Research Article

Evaluating the potency of active compounds from *Eurycoma longifolia* jack roots extract as prostate cancer therapy

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ABSTRACT

Aim and Scope: Prostate cancer will occur when immortal cells develop in the prostate gland. It was frequently diagnosed in men and becomes the fourth most common cancer in the world in 2014. In Indonesia, it has high prevalence of about 16 cases in 100,000 of men. Furthermore, Indonesia has an endemic plant called *Pasak Bumi* (*Eurycoma longifolia* Jack) which is well-known as traditional medicine and cancer therapy. The root extract of *Pasak Bumi* consists of eurycomanone, quassinoid, and canthin. Therefore, this study aimed to predict the potency of *E. longifolia* active compounds as prostate cancer therapy. **Material and Methods:** There are four essential protein targets in prostate cancer such as *Bcl-2, RAS, MAPK,* and *CASP*. The three-dimensional structure of active compounds was retrieved from PubChem, and protein target was downloaded from Protein Data Bank. The interactions were calculated using Pyrx.0.8. Thus, the molecular interaction was analyzed using LigPlus. Moreover, the biological activity of the active compound was analyzed using PASS server. **Results and Discussion:** Results showed that each active compound is strongly bind to *RAS* and only *RAS* -quassinoid complex which has the lowest binding affinity score about −9.3 Kcal/mol. Based on the structure-activity relationship analysis, only eurycomanone and quassinoid which potentially involved in the anticancer mechanism as antineoplastic and apoptosis agonist. It means these compounds are blocking the active site of *RAS* protein to inhibit the cell proliferation. **Conclusion***:* It can be concluded that all active compounds have the lowest binding affinity to *RAS*. Moreover, quassinoid is the most active compounds which have the influence to *RAS*. It shows that quassinoid potentially as a therapeutic agent.

KEY WORDS: Anticancer, Apoptosis, Canthin, Eurycomanone, Herbal compound, Inhibitor, Prostate cancer, Quassinoid

INTRODUCTION

Prevalence of prostate cancer in Indonesia is around 16 cases per 100,000 of men.[1] Moreover, prostate cancer is including to the top 10 most common cancer in men. It according to data collected from 13 national histopathology centers throughout Indonesia.[2] There are several therapy for prostate cancer patients such as hormone therapy and radiation therapy,[3] but they have a lot of side effect and high cost. Therefore, the specific molecular mechanism of prostate cancer becomes a particular concern in research to find the solution.

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

Genes that involved in the prostate cancer were located on the chromosome number 1 such as *p53, Bcl-2, RAS, p21,* and caspase.[4] The family of *Bcl-2* plays an important role in the apoptosis, such as *Bcl-2* and *Bcl-*$_{XL}$ were decreasing the apoptosis, while Bax, Bik, and Bid were increasing the mechanism of apoptosis.[5] There are two pathways of apoptosis such as intrinsic and extrinsic.[6] The intrinsic pathway induced by cellular stress involving *Bcl-2* and *caspase-9* while extrinsic pathway induced by reaction of ligand binding which involving Fas and *caspase-8*.[7] Therefore, *Bcl-2*, *RAS, caspase-9,* and *MAPK* could be the essential protein target in prostate cancer.

Indonesia is well-known as mega biodiversity and has a lot of herbal medicine species. *Pasak Bumi* or *Eurycoma*

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Received on: 14-02-2018; Revised on: 18-04-2018; Accepted on: 26-05-2018

longifolia Jack is one kind of endemic plant in South Borneo, Indonesia. It belongs to Simaroubaceae family[8] and believes have a function as an aphrodisiac. Furthermore, roots of *Pasak Bumi* are known consist of an essential active compound such as eurycomanone, quassinoid, and canthin as anticancer.[9] Based on the recent research, quassinoid known had cytotoxic effect in the colon cancer, breast cancer, lung cancer, and fibrosarcoma[10]. Therefore, this study aimed to predict the potency of *E. longifolia* active compounds as prostate cancer therapy based on *in silico* approach.

MATERIALS AND METHODS

Retrieval Data

Three three-dimensional (3D) structure of an active compound from *Pasak Bumi* roots extract such as eurycomanone (CID 13936691), quassinoid (CID 5458870), and canthin (CID 97176) was collected from PubChem (www.pubchem.ncbi.nlm.nih.gov). Moreover, the 3D structure of protein target in prostate cancer, *Bcl-2* (PDBID 4XLD), *caspase 9* (PDBID 1NW9) *RAS* (PDBID 2CE2), and *MAPK* (PDBID 4QTA) were downloaded from protein data bank (www.rcsb.org/pdb/home.home.do). The 3D structure was provided for molecular docking [Figure 1].

