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Research Article

Antiproliferative Effect of the Aqueous Extract of *Prunus armeniaca* (Apricot) Seeds on the A375 Melanoma Cell Line

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ABSTRACT

Malignant melanoma is one of the most aggressive forms of skin cancer and is associated with high metastatic potential and resistance to conventional therapies. Natural products have attracted considerable attention as alternative sources of anticancer agents due to their bioactive phytochemicals. *Prunus armeniaca* (apricot) seeds contain several biologically active compounds, including amygdalin, flavonoids, and phenolic compounds, which have been reported to exhibit antioxidant and anticancer properties. Therefore, this study aimed to evaluate the antiproliferative effect of the aqueous extract of *Prunus armeniaca* seeds on the A375 melanoma cell line. This study aimed to investigate experimental plant-based therapeutic alternatives to replace drugs and chemical compounds currently applied to cancer patients. Skin cancer is the most aggressive cancer in humans. The A375 line was chosen to test the aqueous extract of *Prunus armeniaca* seeds. The aqueous extract was prepared after collecting and drying the seed pulp and then grinding it well. The results of the chemical analysis of the extract showed the presence of active compounds in good quantities, reaching (48.18) g./mg of flavonoids and phenols, respectively. The results of the antioxidant activity test showed a percentage of (73.8%) with an ROS level of (3.82 mmol/L), while the amount of amygdalin in the seeds reached (683.7 mg/100g) using the HPLC test. The cytotoxic activity of the extract was evaluated using the MTT assay on the A375 melanoma cell line at three exposure times (24, 48, and 72 hours) and five µg/ml concentrations (20, 40, 80, 160, and 320). The results showed a decrease in cell viability, with the 20 µg/ml concentration exhibiting the lowest inhibition rate (6.3%), while the highest concentration (320 µg/ml) showed the lowest cell viability (13.5%). Cell death reached 86.5% at 72 hours, depending on the concentration and exposure time. Microscopic examination of the cells revealed shrinkage, morphological changes, and cell death in cells exposed to the extract compared to the control. These results indicate antiproliferative activity in cancer cells, which may be attributed to the presence of active compounds in the extract and their ability to modulate oxidative stress on cells and induce apoptosis. Apricot kernel extract may represent a promising source of bioactive compounds for further investigation as potential anticancer agents.

Keywords: Cytotoxicity; HPLC; *Prunus armeniaca* A375; amygdalin, antioxidants

INTRODUCTION

Melanoma is responsible for the highest number of fatalities in humans with regard to skin cancers globally. Although there have been many improvements in the diagnosis and treatment of melanoma, the problem of drug resistance still persists [1].

Melanoma is the leading cause of skin cancer mortality worldwide. Melanoma arises from changes and abnormalities in the molecular structure of pigment cells in the skin. It develops as a result of exposure to many risk factors such as ultraviolet radiation and chemicals. It begins to appear as clusters of cells protruding outwards, resembling a scar on the skin [2]. Despite the abundance of treatments of synthetic

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chemical origin and their application in treatment protocols for cancer patients, including melanoma, this is accompanied by many side effects such as anemia, decreased immunity, nausea, severe fatigue, and pain in the joints and muscles [3]. However, there is a significant global trend towards reducing or limiting traditional chemotherapy due to its toxicity compared to drugs of plant origin or immunomodulatory agents. Studies have also indicated the possibility of applying a dual therapy combining both types, which provides an opportunity to control the tumor and prolong survival [4].

Plant extracts have been known for a long time, but in the last decade their potential use in producing drugs and pharmaceutical derivatives targeting cancer cells has expanded, especially since some plants contain active substances that affect cells, such as the apricot tree (*Prunus armeniaca*), particularly the kernel, which contains phenolic antioxidants. Amygdalin is another compound that is biologically active against cancer cells [5]. Extracts from the bitter-tasting kernel of apricot trees were tested on some cancer cell lines and proved effective in inhibiting cell division and inducing programmed cell death [6]. The A375 cancer cell line is derived from melanocytes and is currently being used in many preclinical studies in the laboratory to determine the effectiveness of some plant extracts. Study [7] indicated that apricot kernel extract reduces the growth and division of melanoma cells by inhibiting certain metabolic pathways and receptors on cell surfaces, thus paving the way for its potential use in alternative cancer treatments. This study aimed to investigate experimental plant-based therapeutic alternatives to replace drugs and chemical compounds currently applied to cancer patients.

MATERIALS AND METHODS

SEED COLLECTION AND EXTRACT PREPARATION:

Apricot seeds (*P. armeniaca*) were collected and stored in dry conditions away from sunlight. The kernels were then removed, dried, and ground. Fifty grams of the plant material were used, and half a liter of distilled water was added. The mixture was stirred and concentrated to obtain an aqueous extract of the apricot seeds. It was then placed in plastic containers and stored at 4°C [8].

