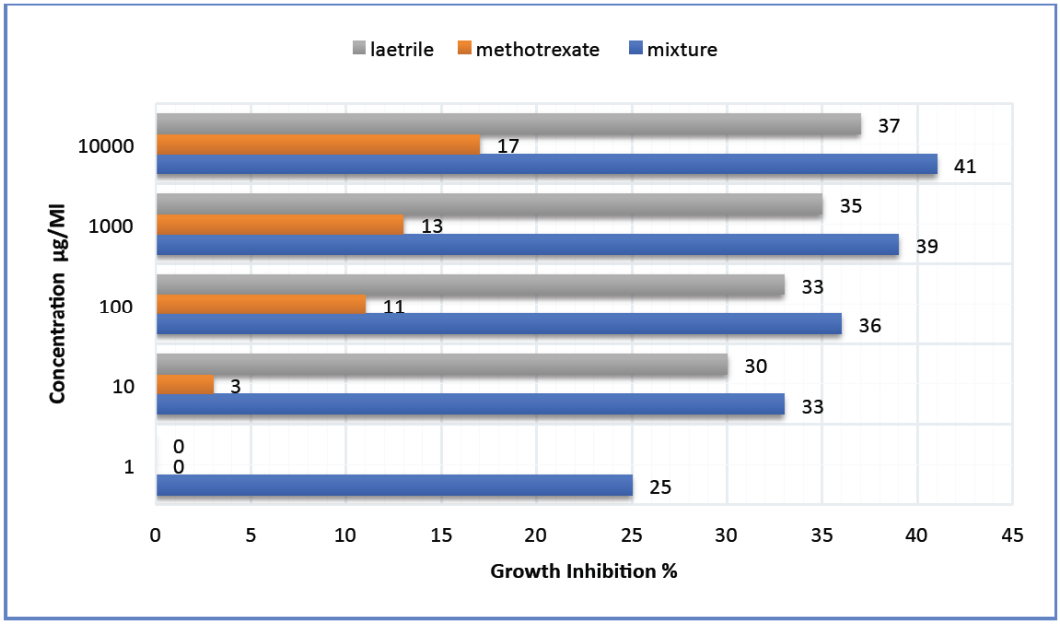


**Figure 5:** Methotrexate, laetrile, and laetrile-methotrexate cytotoxicity on Hela cancer cells at 48 h.

**Table 6: Laetrile, Methotrexate, and Laetrile-Methotrexate Cytotoxicity on Hela Cancer Cells at 72 h. Incubation Period**

| Concentration (µg/ml) | Mean ± SE | | | LSD value |
|---|---|---|---|---|
| | laetrile | MTX | Mixture | |
| 0.1 | C 0 ± 0.0 b | C 0 ± 0.0 b | D 30 ± 1.155 a | 3.76 * |
| 1 | B 32 ± 1.155 b | C 0 ± 0.0 c | C 50 ± 2.887 a | 10.14 * |
| 10 | AB 36 ± 0.577 b | C 0 ± 0.0 c | BC 55 ± 2.887 a | 9.6 * |
| 100 | AB 38 ± 1.732 b | A 15 ± 1.732 c | BC 58 ± 1.155 a | 8.84 * |
| 1000 | A 40 ± 1.155 b | A 25 ± 1.732 c | A 62 ± 1.155 a | 7.76 * |
| LSD value | 6.16 * | 6.9 * | 10.35 * | --- |

*: Significant at (P≤0.05), N. S: Non- Significant).