Molecular Docking

The docking process was carried out using AutoDock Vina in Pyrx 0.8 to compute the binding affinity between ligands (active compounds of *Pasak Bumi*) and protein target. Each of active compound was docked to the specific active site of protein target.[11]

Molecular Interaction

The molecular interaction between active compound and protein target was analyzed by LIGPLUS Program.[12] The result determination was based on the amino acid interaction. The active compound would be determine as potential inhibitor if they had interacted to amino acid in the active site of protein target.

Molecular Visualization

All biomolecules were visualized using CHIMERA 1.8.1 to generate the representative figure.[13]

Analysis of Biological Activity

The active compounds of *Pasak Bumi* were analyzed using PASS online server to determine the biological activity. It depended on the compound structure. The analysis will be resulting probability of active (Pa), and the probability of inactive (Pi) score with a range from 0 to 1. If Pa > Pi mean, it is potential for a specific therapeutic candidate.[14]

RESULTS AND DISCUSSION

Three active compounds of *Pasak Bumi* were able binding to the active site of protein target. Moreover, eurycomanone, quassinoid, and canthin have a strong to *RAS* which indicated by lower binding affinity score than another complex. It indicated by the most minimum binding affinity score which is about −9.3 Kcal/mol [Table 1].

In the normal conditions, activation of *RAS* triggers a cellular response and transformation. Furthermore, it also to be an essential component of signaling network for controlling cell proliferation and survival through the *RAS-MAPK* pathway.[15] Moreover, it is known involving in the prostate cancer development, progression, and metastasis.[16] It proven by recent research which states that *RAS* protein was found in 43% of primary prostate cancer samples and 90% of metastatic samples.[17] It is indicate that *RAS* protein is potentially as therapeutic agent for prostate cancer.

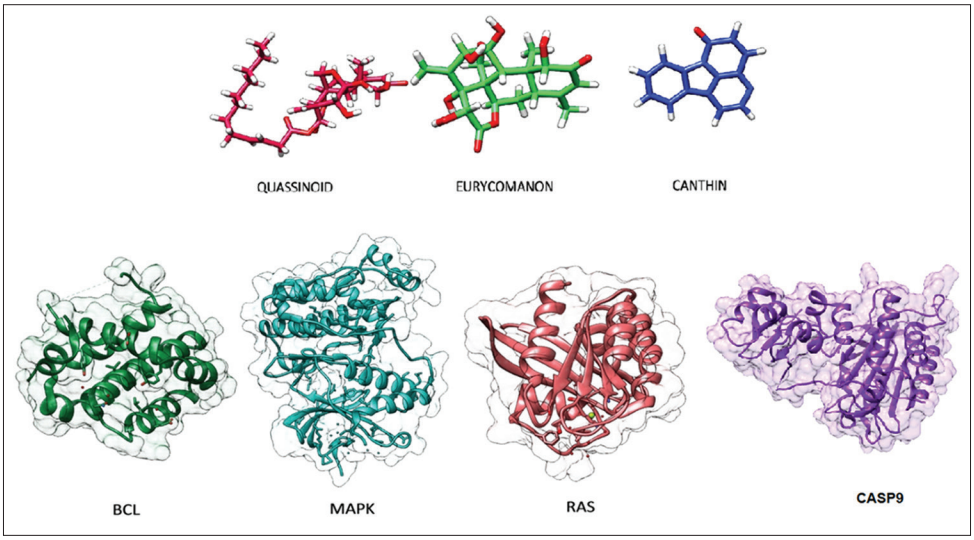

Figure 1: The 3D structure of (a) Active compound of *Pasak Bumi* roots and (b) Protein target of prostate cancer

Table 1: Binding affinity score between protein target and active compound of Pasak Bumi

Protein target	Active compound (Ligand)	Binding affinity (Kcal/mol)
Bcl-2	Eurycomanone	−6.8
	Quassinoid	−8.1
	Canthin	−7.7
RAS	Eurycomanone	−9.2
	Quassinoid	−9.3
	Canthin	−9.1
MAPK	Eurycomanone	−8.1
	Quassinoid	−8.3
	Canthin	−7.6
Casp9	Eurycomanone	−8.2
	Quassinoid	−7.8
	Canthin	−6.8

Based on the UniProt database, gross domestic product was bind to the active site of *RAS* protein. It could be used as a promising template to screen the potential inhibitor for prostate cancer inhibition. The active site of *RAS* protein is around on the Gly15, Lys16, Asn116, Ala146, Ser145, Asp119, Leu120, Asp30, Lys117, Ala18, Cys32, Gly13, Ser17, and Phe25 residues.

