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Unveiling the Anticancer Potential of Pasak Bumi (Eurycoma Longifolia Jack) Root Extract in Prostate Cancer Treatment

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ABSTRACT

Background: Prostate cancer remains a significant global health concern, necessitating the exploration of novel therapeutic avenues to enhance treatment efficacy and mitigate adverse effects. **Objective** This study delves into the potential anticancer properties of Pasak Bumi (Eurycoma longifolia Jack) root extract, a traditional Southeast Asian medicinal plant, against prostate cancer. **Methods:** The research employs a multifaceted approach, encompassing molecular and cellular analyses to unravel the intricate mechanisms underlying Pasak Bumi's effects on prostate cancer cells. Primary focus is given to the PTEN/P13k/Akt pathway, a critical regulator of cell survival and apoptosis. Various concentrations of Pasak Bumi root extract are applied to prostate cancer cell lines, and the impact on apoptosis, cell proliferation, and key molecular targets is assessed. **Results:** Preliminary findings reveal that Pasak Bumi root extract induces apoptosis in prostate cancer cells, evidenced by downstream molecular events associated with programmed cell death. The extract demonstrates concentration-dependent effects, with higher concentrations exhibiting more pronounced anticancer activity. Moreover, Pasak Bumi root extract appears to modulate the PTEN/P13k/Akt pathway, providing a potential mechanistic link to its anticancer effects. **Discussion:** The study's significance lies in its contribution to the evolving landscape of natural compounds as anticancer agents, particularly in the context of prostate cancer. Pasak Bumi's traditional use as a medicinal plant, coupled with emerging scientific evidence, underscores its potential translational value. The observed modulation of the PTEN/P13k/Akt pathway aligns with the current understanding of prostate cancer pathogenesis, offering a plausible explanation for Pasak Bumi's anticancer effects. **Conclusion:** This research sheds light on the promising anticancer potential of Pasak Bumi root extract against prostate cancer. Further exploration of its molecular interactions, synergy with conventional therapies, and efficacy at different stages of cancer progression is warranted. The findings present Pasak Bumi as a nature-inspired candidate for prostate cancer treatment, warranting continued investigation into its therapeutic applications. As the scientific community endeavors to enhance cancer treatment modalities, Pasak Bumi emerges as a captivating subject in the pursuit of effective and minimally invasive prostate cancer therapies.

Keywords: Prostate cancer, Pasak Bumi (Eurycoma longifulia Jack), molecular targets, PTEN, apoptosis

1. BACKGROUND

Prostate cancer is a malignant disease in men and is the second most frequently diagnosed cancer, ranking sixth in the causes of death among men worldwide. The incidence of prostate cancer varies more than 25 times between different regions of the world. Over 670,000 men are diagnosed with prostate cancer each year globally (1). In 2014, approximately 233,000 new cases and 29,480 deaths due to prostate cancer occurred in the United States (2). According to the Indonesian Society of Urologic Oncology (ISUO) data from 2011 covering the period 2006-2010, there were 971 prostate cancer patients, with an average age of 68.3 years. The majority were in the age range of 70-79 years, accounting for 37.6%, and the highest number of patients (490) were at stage 4 (50.5%) (1).

The etiology of prostate cancer remains controversial, with the carcinogenesis process being complex. Genes responsible for familial prostate cancer are located on chromosome (1). Various parts of the human genome are identified as potential areas containing tumor suppressor genes involved in prostate cancer, including mutated genes such as p53, p16(CDKN2A), phosphatase and tensin homolog (PTEN), bcl-2, ras, p27 (CDKN1B), and caspase (3).

PTEN is a tumor suppressor gene that induces cellular apoptosis through modulation of the P13K/Akt pathway. The phosphatidylinositide 3-kinase (PI3K) pathway plays a crucial role in prostate cancer, with estimates suggesting its involvement in 30-50% of cases (4). Specifically, PTEN inhibits Akt phosphorylation, necessary for activation and targeting of many effectors (5). Prostate cancer resistant to treatment and poorly differentiated often experiences PTEN loss, leading to P13K/AKT pathway activation and subsequent apoptosis resistance (6). Restoring PTEN activity in PTEN-deficient prostate cancer cells has been shown to increase sensitivity to FADD-mediated caspase-8, promoting prostate cancer cell apoptosis and facilitating BIDD to allow cytochrome c release from mitochondria, further driving apoptosis (7).

