Investigation of the *in vitro* Anticancer Activity of *Sorbus aria* Extract on Prostate Cancer

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Abstract

Cancer is one of the common diseases observed in our country and worldwide with a high mortality rate. Although cancer has been treated with different applications such as chemotherapy, radiotherapy and surgical operation, the exact remedy of this disease has still not been found, yet. The fact that current treatment methods have severe side effects on patients has increased the interest in natural anticancer agents that are accepted easily and considered as safer by society. The aim of this study is to investigate the anticancer activity of whitebeam (*Sorbus aria*) that grows in the Central Anatolia Region in Turkey, extract on PC-3 human prostate cancer cells *in vitro*. The anticancer activity of the ethanol extract obtained from whitebeam fruits evaluated using MTT cell proliferation assay. Also, Folin-Ciocalteu assay and CUPRAC method were performed to determine total phenol content and antioxidant capacity of whitebeam fruits, respectively. Whitebeam fruit extract showed strong anticancer activity on prostate cancer *in vitro*. The obtained ethanol extract is found to be a promising natural anticancer agent *in vitro*, and our findings need to be verified with *in vivo* studies and clinical trials.

Key words: Prostate cancer, *Sorbus aria,* natural anticancer agents.

1. Introduction

Cancer, caused by uncontrolled proliferation and spread of abnormal cells, is the second most common cause of death worldwide. Generally, all healthy human cells grow and divide to multiply. During the cell cycle, if the cells are damaged or aged, they will go through apoptosis, and old cells are replaced with new ones. However this regular process is sometimes disrupted and abnormal cells, growing uncontrolled manner, form tumors. Some cancer cells can reach distant locations in the body through the lymph system and blood circulation. Thus, new tumors occur away from the original tumor site, which is called metastasis [1]. Prostate cancer, one of the most common type of cancer in men, is the second leading cause of deaths in men older than 65 years of age in western populations [2].

Although the exact solution of cancer is still not fully available, treatments such as surgical removal of the prostate gland, radiotherapy, chemotherapy, and hormone treatment are generally used to save lives [3]. However, those treatment methods possess serious side effects such as hair

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loss, nausea, fatigue, anemia, mucositis, infertility, and diarrhea [4]. Plant-based drug development studies have recently been gaining interest since natural agents are considered as safer with no or less side effects on normal cells.

S. aria, also known as whitebeam, is a deciduous, slow-growing, small to medium-sized shrub or tree (Figure 1). Leaves of whitebeam change color with the season. Light green leaves are seen in spring, dark green leaves are seen in summer, and yellow leaves are observed in fall. Whitebeam has a lifespan of approximately 100-200 years [5].

Figure 1. Leaves and fruits of *S. aria* [4].

In this study, the anticancer effects of whitebeam plant on prostate cancer were investigated. Also, total phenol content and antioxidant capacity of whitebeam were determined. Our findings showed that ethanol extract of whitebeam with a significant phenol content detected displayed a promising anticancer activity on prostate cancer *in vitro* and this plant needs to be further investigated with *in vivo* test and clinical studies.

2. Materials and Method

2.1. Ethanol Extraction from fruits of whitebeam

Fruits of whitebeam, obtained from a local bazaar at Isparta, Turkey, were washed with deionized water upon arrival in the laboratory. Then, 12 grams of fruits were mixed with 70 ml ethanol and the mixture was sonicated for 5 minutes. After sonication, fruits were stirred for 30 minutes at magnetic stirrer. The supernatant was transferred in a clean bottle, and the pellet was mixed with 30 ml of ethanol. This process repeated three times. In the last repeat, 20 ml of ethanol was used. The collected supernatants were filtered with Whatman grade 41 filter paper. 44 ml of deionized water were added into a filtered solution. The final solution was evaporated in a rotary evaporator at 40 °C and 200 rpm for 1 hour until the removal of ethanol [6].

2.2. Determination of total phenol content using Folin-Ciocalteu assay

The amount of total phenol was determined using Folin-Ciocalteu assay. For the total phenolic

capacity, a whitebeam stock solution (1 mg/ml) was prepared using dimethyl sulfoxide (DMSO). First, 20 μl of whitebeam stock solution was pipetted into a well of the 96-well plate. Then, 100 μl of Folin-Ciocalteu phenol reagent was added into the whitebeam stock solution. The mixture of Folin-Ciocalteu phenol reagent and the stock solution was incubated at room temperature for 2.5 minutes in 96-well plates. After incubation, 80 μl of sodium carbonate was added into each well. The final mixture was incubated in 96-well plates at room temperature for 1 hour at dark and then the absorbance of the wells was measured at 775 nm using a plate reader (Multiskan FC, Thermo Fisher Scientific, US).

