

Effect of *Capparis cartilaginea* Fruit Extract Flavonoids on Wound Healing in Human Prostate Cancer Cells

Walaa Najm Abood^{1,2}, Nawal Ahmed Al-Henhena³, Ammar Najim Abood⁴, Mazen M. Jamil Al-Obaidi⁵, Salmah Ismail⁶, Mahmood Abdulla^{3*}, Rami Al Bartan⁷

¹Department of Biomedical Science, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

²Department of Microbiology and Immunology, Faculty of Medicine, University of Diyala, Iraq.

³Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

⁴Department of Surgery, Faculty of Medicine, University of Diyala, Iraq.

⁵Center Study of Periodontology, Faculty of Dentistry, Universiti Teknologi MARA, 40450 Shah Alam, Selangor DarulEhsan, Malaysia.

⁶Biological Institute, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

⁷Tropical Infectious Diseases Research and Education Center, Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

*E-mail ✉ Mahmood955@yahoo.com

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ABSTRACT

Prostate cancer (PCa) is the most common type of cancer among men in developed countries. Despite the progress made in treatments such as surgery, radiation, and hormonal therapies, advanced-stage PCa still lacks effective management strategies. In response, alternative approaches, including dietary cancer-preventive agents, have gained attention, and medicinal plants are considered potential therapeutic options. *Capparis cartilaginea* (*C. cartilaginea*) contains various active compounds such as tannins, alkaloids, steroids, terpenoids, and flavonoids. This study aimed to investigate the cytotoxic effects of the ethanol extract of *C. cartilaginea* fruit on the 22RV1 human prostate cancer cell line. Observations under an inverted light microscope showed that exposure to the extract resulted in dose- and time-dependent changes in cell morphology and migration. The cytotoxicity was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), while the effect on cell migration was assessed through a wound-healing assay. After 72 hours of treatment, the MTT assay showed a significant cytotoxic effect, with a concentration of 0.088 ± 0.031 µg/mL of the extract. Morphological changes in the cells post-treatment indicated that the extract induced substantial cell death, which increased with higher concentrations. These results suggest that the ethanol extract of *C. cartilaginea* fruit may be promising as a potential treatment for prostate cancer.

Keywords: Fruit extract, Prostate cancer, Wound healing, *C. Cartilaginea*, Flavonoids, Scratch assay

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Introduction

Prostate cancer (PCa) contributes to 6.6% of cancer-related deaths in men, ranking as the fifth leading cause of male cancer fatalities [1]. In the Kingdom of Saudi Arabia, the Saudi Cancer Registry recorded a total of 1739 cases over seven years. The highest incidence rate, 10.1 per 100,000 men, was noted in the eastern region, with Riyadh, Makkah, Jizan, and Najran showing rates of 7.1, 5.2, 1.4, and 2 per 100,000 men, respectively [2]. Compared to Western nations, the Middle East sees considerably lower rates of PCa [3]. In Saudi Arabia, PCa ranks as the sixth most common cancer among men of all ages [4]. According to the International Agency for Research on Cancer, in 2018, the age-standardized incidence rate for PCa was 7.7 per 100,000 men, and the

mortality rate was 5.1 per 100,000 men. Compared to countries in Europe, the PCa incidence in Saudi Arabia remains notably low [2].

Early detection of PCa is crucial, as screening for tumor markers can facilitate effective intervention when the tumor is still confined to the prostate [5]. Treatments for localized PCa, such as radical prostatectomy and radiotherapy, may significantly impair quality of life, leading to issues such as sexual dysfunction, urinary incontinence, and disrupted bowel function [6]. Treatment decisions are influenced by clinical staging and the patient's overall health. While low-risk localized PCa may be managed with active surveillance, intermediate and high-risk cases typically require more aggressive treatments, such as radiotherapy or surgery. For metastatic PCa, androgen deprivation therapy (ADT), which targets androgen receptor signaling, is commonly used [7]. PCa progresses slowly, with symptoms emerging only later, providing a window for therapeutic action. A slight delay in progression can markedly enhance the patient's quality of life. Dietary substances have emerged as a viable approach in the development of non-toxic chemopreventive strategies [8]. With advancements in organic chemistry, improvements in extraction methods, and a deeper understanding of plant compounds, plant-based medicines have garnered significant research interest. These plant-derived compounds can also reduce drug production costs, offering a more affordable alternative for the pharmaceutical industry [9, 10].

Capparis cartilaginea (*C. cartilaginea*), including its flowers, fruits, and shoots, has been utilized in traditional medicine to treat a wide array of human diseases for centuries. In addition to its medicinal applications, it is used in cosmetics, food, and animal feed [11, 12]. This plant species is found in regions such as the Arabian Peninsula, parts of North Africa, Western Asia, and the Indian subcontinent [13]. Plants in the *Capparis* genus are typically annual or perennial, with some being climbers or trees [14]. Among the six flavonoids isolated from *C. cartilaginea* are kaempferol-3-O-rutinoside, quercetin-3-O-rutinoside, kaempferol 3-neohesperidoside, kaempferol 3-(2 G-rhamnosylrutinoside), quercetin 3-(2 G-rhamnosylrutinoside), and isorhamnetin-3-O-rutinoside [15]. The plant also contains non-nutritive phytochemicals like glycosides, tannins, flavonoids, alkaloids, saponins, unsaturated sterols, and coumarins [16]. Flavonoids and alkaloids are known for their medicinal properties and are believed to play a role in cancer prevention and inhibition [17]. Research in animal models indicates that these compounds can disrupt the cell cycle, prevent mutations, and halt carcinogenesis by breaking down lipid peroxides. They also neutralize free radicals, such as superoxides and hydroxyl radicals, protecting cells from radiation damage [18]. Flavonoids offer a range of therapeutic benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral effects, and they also provide cardio-protective and neuroprotective properties [19]. Quercetin, in particular, has been shown to inhibit the spread of various cancers, including those of the breast, lung, prostate, cervical, liver, and colon. It works through multiple mechanisms, such as regulating cellular signaling, binding to cellular receptors, and inhibiting enzymes that activate carcinogens [20]. Glucosinolates, another group of secondary metabolites found in plants, are being studied for their anti-carcinogenic potential. Isothiocyanates derived from glucosinolates are being researched for their cytotoxic effects on cancer cells and are currently being explored for the development of new anticancer therapies [21].