The emergence of resistance to hormonal therapy is a significant issue in hormonal therapy administration. The mechanisms of resistance to hormonal therapy are not yet fully understood. Current prostate cancer exhibits heterogeneous cells (androgen-dependent and androgen-independent) (1). Hormone Refractory Prostate Cancer (HRPC) is resistant to all hormonal interventions, requiring expensive chemotherapy. Docetaxel is the chemotherapy drug commonly used (1). However, the success of therapy remains unsatisfactory, and the side effects are substantial, necessitating relatively inexpensive alternative therapies without significant side effects for patients.

Eurycoma longifolia Jack, also known as Pasak Bumi, is a tropical plant belonging to the Simaroubaceae family, distributed in Southeast Asian countries (8). Eurycoma longifolia is native to South Kalimantan, Indonesia, and its roots contain biologically active compounds used for detoxification, free radical antioxidant, and anticancer purposes (9). Scientific data on the anticancer mechanisms of E. longifolia, both in vitro and in vivo, are still limited.

Compounds found in E. longifolia include quassinoids (9, 10) and the alkaloid 9-methoxyxanthone-6-one (11), and canthinone alkaloid (12). Quassinoids have cytostatic effects on colon cancer, breast cancer, lung cancer, skin cancer (melanoma), and fibrosarcoma (13). In vitro and in vivo research using ethanol extracts on breast cancer cells showed inhibition of COX-2 expression, decreased BCl-2 expression, increased Caspase 3, increased p53 expression, increased p21 expression, increased GADD45 expression, and decreased Ras expression (10). This suggests that E. longifolia Jack has the potential as a new active drug for cancer suppression.

Based on the above considerations, the active compounds in Pasak Bumi roots (E. Longifolia Jack) have cytotoxic effects on various cancers such as colon cancer, breast cancer, lung cancer, skin cancer (melanoma), and ovarian cancer. It is essential to determine whether it also exhibits cytotoxicity on androgen-independent prostate cancer cells. Hormone-resistant prostate cancer therapy (HRPC) currently requires expensive chemotherapy with significant side effects for patients. Therefore, alternative therapeutic agents that are relatively inexpensive and have minimal side effects, such as Pasak Bumi (E. Longifolia Jack) roots, are needed.

In prostate cancer, there are changes in the tumor suppressor gene PTEN. The reversible nature of genetic expression changes in cancer allows the possibility of alternative treatment therapy. The goal of gene-targeted therapy is to restore gene expression changes in cancer, bringing it back to a normal genomic condition. Some bioactive components of herbs can inhibit the function of DNA Methyltransferase enzymes, affecting gene expression processes and inhibiting cancer activation through the reactivation of silenced tumor suppressor genes (14-17). Flavonoids can reactivate silenced tumor suppressor genes. Pasak Bumi roots contain triterpenoid compounds with a structure similar to flavonoids, suggesting that Pasak Bumi roots have the potential as an agent for PTEN gene reactivation.

In silico research by Rahman et al. (2020) showed that active compounds in Pasak Bumi roots have the potential to increase p53 expression, which plays a role in DNA damage, thus influencing PTEN expression involved in eliminating prostate cancer through increased apoptosis. Our research indicate that ethanol extract of Pasak Bumi roots can increase apoptosis in PC3 cells.

2. OBJECTIVE

This study is part of the Basic Research Strengthening in the fields of Independence and Food and Health Resilience according to the 2020-2024 ULM Development Plan. The research leverages the potential of the South Kalimantan vegetation, particularly in wetland development, for the development of active compounds in herbal medicine based on local wisdom and ethnopharmacology. Thus, this research can contribute to the fundamental focus of standardized herbal development, functional food and medicine ingredients. Pasak Bumi was chosen because it has been commonly used and consumed by the local population for medicinal purposes. The strategic plan of this research is theoretical and applicative studies in the design of prostate cancer drugs based on local plants, specifically Pasak Bumi roots, leading to the creation of a phytopharmaceutical for prostate cancer.