CHEMICAL ANALYSIS AND MEASUREMENT OF THE ANTIOXIDANT CAPACITY OF THE AQUEOUS EXTRACT

The total phenolic content was estimated using the Folin-Ciocalteu assay and a 762 nm spectrophotometer with calcic acid as a standard [9]. The number of flavonoids in the aqueous extract was calculated using the same method, but with quercetin as the standard flavonoid at 420 nm [10]. The antioxidant activity was calculated using the DPPH assay on a sample of the extract, following the procedure described in [11]. The amount of reactive oxygen species (ROS) in the aqueous extract of *P. armeniaca* was estimated following the procedure described in [12]. The amount of amygdalin was estimated using HPLC, as described in [13]. After preparing the extract, it was filtered using 0.22 µm filters. The sample was then injected into the apparatus using a titration column. The resulting peaks were read and compared with a standard amygdalin solution, and the concentration was calculated from the standard titration curve.

TOXICITY TESTING

The A375 cancer cell line (a melanoma cell line) was prepared after culture and activation for the purpose of performing a toxicity test of the aqueous extract using five µg/ml concentrations (20, 40, 80, 160, and 320). The cells were exposed to all concentrations for three periods (24, 48, and 72 hours). Subsequently, the MTT assay was used to determine cell viability, and the results were read using ELISA at a wavelength of 490 nm. The percentage of cell inhibition was calculated using equation (14):

Percentage of Inhibition = 100 - Cell Viability%

RESULTS

RESULTS OF QUANTITATIVE ESTIMATION OF ACTIVE COMPOUNDS AND ANTIOXIDANT POTENCY

The results of chemical analyses to quantify some active compounds in the aqueous extract of apricot kernels (*P. armeniaca*) showed that flavonoids amounted to 18 mg/g of the extract, while phenolic compounds amounted to 48 mg/g, as shown in Table (1). The presence of amygdalin in the extract was

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determined using HPLC, yielding a concentration of 683.7 mg/100 g. The antioxidant capacity of DPPH and reactive oxygen species (ROS) in the extract was also tested, with values of 73.8% and 3.82 mM, respectively, as shown in Table (1).

Table 1. Some phytochemical properties and antioxidant activity of the aqueous extract of apricot seeds *P. armeniaca*

No.	Parameter	Result	Method
1	Total phenolic content (TPC)	g extract/48 mg	Folin-Ciocalteu assay
2	Amygdalin	683.7 mg/100g extract	HPLC analysis method
3	T. flavonoids (TFC)	gext../ 18 mg	AlCl3 colorimretric Ass.
4	DPPH radical scavenging activity	73.8%	DPPH assay
5	ROS level	3.82 mmol/L	ROS Assay

After conducting statistical analysis of the results from the toxicity tests of the aqueous extract of apricot seeds on the cancer cell line A375 (melanoma), as shown in Table 2, there is variation in the inhibitory effect on cell growth depending on dose and time of exposure. The results recorded after 24 hours of exposure to the extract showed a cell death rate of 6.3% at a concentration of 20 µg/ml, which increased significantly to 55.8% at the highest concentration used, 320 µg/ml for the same period. We note from this that the extract is very effective at high concentrations. In contrast, the inhibition values increased for all concentrations used in the test after 48 hours of treatment with the extract. At the lowest concentrations, the percentage of cancer cell death was approximately 20%, but there were significant differences compared to the 24-hour period, as cell death was recorded at 58% and 69.6% at a concentration of 160 µg/ml. 320 µg/ml respectively.

