



## Short Communication

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# Cytotoxic Effect of Brazilin on DU145 Prostate Cancer Cells Using the WST-8 Assay

Efek Sitotoksik Brazilin terhadap Sel Kanker Prostat DU145 Menggunakan Uji WST-8

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### ABSTRACT

Prostate cancer is characterized by abnormal growth of prostate gland cells due to uncontrolled cellular proliferation. Brazilin, a homoisoflavonoid compound derived from *Caesalpinia sappan* L., is known for its anticancer activity. This study aimed to evaluate the cytotoxic effect of brazilin on DU145 prostate cancer cells through an in vitro assay employing the WST-8 method. The results showed that both brazilin and cisplatin significantly reduced the cancer cell survival rate compared to the negative control group ( $p < 0.05$ ). Although no significant difference was observed between the two compounds at the highest concentration (ns), their  $IC_{50}$  values differed significantly ( $p < 0.05$ ), recorded at 18.92 ppm for brazilin and 0.03569 ppm for cisplatin; both were classified as highly active. These findings suggest that brazilin possesses cytotoxic potential comparable to cisplatin at elevated concentrations and may be developed further as a naturally derived anticancer agent.

### ABSTRAK

Kanker prostat merupakan pertumbuhan abnormal sel jaringan kelenjar prostat akibat proliferasi sel yang tidak terkontrol. Brazilin, senyawa homoisoflavonoid yang diperoleh dari kayu secang (*Caesalpinia sappan* L.), diketahui memiliki aktivitas antikanker. Penelitian ini bertujuan untuk mengevaluasi efek sitotoksik brazilin terhadap sel kanker prostat DU145 secara in vitro menggunakan uji WST-8. Hasil menunjukkan bahwa brazilin dan cisplatin secara signifikan menurunkan tingkat kelangsungan hidup sel kanker dibandingkan dengan kontrol negatif ( $p < 0,05$ ). Meskipun tidak terdapat perbedaan signifikan antara keduanya pada konsentrasi tertinggi (ns), nilai  $IC_{50}$  menunjukkan perbedaan signifikan ( $p < 0,05$ ), yaitu 18,92 ppm untuk brazilin dan 0,03569 ppm untuk cisplatin, keduanya termasuk dalam kategori aktivitas sangat kuat. Temuan ini menunjukkan bahwa brazilin memiliki potensi sitotoksik yang sebanding dengan cisplatin pada konsentrasi tinggi dan dapat dikembangkan lebih lanjut sebagai agen antikanker berbasis bahan alam.

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## 1. INTRODUCTION

Prostate cancer is one of the most prevalent malignancies among men and a leading cause of cancer-related mortality globally. In Indonesia, it accounts for 7.4% of 183,368 reported cancer cases

and contributes to 2.1% of cancer-related deaths (Global Cancer Observatory, 2022). Characterized by uncontrolled cellular proliferation, prostate cancer can invade adjacent tissues and metastasize to distant organs. Effective treatment options are essential, particularly for advanced and aggressive types.



Chemotherapy is widely used in prostate cancer management, but its side effects—including organ toxicity and cognitive impairment—pose serious limitations (Zuriati et al., 2018). As a result, natural compounds have gained interest as alternative or complementary therapies with potentially lower toxicity profiles (Shabrina & Iskandarsyah, 2019).

*Caesalpinia sappan* L. (sappanwood) contains brazilin, a homoisoflavonoid known for its diverse pharmacological activities, including anticancer properties. Studies have demonstrated brazilin's cytotoxicity against several cancer cell lines such as A549 (lung), T47D (breast), and HeLa (cervical), with mechanisms involving apoptosis induction, autophagy activation, and modulation of proteins such as caspases and Bcl-2 family members (Hastuti et al., 2022; Jenie et al., 2020; Suyatmi et al., 2022; Nava-Tapia et al., 2022).

