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Original Article

# Investigation of the Antiproliferative and Genotoxic Effects of *Serenoa repens* (W. Bartram) Small Extracts Available in the Market

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## Abstract

**Introduction:** *Serenoa repens* (W. Bartram) Small (Saw Palmetto), commonly referred to as the small palm, has been used in both traditional and modern medicine for centuries. Known for its diuretic, endocrinological, anabolic, and anti-inflammatory properties, Saw Palmetto is mostly used to treat benign prostatic hyperplasia (BPH). Known for its diuretic, endocrinological, anabolic, and anti-inflammatory properties, Saw Palmetto is mostly used to treat BPH. There has been a recent surge in interest in these products because herbal products are believed to be safer and have fewer side effects. Pharmacies stock many Saw Palmetto products authorized by the USA Department of Agriculture. People have begun to purchase these herbal products online as technology has advanced, especially during the COVID-19 pandemic, due to faster and easier access and cheaper costs. Many counterfeit products originate from popular e-commerce websites that sell goods that lack the necessary safety inspections. A literature review found that these products have adverse outcomes, including significant outcomes that can lead to death, which poses major public health concerns. The aim of this study was to compare the antiproliferative and genotoxic effects of *Serenoa repens* (W. Bartram) Small extracts obtained from two separate commercial sources, purchased over the internet and from a pharmacy, on human prostate cancer cell line (PC-3).

**Methods:** The antiproliferative effect of Saw Palmetto extracts at different doses (10-200 µg/ml) was evaluated with the MTT test and the genotoxic effect was determined by the comet assay.

**Results:** According to MTT results, the extract purchased from the pharmacy did not cause high inhibition in PC-3 cells at he entire dose range, while higher inhibition values were recorded in the extract obtained from the internet. On the other hand, when the genotoxic activities of both extracts were examined, it was determined that the DNA damage caused by the product purchased from the pharmacy in PC-3 cells was lower than the product purchased from the internet.

**Conclusions:** This research highlights the health risks of herbal products purchased online and the need to source dietary supplements from trusted suppliers. It is crucial to change this view and increase public awareness to ensure safer use of herbal products.

**Keywords:** *Serenoa repens*, comet assay, antiproliferative effect, genotoxicity, MTT assay

## 1. Introduction

*Serenoa repens*, which is native to the Americas and belonging to the Arecaceae family, was first used by Native Americans to treat infertility and erectile dysfunction. Later, colonists began using the berries of this plant as a tonic. The earliest reports in the literature on its use for urinary complaints date back to the early 20th century (1). The fruits of *Serenoa repens* are known to grow in clusters

and ripen from October to December, turning dark purple to black color. The extract of this plant is obtained from the ripe, partially dried fruits. Since different manufacturers use different extraction methods, it is unlikely that one product is equivalent to another (2). Today, Saw Palmetto is considered one of the most widely used herbal remedies for benign prostatic hyperplasia (BPH). Traditionally, it has been used to treat chronic or subacute cystitis, genitourinary rhinitis, testicular atrophy, sex hormone imbalances, and most importantly, prostate enlargement. Other traditional indications include mastitis, eczema, bronchial pathologies, and cough. However, there is no scientific evidence to support these traditional uses of this plant (3, 4).