The purpose of this study was to explore the cytotoxic effects of the ethanol extract from *C. cartilaginea* fruit on the 22RV1 human prostate cancer cell line.

Materials and Methods

Fruit preparation

Fresh *C. cartilaginea* fruits were harvested in October 2018 from Wadi Molham, Wadi Abu Al-Haza, and Wadi Al-Quraine, located in the Tuwaiq Mountains of Saudi Arabia. These fruits were immediately freeze-dried using a freeze dryer (ilShin BioBase, Korea), set at -60 °C and 5 mTorr pressure for 24 hours. Afterward, the dried fruits were pulverized with a Waring blender (USA). The resulting powder was stored in an airtight container at -80 °C for later use. To prepare the extract, the powdered fruits underwent ethanol extraction following the procedure established by Al-Goufi and Sonbol [22]. The extract was then prepared at concentrations ranging from 0.5 to 20 µg/mL by diluting stock solutions with RPMI-1640 medium to a final volume of 100 µL. According to the US National Cancer Institute's guidelines, extracts are considered cytotoxic if their IC₅₀ is under 20 µg/mL in preliminary assays [23].

Cell culture and subculture procedures

The prostate cancer cell line 22RV1 was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin. These cells were cultured in 25 cm² tissue culture flasks in a humidified incubator with 5% CO₂ at 37 °C. Upon reaching approximately 80% confluence, the medium was removed, and the cells were washed with PBS. To detach the cells, trypsin-EDTA (1 mL) was added for 5–7 minutes. Observations were made under an inverted light microscope (Nikon Instruments, Melville, NY, USA) to ensure full detachment. Following detachment, 3 mL of pre-warmed complete medium was added, and the cells were pipetted gently to break up clumps. The suspension was transferred to a 15 mL tube and centrifuged at 1500 rpm for 5–10 minutes. The pellet was then resuspended in fresh medium, and approximately $0.5-1 \times 10^6$ cells were transferred to new 25 cm² flasks with 8 mL of medium. Changes in cell morphology indicative of cell death were monitored under an inverted light microscope (Nikon Instruments, Melville, NY, USA).

Determination of cell viability and proliferative activity

The effects of *C. cartilaginea* fruit extract on the 22RV1 prostate cancer cell line were assessed using the MTT assay. This assay detects mitochondrial dehydrogenase activity, which converts MTT (a yellow soluble compound) to purple formazan crystals in live cells. Cells were plated at densities of 5000 and 10,000 cells/100 µL in 96-well plates (PermanoxTM, City) and incubated overnight at 37 °C with 5% CO₂. After 24, 48, and 72 hours of treatment with different concentrations of the extract (0.5, 5, 10, 15, and 20 µg/mL), the cells were subjected to MTT solution (3 mg/mL, 10 µL per well). After incubating the cells with MTT for 4 hours, the supernatant was removed, and 100 µL of DMSO was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using an ELISA reader (BioTek Instruments, Winooski, VT, USA). Cell viability was calculated following Kamiloglu *et al.*'s method [24], while cytotoxicity was calculated using the formula by Choudhury *et al.* [25].

Cells seeded at densities of 5000 and 10,000 cells/well were treated with varying concentrations of the fruit extract for different time points (24, 48, and 72 hours). Morphological changes related to cell death were assessed at each interval. Cell density optimization led to the selection of a 10,000 cells/well density, which provided reliable data on the extract's effect on cell viability over time.

Wound-healing scratch assay

The migration of 22RV1 prostate cancer cells was assessed using a wound-healing scratch assay, as described by Cormier *et al.* [26]. Cells were seeded in 12-well plates and grown to 75% confluence at 37 °C with 5% CO₂. A sterile 10 µL pipette tip was used to create uniform scratches across each well, leaving a cell-free area. After scraping, the wells were washed with PBS to remove detached cells. Cells were then exposed to varying concentrations of *C. cartilaginea* fruit extract (0.5, 5, 10, 15, and 20 µg/mL), with control cells being scratched, washed, and maintained in the growth medium containing 10% FBS. The cultures were incubated at 37 °C with 5% CO₂. Images of the wound area were captured at 0, 24, 48, and 72 hours using a DS-U3 digital camera controller (Nikon Instruments, USA) and analyzed with NIS-Elements version 4.0 software. Two independent experiments were performed with two wells for each concentration, and the same wound area was imaged at each time point for consistency [26, 27].