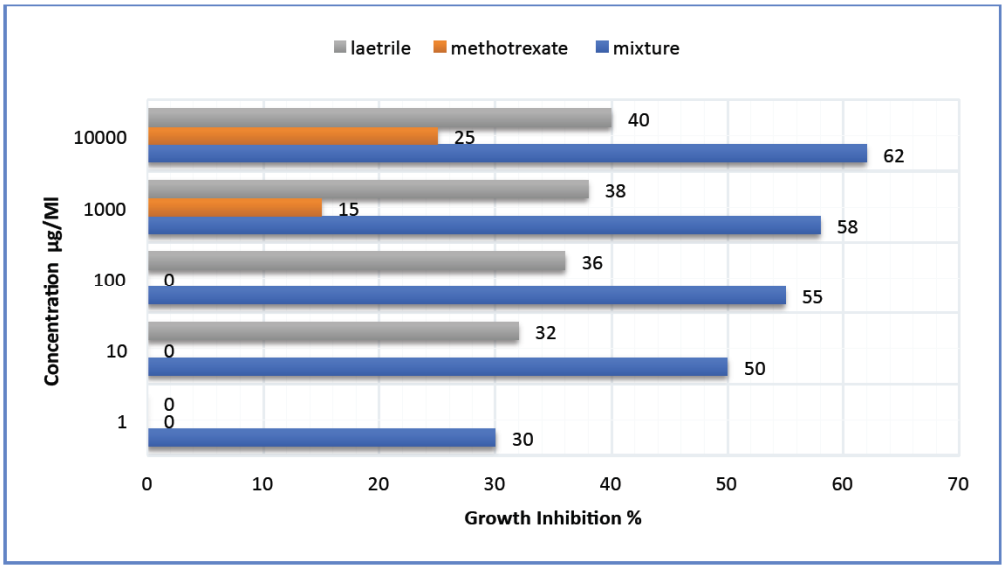


**Figure 6:** Laetrile, methotrexate, and laetrile-methotrexate cytotoxicity on Hela cancer cells at 72 h. incubation period.

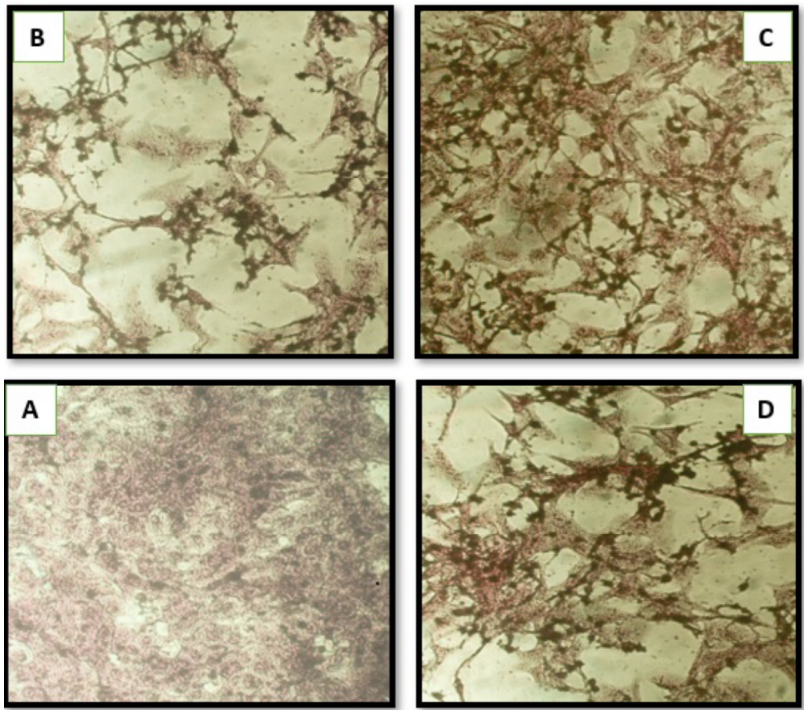


**Figure 7:** HeLa cells Morphology. (B) HeLa cells treated with 10000 (µg/ml) of (laetrile, MTX combination) at 72hr, (C) HeLa cells treated with 10000 (µg/ml) of (MTX) at 72hr, (D) HeLa cells treated with 10000 (µg/ml) of (laetrile) mixture at 72hr, (A) HeLa cells treated with treatment (control).