Saw Palmetto preparations are available in various formulations, including liquids, tablets, capsules, and herbal teas. The plant contains polysaccharides with acidic character and a molecular weight of approximately 10,000 daltons (galactose (38.4%), arabinose (18.7%), uronic acid (14%), invert sugar (28.2%) and mannitol); plant sterols such as campesterol, stigmasterol, β-sitosterol, β-sitosterol-3-O-β-D-diglucoside and several β-sitosterol esters with saturated fatty acids; flavonoids such as isoquercitrin, rutin, kaempferol-3-O-β-D-glucoside, and rhoifolin; triglycerides and fatty acids including capric, caproic, caprylic, lauric, myristic, oleic (oleic and myristoleic acid), and palmitic acid; as well as tannins, volatile oils, resin, carotene, and anthranilic acid (5). Fatty acids are considered the main active components (6). The active ingredients of Saw Palmetto extracts, volatile oils and free fatty acids, are thought to have inhibitory effects on 5-alpha-reductase which blocks the conversion of testosterone to its active metabolite, dihydrotestosterone. The plant is also known to have diuretic, endocrinological, anabolic and anti-inflammatory properties. In addition, rare and generally modest adverse effects of Saw Palmetto include dizziness, headache, nausea, vomiting, constipation and diarrhea. Saw palmetto has been among the top 10 best-selling herbal medicines in the United States since the 1990s, generating approximately $700 million in annual revenue worldwide from *Serenoa repens* preparations (7). With data from more than 35 clinical studies supporting the use of standardized Saw Palmetto extracts, it is the most commonly used herbal treatment for urological symptoms associated with BPH in Europe. It was reported that the use of the extract in the treatment of lower urinary tract symptoms (LUTS) related with BPH has increased peak urine flow and reduced nocturia without elevating serum prostate-specific antigen (PSA) levels (8).

It's known that BPH develops from the proliferation of stromal and epithelial cells in the transition zone of the prostate surrounding the urethra (9). This expansion squeezes the urethra and obstructs the bladder outlet, leading to clinical symptoms such as LUTS, urinary retention, or incomplete bladder emptying, which could cause an infection. The most common clinical presentation of BPH involves LUTS, a group of symptoms that include both obstructive symptoms such as hesitancy, weak stream, incomplete voiding, urinary retention, and overflow incontinence and irritative symptoms such as frequency and urgency (10). If left untreated, the chronic condition may result in high-pressure retention (a potentially life-threatening complication) and irreversible changes in the bladder detrusor muscle (11). Treatment options include pharmacological and surgical interventions. For mild to moderate BPH, pharmacological therapy has become the standard of care, as well-designed clinical studies have shown that 5-alpha-reductase inhibitors (5-ARIs) such as finasteride and alpha-blockers such as tamsulosin, significantly improve LUTS and increase peak urine flow in men with BPH. Subsequently, numerous clinical studies have confirmed the efficacy of two 5-ARIs (finasteride and dutasteride) and five alpha-blockers (including tamsulosin, alfuzosin, and silodosin) approved by the U.S. Food and Drug Administration for the treatment of BPH (12). BPH is a disease whose prevalence increases with age and is emerging as a common health problem in the general population. In response to health concerns, people have increasingly turned to herbal supplements because they believe they have fewer side effects than traditional medications. However, these herbal remedies are sold under numerous brand names by various manufacturers, often lacking adequate

safety assessments. In addition to pharmacies, similar products are also available on reputable internet platforms, but some of these may be illegal, counterfeit, or produced by unauthorized individuals or manufacturers.

In a study examining the inhibition of 5α-reductase type I and II enzymes, the inhibitory capacity of 10 commercially available *Serenoa repens* products was determined. The study revealed that hexanic extracts showed better biological activity compared to other extracts. The findings of this study showed that pharmacological effects differed among drugs of various commercial brands, and this may be important in determining the treatment outcome. The efficacy of the products depends not only on the presence of the plant, but also on the extraction technique used and differences in formulation (13). The effects of *Serenoa repens* extracts of various commercial brands on prostate epithelial and fibroblast cells were subjected to a comparative study. The study showed significant differences in 5α-reductase inhibitory activity among the products, thus emphasizing that these differences may affect their biological activity. It was also noted that low-quality, non-standardized products would not work; this scenario emphasizes the need for quality control in evaluating product efficacy (14).

Recently, online shopping has become more popular due to its affordability and easy accessibility. However, fake products can be found even on highly advertised and reputable websites. Nowadays, numerous reports in both broadcast and print media have highlighted the rise of fake health products (16). To prevent the rise of such incidents and to promote public health protection, stricter regulatory controls should be implemented and the sale of these products through online platforms should be banned.

The aim of this study was to investigate the safety of two *Serenoa repens* extracts, one approved by the Ministry of Agriculture and obtained from a pharmacy, and the other purchased from a well-known e-commerce site in Türkiye, by *in vitro* cell culture techniques using the MTT test for antiproliferative effect and the comet test for genotoxic potential. Although existing studies have addressed the efficacy, biological activity and toxic consequences of *Serenoa repens* and compared various preparations, none of them have evaluated the toxicity of pharmacy and internet-sourced products. By emphasizing the need for regulatory control and the need to restrict uncontrolled internet sales of such products, this study aims to fill this gap and provide information.