Wound area calculation

The wound area was determined by outlining the exposed area in the captured images taken at different time intervals, using ImageJ software (Fiji-ImageJ version 1.51 h) [28]. The software was calibrated to measure the area in micrometers rather than pixels, utilizing a scale slide (Graticules LTD, Tonbridge, UK) for proper scaling. As the experiment progresses, the wound area naturally shrinks, and the migration rate of cells can be assessed by tracking the changes in the wound's size over time. The wound closure percentage was determined by calculating the decrease in the area, with higher closure percentages corresponding to greater cell migration and wound closure over time [29].

$$\text{Wound closure (\%)} = \left[\frac{A_{t=0h} - A_{t=\Delta h}}{A_{t=0h}} \right] \times 100\% \quad (1)$$

$A_{t=0h}$ is the area of the wound measured immediately after scratching ($t = 0$ h).

$A_{t=\Delta h}$ is the area of the wound measured h hours after the scratch is performed.

Statistical analysis

The statistical analysis was performed using GraphPad Prism version 8.2.1 (Prism 8). To compare the treated groups with the control, one-way ANOVA followed by Dunnett's multiple comparisons test was used. Data are presented as the mean $\pm$ standard deviation (SD), with statistical significance set at $P < 0.05$.

Results and Discussion

Impact of *C. cartilaginea* fruit extract on proliferation of 22RV1 cells

The effect of *C. cartilaginea* fruit extract on the growth of 22RV1 prostate cancer cells (10,000 cells per well) was assessed via the MTT assay, as summarized in **Table 1**. At the 24-hour mark, there was a slight decrease in cell viability, even though the cells exhibited some resistance to the extract. As the concentration of the extract was raised from 0.5 to 20 $\mu\text{g/mL}$, the proportion of viable cells showed a gradual reduction. Specifically, the viability dropped from 100% in the untreated cells to 63.86% at the highest concentration of 20 $\mu\text{g/mL}$. This was accompanied by a corresponding rise in cytotoxicity, from 4.66% at the lowest dose to 36.14% at 20 $\mu\text{g/mL}$, as depicted in **Table 1** and **Figure 1**. The findings showed a clear inverse relationship between extract concentration and cell viability.

At the 48- and 72-hour time points, the control cells exhibited an increase in cell viability, while the treated cells showed an even more pronounced decrease in viable cells. At 20 $\mu\text{g/mL}$, the viability of the treated cells dropped to 17.54%, with cytotoxicity reaching 82.46%, as indicated in **Table 1** and **Figure 1**. The lowest concentration of 0.5 $\mu\text{g/mL}$ did not result in significant differences when compared to the control. These results emphasize a strong correlation between the extract concentration and cell viability reduction, with further evidence presented in **Figure 1** for the 24, 48, and 72-hour intervals.

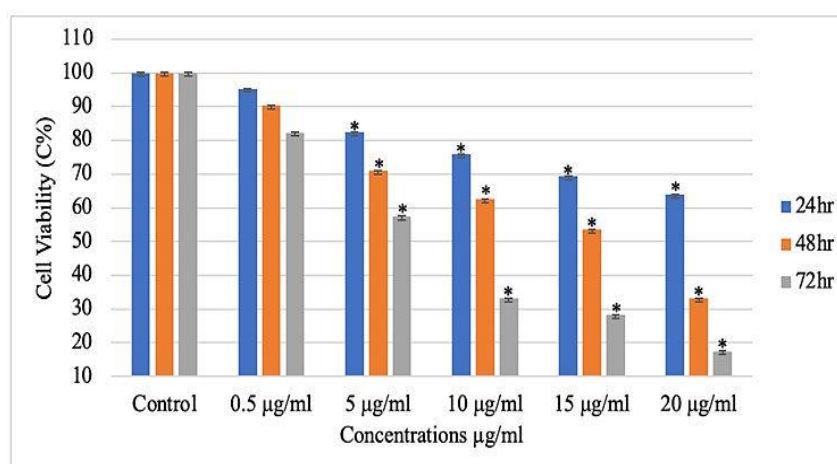


Figure 1. Effect of the *C. cartilaginea* fruit extract on the cell viability at 24, 48, and 72 h post-treatment, at a cell density of 10,000 cells/well; bars represent the mean $\pm$ SD; * is a significant difference at the level of $P < 0.05$.

Table 1. Effect of the *C. cartilaginea* fruit extract on the cell viability at 24, 48, and 72 h post-treatment, at a cell density of 10,000 cells/well.

24-hour post-treatment				
Concentrations	Mean $\pm$ SD	C (%)	Cell cytotoxicity (%)	Adj. P-value
Control	0.4637 $\pm$ 0.5087	100	-	-
0.5 $\mu\text{g/ml}$	0.4421 $\pm$ 0.03840	95.34	4.66	0.6667
5 $\mu\text{g/ml}$	0.3818 $\pm$ 0.03367	82.34	17.66	* 0.0003
10 $\mu\text{g/ml}$	0.3522 $\pm$ 0.02575	75.95	24.05	* < 0.0001
15 $\mu\text{g/ml}$	0.3212 $\pm$ 0.03203	69.27	30.73	* < 0.0001
20 $\mu\text{g/ml}$	0.2961 $\pm$ 0.04991	63.86	36.14	* < 0.0001
48-hour post-treatment				
Concentrations	Mean $\pm$ SD	C (%)	Cell cytotoxicity (%)	Adj. P-value
Control	0.4637 $\pm$ 0.5087	100	-	-
0.5 $\mu\text{g/ml}$	0.4421 $\pm$ 0.03840	95.34	4.66	0.6667
5 $\mu\text{g/ml}$	0.3818 $\pm$ 0.03367	82.34	17.66	* 0.0003
10 $\mu\text{g/ml}$	0.3522 $\pm$ 0.02575	75.95	24.05	* < 0.0001
15 $\mu\text{g/ml}$	0.3212 $\pm$ 0.03203	69.27	30.73	* < 0.0001
20 $\mu\text{g/ml}$	0.2961 $\pm$ 0.04991	63.86	36.14	* < 0.0001

