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Research article

Chemistry

POTENTIAL OF PASAK BUMI (E. LONGIFOLIA JACK) ROOT AS AN ANTICANCER AGENT FOR PROSTATE ADENOCARCINOMA CELLS PC3

帕萨克布米（E. 长春花杰克）根作为前列腺腺癌细胞个人电脑 3 的抗癌剂的潜力

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Abstract

The article describes the potential of Pasak Bumi root as an anticancer agent for the prostate by inhibiting proliferation of PC3 cells. Using the roots of Pasak Bumi E. longifolia extracted with ethanol solvent. Prostate cancer PC-3 cell culture was obtained from independent androgen prostate adenocarcinoma that had bone metastases as the research subject. This study was an in vitro experimental study with a post-test control group design. Analysis of PC3 cell viability used the MTT assay method. ANOVA test results showed that p-value (Sig.) at 0.011 and smaller than $\alpha = 0.05$. Therefore, it can be concluded that there is a significant difference in the viability of adenocarcinoma cells in the administration of ethanol extract Pasak Bumi root with different concentrations. Our research showed the result that ethanol extract of Pasak Bumi root has the ability to inhibit the proliferation of PC-3 prostate cancer cells. The higher the ethanol extract concentration of the Pasak Bumi root, the lower the viability of PC-3 cells. Our research proposed the ethanol extract of Pasak Bumi root can be used as an anticancer agent for the prostate through a proliferation inhibition mechanism.

Keywords: Pasak Bumi, Anticancer Agent, Prostate Cancer

摘要 该文章描述了帕萨克布米根通过抑制个人电脑 3 细胞增殖作为前列腺抗癌剂的潜力。使用用乙醇溶剂提取的帕萨克布米 E. 长叶的根。前列腺癌个人电脑-3 细胞培养物取自具有骨转移的独立雄激素前列腺腺癌作为研究对象。本研究是一项体外实验研究，采用后测对照组设计。个人电脑 3 细胞活力的分析使用 MTT 测定法。方差分析检验结果显示 p 值 (签名。) 为 0.011 且小于 α = 0.05。因此，可以得出结论，在不同浓度的乙醇提取物帕萨克布米根给药中，腺癌细胞的活力存在显着差异。我们的研究表明，帕萨克布米根的乙醇提取物具有抑制个人电脑-3 前列腺癌细胞增殖的能力。帕萨克布米根的乙醇提取物浓度越高，个人电脑-3 细胞的活力越低。我们的研究表明，帕萨克布米根的乙醇提取物可通过增殖抑制机制用作前列腺的抗癌剂。

关键词: 帕萨克布米，抗癌剂，前列腺癌

I. INTRODUCTION

Prostate cancer is one of the malignancies in men worldwide and the 4th most common cancer as well as the 6th leading cause of death in men in the world [1]. Based on data from the Indonesian Society of Urologic Oncology (ISUO) 2006-2010, there were 971 prostate cancer sufferers. The mean age was 68.3, most of whom were between 70 and 79 years old (37.6%). The most common stage of cancer was stage 4 (490 patients, 50.5%) [2]. Management of advanced stage of prostate cancer such as mCRPC demands chemotherapy, which requires high costs and may bring quite severe side effects to the patient. Pasak Bumi E. longifolia can be a worthy alternative herbal therapy. Pasak Bumi are easy to find and unique in South Kalimantan. The active compound of Pasak Bumi root has a cytotoxic effect on various types of cancer such as colon cancer, breast cancer, lung cancer, skin cancer, ovarian cancer. The question raised here is whether it is also cytotoxic in prostate cancer.

The aim of this research was to determine the potential of Pasak Bumi root as an anticancer agent for prostate adenocarcinoma cells through inhibition of proliferation.

II. METHODS

The research design used was an in vitro experiment with a post-test only design, treatment by subject, random allocation. In this study, control and treatment groups were given a certain dose of ethanol extract of Pasak Bumi root.