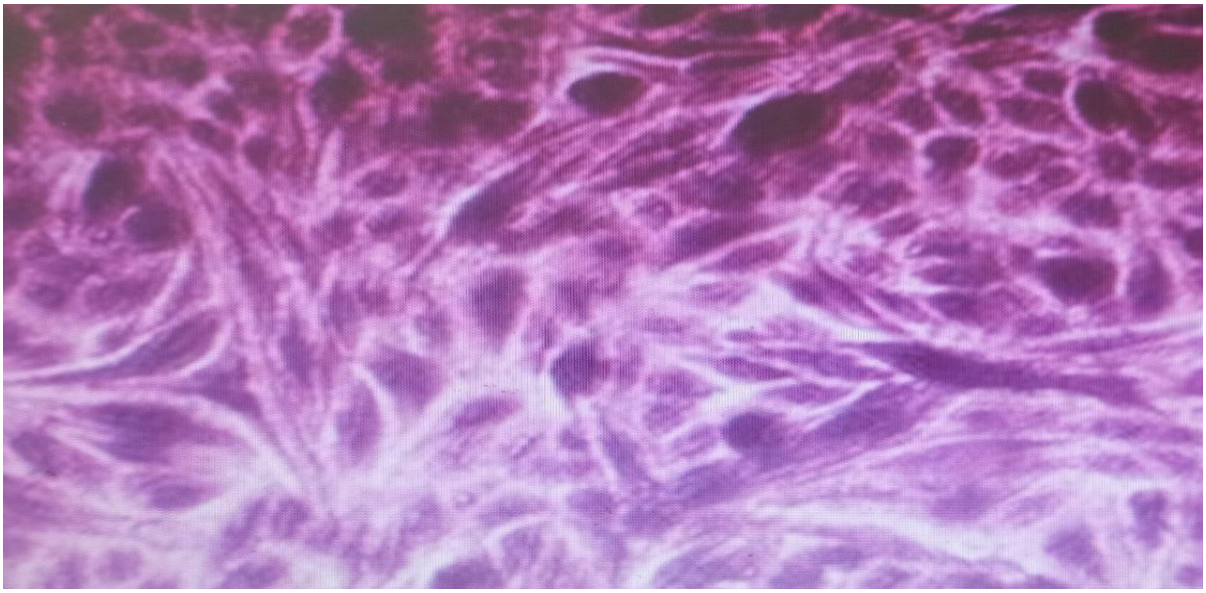

Figure 1. A375 melanoma cells not exposed to the aqueous extract of *Prunus armeniaca* (apricot) seeds.

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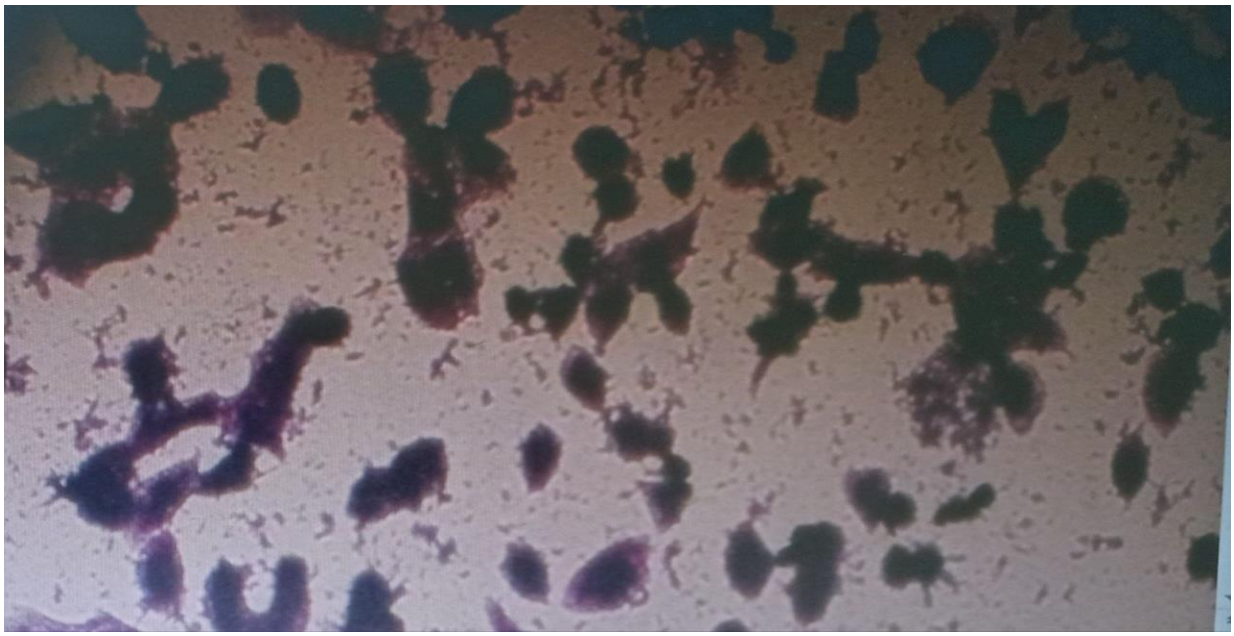

Figure 2. A375 melanoma cells exposed to the aqueous extract of *Prunus armeniaca* seeds (320 µg/mL) for 72 hours

Results indicated that the highest rate of cancer cell death occurred following 72 hours of incubation with the extract at these concentrations. Very few cells survived with an estimated rate of 13.5%, whereas 86% of cancer cells died when the cancer cells were subjected to a 320 µg/ml concentration. This indicates that cell inhibition increases gradually as the concentration and time are increased (Refer to Figures 1, 2 and Table 2).

