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Research Article

STUDY OF THE PHYSIOLOGICAL ROLE OF *STREBLUS ASPER* AS A CHEMOPREVENTIVE AGENT ON HUMAN PROSTATE CANCER (DU-145) CELL LINE

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ABSTRACT

Objectives: This study aims to explore the chemopreventive potential of *Streblus asper* extracts against prostate cancer cells. Specifically, it seeks to assess the efficacy of these extracts in inducing apoptosis and inhibiting cell proliferation within these cancer cells.

Methods: The inhibitory effects of *S. asper* extracts were evaluated using the TBEA technique to determine the IC_{50} values. Morphological changes were indicative of apoptosis, such as cell shrinkage, rounding, vacuolization, formation of apoptotic bodies, membrane blebbing, and cell elongation were observed following treatment. An 8-day cell proliferation assay was conducted to examine the effects on prostate cancer cell line proliferation. In addition, the safety of *S. asper* root extracts was assessed *in vitro* using a Mouse fibroblast cell line to ensure no cytotoxic effects were present on normal cells.

Results: *Streblus asper* extracts demonstrated a significant inhibitory effect on the proliferation of prostate cancer cells, with no observed cytotoxicity on normal Mouse fibroblast cell lines. The results from the TBEA technique confirmed the extract's potency, with noticeable morphological changes supporting the induction of apoptosis in the treated cancer cells.

Conclusion: The findings from this study offer promising insights into the potential of *S. asper* extracts as chemopreventive agents against prostate cancer. By exhibiting significant inhibitory effects on cancer cell proliferation and inducing apoptosis without harming normal cells, *S. asper* presents a viable, natural alternative in the fight against prostate cancer. Further research and clinical trials are warranted to fully elucidate its therapeutic potential and application in cancer prevention strategies.

Keywords: Chemoprevention, *Streblus asper,* Prostate cancer.

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INTRODUCTION

Prostate cancer poses a significant health concern among males, particularly among older men, with elevated rates of occurrence and mortality in African American males [1]. Prostate cancer is the second most prevalent malignancy among men globally, surpassed only by lung cancer. In 2018, there were 1,276,106 newly diagnosed cases of prostate cancer and 358,989 fatalities. This corresponds to 3.8% of the total number of fatalities caused by cancer in males. The incidence and mortality rates of prostate cancer exhibit a positive association with age, as seen by a median age of diagnosis of 66. Prostate cancer disproportionately affects African American men, with an incidence rate of 158.3 new cases per 100,000, considerably higher than that of white men. Additionally, the mortality rate among African American men is nearly double that of their white counterparts [2].

Although there have been advancements in the detection and treatment of prostate cancer, there is still a requirement for alternative therapy. Chemoprevention, a strategy that seeks to impede or postpone the advancement of cancer, exhibits considerable potential. Chemoprevention includes the utilization of hormones, medicines, dietary components, and vaccinations. The perceived safety and availability of natural materials make them appealing options for chemoprevention. Cancer chemoprevention involves the utilization of therapeutic medicines to inhibit, delay, or reverse the process of carcinogenesis before it progresses to invasion. This study focuses on the agents that have been investigated in the context of cancer chemoprevention. These agents are categorized into four main groups: hormones, pharmaceuticals, diet-related agents, and vaccinations. The article presents the primary representatives of each category [3].

Malaysia boasts a diverse array of tropical plants that possess longstanding therapeutic properties. *Streblus asper* plant is abundant in cardiac glycosides, which are bioactive compounds that exert an impact on the cardiovascular system. The root cortex of *S. asper* has yielded over 20 cardiac glycosides that have been successfully isolated. The structure of 15 of these compounds was successfully identified by the researchers. These chemicals aid in comprehending the therapeutic mechanisms of *S. asper*. The plant has been shown to contain many chemical components, including steroids, alcohols, and a diol (Fig. 1). The steroids were extracted from the bark of the stem and the aboveground portions of the plant. The passage closes by indicating that the diagram illustrates the structures of several biologically active chemicals that have been extracted from *S. asper* [4].