## 2. Methods

### 2.1. Preparation of plant extract samples

Two separate capsules of *Serenoa repens* extract were used in the study: one purchased from a pharmacy and the other from an online retailer. First, 10 grams of each product were weighed and macerated with ethanol. Then, the liquid phase was filtered with filter paper following maceration; the solvent was evaporated at reduced pressure using a rotary evaporator (Fig 1). The extracts produced were stored in a +4°C refrigerator until further investigation.

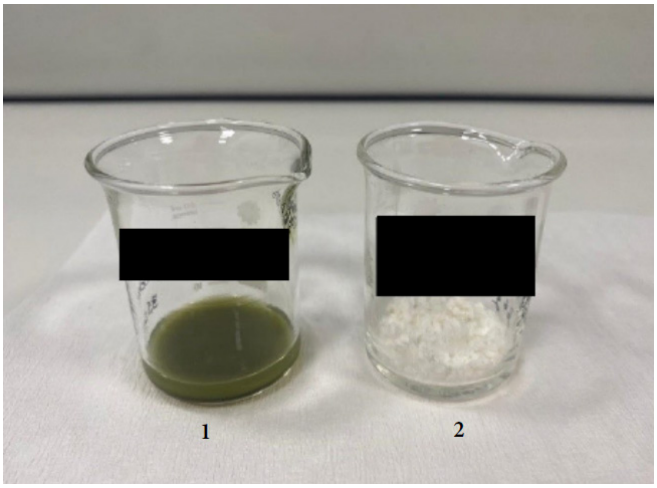


**Figure 1.** Photograph of the extracts after solvent evaporation using rotary evaporation (number 1 is the herbal supplement purchased from a pharmacy, and number 2 is the one purchased from an online retailer).

### 2.2. Cell culture studies

In this study, a prostate cancer cell line (PC-3, CRL-1435, ATCC) was used. Under standard incubation conditions at 37°C in a humidified atmosphere containing 5% $CO_2$, the cells were grown in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS) and 1%

penicillin-streptomycin. Once the cells reached 80–90% confluency, Trypsin-EDTA was used to extract them from the flask surface and subculture them into 25 cm² flasks.

### 2.3. Cell viability of *Serenoa repens* extracts on prostate cancer cells

A colorimetric technique based on the measurement of cellular metabolic activity, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate antiproliferative effects (17). The impact of *Serenoa repens* extracts, obtained from two different sources (an online and a pharmaceutical product), on cell viability was evaluated using the MTT assay (18). PC-3 cells were seeded into 96-well plates at a density of $1 \times 10^4$ cells per well and incubated overnight. The following day, cells were treated with varying concentrations (10, 50, 100, and 200 µg/mL) of each extract for 24 hours. After the incubation period, MTT was added to each well at a final concentration of 0,5 mg/mL, and the cells were incubated for an additional 4 hours. The medium was then aspirated, and 100 µL of SDS buffer was added to each well to solubilize the purple formazan crystals. Absorbance values were measured at 570 nm and 630 nm using a microplate reader (BioTek, Winooski, USA). Cell viability was calculated according to the following equation:

$$\% \text{ Cell Viability} = [(\text{Mean OD of treated cells}) / (\text{Mean OD of control cells})] \times 100$$

### 2.4. Comet Assay

A modified version of the alkaline single-cell gel electrophoresis technique (Comet Assay), first established by Singh et al., was used to assess the genotoxic effects of the produced *Serenoa repens* extracts on PC-3 cells (19). Control, positive control (hydrogen peroxide), and plant extract-treated groups were used for *in vitro* assay. The positive control was hydrogen peroxide ($H_2O_2$) at 100 µM, known to cause DNA damage.