The subject of the study was androgen-independent PC-3 prostate cancer cell line with bone metastases from ATCC. Calculation of cell proliferation used the MTT method (3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide). MTT was dissolved in PBS at a concentration of 5 mg/ml and filtered (Millipore, Bedford, MA). As much as 10 µl/100 µl of the available solution was added to the medium, which then was shaken and incubated at 37^0 for 4 hours. The result was a dark blue formazan product. Afterwards, the medium was replaced with 100 µl β-isopropanol (0.04 M KCL) in an acidic atmosphere and placed at room temperature for 20-30 minutes. The 96 plates were read using an enzyme-linked immunosorbent assay reader (570 nm). To measure the effect of the Pasak Bumi root extract, cell cultures were stimulated with 10% fetal calf serum (FCS) for 48 hours. After the incubation, the cells were washed twice with PBS and cell proliferation was calculated by the MTT method.

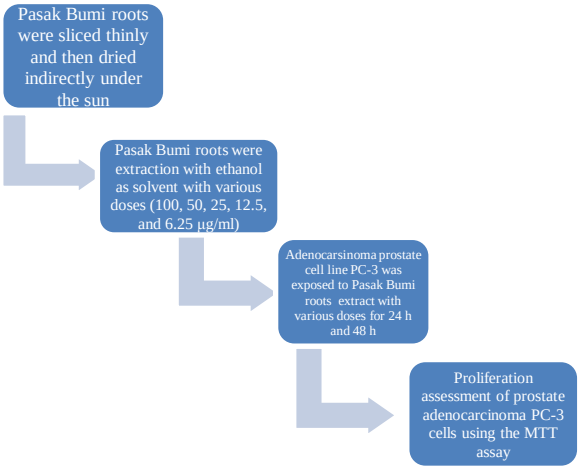

Figure 1. Flowchart of the research methods

III. RESULT AND DISCUSSION

In this study, the percentage of living adenocarcinoma cells in the treatment by ethanol extract of Pasak Bumi root (PBR) with various concentrations for 48 hours based on the absorbance value is shown in Table 1.

Table 1.
Percentage (%) of living adenocarcinoma cells in the treatment by ethanol extract of PBR with various concentrations for 48 hours based on the absorbance value

Treatment	Absorbance					Mean of Absorbance	Percent of Living PC3 Cells
	1	2	3	4	5		
CC	0,312	0,488	0,444	0,405	0,562	0,442	100
SC	0,138	0,143	0,161	0,139	0,139	0,144	0,9
Dose 1	0,373	0,465	0,345	0,257	0,373	0,363	73,6
Dose 2	0,342	0,361	0,345	0,254	0,258	0,312	56,7
Dose 3	0,235	0,335	0,298	0,212	0,375	0,291	49,8
Dose 4	0,328	0,196	0,321	0,201	0,196	0,248	35,6
Dose 5	0,146	0,162	0,231	0,297	0,181	0,203	20,7
MC	0,162	0,125	0,127	0,140	0,152	0,141	

The potential of the Pasak Bumi root extract in inhibiting adenocarcinoma cell proliferation is depicted in a graph of the percent of living cells (Figure 2).

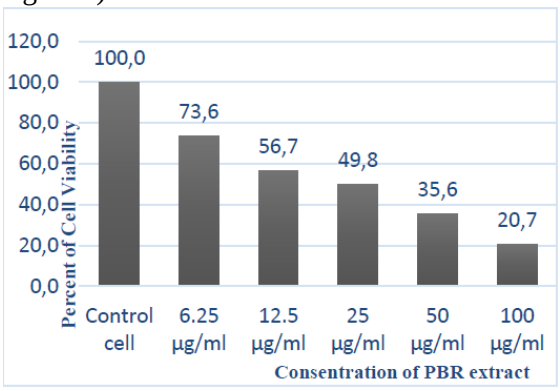

Figure 2. Graph of percentage (%) of living adenocarcinoma (PC3) cells in the administration of PBR extract with various concentrations for 48 hours