Despite its promise, limited studies have explored brazilin's effect on prostate cancer, especially in DU145 cells, which model androgen-independent and metastatic disease. This study aimed to investigate the cytotoxic effect and determine the IC<sub>50</sub> value of brazilin on DU145 prostate cancer cells using the WST-8 assay, with cisplatin as a reference chemotherapeutic agent.

## 2. METHODS

### 2.1. Chemicals and Reagents

Brazilin (purity <95%) was obtained from previous isolation (Fadhila et al., 2022; Sriwidodo et al., 2022). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, dimethyl sulfoxide (DMSO), and WST-8 reagent (Dojindo®) were used in this study. All reagents were of analytical grade.

### 2.2. Preparation of Stock and Working Solutions

A 10,000 ppm stock solution of brazilin was prepared by dissolving 10.53 mg of the compound (adjusted for purity) in 1 mL of DMSO. The solution was mixed using a vortex and sonicated for 10 minutes at room temperature. Aliquots were stored at –20 °C in the dark. Working concentrations of 12.5, 25, and 50 ppm were prepared by serial dilution in DMEM, maintaining a final DMSO concentration below 0.5% (v/v).

### 2.3. Cell Line and Culture Conditions

The DU145 human prostate cancer cell line (ATCC® HTB-81™) was cultured in DMEM supplemented with 10% FBS, 100 IU/mL penicillin, and 10 µg/mL streptomycin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. Media were replaced every 2–3 days. Cells were harvested at 70–90% confluency using 0.025% trypsin-EDTA, incubated for 3 minutes, neutralized with DMEM, and centrifuged at 200 × g for 5 minutes. Viability was confirmed using trypan blue exclusion, and only cultures with >90% viability were used.

### 2.4. Cell Seeding and Drug Treatment

Cells were seeded at a density of 5 × 10<sup>4</sup> cells per well into 96-well plates (100 µL/well) and incubated for 24 hours. The medium was then replaced with treatment medium containing brazilin (12.5, 25, 50 ppm), cisplatin (1, 5, 10 ppm as positive control), or 0.5% DMSO (vehicle control). Treatments were conducted in triplicate and repeated three times independently (n = 3).

### 2.5. Cytotoxicity Assay

Following 24-hour treatment, 10 µL of WST-8 reagent was added to each well and incubated for 3 hours at 37 °C. Absorbance was measured at 450 nm using a microplate reader (Tecan Infinite®). Wells without cells served as blanks to correct background absorbance.

### 2.6. IC<sub>50</sub> Determination

Cell viability data were used to calculate IC<sub>50</sub> values using a four-parameter logistic regression model in GraphPad Prism v9.0.0 (GraphPad Software, USA). The equation applied was:

$$Y = Bottom + \frac{(Top - Bottom)}{1 + 10^{(X - \log IC_{50}) \times Hill Slope}}$$

where Top and Bottom refer to the maximum and minimum viability values, respectively.

### 2.7. Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). Normality was tested using the Shapiro–Wilk test, and homogeneity of variance using Levene's or Bartlett's test. For normally distributed and homogenous data, one-way ANOVA followed by Tukey's post hoc test (α = 0.01) was applied. For non-parametric data, the Kruskal–Wallis test followed by Dunn's test was used. Analyses were performed using GraphPad Prism v9.0.0 and IBM SPSS Statistics v26.

### 2.8. Experimental Validation

The assay was considered valid if the coefficient of variation (CV) between technical replicates was <20% and the positive control (cisplatin) consistently demonstrated cytotoxic activity. All raw data and analytical results were documented in accordance with laboratory protocols.

## 3. RESULTS AND DISCUSSION

### 3.1. Cytotoxic Activity Evaluation

The cytotoxic activity of brazilin and cisplatin on DU145 prostate cancer cells was evaluated using the WST-8 assay. This colorimetric method measures mitochondrial succinate dehydrogenase activity, which converts the WST-8 substrate into an orange-colored formazan product, indicative of viable cells (Aslantürk, 2018). DU145 cells were selected as they represent an androgen-independent, highly aggressive prostate cancer phenotype often used in chemotherapeutic screening (ATCC, 2024).