3. MATERIAL AND METHODS

Research Design

The research design used was an in vitro experiment on PC-3 prostate cancer cells with a post-test only group design, random allocation. The study consisted of a con-

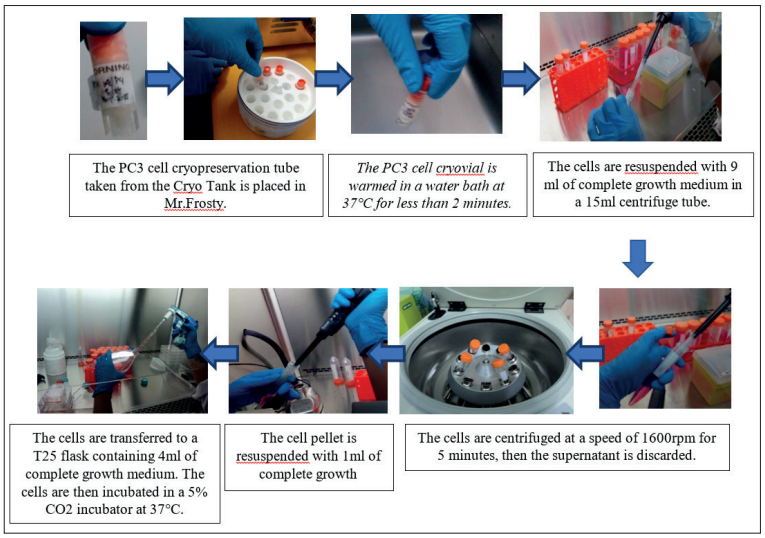


Figure 1. Thawing procedure of PC3 cells.

trol group and a treatment group given ethanol extract of Pasak Bumi roots with 5 different dosage variations.

Research Sample

The research sample was PC-3 cells, which are androgen-independent prostate adenocarcinoma cells. PC-3 cells were obtained from ATCC and stored in frozen form at -80°C in liquid nitrogen tanks to avoid multiple thawing. The cells were stored in conical tubes in aliquot form.

The study consisted of 6 groups, and the sample size was calculated based on the number of groups using the Federer formula (1963) for experimental research.

$(t - 1)(r - 1) \geq 15$

$t = 13$

Thus, it was obtained:

$(6 - 1)(r - 1) \geq 15, 5r - 5 \geq 15, 5r \geq 20, r \geq 4$

Explanation: t = number of treatments (groups); r = number of replications (samples)

The groups in this study included:

1. P0: Control: PC-3 prostate cancer cells without treatment
2. P1: PC-3 cells + Pasak Bumi root ethanol extract (EAPB) at a dose of 6.25 µM
3. P2: PC-3 cells + EAPB at a dose of 12.5 µM
4. P3: PC-3 cells + EAPB at a dose of 25 µM
5. P4: PC-3 cells + EAPB at a dose of 50 µM
6. P5: PC-3 cells + EAPB at a dose of 100 µM

Variables and Operational Definitions of Research Variables

Independent variable is Ethanol extract of Pasak Bumi roots (E. Longifolia Jack) at various doses. Dependent variables are Expression of PTEN gene and protein in PC-3 Prostate Adenocarcinoma.

Research Procedure

In the extraction of Pasak Bumi roots (Eurycoma longifolia Jack), the process involved meticulous steps to ensure the efficacy of the subsequent experiments. The roots were delicately sliced, dried thoroughly, and processed into a fine powder, resulting in 100 grams. This powdered form was immersed in ethanol solvent for approximately a week, and the subsequent solution was separated from the Pasak Bumi root using a rotary evaporator under a stream of nitrogen gas.

For the PC-3 cell culture, the Prostate Cancer Cell-line was cultivated in Eagle's Minimum Essential Medium supplemented with Fetal Bovine Serum and L-glutamine. The cells were meticulously maintained in a controlled atmosphere, with regular changes in the culture medium to achieve the desired growth rate. Subsequent cell harvests were conducted after the cells reached 80% confluence, involving trypsinization, resuspension, and subculturing.

The examination of PTEN protein expression utilized flow cytometry and specific materials and tools, including PC-3 cells, antibodies, and a flow cytometer. The cells were washed, stained with antibodies, and analyzed to determine PTEN protein expression percentages.

In analyzing PTEN gene expression in the PC-3 cell line, real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was employed. This involved RNA isolation, cDNA synthesis, and a quantification test of gene expression, including the preparation of a PCR reaction mix and subsequent qRT-PCR.