**Table 7: The Combination Pattern of Laetrile and Methotrexate on Hela Cancer Cells after 24 Hours of Incubation**

| Reagents concentration µg/ml | | Con. ratio | CI value | Combination pattern | Dose reduction index value | |
|---|---|---|---|---|---|---|
| Laetrile | MTX | | | | Laetrile | MTX |
| 0.5 µg/ml | 0.5 µg/ml | 1:01 | 0.00102 | Very Strong Synergism | 2411.52 | 1639.2 |
| 5 µg/ml | 5 µg/ml | | 0.00923 | Very Strong Synergism | 264.615 | 183.393 |
| 50 µg/ml | 50 µg/ml | | 0.07611 | Very Strong Synergism | 31.4279 | 22.5788 |
| 500 µg/ml | 500 µg/ml | | 0.39429 | Synergism | 5.65813 | 4.59666 |
| 5000 µg/ml | 5000 µg/ml | | 3.26295 | Strong Antagonism | 0.67055 | 0.56445 |

The CI and DRI values were calculated utilizing Compusyn software. A CI value higher than one can detect an antagonistic effect, a CI value of 1 suggests an additive impact, and a CI value less than 1 shows a synergistic effect. A dose reduction index (DRI) exceeding one corresponds to reduced toxicity [32].

**Table 8: The Combination Pattern of Laetrile and Methotrexate on Hela Cancer Cells after 48 Hours of Incubation**

| Reagents concentration µg/ml | | Con. ratio | CI value | Combination pattern | Dose reduction index value | |
|---|---|---|---|---|---|---|
| Laetrile | MTX | | | | Laetrile | MTX |
| 0.5 µg/ml | 0.5 µg/ml | 1:01 | 0.00098 | Very Strong Synergism | 1324.91 | 4323.58 |
| 5 µg/ml | 5 µg/ml | | 0.00703 | Very Strong Synergism | 185.419 | 610.67 |
| 50 µg/ml | 50 µg/ml | | 0.06267 | Very Strong Synergism | 20.7879 | 68.6789 |
| 500 µg/ml | 500 µg/ml | | 0.56085 | Synergism | 2.32105 | 7.69144 |
| 5000 µg/ml | 5000 µg/ml | | 5.21785 | Strong Antagonism | 0.24937 | 0.82798 |

The CI and DRI values were calculated utilizing Compusyn software. A CI value higher than one can detect an antagonistic effect, a CI value of 1 suggests an additive impact, and a CI value less than 1 shows a synergistic effect. A dose reduction index (DRI) exceeding one corresponds to reduced toxicity [32].

**Table 9: The Combination Pattern of Laetrile and Methotrexate on Hela Cancer Cells after 72 Hours of Incubation**

| Reagents concentration µg/ml | | Con. ratio | CI value | Combination pattern | Dose reduction index value | |
|---|---|---|---|---|---|---|
| Laetrile | MTX | | | | Laetrile | MTX |
| 0.5 µg/ml | 0.5 µg/ml | 1:1 | 0.00068 | Very Strong Synergism | 1528.5 | 30316 |
| 5 µg/ml | 5 µg/ml | | 0.00339 | Very Strong Synergism | 311.534 | 5703.75 |
| 50 µg/ml | 50 µg/ml | | 0.02957 | Very Strong Synergism | 35.6981 | 643.655 |
| 500 µg/ml | 500 µg/ml | | 0.26676 | Strong Synergism | 3.95904 | 70.5581 |
| 5000 µg/ml | 5000 µg/ml | | 2.31793 | Antagonism | 0.45601 | 7.99901 |

The CI and DRI values were calculated utilizing Compusyn software. A CI value higher than one can detect an antagonistic effect, a CI value of 1 suggests an additive impact, and a CI value less than 1 shows a synergistic effect. A dose reduction index (DRI) exceeding one corresponds to reduced toxicity [32].

cause cytotoxicity were lower at all time intervals (24, 48, and 72 hours of incubation), except for a concentration of 1000 µg/ml. This reduction in required concentration was significant for both laetrile and MTX, demonstrating a favorable dose reduction. An unfavorable dose reduction was seen in 1000 µg/ml at all incubation periods Tables **7**,**8**,**9**.

## 4. DISCUSSION

The findings of the laetrile cytotoxicity study indicate that laetrile toxicity escalates with higher concentrations and longer incubation periods. It exhibits a cytotoxicity pattern that relies on both the concentration and duration. However, the cytotoxicity mechanism was specific to the cell cycle, and the cell cycle was nonspecific.