The statistical test of the effect of PBR ethanol extract at various doses on the viability of adenocarcinoma cells was carried out using ANOVA. Therefore, to find out whether there is a difference in the viability of adenocarcinoma cells at each concentration, the one-way ANOVA test, namely LSD, was used. LSD test results obtained p-value (Sig.) at 0.011 and smaller than $\alpha = 0.05$. Hence, it can be concluded that there is a significant difference in the viability of adenocarcinoma cells in the ethanol extract of PBR with different concentrations. The results of multiple comparisons using [Ri-Rj] 5% showed that giving PBR ethanol extract at various concentrations showed a significant difference in the viability of adenocarcinoma cells. By using a concentration of 100 µg/mL as a reference, the viability of adenocarcinoma cells at concentrations of 6.25 µg/mL, 12.5 µg/mL and 25 µg/mL showed a significant difference with the concentration of 100 µg/mL. Meanwhile, the concentration of 100 µg/mL with a concentration of 50 µg/mL did not show a significant difference.

The administration of PBR ethanol extract with various concentrations showed a significant difference in the viability of PC3 adenocarcinoma cells. The higher the ethanol extract dose of the Pasak Bumi root, the lower the viability of PC3 cells, which means that PC3 cell proliferation is further inhibited with increasing doses. The study by Hajjouli et al. [3] shows that the active compounds of Tongkat Ali are able to inhibit proliferation by preventing the induction of NF-κB and MAPK. According to this study, E. longifolia Jack can perform regulation in the proliferation, apoptosis, and inflammation mechanisms. The protein that plays a role in this mechanism is MAPK [3].

The research of Tong et al. [4] demonstrated the in vitro selective cytotoxic activity of quasionoid compounds from E. longifolia (Tongkat Ali) on LNCaP prostate cancer cells. The quasinoid from E. longifolia (Tongkat Ali) was shown to downregulate the expression level of the G1-to-S phase transition's regulatory proteins, namely cyclin D1, CDK4, and CDK2, and up-regulate the cyclin-inhibiting protein, p21Waf1/Cip1, which then caused cell cycle termination in G0/G1 phase [4].

Nurani's in vitro and in vivo research using ethanol extract of Pasak Bumi root on breast cancer cells resulted in inhibitory activity of COX-2 expression, decreased BCl-2 expression, increased Caspase-3, increased p53 expression, increased p21 expression, increased GADD45 expression, and decreased Ras [5]. The study showed that the ethanol extract of the roots of Pasak Bumi could act as a chemopreventive for breast cancer cells by inhibiting proliferation mechanisms.

Proteins that have a high affinity for quasinoids are mitogen-activated protein kinase (MAPK). MAPK is involved in the processes of proliferation, cell cycle regulation, and apoptosis. MAPK is a trigger for proliferation, hence it will inhibit the function of MAPK in the anticancer therapy mechanism [6].

The process of cell proliferation is triggered by MAPK and Ras. This study is in accordance with the in silico research results by Rahman et al. [7], which showed the affinity of the active

compound content of Pasak Bumi root extract on MAPK and Ras. The active compounds of the quasinoid and canthin groups from the extract can inhibit proliferation based on in silico analysis [7].

The Ras mechanism involves in the regulation of the cell cycle. The cell cycle in question is cell division, causing cell proliferation [8]. Negative feedback on the cell proliferation by RAS is required in the G1 phase. Extension of this phase will inhibit proliferation [8]. This is in accordance with the results of research by Tong et al. [4] on the active compounds of Pasak Bumi roots that have a cytotoxic effect. The cytotoxic effect of E. longifolia is to inhibit cell growth by resting / prolonging the G0/G1 phase in the cell division process. Quacinoids can also trigger the cessation of the G2/M phase in the cell cycle and then cause cell death [4].