Streblus asper exhibits potential anticancer effects through several actions. It contains compounds that can directly kill cancer cells by activating processes such as apoptosis. The plant's antioxidant

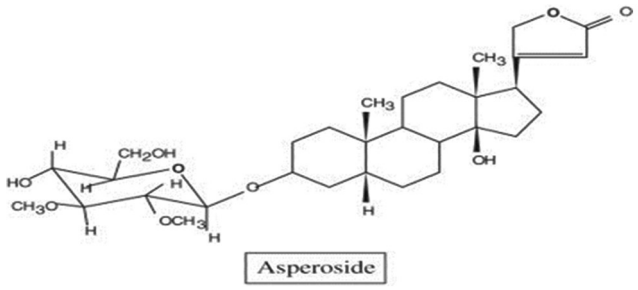

Fig. 1: Chemical structure of *Streblus asper*

properties help combat harmful free radicals linked to cancer development and may lessen chemotherapy side effects. In addition, *S. asper* potentially boosts the immune system's ability to fight tumors. Laboratory research demonstrates its activity against various cancer types (cervical, liver, lung, leukemia), and animal studies show promise in inhibiting tumor growth, improving survival, and reducing the toxicity of certain chemotherapy drugs. The present study investigates the underlying mechanisms by which SA extracts exert inhibitory effects on the proliferation of prostate cancer cells, with a specific emphasis on their impact on cell death pathways. Comprehending these pathways is of utmost importance in the development of chemoprevention methods based on SA for the treatment of prostate cancer [5].

METHODS

Selection and extraction of plant

The specimen of the plant was acquired from a nursery situated in TasekGelugor, Penang, Malaysia, and has been authenticated by the Malaysian Institute of Pharmaceuticals and Neutraceuticals. The roots were divided into pieces larger than 2 mm and then washed with distilled water. They were then dried at room temperature for a period of 2 weeks. Subsequently, the dried root material was pulverized using a Retstch SM 100 grinder. The process of triplicate extractions involved the boiling of pulverized root powder with distilled water for duration of 30 min, followed by filtration using a 0.75-micron filter. The merged filtrates were subsequently lyophilized (freeze-dried) for subsequent utilization. Before doing the experiment, the powder was accurately measured and then diluted to the necessary quantity using ultrapure, double-deionized distilled water [6].

Cell culture

The research conducted in this study utilized the DU-145 prostate cancer cell line, which was acquired from the American Type Culture Collection (ATCC, Manasus, VA, USA; Catalog No. CCL-2 TM). The cells were cultured in a mixture consisting of DMEM (Life Technologies, USA) supplemented with 10% fetal bovine serum (FBS), 1% sodium pyruvate, and 1% penicillin-streptomycin. The incubation circumstances consisted of a climate with 5% CO_2 and 95% air humidity, maintained at a temperature of 37°C. To maintain uniformity, the media was replenished every 72 h, and cells were sub-cultured when they reached 80–90% confluency [7]. The experiment was limited to ten passes to reduce experimental variability.

Cell proliferation assay

Upon reaching 80-90% confluency, DU-145 cells were harvested using 0.25% trypsin/EDTA, and the old medium was replaced. For the cytotoxic assay, cells were seeded into 96-well culture plates at a density of 1×10^5 cells per well, using 100 µl of complete medium. After 24 h, the cells were exposed to *S. asper* root extract at varying concentrations (0.025 mg/ml–0.125 mg/ml) and incubated for 72 h at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air.

Following the treatment period, cell viability was assessed using the AlamarBlue® assay according to the manufacturer's instructions. The AlamarBlue® solution was pre-warmed to 37°C, and control wells (without cells) containing the dye were included in triplicate to account for background fluorescence. After a 4-h incubation at 37°C in the 5% CO_2 humidified incubator (with minimal light exposure), fluorescence (excitation 530 nm, emission 590 nm) was measured using a microplate reader, with background control wells used for blanking. The half-maximal inhibitory concentration (IC_{50}) value, representing the concentration at which 50% cell growth inhibition occurs, was determined from the results of this cell proliferation assay and the dose was 0.1 mg/dl [8].