PC-3 cells were seeded onto 6-well sterile plates at a density of $2\times10^4$ cells per well and cultured for 24 hours at 37°C in a humidified environment containing 5% $CO_2$. The extracts were dissolved in RPMI medium at a stock concentration of 0.5 mg/mL and administered to the cells at 50, 100, and 200 µg/mL after 24 hours. The cells were then cultured for further 24 hours.

At the end of the incubation period, the culture medium was discarded, the cells were trypsinized and then centrifuged at 1800 rpm for ten minutes. The cell pellet was resuspended in PBS and the supernatant was discarded. This washing procedure was repeated two more times. The supernatant was removed after the final wash using cold PBS and the cell concentration was adjusted to $1\times10^6$ cells/mL.

10 µL aliquot of the cell suspension was combined with 90 µL of 0.6% low-melting-point agarose (LMA) and layered onto slides pre-coated with 1% normal-melting-point agarose. The slides were submerged in cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, and 10% DMSO, pH 10) at 4°C for 1 hour after solidification on ice. The slides were rinsed with cold PBS and put in a horizontal electrophoresis tank following lysis.

All slides were covered with cold alkaline electrophoresis buffer (300 mM NaOH, 10 mM $Na_2EDTA$, pH 13.0) for 20 minutes. Electrophoresis was performed in the same solution at 4°C, 25 V, and 300 mA for a duration of 20 minutes. After electrophoresis, the slides were neutralized by washing three times with neutralization buffer (0.4 M Tris, pH 7.5). After electrophoresis, the slide was drained, neutralized with neutralization buffer (0.4 M Tris-HCl, pH 7.5) for 5 minutes and stained with ethidium bromide (20 µg/mL in distilled water; 50 µL per slide) and covered with a coverslip. Imaging was performed using a fluorescence microscope (Olympus BX51, Japan) at 400× magnification.

The percentage of DNA in the tail (% $DNA_T$) was quantified using the BAB Bs200Pro image analysis software (BAB LTD., Ankara, Turkey). A total of 100 cells per group (from two slides) were analyzed, and all experiments were performed in triplicate.

### 2.5. Statistics Analysis

All data were expressed as the mean ± standard error of the mean (SEM), based on at least three independent replicates of each experiment. Statistical analyses were performed using SPSS version 25.0 software. The $IC_{50}$ values obtained

from the MTT assay were evaluated using the Student's t-test. The comet assay findings were evaluated using the Mann-Whitney U test for non-normally distributed data when comparing two groups, and the Kruskal-Wallis analysis of variance for comparisons across many groups. A p-value below 0.05 was deemed statistically significant.

## 3. Results

### 3.1. MTT results of *Serenoa repens* extracts

In the present study, the effect of *Serenoa repens* extracts obtained from two different commercial sources (a pharmacy and an online retailer) on the viability of PC-3 cells was evaluated using the MTT assay for 24 hour (Fig 2).

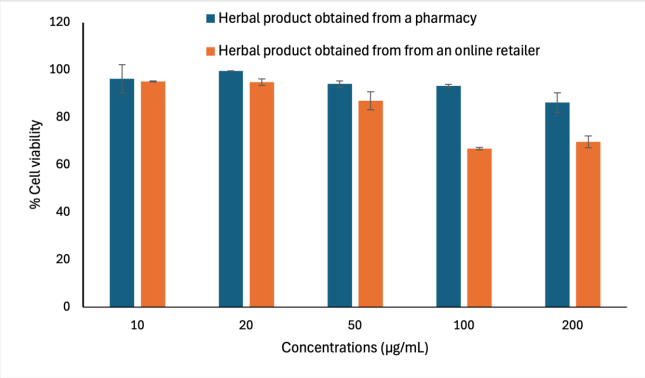


**Figure 2.** Effect of *Serenoa repens* on the 24-hour viability of PC-3 cells at 24 h.

According to our results, the extract obtained from the pharmacy product did not cause significant inhibition of PC-3 cell viability across the entire concentration range, with 86.25% of the cells remaining viable even at the highest concentration tested (200 µg/mL). In contrast, the extract obtained from the online retailer showed a higher inhibitory effect. Although both extracts produced similar results at lower concentrations (10, 20, and 50 µg/mL), maintaining cell viability around 90%, the online product extract significantly reduced viability at 100 and 200 µg/mL, leading to 32% inhibition.