The results of this study are in line with the research of Al-Salahi et al. [9], who stated that E. longifolia Jack has proapoptotic and anti-proliferative effects on HL-60 cell line. Research by Al-Salahi et al. [10] showed that intraperitoneal administration of E. longifolia jack extract (50 mg/kg) resulted in significant growth inhibition of subcutaneous tumors compared to control mice. Longifolia jack root extract exhibits strong antiproliferative activity in an in vivo model of leukemic cells [10]. With these effects, E. longifolia Jack can be used as a candidate for anti-prostate cancer therapy.

IV. CONCLUSION

Ethanol extract of Pasak Bumi root has the ability to inhibit the proliferation of PC-3 prostate cancer cells. The higher the ethanol extract concentration of Pasak Bumi root, the lower the viability of PC-3 cells. The concentration of PBR extract of 50 µg/mL was an effective dose to inhibit proliferation of PC3 prostate adenocarcinoma cells.

The results of this study are in line with the research of Al-Salahi et al. [9], who stated that E. longifolia Jack has proapoptotic and anti-proliferative effects on HL-60 cell line. This study is in line with the research of Hajjouli et al. [3], stating that E. longifolia Jack can perform regulation in the proliferation, apoptosis, and inflammation mechanisms.

The ethanol extract of Pasak Bumi root has the potential as an anticancer agent for the prostate through a proliferation inhibition mechanism.

REFERENCES

[1] BRAY, F., FERLAY, J., SOERJOMATARAM, I., SIEGEL, R.L., TORRE, L.A., and JEMAL, A. (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 68 (6), pp. 394-424.

[2] UMBAS, R., HARDJOWIJOTO, S., MOCHTAR, C.A., SAFRIADY, F., DJATISOESANTO, W., SOEDARSO, M.A., et al. (2011) *Panduan penanganan kanker prostat.* IAUI.

[3] HAJJOULI, S., CHATEAUVIEUX, S., TEITEN, M.H., ORLIKOVA, B., SCHUMACHER, M., DICATO, M., CHOO, C.Y., and DIEDERICH, M. (2014) Eurycomanone and eurycomanol from *Eurycoma longifolia* jack as regulators of signaling pathways involved in proliferation, cell death and inflammation. *Molecules*, 19 (9), pp. 14649-14666.

[4] TONG, K.L., CHAN, K.L., ABU BAKAR, S., LOW, B.S., MA, H.Q., and WONG, P.F. (2011) The in vitro and in vivo anti cancer activities of a standardized quassinoid composition from Eurycoma longifolia on LNCap human prostate cancer cells. *PLOS ONE*, 10 (3), pp. 1-21.

[5] NURANI, L.H., PRAMONO, S., and MUSTOFA, M. (2008) The Cytotoxicity of Extract of *Eurycoma Longifolia* Jack Root on T47D Cell Line. In: *Proceedings of the International Symposium on Cancer, Yogyakarta.* Yogyakarta: Ahmad Dahlan University.

[6] ZHOU, G., YANG, J., and SONG, P. (2019) Correlation of ERK/MAPK signaling pathway with proliferation and apoptosis of colon cancer cells. *Oncology Letter*, 17, pp. 2266-2270.

[7] RAHMAN, E.Y., UTOMO, D.H., ALI, M., PURNOMO, B.B., and KANIA, N. (2018) Evaluating the Potency of Active Compounds from *Eurycoma Longifolia* Jack Roots Extract as Prostate Cancer Therapy. *Drug Invention Today*, 10 (12), pp. 2374-2377.