Cell morphology analysis

DU-145 cells were cultured at a density of 1×10^5 cells per well in 6-well plates and let to grow for 24 h to evaluate morphological changes. Afterward, the samples were subjected to the IC_{50} concentration of *S. asper* root extract and put in a humidified atmosphere with 5% CO_2 for an additional 72 h at 37°C. The phase-contrast inverted microscope (Carl Zeiss, USA) was used to assess the morphological changes in both untreated and *S. asper*-treated cells. The Live/Dead Viability/Cytotoxicity Kit (Molecular Probes, USA) was used to conduct a live/dead fluorescence staining experiment to determine the populations of viable and dead cells. The cells were cultivated for duration of 24 h by seeding them on glass slides that were positioned within 6-well plates. The cell density in each well was set at 1×10^5 cells. Following the 72-h treatment period, the slides were rinsed with phosphate-buffered saline (PBS) and stained with a solution containing ethidium homodimer and 0.4 mM calcein. After the staining process, a coverslip was provided to the slide to facilitate visibility using a spectrum confocal laser scanning microscope (CLSM) manufactured by Leica, namely the TSC SP2. The green fluorescence exhibited by live cells can be attributed to their uptake and subsequent processing of calcein. On the other hand, deceased cells exhibited red fluorescence due to the penetration of ethidium homodimer into damaged membranes and its attachment to nucleic acids. In addition, their failure to retain calcein further confirmed cellular death [9].

Quantification of apoptosis through Annexin V/PI staining

The FITC Annexin V Apoptosis Detection Kit II (Becton-Dickinson, USA) was used to measure the extent of apoptosis induction. The purpose of this kit is to detect apoptotic cells by utilizing FITC Annexin V, which attaches to phosphatidylserine (PS) that is transported to the outer layer of the cell membrane during apoptosis. Dead cells are stained with propidium iodide [3].

In summary, a total of 1×10^5 cells were distributed onto 6-well plates and subjected to a 24-h incubation period. The cells were subsequently subjected to 24 h of treatment with either DMEM alone (control) or the IC_{50} concentration of *S. asper* root extract. After undergoing treatment, cells that were either adherent or non-adherent were gathered, rinsed with ice-cold phosphate-buffered saline (PBS), and then reconstituted in Annexin binding buffer with a final concentration of 1×10^5 cells per milliliter. A total of around 1×10^5 cells were cultured in a 100 µl buffer solution. These cells were then exposed to 5 µl of Annexin V and 1 µl of 100 µg/ml PI for 15 min at room temperature, while being shielded from light. Following the incubation period, a volume of 400 µl of Annexin binding buffer was introduced. Subsequently, flow cytometry analysis was conducted utilizing a Becton Dickinson FACScan flow cytometer equipped with CellQuest Software (BD Biosciences, USA) to estimate the proportion of apoptotic cells. The results are displayed as the mean±standard error of the mean [10].

Statistical analysis

To ensure reproducibility, each experimental condition was conducted in triplicate. The findings are presented in the form of the mean value±standard deviation (SD). The study employed a statistical analysis technique known as one-way analysis of variance (ANOVA) to examine potential variations among groups. Subsequently, the Bonferroni *post hoc* test was conducted to find particular comparisons that achieved statistical significance. The criteria for statistical significance was set at a value of $p<0.05$.

RESULTS

Effect of Streblus aspera on the proliferation of cervical cancer

The impact of *S. asper* extracts on DU-145 cells was assessed in a cell proliferation experiment utilizing an AlamarBlue® assay. The experiment utilized five different concentrations of the extract: 0.00 mg/ml (control), 0.025 mg/ml, 0.50 mg/ml, 0.75 mg/ml, 0.1 mg/ml, and 0.125 mg/ml. Cell proliferation activity was assessed by employing AlamarBlue® and quantified using a spectrophotometer at a wavelength of 570 nm after a 72-h incubation period, as illustrated in Fig. 2.

After 72 h of exposure to *S. asper* extract, there was a concentration-dependent decrease in the viability of HeLa cells. The viability rates were as follows: 75.08% (0.025 mg/ml), 68.37% (0.50 mg/ml), 59.65% (0.75 mg/ml), 50.69% (0.1 mg/ml), and 38.24% (0.125 mg/ml). The control had a viability rate of 100.00%. 0.1 mg/ml was determined to be the half-maximal inhibitory concentration (IC_{50}).

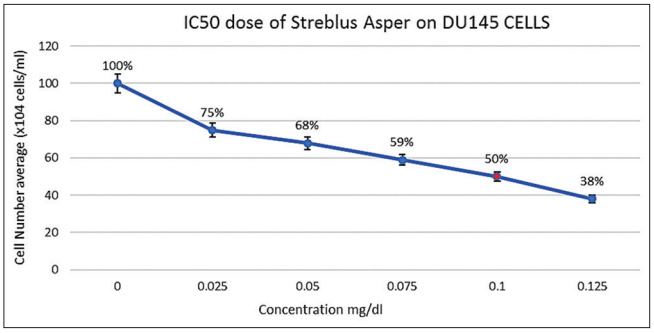

Fig. 2: Cell viability of DU 145 cells treated with *Streblus asper* extract

Effect of *S. asper* root extract on morphological analysis

On microscopic inspection, morphological changes induced by the treatment were identified, such as membrane blebbing, rounding, and cell contraction. In contrast to untreated controls (Fig. 3a), the viability of HeLa cells treated with *S. asper* demonstrated a substantial decrease (Fig. 3b). The results obtained from these studies cumulatively suggest that the growth of HeLa cells is inhibited by the extract of *S. asper*.