Laetrile dosages ranging from 0.1 to 1000 µg/ml yielded the same results in additional research. This research confirms previous findings that laetrile may kill prostate cancer cells, with the amount of damage being dose-dependent. This is achieved by boosting the proapoptotic Bax protein levels and activating Caspase-3, suppressing the anti-apoptotic Bcl-2 protein, and triggering apoptotic cell death in prostate cancer cells [32]. According to new research, Laetrile blocks the development and multiplication of three distinct bladder cancer cell types in a dose-dependent manner. Because of this, the cell cycle development is

significantly prolonged, and the G0/G1 phase is halted [33].

The cytotoxicity of laetrile is mainly associated with the cellular level of hydrocyanic acid and benzaldehyde, which are formed intracellularly due to the glucosidase catalytic role [34].

Hydrocyanic acid's anticancer impact is attributed to its ability to inhibit cytochrome C oxidase activity, which is considered a crucial element of the electron transport chain in mitochondrial respiration; by inhibiting it, both the oxidative metabolism process and the following oxidative phosphorylation mechanism also inhibited, leading to a decline in energy reserves. The cytotoxic outcomes of benzaldehyde are attributed to its capacity to stimulate the activation of caspase 3, 8, and 9, causing apoptosis. Likewise, laetrile's possible impact on prostate cancer cells is due to its capacity to initiate apoptosis by activating caspase-3. The extent of this phenomenon depends on factors such as the quantity and length of the exposure [35].

The results of the MTX cytotoxicity assay demonstrated that the highest level of cytotoxicity was detected after a 24-hour incubation period, as compared to 48 hours and 72 hours. Cancer cells' resistance to the effects of methotrexate may be the underlying reason. Methotrexate resistance can be attributed to various factors, such as inadequate drug transportation, DHFR mutations that decrease the protein's susceptibility to inhibition, elevated intracellular DHFR levels due to gene amplification or alterations in gene regulation, and a diminished capacity to produce methotrexate polyglutamate [10, 36].

On the other side, mixture cytotoxicity results demonstrate a positive association between the rise in mixture concentration, incubation duration, and the escalation of cytotoxicity on cancerous cells. Moreover, the cytotoxicity of the laetrile and methotrexate mixture exceeded the cytotoxicity of each chemical independently in all the concentrations except the higher one and in all the incubation periods.

The combination of laetrile and MTX resulted in three positive effects. Firstly, based on the combination index value, the mixture exhibited a synergistic effect based on cytotoxicity at all studied concentrations, except for the highest one, and during all incubation periods, suggested synergism mechanism between mixture constituents may related to the multiple modes of anticancer activity that exhibited by each compound (laetrile and methotrexate) without interfering with the mechanisms involved (cyanide, benzaldehyde, and methotrexate).

The second advantage of the mixture is that it reduces the development of cancer cell resistance to the cytotoxicity of mixture MTX. This increase in MTX cytotoxicity may be due to the decreased ability of cancer cells to synthesize the DHFR enzyme, which is a pathway for cancer cell resistance to MTX cytotoxicity. This decrease in synthesis ability is related to a reduction in cancer cell mitochondrial activity, which is, in turn, caused by laetrile's Hydrocyanic acid. This acid restricted the function of cytochrome C oxidase, which is considered a vital element of the electron transport chain in mitochondrial respiration [37, 38]. Another mechanism ascribed to the decrease in the development of resistance to the combination MTX cytotoxicity involved a reduced concentration of methotrexate in the combination compared to when it is used alone. Gene amplification is a commonly used technique by cancer cells to acquire tolerance to high concentrations of methotrexate [39]. The rise in DHFR activity in methotrexate-resistant cancer cells is due to gene amplification [38, 40].

The third advantage of the mixture is that, based on the dose reduction index value, it showed a decrease in the concentration of the components needed to produce significant cytotoxicity compared to the concentration of each element alone. This indicates that the mixture has a high level of safety and a reduced likelihood of side effects or adverse reactions compared to the individual components of the mix.

A study limitation was that the concentration ranges of the reagents were not constrained to a specific concentration. Instead, we used a wide range of concentrations to gain knowledge regarding the effective concentrations of the laetrile MTX combination.

## 5. CONCLUSION

This study found that combining laetrile and MTX effectively suppressed cervical cancer growth in a time- and concentration-dependent manner. The mixture demonstrated synergistic cytotoxicity, as confirmed by the combination index score. Additionally, the required dosage for significant cell toxicity was lower when using the combination compared to either compound alone, as shown by the dose reduction index, suggesting improved safety.