Control	0.6857 ± 0.09010	100	-	-
0.5 µg/ml	0.6192 ± 0.1304	90.30	9.70	0.2969
5 µg/ml	0.4863 ± 0.07583	70.92	29.08	* < 0.0001
10 µg/ml	0.4293 ± 0.06511	62.61	37.39	* < 0.0001
15 µg/ml	0.3679 ± 0.05027	53.65	46.35	* < 0.0001
20 µg/ml	0.2272 ± 0.04441	33.13	66.87	* < 0.0001
72-hour post-treatment				
Concentrations	Mean ± SD	C (%)	Cell cytotoxicity (%)	Adj. P-value
Control	0.9101 ± 0.1257	100	-	-
0.5 µg/ml	0.7492 ± 0.2089	82.32	17.68	0.1568
5 µg/ml	0.5214 ± 0.3170	57.29	42.71	* < 0.0001
10 µg/ml	0.3010 ± 0.02190	33.07	66.93	* < 0.0001
15 µg/ml	0.2578 ± 0.01255	28.33	71.67	* < 0.0001
20 µg/ml	0.1596 ± 0.03138	17.54	82.46	* < 0.0001

Data are expressed as mean ± SD, C (%): cell viability, and Adj. P-value: Adjusted P-value, SD: standard deviation, *P < 0.05

Morphological changes induced by *C. cartilaginea* fruit extract in 22RV1 cells

The morphological effects of various concentrations of *C. cartilaginea* fruit extract (0.5, 5, 10, 15, and 20 µg/mL) on the 22RV1 prostate cancer cell line were observed using an inverted light microscope at a magnification of 20x over different time intervals. The observations revealed a concentration-dependent increase in cell death, as indicated by visible morphological alterations.

After 24 hours of treatment, the control cells exhibited typical characteristics of cell growth, such as intact form, uniform size, clear outlines, and active proliferation, all of which were adherent to the culture plate. No contact inhibition was noted, reflecting healthy cells (**Figure 2a; 24h**). Cells treated with 0.5 µg/mL *C. cartilaginea* fruit extract showed no major differences in morphology, remaining similar to untreated cells (**Figure 2b; 24h**). However, when treated with 10, 15, or 20 µg/mL concentrations, cells became more heterogeneous. Some cells adhered tightly to the plate, while others were less attached and more rounded. A decrease in cell size suggested the onset of early necrosis (**Figures 2d-2f; 24h**).

At 48 hours, control cells were healthy, with around 70% confluence. They appeared bright with clear, well-defined edges and spread extensively on the culture surface (**Figure 2a; 48h**). Cells treated with 0.5 and 5 µg/mL *C. cartilaginea* fruit extract showed minimal signs of shrinkage, rounding, or detachment (**Figures 2b and 2c; 48h**). However, those exposed to 10 µg/mL began to show significant detachment and became flat and shriveled (**Figure 2d; 48h**). When treated with 15 µg/mL, most cells lost their typical shape and swelled (**Figure 2e; 48h**). At 20 µg/mL, the effects were more pronounced, with cells showing a similar pattern to those treated with 15 µg/mL (**Figure 2f; 48h**).

After 72 hours, the control cells had almost reached 90% confluence and showed signs of over-confluence, with cells competing for nutrients (**Figure 2a; 72h**). At 0.5 µg/mL, some cells displayed shrinkage and partial detachment (**Figure 2b; 72h**), and at 5 µg/mL, this effect was more noticeable, with more cells detaching and shrinking (**Figure 2c; 72h**). The 10 µg/mL treatment led to the cells detaching from the plate, floating in the medium, and disrupting the extracellular matrix, inhibiting cell-to-cell interaction (**Figure 2d; 72h**). At the higher concentrations of 15 and 20 µg/mL, almost all cells appeared circular and exhibited a cloudy, unhealthy appearance (**Figures 2e and 2f; 72h**). These cells had a reduced volume, rounded shape, and significant shrinkage, with nearly no viable cells left (**Figures 2e and 2f; 72h**).