### 3.2. Effect on Cell Viability

Both brazilin and cisplatin significantly reduced DU145 cell viability in a dose-dependent manner compared to the negative

control ( $p < 0.05$ ). At the highest concentration tested, no statistically significant difference was observed between the cytotoxic effects of brazilin and cisplatin, indicating that brazilin exhibits comparable potency at elevated concentrations (Table 1).

**Table 1.** DU145 Cell Survival Rates (%) After Treatment

Concentration (ppm)	Brazilin (%)	Cisplatin (%)
50	8.57 ± 0.87	—
25	6.62 ± 1.06	—
12.5	5.15 ± 0.21	—
20	—	5.76 ± 0.15
4	—	3.37 ± 0.72
0.8	—	3.36 ± 0.62

### 3.3. IC<sub>50</sub> Determination

Based on cell viability data, IC<sub>50</sub> values were calculated to determine cytotoxic potency. Brazilin exhibited an IC<sub>50</sub> of 18.92 ppm, while cisplatin displayed a markedly lower IC<sub>50</sub> of 0.03569 ppm, indicating higher potency (Table 2). Nonetheless, both compounds were classified as having very strong cytotoxic activity.

**Table 2.** IC<sub>50</sub> Values of Brazilin and Cisplatin on DU145 Cells

Compound	IC <sub>50</sub> (ppm)	Cytotoxic Activity
Brazilin	18.92	Very strong
Cisplatin	0.03569	Very strong

### 3.4. Mechanistic Considerations and Comparative Analysis

The disparity in IC<sub>50</sub> values may be attributed to differing mechanisms of action. Cisplatin functions primarily through DNA crosslinking, which inhibits replication and induces cell death via DNA damage (Wang et al., 2021). In contrast, brazilin exhibits multifaceted mechanisms, including inhibition of proliferation, apoptosis induction, and autophagy activation. It also upregulates pro-apoptotic proteins (e.g., Bcl-2, Bcl-XL), activates caspases-3 and -7, and suppresses Bax expression, thereby overcoming apoptosis resistance (Nava-Tapia et al., 2022).

### 3.5. Potential for Combination Therapy

Previous studies suggest that brazilin may enhance the cytotoxic effect of chemotherapeutic agents such as cisplatin when used in combination (Jenie et al., 2019). This synergism could be particularly beneficial in drug-resistant cancers. Although brazilin's IC<sub>50</sub> is higher than cisplatin's, its natural origin and diverse biological activity make it a promising candidate for further development with potentially lower toxicity.

### 3.6. Study Limitations and Future Directions

This study was limited to a single cell line (DU145) and did not directly evaluate mechanisms of apoptosis. Future studies should include apoptosis assays (e.g., flow cytometry), protein expression analysis, and in vivo models to further elucidate brazilin's anticancer potential and confirm its safety and efficacy.

## 4. CONCLUSION

Brazilin demonstrated strong cytotoxic activity against DU145 prostate cancer cells, with an IC<sub>50</sub> value in the very strong category. Although less potent than cisplatin, brazilin's comparable efficacy at higher concentrations and its diverse biological actions support its potential as a natural-based anticancer candidate. Further research using in vivo models and clinical trials is necessary to validate these findings. Additionally, formulation improvements such as nanoparticle or targeted delivery systems are recommended to enhance its bioavailability and therapeutic index.

## AUTHOR CONTRIBUTIONS

Conceptualization, S.A. and N.P.D.; Methodology, S.A.; Validation, S.A., N.P.D., and D.P.; Investigation, S.A.; Writing—original draft preparation, S.A.; Writing—review and editing, S.A. and D.P.; Supervision, N.P.D. All authors have read and agreed to the published version of the manuscript.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the author(s) used ChatGPT (OpenAI) to assist in improving the clarity, structure, or readability of the text. The author(s) reviewed, edited, and verified all content to ensure accuracy and take full responsibility for the published work.

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