[8] MALUMBERS, M. and PELLICER, A. (2014) RAS Pathway to cell cycle control

and cell transformation. *Frontiers in Bioscience*, 3, pp. 887-912.
[9] AL-SALAHI, O.S.A., ZAKI, A.H., CHAN, K.L., SHAH, A.M., AL-HASSAN, F., ABDULLAH, W.Z., and YUSOFF, N.M. (2013) In Vitro Anti Proliferative and Apoptotic Activities of Eurycoma Longifolia Jack (Simaroubaceae) on HL-60 Cell Line. *Tropical Journal of Pharmaceutical Research*, 12 (1), pp. 57-61.
[10] AL-SALAHI, O.S., JI, D., MAJID, A.M., KIT-LAM, C., ABDULLAH, W.Z., ZAKI, A., JAMAL DIN, S.K., YUSOFF, N.M., and MAJID, AS. (2014) Anti-tumor activity of Eurycoma longifolia root extracts against k-562 cell line: in vitro and in vivo study. *PLOS ONE*, 9 (1), 83818.

参考文:

[1] BRAY, F., FERLAY, J., SOERJOMATARAM, I., SIEGEL, R.L., TORRE, L.A., 和 JEMAL, A. (2018) 2018 年全球癌症统计数据：环球影业估计全球 36 种癌症的发病率和死亡率 185 个国家。加州：临床医生癌症杂志，68 (6)，第 394-424 页。
[2] UMBAS, R.、HARDJOWIJOTO, S.、MOCHTAR, C.A.、SAFRIADY, F.、DJATISOESANTO, W.、SOEDARSO, M.A. 等。(2011) 癌症治疗指南前列腺。国际原子能机构。
[3] HAJJOULI, S., CHATEAUVIEUX, S., TEITEN, MH, ORLIKOVA, B., SCHUMACHER, M., DICATO, M., CHOO, CY 和 DIEDERICH, M. (2014) 来自长叶芨芨草的欧马酮和广藿香醇杰克作为参与增殖、细胞死亡和炎症的信号通路的调节剂。分子，19 (9)，第 14649-14666 页。
[4] TONG, K.L., CHAN, K.L., ABU BAKAR, S., LOW, B.S., MA, H.Q. 和 WONG, P.F. (2011) 长叶芨芨草的标准化类固醇组合物对 LN 帽人前列腺癌细胞的体外和体内抗癌活性。PLOS 一，10 (3)，第 1-21 页。
[5] NURANI, L.H., PRMONO, S. 和 MUSTOFA, M. (2008) Eurycoma 长叶杰克根提取物对吨 47D 细胞系的细胞毒性。在：国际癌症研讨会论文集，日惹。日惹：艾哈迈德达兰大学。
[6] ZHOU, G., YANG, J., 和 SONG, P. (2019) ERK/MAPK 信号通路与结肠癌细胞增殖和凋亡的相关性。肿瘤学信函，17，第 2266-2270 页。
[7] RAHMAN, E.Y., UTOMO, D.H., ALI, M., PURNOMO, B.B. 和 KANIA, N. (2018) 评估长叶金刚根提取物作为前列腺癌治疗的活性化合物的效力。今日药物发明，10 (12)，第 2374-2377 页。
[8] MALUMBERS, M. 和 PELLICER, A. (2014) 细胞周期控制和细胞转化的 RAS 途径。生物科学前沿，3，第 887-912 页。
[9] AL-SALAHI, O.S.A., ZAKI, A.H., CHAN, K.L., SHAH, A.M., AL-HASSAN, F., ABDULLAH, W.Z., 和 YUSOFF, N.M. (2013) Eurycoma 长叶杰克的体外抗增殖和凋亡活性（苦瓜科）在 HL-60 细胞系上。热带药物研究杂志，12 (1)，第 57-61 页。
[10] AL-SALAHI, O.S., JI, D., MAJID, A.M., KIT-LAM, C., ABDULLAH, W.Z., ZAKI, A., JAMAL DIN, S.K., YUSOFF, N.M. 和 MAJID, A.S。(2014) 长叶芨芨草根提取物对 k-562 细胞系的抗肿瘤活性：体外和体内研究。公共科学图书馆一号，9 (1)，83818。