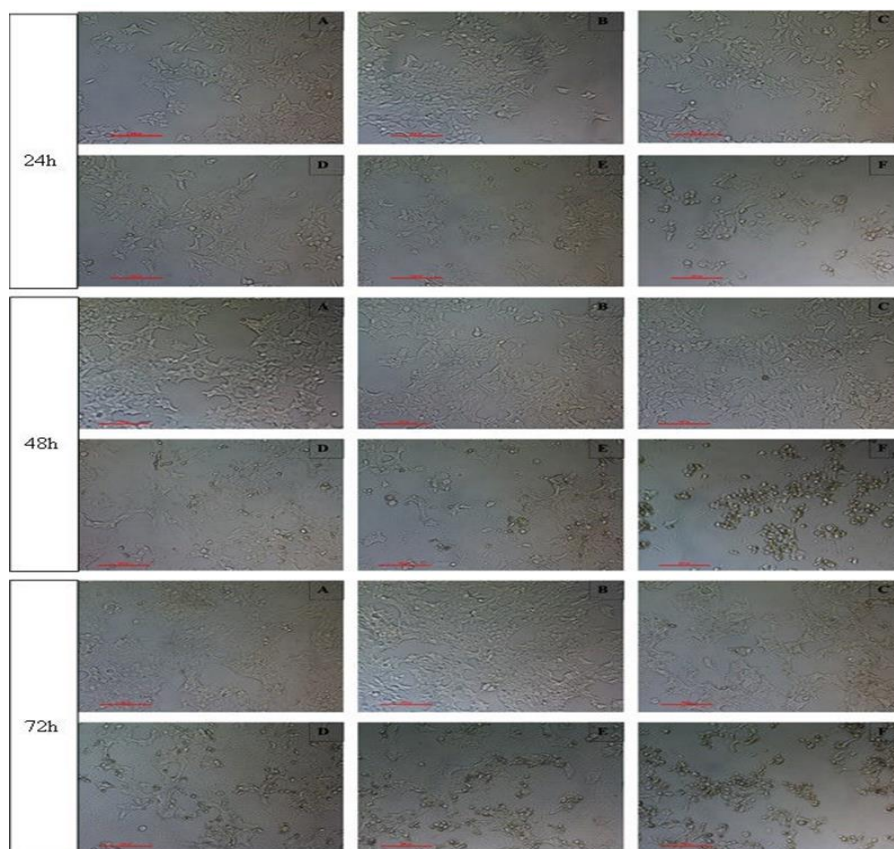


Figure 2. Morphological changes observation 24, 48, and 72 h post-treatment, A) a control untreated cells, B) 0.5 µg/ml, C) 5 µg/ml, D) 10 µg/ml, E) 15 µg/ml, and F) 20 µg/ml (magnification 20x); bar = 500 mm

Effect of C. cartilaginea Fruit extract on migration of 22RV1 prostate cancer cells over 24, 48, and 72 hours

To assess the migration ability of prostate cancer 22RV1 cells, cultures were scratched and treated with different concentrations of *C. cartilaginea* fruit extract. The reduction in the scratched area was measured at three time points: 24, 48, and 72 h. Cells were grown to 70% confluence to ensure proper measurement of the scratch area using imaging software.

At the start (0 h), untreated cells had an initial scratch area of 315,688.3 µm, with a mean ± SD of 47.40 ± 99.2 µm. By the end of 72 h, the scratch area decreased to 84,422.9 µm, with a mean ± SD of 105.24 ± 125.5 µm. For cells treated with 0.5 µg/mL of *C. cartilaginea* fruit extract, the initial scratch area was 305,253.3 µm (mean ± SD of 105.52 ± 125.6 µm), which reduced over time to 259,266.4 µm (mean ± SD of 92.77 ± 122.7 µm). At a concentration of 5 µg/mL, the initial area was 230,996.8 µm (mean ± SD of 108.02 ± 126.0 µm), and after 72 hours, it reduced at a slower pace to 204,002.5 µm (mean ± SD of 100.11 ± 124.5 µm).

With 10 µg/mL, the scratch began at 300,977.6 µm (mean ± SD of 94.97 ± 123.3 µm). However, over the 72 hours, the scratch area increased, eventually reaching 391,211.8 µm (mean ± SD of 65.82 ± 111.6 µm). At a concentration of 15 µg/mL, the initial area was 320,546.0 µm (mean ± SD of 73.73 ± 115.6 µm), and over time, the scratch area expanded to 410,022.7 µm (mean ± SD of 61.36 ± 109.0 µm). This treatment also led to a noticeable reduction in cell density across the scratch, with fewer viable cells, necessitating media removal to obtain a clear image of the area (**Figure 3**).

When treated with the highest concentration of 20 µg/mL, the initial scratch area measured 388,649.9 µm (mean ± SD of 82.07 ± 119.1 µm). Over 72 hours, the area expanded to 475,422.7 µm (mean ± SD of 73.81 ± 115.6 µm), accompanied by a marked increase in cell death. Most cells did not migrate and instead died in place, requiring media withdrawal to capture images of the scratch area (**Figures 3 and 4; Table 2**).

Table 2. The change in the wound area overtime

Concentrations	Total area (µm)	Mean ± SD
Control (0 h)	315688.3	47.40 ± 99.2

Control (24 h)	127933.8	99.50 ± 124.4
Control (48 h)	89097.1	106.56 ± 125.8
Control (72 h)	84422.9	105.24 ± 125.5
0.5 µg/ml (0 h)	305253.3	105.52 ± 125.6
0.5 µg/ml (24 h)	289319.5	99.78 ± 124.5
0.5 µg/ml (48 h)	260139.9	97.80 ± 124.0
0.5 µg/ml (72 h)	259266.4	92.77 ± 122.7
5 µg/ml (0 h)	230996.8	108.02 ± 126.0
5 µg/ml (24 h)	213715.9	103.02 ± 125.1
5 µg/ml (48 h)	212547.4	101.09 ± 124.7
5 µg/ml (72 h)	204002.5	100.11 ± 124.5
10 µg/ml (0 h)	300977.6	94.97 ± 123.3
10 µg/ml (24 h)	324596.2	73.18 ± 115.3
10 µg/ml (48 h)	346573.9	63.93 ± 110.5
10 µg/ml (72 h)	391211.8	65.82 ± 111.6
15 µg/ml (0 h)	320546.0	73.73 ± 115.6
15 µg/ml (24 h)	350285.5	54.75 ± 104.7
15 µg/ml (48 h)	381066.4	57.88 ± 106.8
15 µg/ml (72 h)	410022.7	61.36 ± 109.0
20 µg/ml (0 h)	388649.9	82.07 ± 119.1
20 µg/ml (24 h)	420741.5	67.13 ± 112.3
20 µg/ml (48 h)	456377.8	56.68 ± 106.0
20 µg/ml (72 h)	475422.7	73.81 ± 115.6

Data are expressed as the total area in microns (µm) and mean ± SD, SD: standard deviation.