2.3. Determination of antioxidant activity using CUPRAC method

Copper (II) chloride dihydrate solution (10 mM), Neocuproine hydrochloride monohydrate (Nc) solution (7.5 mM), and ammonium acetate solution (1 M) were utilized in the Cupric Reducing Antioxidant Capacity (CUPRAC) batch methodology. Copper solution and ammonium acetate solution (pH 7.0) were prepared in water. Neocuproine solution was prepared fresh in ethanol before each experiment. In a glass test tube, 1 ml of Copper solution, 1 ml of Neocuproine, and 1ml of ammonium acetate were mixed gently. Then, 1.1 ml of 10 mg/ml whitebeam solution was also added into the same test tube. In a separate glass test tube, all reagents aforementioned were mixed except that DMSO was used in place of plant extract as control. After 30 minutes of incubation at dark, the absorbance of the solutions was measured at 450 nm using a spectrophotometer (Evolution 220, Thermo Fisher Scientific, US). Total antioxidant capacity was calculated using the following equation (1):

Total antioxidant capacity (mM Trolox / g sample) = (*Absorbance* / 16700) x (*Total volume* (ml) / *Sample volume* (ml)) x (*Dilution factor*) x (*Extraction volume* (ml)/ *Sample weight* (g))   (1)

2.4. MTT cell proliferation assay

For the MTT assay, the stock solution of the extract (10 mg/ml) was prepared in DMSO. The stock solution was then diluted with Dulbecco's Modified Eagle Media supplemented with %10 FBS and %1 antibiotic/antimycotic (DMEM-10) to sub-concentrations of 100 μg/ml, 50 μg/ml, 25 μg/ml, 10 μg/ml. PC-3 human prostate cancer cells (1 x 10^4 cell/well) diluted in DMEM-10 were incubated in wells of 96-well plates in an incubator at 37 °C and 5% CO_2 for 24 hours. After incubation, the previously diluted extract solutions were replaced with DMEM-10. Later, the plates were incubated in the CO_2 incubator. On the day of the test, 10 μl of MTT dye solution (5 mg/ml in PBS) was added into each well and the plate was incubated in an incubator for an additional 2 hours. The test was repeated for 24, 48, and 72 hours and the absorbance of plates was measured at 570 and 690 nm wavelengths. In cell viability calculations, the values obtained by subtracting the absorbance value measured at 690 nm from the absorbance value measured at 570 nm were used. Cell viability (%) was calculated using the following equation (2):

% Cell viability = (*Sample absorbance* / *Control absorbance*) × 100   (2)

3. Results

3.1. Total phenol content of whitebeam fruits extract

Folin-Ciocalteu assay is widely used to measure the total phenolic contents of plant extracts. The total phenol content measured as gallic acid equivalent (GAE). The total phenol content of whitebeam fruits extract was determined 30.47 mg gallic acid equivalent phenolic compound per g sample.

3.2. Antioxidant capacity of whitebeam fruits extract

To determine the amount of antioxidant in a biological sample, Total Antioxidant Capacity (TAC) assay is one of the most common tests. TAC is given as Trolox Equivalent Antioxidant Capacity (TEAC). CUPRAC assay results showed that ethanol extract of whitebeam fruits has 469.77 µmol Trolox per g sample.

3.3. Anticancer activity of whitebeam fruits extract

The graph showing the anticancer activity of whitebeam fruits extract for 24-48-72 hours was presented in Figure 2. Overall, the cell viability was reduced upon treatment with whitebeam fruits extract in a dose-dependent manner. At high concentrations, 100 µg/ml, and 50 µg/ml, cell viability was determined below %10. For 25 µg/ml and 10 µg/ml concentrations of the extract, the highest inhibition was achieved at 24 hours incubation.
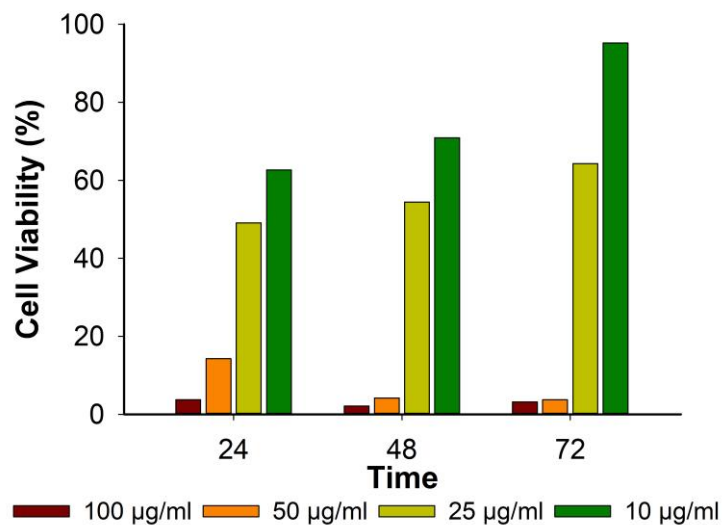

Figure 2. MTT cell viability test results.