According to the findings of the study, which were based on the MTT assay, CI, and DRI, the study suggested that laetrile helps lower the resistance of cervical cancer cells to MTX, which enhances the therapeutic benefits of the pharmaceutical. For the treatment of cervical cancer, this opens the door to the possibility of reintroducing MTX in a way that is both effective and safe.

## AUTHOR CONTRIBUTIONS

Design and development: Kawakeb N Abdulla, Rasha Kareem khudhur, Youssef Shakuri Yasin

Data collection and organization: Kawakeb N Abdulla, Ali Muafaq Said, Youssef Shakuri Yasin

Data analysis and interpretation: Rasha Kareem khudhur, Ali Muafaq Said, Youssef Shakuri Yasin

Composition of the article: Kawakeb N Abdulla, Rasha Kareem khudhur, Youssef Shakuri Yasin

Reviewing the essay critically for key conceptual points: Ali Muafaq Said, Youssef Shakuri Yasin

Proficiency in statistical analysis: Kawakeb N Abdulla, Rasha Kareem Khudhur, Youssef Shakuri Yasin

Ultimate endorsement and guarantee of the article: Rasha Kareem Khudhur, Youssef Shakuri Yasin

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## CONFLICTS OF INTEREST

Any potential bias or prejudice is missing.

## ABBREVIATIONS

ICCMGR = The Iraqi Centre for Cancer and Medical Genetics Research

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide stain

RPMI = Roswell Park Memorial Institute medium

SAS = Statistical Analysis System

LSD = Least Significant Difference

MTX = methotrexate

DRI = dose reduction index

CI = combination index

SE = Standard error

LSD = Least Significant Difference

## REFERENCES

[1] Rana RM, Rampogu S, Abid NB, Zeb A, Parate S, Lee G, *et al*. In silico study identified methotrexate analog as potential inhibitor of drug resistant human dihydrofolate reductase for cancer therapeutics 2020; 25(15): 3510. https://doi.org/10.3390/molecules25153510

[2] Lee HM, Moon AJB, therapeutics. Amygdalin regulates apoptosis and adhesion in Hs578T triple-negative breast cancer cells 2016; 24(1): 62. https://doi.org/10.4062/biomolther.2015.172

[3] Khashman BM, Abdulla KN, Karim S, Alhashimi S, Mohammed ML, Sarah NA. The Effect of Twist Expression On The Development Ofcervical Carcinoma in A Group Of Iraqi Women Infected with Hpv. Biochem Cell Arch 2019; 19(2): 3913-6.

[4] Abdulla KN, Khashman BM, Alheshimi SJ. The Immunohistochemical Evaluation of BRCA2 Expression with HPV Infection in a Group of Iraqi Women with Cervical Carcinoma. Indian Journal of Public Health Research & Development 2019; 10(9). https://doi.org/10.5958/0976-5506.2019.02590.7

[5] Guo M, Kong Q, Wang W, Yu HJPB. Biotransformation of amygdalin by lactic acid bacteria fermentation 2023; 132: 221-7. https://doi.org/10.1016/j.procbio.2023.07.022

[6] Ali B, Rawal YK, Thayes CJAJoM, Plants A. Nutritional composition, phytochemical screening, antioxidant, GC-MS, and FTIR analysis of methanolic extract of apricot, Prunus armeniaca kernel from Ladakh, India 2022; 8(3): 149-69.

[7] Qi X, Wong BL, Lau SH, Ng KT-P, Kwok SY, Sun CK-W, *et al*. A hemoglobin-based oxygen carrier sensitized Cisplatin based chemotherapy in hepatocellular carcinoma 2017; 8(49): 85311. https://doi.org/10.18632/oncotarget.19672

[8] Popa V-M, Raba D-N, Moldovan C, Poiana M-A, Misca C-D, Dumbrava D-G, *et al*. A review of amygdalin characteristics 2021; 27(4): 409-15.