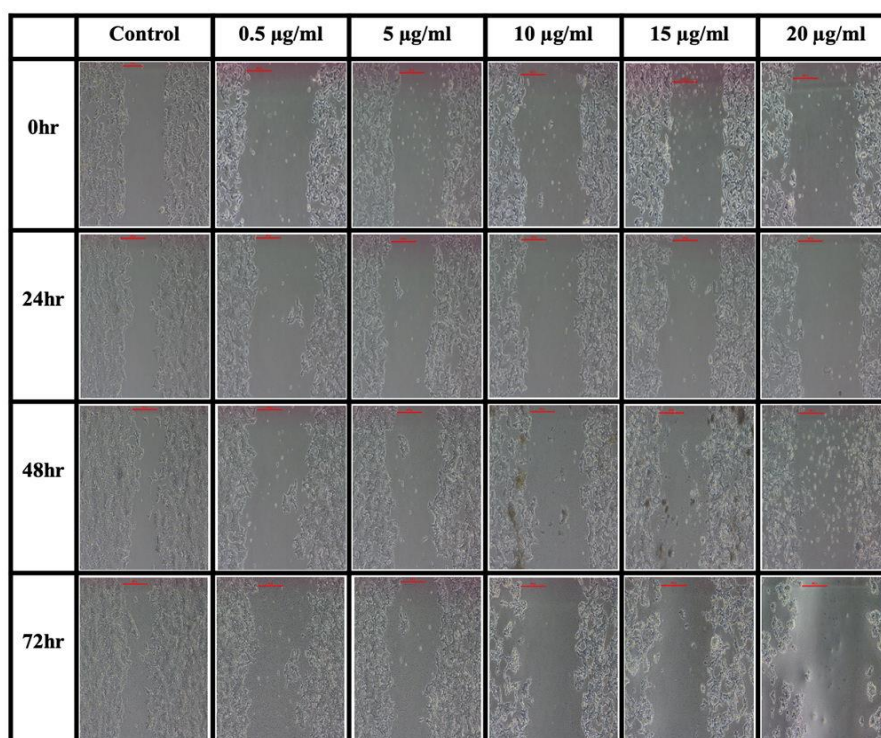


Figure 3. Effect of the *C. cartilaginea* fruit extract on prostate cancer 22RV1 cells migration; the pictures demonstrate the wound-healing assay at 0, 24, 48, and 72 h of exposure to 0.5, 5, 10, 15, and 20 µg/ml *C. cartilaginea* fruit extract; the control indicates untreated cells; the magnification is 10x.

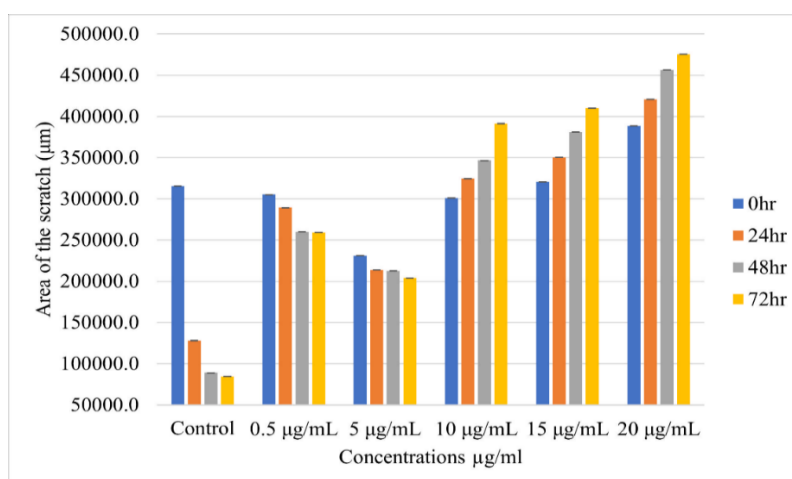


Figure 4. The change in the wound area overtime

The evaluation of cell migration within the wound borders was performed by measuring the percentage reduction in the wound area. The change in wound closure percentage over time was analyzed for both control and treated cells exposed to various concentrations of *C. cartilaginea* fruit extract. Over time, the wound closure for the untreated control increased from 59.47% to 73.26%. For cells treated with 0.5 μg/mL of the extract, the closure percentage rose from 5.22% to 15.07%, though at a slower pace. At 24 h, the closure for the 5 μg/mL treated cells was 7.48%, and by 72 hours, it had increased to 11.69%. However, when treated with 10 μg/mL, the wound closure percentage decreased from -7.85% at 24 hours to -29.98% by 72 hours. Similarly, cells treated with 15 μg/mL exhibited a decrease from -9.28% at 24 hours to -27.91% at 72 hours. Finally, cells exposed to 20 μg/mL showed a starting closure percentage of -8.26%, which declined to -22.33% by 72 hours (**Figure 5**).