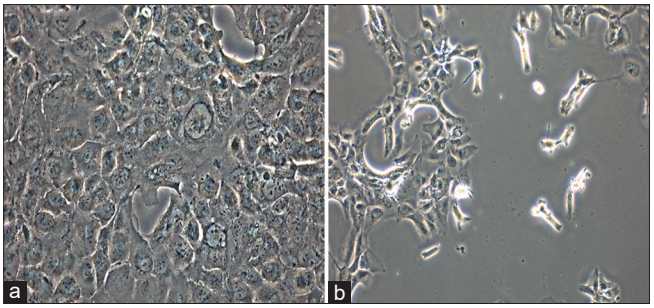

Fig. 3: (a) Healthy, full confluent DU 145 cells before treatment. (b) Light micrograph of DU 145 cells with 0.1 mg/ml *S. asper* compound treatment for 72 h. Some of the morphological changes, as shown in this figure, such as vacuolization, cell elongation, cell shrinkage, and rounding of cells. Pictures were in ×200 magnification

Human osteoblast cell lines cytotoxicity assay

The growth of normal cells may be influenced by *S. asper* subsequent to IC50 therapy. A cytotoxicity experiment was conducted on mouse fibroblast cell lines to evaluate this. The purpose of conducting the cytotoxicity experiment was to validate that the concentration of *S. asper* at the defined IC_{50} value of 0.1 mg/ml would not exert any detrimental impact on the viability and survival of mouse fibroblast cell lines.

The findings indicated that the cellular morphology remained unaltered following treatment with *S. asper*, in contrast to the control cells that were not subjected to any therapy. The DU 145 cells exhibited a mortality rate of roughly 50% due to the IC_{50} value of *S. asper*. Nevertheless, Fig. 4a,b verifies that the extract caused the destruction of fewer than 10% of the Mouse fibroblast cell lines. These findings indicate that *S. asper* does not induce cytotoxicity in mouse fibroblast cell lines, therefore establishing its safety for application in normal cells.

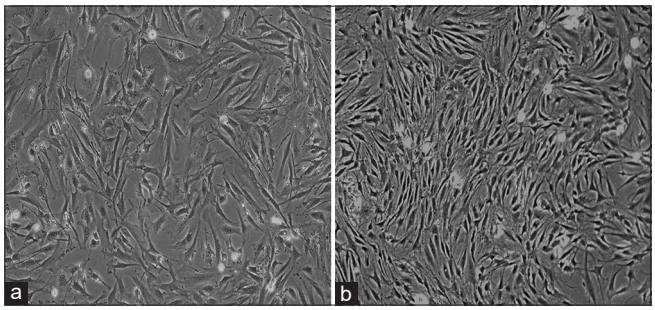

Fig. 4: Effect of *S. asper* one of normal Mouse fibroblast cell line. Cells were treated at IC_{50} of *S. asper* for 72 h. (a) Morphological changes upon treatment with media only (b) Percentages of cell viability upon treatment with *S. asper*

Apoptotic effect of *Streblus asper* compound on DU145 cells

The inhibitory effect was further confirmed with apoptosis analysis with flow cytometry. The result showed that the cells analyzed for apoptotic rate clearly showed significant differences after 72-h incubation. Table 1, summarizes the percentage of apoptotic cells in the *S. asper* compound treated and untreated DU 145-cell population obtained after data analysis from the experiments.