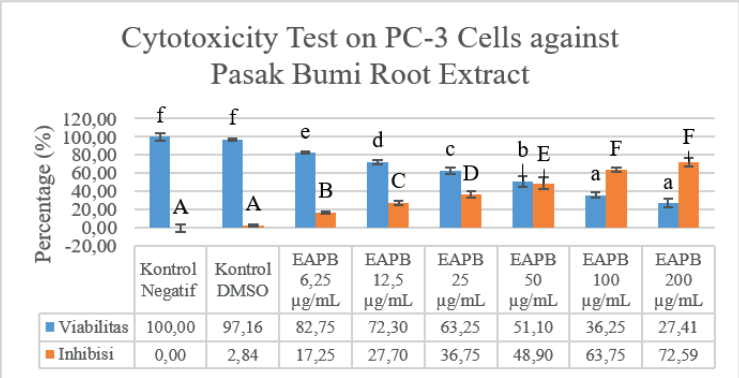
The data processing and analysis included the calculation of PTEN protein expression as an average percentage of cells expressing PTEN protein, determined through immunofluorescence. Additionally, the PTEN gene expression was assessed and calculated through qRT-PCR, providing comprehensive insights into the cellular responses and molecular mechanisms under investigation. These meticulous procedures and analyses constitute a robust methodology to explore the effects of Pasak Bumi root extracts on PC-3 cell lines, shedding light on potential therapeutic implications.

Data Analysis

In this study, data analysis was performed in four stages of calculation, namely normality test of sample data using the Shapiro-Wilk test, One-Way Anova test (F test), and Partial Least Square (PLS) test. The Shapiro-Wilk test, One-Way Anova test (F test) used the SPSS software version 22. The PLS test used SmartPLS 2.0 software.

4. RESULTS

The results of the cytotoxicity test on PC-3 cells exposed to Pasak Bumi Root Extract (EAPB) revealed a noticeable downward trend in cell viability with higher doses of EAPB compared to the negative control and DMSO, as illustrated in Figure 1's blue bars. The cytotoxicity test on PC-3 cells against Pasak Bumi Root Extract demonstrated an increase in the main molecular inhibition, indicated by the orange bars, corresponding to the inhibitory effects of EAPB, correlating with the escalating doses ranging from 6.25 micrograms/mL to 200 micrograms/mL. The correlation showed a positive and exponential relationship. Although the inhibitory effects remained below 80%, it proved to be



	Kontrol Negatif	Kontrol DMSO	EAPB 6,25 µg/mL	EAPB 12,5 µg/mL	EAPB 25 µg/mL	EAPB 50 µg/mL	EAPB 100 µg/mL	EAPB 200 µg/mL
Viabilitas	100,00	97,16	82,75	72,30	63,25	51,10	36,25	27,41
Inhibisi	0,00	2,84	17,25	27,70	36,75	48,90	63,75	72,59

Figure 2. Cytotoxicity Test on PC-3 Cells against Pasak Bumi Root Extract

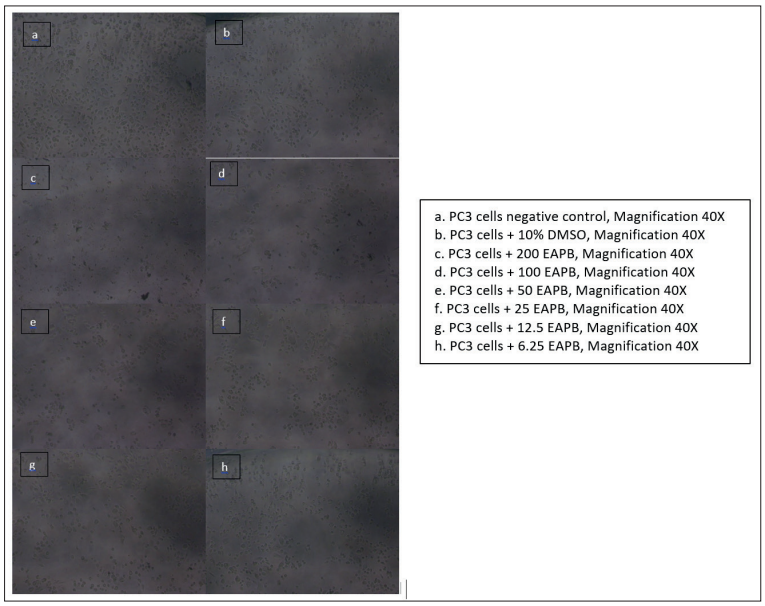


Figure 3. PC3 cells magnification on microscope 40x.