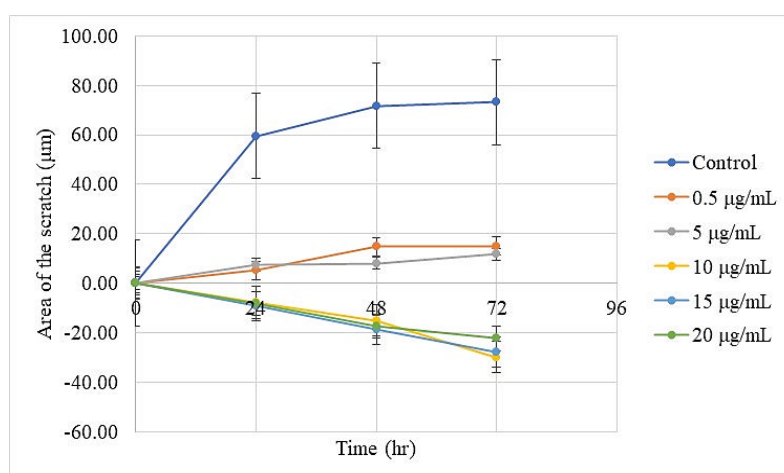


Figure 5. Wound closure percentage overtime

Phenolic compounds and flavonoids are widely recognized for their anticancer effects, particularly in inducing apoptosis in a variety of cancer cell types [30]. The methanolic extract of *C. cartilaginea* fruit contains several bioactive components, including steroids, carbohydrates, alkaloids, saponins, cardiac glycosides, flavonoids, and phenolics [14, 31, 32]. To better understand the potential therapeutic benefits of this plant, the phytochemical composition of the fruit extract was analyzed, focusing on determining the optimal consumption levels that could lead to significant pharmacological effects in human tissues and fluids. In the current study, we evaluated the anticancer potential of the ethanolic extract of *C. cartilaginea* fruit and its role in apoptosis in the 22RV1 human prostate cancer cell line. Our results indicate that *C. cartilaginea* fruit extract has a marked impact on reducing the migratory capacity of prostate cancer cells, as evidenced by a significant widening of the scratch area and an increase in cell death by approximately 70%.

We observed variations in cell density and viability, but no consistent pattern of cell viability was noted. The MTT assay, which measures mitochondrial activity by converting tetrazolium salts into formazan, revealed potential

fluctuations in cell metabolism or slight errors in cell seeding. In a dose- and time-dependent manner, the extract from *C. cartilaginea* fruit inhibited the proliferation of 22RV1 prostate cancer cells. As both the concentration and treatment time increased, the cytotoxic effect on the prostate cancer cells was more pronounced. Significant differences in cell viability were noted between control and treated groups at concentrations of 10, 15, and 20 $\mu\text{g/ml}$ ($P < 0.0001$). This finding is novel since earlier studies have focused on different species within the Capparidaceae family. However, similar cytotoxic effects were observed by Khodaei *et al.* [33], who demonstrated significant cytotoxicity in HT-29 colon cancer cells following caper extract treatment. They attributed these effects to mitochondrial dysfunction, elevated reactive oxygen species, alterations in sirtuin-3 activity, and eventual cell death. Khodaei *et al.* [33] used 1, 5, and 10 mg/ml concentrations of caper extract over 24, 48, and 72 hours, with the maximum cytotoxic effect noted at 0.1 mg/ml . In alignment with these findings, our study showed a significant reduction in 22RV1 prostate cancer cell viability after 24 hours of exposure to *C. cartilaginea* fruit extract at concentrations of 5 $\mu\text{g/ml}$ ($P < 0.0003$) and 10, 15, and 20 $\mu\text{g/ml}$ ($P < 0.0001$). This reduction was also significant after 48 and 72 h at these concentrations ($P < 0.0001$).

Additionally, the oil extracted from *C. cartilaginea* contains several isothiocyanates, such as methyl isothiocyanate, isopropyl isothiocyanate, isobutyl isothiocyanate, ethyl isothiocyanate, and butane-1-isothiocyanate [31, 34]. Crowley *et al.* [35] investigated the anticancer properties of various natural and synthetic isothiocyanates, including allyl-isothiocyanate, benzyl-isothiocyanate, and phenylethyl-isothiocyanate, on prostate cancer cells. Their study found that treatment with isothiocyanates led to a dose-dependent decrease in cell viability and cytotoxicity in both androgen-dependent (22RV1) and androgen-independent (DU145) prostate cancer cells. A range of isothiocyanate concentrations (0-15 μM) was applied over 24 and 48 hours, with MTT assays revealing a significant reduction in cell viability at 10 and 15 μM ($P < 0.05$, $P < 0.0001$) [35]. In line with this, our study also observed a notable reduction in 22RV1 cell viability after 24 hours of exposure to *C. cartilaginea* fruit extract at 5 $\mu\text{g/ml}$ ($P < 0.0003$) and 10, 15, and 20 $\mu\text{g/ml}$ ($P < 0.0001$). The cytotoxic effect persisted after 48 and 72 h of exposure at these concentrations ($P < 0.0001$).