In addition, Fig. 5, a flow cytometry analysis experiment was designed to assess apoptosis, or programmed cell death, in a control group and a treated group. Flow cytometry is a technique that uses lasers to measure the properties of individual cells as they flow in a stream past a detector. In this case, the cells were likely stained with fluorescent dyes that target specific markers of apoptosis. Here, the treated group has a significantly higher percentage of apoptotic cells compared to the control group. Here is a breakdown of the results:

1. Live cells: The percentage of live cells in the control group (72.3%) is significantly higher than in the treated group (53.0%). This indicates that the treatment induced cell death.
2. Early apoptosis: The treated group (12.0%) has a higher percentage of cells in early apoptosis compared to the control group (7.4%). Early apoptosis is the initial stage of programmed cell death.
3. Late apoptosis: The percentage of cells in late apoptosis is also higher in the treated group (13.0%) compared to the control group (8.3%). Late apoptosis is the final stage of cell death before the cell is broken up into fragments.
4. Total apoptosis: The total percentage of apoptotic cells (sum of early and late apoptosis) is significantly higher in the treated group (25.0%) compared to the control group (15.7%).

DISCUSSION

This study investigates the chemopreventive properties of *S. asper*, a plant that has been historically employed in some cultures for the treatment of several diseases, including cancer. Scientists are investigating the potential of *S. asper* to create novel chemopreventive medicines that can impede or decelerate the progression of cancer [4]. Cancer is a significant public health issue, and despite the existence of accessible therapies, the rate of occurrence is not declining. Research has been carried out on prostate cancer cells using *S. asper*. Chemoprevention refers to the application of natural or synthetic substances to avert or postpone the development of cancer [11]. This emerging topic of study is centered around the identification of biomarkers that can be utilized to forecast

Table 1: The percentage of apoptotic cells distribution on DU145

Intervention	Live cells %	Early apoptosis %	Late apoptosis %	Total apoptosis %
Control group	72.3±2.0	7.4±0.4	8.3±0.31	12.053±0.31
Treated group	53.0±1.1***	12.0±0.2***	13.0±0.6**	22.0±0.31**

**Significant differences (p<0.01) with respect to untreated control groups. The cells were treated with IC_{50} of media only and *Streblus asper* at 72 h. Data represents percentages of the population of dead cells, live cells, and cells undergoing apoptosis. Data shown are mean±SD of triplicate experiments. SD: Standard deviation

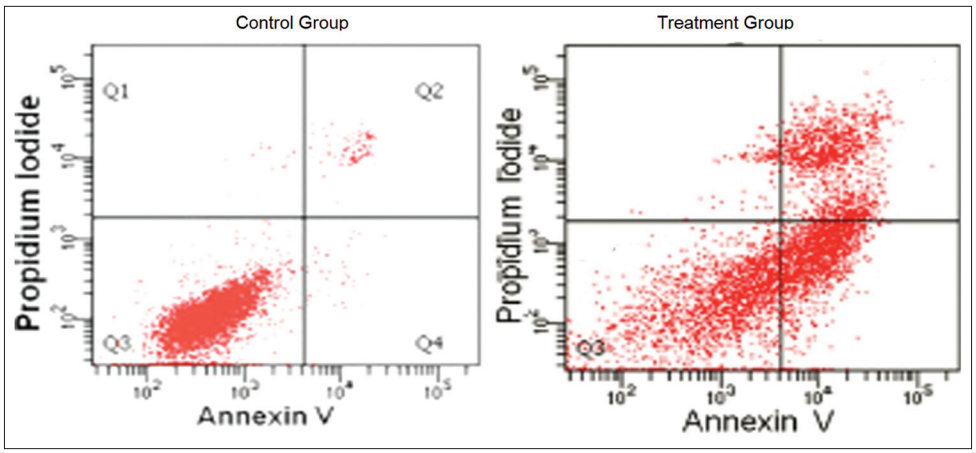

Fig. 5: Effect of *S. asper* on apoptosis induction of DU-145. The cells were treated with *S. asper* at IC_{50} for 72 h. The upper left, lower left, lower right, and upper right quadrants distinguished between necrotic/debris cells, viable cells, and early apoptotic and late apoptotic cells respectively

the efficacy of chemoprevention drugs. The efficacy of *S. asper* in the prevention of prostate cancer cells has been demonstrated, suggesting the presence of phytochemicals with potential anticancer properties.

The results indicate that the administration of *S. asper* extracts has a substantial inhibitory effect on the growth and division of prostate cancer cells. The level of inhibition was influenced by both the dosage and duration. The IC_{50} value of SA root extracts was determined to be 0.1 mg/ml, suggesting that this concentration has the ability to eliminate 50% of prostate cancer cells. A previous investigation demonstrated that the root of *S. asper* is abundant in cardiac glycosides, potentially playing a role in the observed anti-cancer properties [12]. Cardiac glycosides are commonly employed in clinical practice for the treatment of heart disease. Recent research has revealed their involvement in the regulation of cellular processes that may enhance the vulnerability of cancer cells to apoptosis [13].