[9] Jumaa AH, Hussein SMJIJoC, Genetics M. Study the *in vitro* effect of alcoholic extract of Prunus aremasia kernels, Methotreaxte, amygdalin and the combination between them on Hela cancer cell line 2015; 8(2). https://doi.org/10.29409/ijcmg.v8i2.152

[10] Jarad A. Diabetic wound healing enhancement by tadalafil 2020.

[11] Mohammad NSK, Ali AH, Salah LDFSJJoSiFS. Prunus armeniaca Seeds Extract Inhibits Cell Proliferation and Enhances Cell Death in Cancer Cells 2023; 10(1S): 4178-95.

[12] Elad S, Ranna V, Ariyawardana A, Correa MEP, Tilly V, Nair RG, *et al*. A systematic review of oral herpetic viral infections in cancer patients: commonly used outcome measures and interventions 2017; 25: 687-700. https://doi.org/10.1007/s00520-016-3477-7

[13] Said AM, Abdulla KN, Ahmed NH, Yasin YS. Antiproliferative Impact of Linagliptin on the Cervical Cancer Cell Line. Asian Pacific Journal of Cancer Prevention: APJCP 2024; 25(9): 3293. https://doi.org/10.31557/APJCP.2024.25.9.3293

[14] Jumaa AH, Al Uboody WSH, Hady AMJJoPS, Research. Esomeprazole and Amygdalin combination cytotoxic effect on human cervical cancer cell line (Hela cancer cell line) 2018; 10(9): 2236-41. https://doi.org/10.29409/ijcmg.v9i2.187

[15] Jumaa AH, Jarad AS, Al Uboody WSHJM-lU. The effect of esomeprazole on cell line human cervical cancer 2020; 20(1).

[16] Jumaa AHJIJoC, Genetics M. The cytotoxic effect of Vincristine-Amygdalin combination on human cervical cancer cell line (Hela cancer cell line) 2016; 9(2). https://doi.org/10.29409/ijcmg.v9i2.187

[17] Yasin YS, Jumaa AH, Jabbar S, Abdulkareem AHJAPJoCPA. Effect of Laetrile Vinblastine Combination on the Proliferation of the Hela Cancer Cell Line 2023; 24(12): 4329. https://doi.org/10.31557/APJCP.2023.24.12.4329

[18] Wang L, Huang X, Chai Y, Zou L, Chedrawe M, Ding YJOR. Octreotide inhibits the proliferation of gastric cancer cells through P300-HAT activity and the interaction of ZAC and P300 2017; 37(4): 2041-8. https://doi.org/10.3892/or.2017.5451

[19] Almahdawy S, Said AM, Abbas IS, Dawood AH. The evaluation of antimicrobial and cytotoxic activity of the essential oil extracted from the aerial parts of southernwood herb (Artemisia abrotanum L.) that recently grown in Iraq. Asian Journal of Pharmaceutical and Clinical Research 2017; 10(10). https://doi.org/10.22159/ajpcr.2017.v10i10.21725

[20] Mensoor M, Said A. Determination of heavy metals in freshwater fishes of the Tigris River in Baghdad. Fishes 2018; 3(2): 23. https://doi.org/10.3390/fishes3020023

[21] Said AM, Numan IT, HAMAD MN. Study of the cytotoxic and genotoxic effects for fractioniated extracts of convolulus arvensis on bone marrow in mice. International Journal of Pharmacy and Pharmaceutical Sciences Received 2013; 18: 303-5.

[22] Milazzo S, Ernst E, Lejeune S, Boehm K. Laetrile treatment for cancer. Cochrane Database of Systematic Reviews 2006(2). https://doi.org/10.1002/14651858.CD005476.pub2

[23] Dolgin JL. Challenging Medicine: Ivermectin Cases, Medical Futility Cases and the Patient-Physician Relationship. Quinnipiac L Rev 2023; 42: 363.

[24] Rahman AJMAPP. A review on medicinal plants with anticancer activity available in Bangladesh 2018; 1(4). https://doi.org/10.31031/MAPP.2018.01.000516

[25] Al-Hraishawi H, Al-Saadi N, Jabbar S. *In vitro* analysis: the anticancer activity of zinc oxide nanoparticles from Cinnamomum verum. Journal of Nanostructures 2023; 13(1): 146-50.