Furthermore, Moharram *et al.* [34] assessed the cytotoxicity of methanolic extracts from *C. cartilaginea* leaves, stems, and twigs against the A549 human lung carcinoma cell line. They found that the twigs had the strongest cytotoxic effect at 57.5 $\mu\text{g/ml}$, followed by the stem extract at 240 $\mu\text{g/ml}$ [34].

Studies have consistently focused on the cytotoxicity of plants from the Capparidaceae family against different cancer cell lines. For instance, Mansour *et al.* evaluated the hydroethanolic extract of *C. spinosa* L. for its effect on the HeLa cervical cancer cell line, finding that the extract inhibited cell growth in a dose-dependent manner [36]. Similarly, Moghadamnia *et al.* [37] tested *C. spinosa* L. extracts on various cancer cell lines, including MCF-7 (breast carcinoma), Saos (human osteosarcoma), HeLa, and Fibroblast, determining that a 250 $\mu\text{g/ml}$ dose was effective 72 h after treatment compared to normal cells. Kulisic-Bilusic *et al.* [38] examined the effects of both aqueous infusions and essential oil of *C. spinosa* L. on HT-29 colon cancer cells over 15 and 72 hours. They found that at 0.1 g/l , the aqueous infusion had a greater antiproliferative impact than the essential oil at both time intervals [38]. In another study, Sheikh *et al.* [39] tested the ethanolic extract of *C. spinosa* L. on the PLC/PRF/5 liver cancer cell line and found the highest growth inhibition at a concentration of 5000 $\mu\text{g/ml}$. These variations in cytotoxic effects can be attributed to factors such as the type of extract, geographical origin, plant part used, and other experimental conditions, including cell line type, seeding densities, incubation periods, and concentration levels.

In the present study, we examined the morphological alterations in 22RV1 prostate cancer cells after treatment with varying concentrations of *C. cartilaginea* fruit extracts (0.5, 5, 10, 15, and 20 $\mu\text{g/ml}$) over 24, 48, and 72 h, under an inverted light microscope. Cells treated with 10, 15, and 20 $\mu\text{g/ml}$ exhibited noticeable shrinkage and flattening, with early signs of necrosis appearing, along with substantial circularization of cells by 72 h. In a similar study, Sheikh *et al.* [39] investigated the effects of varying concentrations of *C. spinosa* L. ethanolic extract (150, 310, 620, 1250, 2500, and 5000 $\mu\text{g/ml}$) on PLC/PRF/5 liver cancer cells, noting significant morphological changes at a concentration of 1250 $\mu\text{g/ml}$ after 48 hours. These changes included cell shrinkage, irregular shapes, plasma membrane blebbing, nuclear condensation, and apoptotic body formation, typical markers of apoptosis [39]. Zhang *et al.* [40] also evaluated the effects of three flavonoids—genistein, luteolin, and quercetin—on DU145 cell morphology, noting that treated cells exhibited swelling and membrane damage after 24 h at various concentrations (20, 40, 80, and 100 μM). Similarly, our results indicate that *C. cartilaginea* fruit extract effectively induced cell death, with increasing mortality corresponding to higher concentrations. These morphological changes may result from disruptions to the extracellular matrix and inhibition of cell-to-cell contact [40, 41].

Additionally, we assessed the impact of *C. cartilaginea* fruit extract on the migration of 22RV1 prostate cancer cells using a wound-healing assay, measuring the effect over time and at different concentrations. A similar study by YangArumugam and Abdull Razis [42] examined the effect of quercetin on the migration of PC-3 prostate cancer cells, reporting that quercetin significantly reduced the migration rate of PC-3 cells ($P < 0.05$). In line with these findings, our study showed that *C. cartilaginea* fruit extract also significantly inhibited the migration of prostate cancer 22RV1 cells. At higher concentrations, we observed an increase in the number of dead cells in the cell-free area, and the cell density across the scratch decreased over time. In untreated cells, the scratch area eventually closed.

Conclusion

Cancer, being a leading cause of mortality globally, often progresses due to abnormalities in the apoptotic pathways. Therefore, we propose that substances like medicinal plants, which can influence apoptosis in prostate cancer (PCa) cells, may hold promise for therapeutic applications in treating PCa. Phytochemicals, known for their significant therapeutic potential, have gained attention for their role in combating various diseases, including cancer. *C. cartilaginea* is particularly rich in flavonoids such as kaempferol-3-O-rutinoside, quercetin-3-O-rutinoside, quercetin-7-O-rutinoside, and quercetin-3-glucoside-7-O-rhamnoside, which have garnered significant interest due to their possible health benefits, particularly their antioxidant effects. However, the overall biological effects of these compounds remain uncertain, mainly due to limited knowledge about their bioavailability, metabolism, and how different conjugates contribute to their activity in humans and animals. Our findings demonstrate that the fruit extract of *C. cartilaginea* effectively inhibits the migration of 22RV1 prostate cancer cells and impairs their wound healing capacity. Nonetheless, further research is necessary to determine the precise mechanisms through which this extract operates before considering it as a potential anticancer treatment. Some limitations in our study include the lack of comparison with normal cell lines, although we did use a positive control group for *C. cartilaginea* fruit extract. Additionally, the seasonal nature of the fruit posed challenges, as many of the fruits were already deteriorating by the time they arrived at the laboratory. Future studies could explore the effects of other parts of the *C. cartilaginea* plant, such as the stem, roots, and leaves, on both normal and cancerous human prostate cells. Moreover, the impact of *C. cartilaginea* fruit extract on gene expression levels should be further investigated.

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