Numerous cancer treatment medications exhibit toxicity toward both cancerous and non-cancerous cells. The potential of *S. asper* extracts as a natural chemopreventive agent is promising, primarily because of its potential for reduced toxicity. The utilization of the cytotoxicity test is a dependable approach for evaluating the existence of deleterious constituents within *S. asper* extracts [14]. The choice of the cell line for the cytotoxicity assay is not of utmost importance; nonetheless, it is advisable to utilize human or rodent cells for subsequent research. *S. asper* compound's antiproliferative effect was initially found by testing the IC_{50} value of several concentration ranges in prostate cancer cell lines. The growth inhibitory effect of treated prostate cancer cells was higher than untreated cells. The IC_{50} value of 0.1 mg/ml demonstrated that the chemical inhibits prostate cancer cells. The IC_{50} value was larger than in the prior study.

This study's higher IC_{50} value may be attributable to cell line differences. The IC_{50} value must take into account that inhibitory effects vary by chemical and cell line. This study found the first IC_{50} value of *S. asper* chemical on DU 145 prostate cancer cells. Growth inhibition was affected by 0.1 mg/ml *S. asper* chemical in this cell line investigation. Tumor cells develop quickly in DU145 lines. *S. asper* high root chemical at concentrations above 0.1 mg/ml inhibited more than lower quantities. Fast-growing tumors react better to conventional cytotoxic chemotherapy than slow-growing tumors.

Extensive research has been conducted on the underlying mechanism of targeted chemicals and natural products employed in cancer treatment, with apoptosis being among the mechanisms investigated. The identification of the apoptotic agent has played a pivotal role in the advancement of cancer therapeutic research. The efficacy of inducing apoptosis in cancer cells can be significantly enhanced if the apoptotic agent can impede proliferation and progression through pathways that induce apoptosis [15].

SA root extracts may cause DU-145 cell death or growth suppression through apoptosis. The morphological alterations of DU-145 cells before and after therapy were examined under a microscope. Fig. 5 shows apoptosis-related elongation, cell contraction, and membrane blebbing. SA root extracts caused DU-145 cell death via flow cytometry. SA root treatment increased early DU-145 cell apoptosis. The result showed that therapy did not create significant necrotic cells, ruling out necrosis-induced cell death. We investigated the mechanism of action and proliferation suppression and apoptosis promotion of the *S. asper* root product in human prostate cancer DU-145 cells. We found that IC_{50} concentrations of *S. asper* product inhibited DU-145 cell growth and induced apoptosis. The cell proliferation assay showed that water and aqueous methanol extracts inhibited DU-145 cell proliferation by reducing viable cell count. The cell proliferation assay showed that *S. asper* extracts inhibited proliferation better. Reversed-phase contrast microscopy may show apoptosis-enhanced cell death and reduced cell proliferation. Early apoptosis was seen in inverted phase-contrast microscopy with cell shrinkage, membrane blistering, and apoptotic bodies. These morphological observations of apoptotic cells were corroborated by prior research and commonly utilized to identify apoptosis. Analysis demonstrated that *S. asper* root extracts induce apoptosis. Thus, *S. asper* root product may induce apoptosis in prostate cancer cells.

CONCLUSION

This *in vitro* study showed that *S. asper* root product inhibited the prostate cancer cell line (DU145). Inhibiting DU145 cell proliferation

with double distilled water and *S. asper* root extract was more effective. The DU145 cell line was cultured *in vitro* to test the *S. asper* chemical effect. DU145 cells were shown to be susceptible to *S. asper* chemical in this investigation. The *S. asper* root component inhibits prostate cancer cell growth and proliferation. One promising option is inhibiting cancer cell multiplication without harming normal cells. To the best of our knowledge, no other scientific study has examined the effects of *S. asper* on DU145 cells. The inhibitory capability of *S. asper* compound on DU145 cells is important for developing new cancer treatments. In conclusion, *S. asper* chemical may be a cancer treatment target, particularly for prostate cancer.

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CONFLICTS OF INTEREST

In this original article, there are no potential conflicts of interest.

ETHICAL CLEARANCE

Approved by the University of Cyberjaya, ethical approval committee.

AUTHORS CONTRIBUTION

All the authors contributed equally to this review paper.

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