Table 2. Cytotoxic activity of the aqueous extract of *Prunus armeniaca* seeds against the A375 melanoma cell line

Time	24 h		48 h		72 h	
Con... µg\ml	Cell viability%	Inhibition%	Cell viability%	Inhibition%	Cell viability%	Inhibition%
20	93.7	6.3	80.1	19.9	73.4	26.6
40	81.3	18.7	66.3	33.7	49.5	50.5
80	67.8	32.2	52.6	47.4	38.6	61.4
160	60.5	39.5	41.9	58.1	22.7	77.3
320	44.2	55.8	30.4	69.6	13.5	86.5

Values are expressed as mean ± standard error (SE).
Standard error (SE) = 3.623.

DISCUSSION

Many studies and reviews published in peer-reviewed journals, including (15), have reported the active compounds in apricot kernel seed (*P. armeniaca*) extracts and their antioxidant activity. The results showed a relatively high number of flavonoids and phenols in the extract. Furthermore, DPPH tests demonstrated anti-free radical activity. Thus, this plant is considered promising in combating ions and oxidative radicals within living cells.

Given the side effects caused by chemotherapy drugs used in cancer treatment, such as nausea, vomiting, and weight loss due to decreased appetite, in addition to the cytotoxicity to the immune system of patients, attention has turned to researching and investigating naturally occurring plant-derived compounds. Among

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the promising plants in this field is the apricot, whose seeds contain unique compounds with properties toxic to cancer cells. This aspect has been supported by many studies, including [16], which documented the presence of the amygdalin compound in the seeds of this plant and showed its ability to inhibit cancer cells at different times and doses.

Apricot seed extracts from *P. armeniaca* contain phenolic antioxidants and flavonoids, in addition to amygdalin, which is associated with its toxicity to cancerous tissues due to disrupting some metabolic pathways in cells [17]. Apricot seeds have been studied in many studies on several cell lines, but they have not been studied adequately on human skin cancer. This necessitated testing the toxicity of aqueous extracts of this plant on the A375 cell line to fill a gap in this field. As confirmed by [18], there is a significant decrease in the viability of melanoma cell lines when exposed to extracts prepared from apricot seeds. This may be due to the induction of programmed cell death due to disruptions in some JNK and ERK pathways that provide energy and oxidize glucose for cell survival. Based on this, the extract showed promising activity on skin cancer cells.

Approximately 11 phenolics were identified in apricot kernel extracts, which are characterized by good antioxidant activity and have considerable biological activity. Among these are those that function as both antioxidants in healthy cells and induce oxidative stress in tumor cells, resulting in mitochondrial dysfunction, which triggers apoptosis-related enzymes like caspase-3 in certain cancer cell lines [19].

The antioxidant effects observed in the *P. armeniaca* fruits are attributed to their capacity to neutralize ROS, thus making them ideal experimental substitutes for the treatment of cancer without the side effects experienced when using chemotherapy drugs. It has been established [20] that the *P. armeniaca* plant contains medicinal properties such as inhibition of cancer cells because it contains amygdalin, which encourages cellular lysis by stimulating the synthesis of enzymes that induce cell lysis and cytokinesis leading to the interruption of cell division cycles.

In many scientific articles from around the world, the pulp extract of the seeds and bitter almonds of *P. armeniaca* has shown a highly inhibitory impact on the development of tumor cells. According to these sources [21], in contrast to normal cells, the influence is practically non-existent. It is assumed that the active ingredient of this substance is amygdalin, which occurs widely in these fruits and is quickly degraded to glucose and cyanide under the action of glucokaidase enzymes. Due to the fact that there is a greater number of this enzyme in cancer cells, death comes to them faster. It is this feature that allows us to say that the extracts have a high therapeutic potential, but it requires serious studies to prove their harmlessness for people. Also, there are cofactors that cause synergistic activation of this property; namely, ROS and antioxidants cause oxidative stress, leading to mitochondria damage and subsequent cell death [22].

The objective of research is to discover alternatives to conventional therapies and examine plants for their bioactive properties against diseases like cancer, without causing adverse reactions, which are associated with chemotherapy drugs and radiation therapy, which cannot distinguish between normal and cancerous cells. Synergistic or complementary therapies have already been used with success as adjunctive treatment strategies for chemotherapy globally, causing minimal side effects [23].

CONCLUSIONS

The aqueous extract of *Prunus armeniaca* seeds exhibited notable cytotoxic and antiproliferative effects against A375 melanoma cells, accompanied by strong antioxidant activity and significant morphological changes indicative of cell death. The observed effects may be associated with the presence of bioactive phytochemicals, particularly phenolic compounds, flavonoids, and amygdalin. These results highlight the potential of apricot seed extract as a candidate for further investigation in melanoma research. Nevertheless, additional mechanistic studies, as well as in vivo and clinical evaluations, are essential to confirm its therapeutic potential and safety profile.

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