[26] Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari FJB. The role of plant-derived natural antioxidants in reduction of oxidative stress 2022; 48(3): 611-33. https://doi.org/10.1002/biof.1831

[27] Rahbari R, Sheahan T, Modes V, Collier P, Macfarlane C, Badge RMJB. A novel L1 retrotransposon marker for HeLa cell line identification 2009; 46(4): 277-84. https://doi.org/10.2144/000113089

[28] Bor T, Aljaloud SO, Gyawali R, Ibrahim SA. Antimicrobials from herbs, spices, and plants. Fruits, vegetables, and herbs: Elsevier; 2016. p. 551-78. https://doi.org/10.1016/B978-0-12-802972-5.00026-3

[29] Zhang Y, Qi D, Gao Y, Liang C, Zhang Y, Ma Z, *et al*. History of uses, phytochemistry, pharmacological activities, quality control and toxicity of the root of Stephania tetrandra S. Moore: A review. Journal of ethnopharmacology 2020; 260: 112995. https://doi.org/10.1016/j.jep.2020.112995

[30] Meyer CT, Wooten DJ, Lopez CF, Quaranta V. Charting the Fragmented Landscape of Drug Synergy. Trends Pharmacol Sci 2020; 41(4): 266-80. https://doi.org/10.1016/j.tips.2020.01.011

[31] Mohammed BJ, AL-Thwani AN, Kadhim AAAJIJE. Effect of Nicotine Injection on Tumor Suppressor Gene P53 Expression in Lung of Mice 2020; 47(12): 41-4.

[32] Ma M, Liang J, Zhang D, Xu X, Cheng Q, Xiao Z, *et al*. Monitoring treatment efficacy of antiangiogenic therapy combined with hypoxia-activated prodrugs online using functional MRI 2021; 11: 672047. https://doi.org/10.3389/fonc.2021.672047

[33] Giannenas I, Sidiropoulou E, Bonos E, Christaki E, Florou-Paneri P. The history of herbs, medicinal and aromatic plants, and their extracts: Past, current situation and future perspectives. Feed additives: Elsevier; 2020. p. 1-18. https://doi.org/10.1016/B978-0-12-814700-9.00001-7

[34] Sekino Y, Teishima JJCDR. Molecular mechanisms of docetaxel resistance in prostate cancer 2020; 3(4): 676. https://doi.org/10.20517/cdr.2020.37

[35] Makarević J, Tsaur I, Juengel E, Borgmann H, Nelson K, Thomas C, *et al*. Amygdalin delays cell cycle progression and blocks growth of prostate cancer cells *in vitro* 2016; 147: 137-42. https://doi.org/10.1016/j.lfs.2016.01.039

[36] Lorestani F, Movahedian A, Mohammadalipour A, Hashemnia M, Aarabi MHJCJoP, Pharmacology. Astaxanthin prevents nephrotoxicity through Nrf2/HO-1 pathway 2023; 102(2): 128-36. https://doi.org/10.1139/cjpp-2023-0015

[37] Alonzo JR, Venkataraman C, Field MS, Stover PJJJoBC. The mitochondrial inner membrane protein MPV17 prevents uracil accumulation in mitochondrial DNA 2018; 293(52): 20285-94. https://doi.org/10.1074/jbc.RA118.004788

[38] Khalifa MF, Ghadhban AGZA, Hade IM, Ali MM. Evaluation of quality of life for women with breast cancer. Scripta Medica 2024; 55(1): 115-8. https://doi.org/10.5937/scriptamed55-47068

[39] Joo W-S, Jeong J-S, Kim H, Lee Y-M, Lee J-H, Hong S-PJAopr. Prevention of epimerization and quantitative determination of amygdalin in Armeniacae Semen with Schizandrae Fructus solution 2006; 29: 1096-101. https://doi.org/10.1007/BF02969298

[40] Barakat H, Aljutaily T, Almujaydil MS, Algheshairy RM, Alhomaid RM, Almutairi AS, *et al*. Amygdalin: a review on its characteristics, antioxidant potential, gastrointestinal microbiota intervention, anticancer therapeutic and mechanisms, toxicity, and encapsulation 2022; 12(10): 1514. https://doi.org/10.3390/biom12101514

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