### 3.2. Comet assay results

As shown in Table 1, a statistically significant, concentration-dependent increase in DNA damage was observed in cancer cells 24 hours after treatment with *Serenoa repens*, compared to the untreated control group. However, all extracts induced significantly lower levels of DNA damage than the positive control group ($p < 0.001$).

The statistical analysis revealed that both formulations of *Serenoa repens* induced DNA damage in PC-3 cells in a concentration-dependent manner. Upon comparison of the genotoxic effects of the two extracts—one procured from a pharmacy and the other from an internet source—statistically significant differences were observed at all tested doses. The extract from the internet source induced significantly more DNA damage compared to the pharmacy extract at a dose of 50 µg/mL ($p < 0.05$).

The online-sourced extract at doses of 100 and 200 µg/mL induced significantly more DNA damage compared to the pharmacy-derived product ($p < 0.001$ for both concentrations). The results indicate that alterations in the source or formulation of *Serenoa repens* extracts may influence their biological activity, particularly regarding their capacity to induce DNA damage.

**Table 1.** Genotoxic effects of *Serenoa repens* extracts on PC-3 cells

| | | $\%DNA_T$ |
|---|---|---|
| **Control** | | 25.22 ± 0.34 |
| **Positive control (100 µM $H_2O_2$)** | | 65.69 ± 0.81 |
| **Herbal product obtained from a pharmacy** | **50 µg/mL** | $30.03 \pm 0.65^{***,+++,¶}$ |
| | **100 µg/mL** | $35.01 \pm 0.62^{***,+++,¶¶}$ |
| | **200 µg/mL** | $37.24 \pm 0.75^{***,+++,¶¶}$ |
| **Herbal product obtained from an online retailer** | **50 µg/mL** | $31.78 \pm 0.65^{***,+++}$ |
| | **100 µg/mL** | $38.71 \pm 0.67^{***,+++}$ |
| | **200 µg/mL** | $43.87 \pm 0.81^{***,+++}$ |

The results are expressed as the mean ± standard deviation of three independent measurements.
$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to the control group; $^{+}p < 0.05$, $^{++}p < 0.01$, and $^{+++}p < 0.001$ compared to the positive control group.
¶$p < 0.05$, ¶¶$p < 0.01$, and ¶¶¶$p < 0.001$ compared to the online retailer group
$\%DNA_T$: Percentage of DNA in the tail

## 4. Discussion

In this study, the antiproliferative and genotoxic effects of *Serenoa repens* (Saw Palmetto) extracts obtained from pharmacy and online retailer were investigated on human prostate cancer cells (PC-3) using MTT and comet assays, respectively. Our

findings revealed significant differences in both antiproliferative and genotoxic profiles between the two products, highlighting potential public health concerns regarding the unregulated online sale of herbal supplements.

The MTT test results revealed that the product extract purchased from a pharmacy did not exhibit antiproliferative activity even at the highest concentrations tested. In contrast, the product extract purchased from an online retailer showed significantly higher cytotoxicity, indicating a stronger inhibitory effect on the cell viability. While moderate cytotoxicity may be interpreted as a desirable antiproliferative activity in the context of prostate cancer, the unregulated nature of products purchased online raises concerns regarding product composition, concentration of active compounds, and the presence of toxic imposters.

The observed differences in antiproliferative activity can be attributed to several factors, including differences in extraction methods, raw material quality, manufacturing standards, and potential contamination or counterfeiting. The fact that both products claim to contain the same active ingredient highlights the lack of standardization in the herbal supplement market.

Booker et al. examined the chemical components of Saw Palmetto products from various brands using gas chromatography and and $^{1}$H NMR metabolomic methods (6). Despite coming from the same plant source, significant differences were found in the amounts of important components such as free fatty acids, phytosterols, and volatile chemicals. These variations in composition have a direct impact on the biological activity and bioavailability of the product. The article also emphasizes that marketed products should be evaluated not only by their botanical names but also by their structures.