Control group, 1% DMSO, showed 86.3% cell viability at 24 hours, 77.76% cell viability at 48 hours, and %99.99 cell viability at 72 hours. The average IC_{50} value for 24th hours was

calculated as 25.4 µg/ml for the agent. It was clearly observed that the ethanol extract of whitebeam fruits has a significant anticancer effect against prostate cancer cell line PC-3 *in vitro*.

4. Discussion

According to the literature, the phenolic amount of whitebeam fruits was found significantly low compared to other well-known plants analyzed. On the other hand, its antioxidant capacity was determined better than the phenolic amount (Table 1). TEAC values showed that the extract of whitebeam fruits presents a significant antioxidant capacity compared to some plants which are commonly consumed as food such as mustard and white mulberry. However, some growing conditions such as soil type, nutrient amount of soil, rainfall, etc are known to affect the phenolic amount and antioxidant capacity of plant extracts. [7]

Table 1. Comparisons of GAE and TEAC values for *S. aria* with other plants' extracts.

Plant	Common Name	Part of Plant	GAE (mg/g)	TEAC (µmol/g)	References
Sorbus aria	Whitebeam	Fruit	30.47	469.77	This study
Cnicus benedictus	Cnicus	Root	1.57	17.61	[8]
Ocimum basilicum L.	Basil	Stem	28.90	266.42	[8]
Morus alba L.	White mulberry	Fruit	19.02	233.91	[9]
Salvia officinalis	Sage	Leaf	26.89	231.60	[10]
Brassica hirta	Mustard	Seed	20.40	45.90	[10]
Matricaria chamomilla	Chamomile	Flower	16.77	153.94	[10]
Lonicera japonica	Japanese honeysuckle	Flower	27.36	121.97	[11]
Murraya koenigii	Curry	Leaf	1.18	1.13	[12]

According to the MTT cell proliferation test results, the fruits of whitebeam showed significant anticancer activity on prostate cancer, and the highest anticancer activity was reached at the 24th hour. When the concentration groups were compared, higher anti-cancer activity was determined at the concentration of 50 µg/ml and above. As a result, it has been observed that whitebeam is a highly effective anticancer agent on PC-3 cancer cells *in vitro*. In addition, IC_{50} value was calculated as 25.4 µg/ml and it is shown in Table 2 by comparing it with other similar studies in the literature. The IC_{50} value obtained in this study is one of the lowest values when compared with similar studies in the literature.

Table 2. IC_{50} values of various plant extracts on cancer cells.

Plant	Common Name	Part of Plant	Cell Line	IC_{50} (μg/ml)	References
Sorbus aria	Whitebeam	Fruit	PC-3	25.4	This study
Moringa peregrina	Miracle tree	Seed	PC-3	600.42	[13]
Diospyros kaki	Persimmon	Leaf	PC-3	57.7	[14]
Azadirachta indica	Nimtree	Leaf	PC-3	100	[15]
Carica papaya	Papaya	Leaf	PC-3	950	[16]
Daniellia oliveri	African Copaiba Balsam Tree	Leaf	PC-3	130	[17]
Vitex doniana	Black plum	Root	PC-3	177.3	[17]
Cajanus cajan	Pigeonpea	Leaf	MCF-7	52	[18]
Lophira alata	Azobé	Tree bark	HepG2	32.98	[19]
Fagara xanthoxyloides	Artar root	Root	CWR-22	44	[20]
Morinda lucida	Brimstone tree	Root	HL-60	171	[21]

Conclusions

Anticancer activity was investigated by performing the ethanol extraction method for whitebeam fruits. MTT cell proliferation test was applied to evaluate its anticancer effect on prostate cancer. From the MTT cell viability test, the IC_{50} value was calculated as 25.4 μg/ml for the extract of whitebeam fruit, which is one of the lowest values in the literature. In this study, other biological activities of whitebeam, such as total phenol and antioxidant content, were also investigated. Folin-Ciocalteu method was used for total phenol analysis and the CUPRAC method was used for antioxidant analysis. Significant antioxidant activity was also determined from the extract. In conclusion, the ethanol extract of whitebeam fruits was found as a promising antioxidant and anticancer agent. The *in vitro* findings of this work should be expanded to *in vivo* test and clinical trials to develop an anticancer drug.

Acknowledgements

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