quite promising for PC-3 testing. The findings suggest a dose-dependent impact on cell viability, with a more pronounced inhibitory effect at higher concentrations of EAPB. This observation provides valuable insights into the potential cytotoxic properties of Pasak Bumi Root Extract on PC-3 cells, indicating a significant molecular inhibitory response that warrants further investigation. The positive correlation between dosage and inhibitory effects highlights the need for a comprehensive analysis of the molecular pathways affected by EAPB, paving the way for a more in-depth understanding of its potential therapeutic applications in prostate cancer treatment.

5. DISCUSSION

In the discussion of Chapter 3's literature review, various critical aspects of apoptosis mechanisms in prostate cancer carcinogenesis were explored. The dominant role of apoptosis, triggered by androgen ablation and chemotherapy agents, underscores its significance in prostate cancer treatment (19, 20). The extrinsic and intrinsic pathways were detailed, highlighting the intricate cascades of events leading to programmed cell damage (21-22).

The discussion then delved into the role of PTEN/PI3K/Akt in prostate cancer apoptosis, emphasizing PTEN's tumor suppressor function and its regulation of the PI3K/Akt pathway5. The loss of PTEN, observed in resistant and poorly differentiated prostate cancer, contributes to apoptosis resistance6. The intricate interplay of molecules like caspases, cytochrome c, and APAF-1 in these pathways was elucidated, providing a comprehensive understanding of the molecular landscape (21, 22).

The management of metastatic prostate cancer through Androgen Deprivation Therapy (ADT) was explored as the gold standard, with intermittent drug administration recommended to address the development of androgen-independent cells (1). The emergence of resistance to hormonal therapy was acknowledged, posing challenges in the administration of ADT (1).

Moving on to Pasak Bumi (E. longifolia Jack), its classification, distribution, and traditional uses in various parts of Southeast Asia were discussed (23). The plant's chemical composition, including quassinoids and alkaloids, was highlighted, underscoring its diverse medicinal properties (24). The effects of Pasak Bumi root as an anticancer agent were explored through studies demonstrating its anti-malarial, anti-ulcer, aphrodisiac, and antiproliferative properties (9, 13). Notably, quassinoids such as (14),15 -dihydroxyklaineanone showed potent antitumor activity.

The discussion also touched on in vitro and in vivo studies demonstrating the inhibitory effects of Pasak Bumi root extract on breast cancer cells through apoptosis induction and proliferation inhibition (10). Compounds like eurycomanone were shown to induce apoptosis in various cancer cell lines, including breast cancer (MCF-7) and cervical carcinoma (HeLa) (11). The promising potential of eurycomanone as a new chemotherapy agent derived from medicinal plants was emphasized (24).

In a recent in silico study, the active compounds eurycomanone and canthin in Pasak Bumi root were predicted to enhance apoptosis through the PTEN pathway. These compounds were found to increase p53 expression, thereby enhancing PTEN expression and contributing to the elimination of prostate cancer cells.

In summary, this comprehensive literature review provides a thorough understanding of apoptosis mechanisms, the role of PTEN/PI3K/Akt, management strategies for metastatic prostate cancer, and the medicinal properties of Pasak Bumi, with a focus on its potential as an anticancer agent. The findings set the stage for further research and exploration in the field of prostate cancer treatment and the development of novel therapeutic agents derived from natural sources.

6. CONCLUSION

This research sheds light on the promising anticancer potential of Pasak Bumi root extract against prostate cancer. Further exploration of its molecular interactions, synergy with conventional therapies, and efficacy at different stages of cancer progression is warranted. The findings present Pasak Bumi as a nature-inspired candidate for prostate cancer treatment, warranting continued investigation into its therapeutic applications. As the scientific community endeavors to enhance cancer treatment modalities, Pasak Bumi emerges as a captivating subject in the pursuit of effective and minimally invasive prostate cancer therapies.

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