Another study compared the fatty acid and sterol levels of *Serenoa repens* extracts from various commercial brands. The results revealed significant differences in chemical content between the products, and the researchers suggested that these differences may be important for pharmacological outcomes. Some brands of products showed low levels of active chemicals and reported that these products did not provide the desired effects. In line with our findings, the results of this study suggest that therapeutic efficacy depends on research on product quality and content (15). Previous studies have emphasized that different extraction techniques may lead to different concentrations of bioactive fatty acids and sterols, which may directly affect the biological activity (6, 2).

De Monte et al (2014) also examined how various extraction methods, including supercritical $CO_2$, hexane, and ethanol, affected *Serenoa repens* extracts. The researchers found that extracts from the supercritical $CO_2$ method exhibited less antiandrogenic activity compared to the other techniques. Hexanic extracts produced better biological effects, leading to improved therapeutic efficacy. These findings highlight how the extraction process affects the biological efficacy of the product (20).

In the comet assay, which evaluates DNA damage at the single-cell level, the extract purchased from pharmacy caused significantly less genotoxicity compared to the extract purchased from an online retailer. The DNA tail intensity was markedly increased in cells treated with the online-purchased extract, suggesting higher levels of strand breaks and overall genomic instability. This outcome further supports the hypothesis that products sourced from unverified online sellers may contain impurities or unregulated additives that pose a genotoxic risk.

Interestingly, despite the increasing popularity of *Serenoa repens* in the treatment of BPH, most of the existing studies have focused on efficacy rather than safety, especially with regard to genotoxicity. Our study addresses this gap by providing comparative toxicity data and also highlights that perceived naturalness is not the same as safety. It also highlights the critical importance of post-market surveillance and quality control, especially in light of recent reports of counterfeit and adulterated health products sold online.

A study conducted in Italy between 2011 and 2013 assessed the health risks of pharmaceuticals and nutritional supplements sold illegally or online. The results showed that supplements sold online were highly risky, both in terms of the health of

customers and the safety of the ingredients. The researchers reported that inadequate regulatory oversight may negatively impact public health and that stricter regulation was needed (21).

Veatch-Blohm et al (2021) evaluated the levels of contamination and consistency of content in 29 herbal supplements commonly used in the United States. The study revealed significant variability in antioxidant activity, phenolic and flavonoid content, and undesirable impurities among different supplements. The findings emphasize the necessity for government regulation of these products in order to avoid harming public health (22).

Researchers at King Saud University's College of Pharmacy investigated the frequency of online purchases of health products, herbs, and medicines in Saudi Arabia, the motivations behind these purchases, and perceptions of product safety. While the online health product market is rapidly expanding, individuals have expressed concerns about the safety and quality of these products. Most of the participants agreed that purchasing products online is easy; however, many expressed uncertainty about the reliability and accuracy of product information. The study highlights the importance of safety guidelines for online health product sales and consumer awareness of these regulations (23).

The PC-3 cell line used in this study provides a relevant model to evaluate the potential therapeutic and toxicological effects of prostate-targeting agents. Previous findings emphasize the importance of ongoing research comparing online purchases with those from pharmacies, underscoring their public health importance. There are very few studies in the literature comparing internet products from online and pharmacy sources, as in our study. The previous studies support the importance of our study, as it is the first research of its kind in this area. However, it should be noted that *in vitro* assays have limitations and may not fully replicate *in vivo* responses. Therefore, future studies involving animal models or clinical samples may further confirm the findings and provide insight into pharmacokinetic and metabolic parameters.

## 5. Conclusion

In conclusion, the significant antiproliferative and genotoxic differences observed between *Serenoa repens* extracts purchased from pharmacies and online sources highlight the urgent need for stricter regulation and monitoring of online herbal supplement sales. Public awareness campaigns and strict regulatory regulations are essential to protect consumers from potentially harmful products and to ensure that herbal therapies maintain both efficacy and safety standards. On the other hand, herbal medicines are becoming more important in phytovigilance systems due to their therapeutic potential. More comprehensive studies on a wider range of brands and formulations are needed to support regulatory decision-making and public health recommendations.

**Conflicts of interest:** The authors declare no conflicts of interest related to this work.

**Ethics approval:** Not applicable.

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