Figure 2 shows that each active compound was bind to inhibition site of *RAS* protein and interact to several amino acids around of that site. However, only quassinoid which has the appropriate bond to inhibition site of *RAS* protein on the residues Gly15, Lys16, Gly13, Asp30, Phe28, Lys117, Glu31, Pro34, Gly12, Ser17, and Asp33 through 2 hydrogen bonding and 9 hydrophobic bonding.

Furthermore, the determination of biological activity shows that eurycomanone and quassinoid have Pa > Pi for antineoplastic and apoptosis agonist with the values ranges from 0.8 to 1 [Figure 3]. However, canthin shows antineoplastic activity with lower value ranges from 0.2 to 0.4, then it has not apoptosis agonist activity. The value of Pa and Pi can be considered to be measures of the compound to study belonging to active and inactive compounds, respectively.[18] However, among the three active compounds of *Pasak Bumi*, eurycomanone, and quassinoid are the most potential for therapeutic agent. These compounds may suppress the prostate cancer development by blocking the active site of *RAS* protein for decreasing the proliferation rate.

CONCLUSION

Active compounds of *E. longifolia* Jack root extract (quassinoid, canthin, and eurycumanon) predicted to have an effect to inhibit the cell proliferation in prostate cancer which is involving as an inhibitor of *Bcl-2, RAS,* and *MAPK*. Furthermore, they are triggering the activation of *caspase-9* for increasing the process of apoptosis. All of the active compounds have the lowest binding affinity to *RAS*. Moreover,

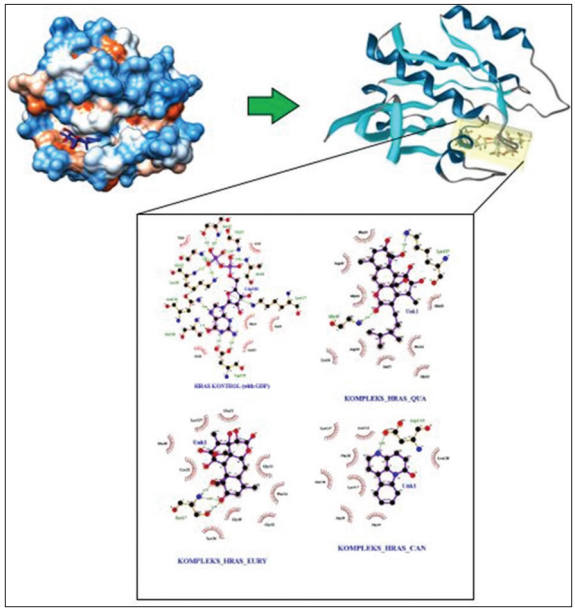

Figure 2: Molecular interaction of active compound in *Pasak Bumi* roots on the active site of RAS

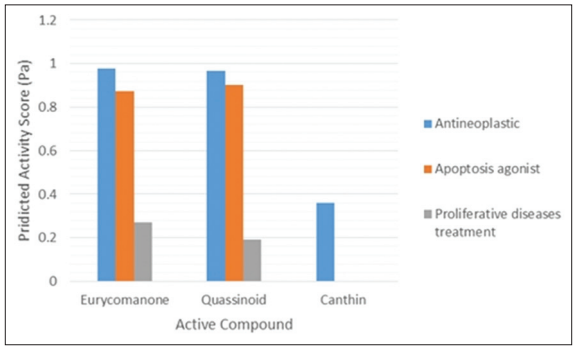

Figure 3: Biological activity of active compound in *Pasak Bumi* roots for prostate cancer

quassinoid is the most active compounds which have influence to *RAS*. It shows that quassinoid potentially as a therapeutic agent.

ACKNOWLEDGMENT

This work supported by the Laboratory of Bioinformatics, Department of Biology, Brawijaya University.

REFERENCES

1. Baade PD, Youlden DR, Cramb SM, Dunn J, Gardiner RA. Epidemiology of prostate cancer in the Asia-pacific region. Prostate Int 2013;1:47-58.
2. Umbas R. Penanganan kanker prostat stadium II pada penderita berusia 70 tahun atau lebih: pengalaman dua Rumah Sakit Tersier di Jakarta. Indones J Cancer 2009;3:133-7.
3. Martin NE, D'Amico AV. Progress and controversiers: Radiation therapy for prostate cancer. CA Cancer J Clin 2014;64:189-407.
4. Dharmayanti NL. Kajian biologi molekuler: gen suppressor tumor (p53) sebagai target gen dalam pengobatan kanker. Wartazoa 2003;13:99-107.

5. Finnegan NM, Curtin JF, Prevost G, Morgan B, Cotter TG. Induction of apoptosis in prostate carcinoma cells by BH3 peptides which inhibit Bak/Bcl-2 interactions. Br J Cancer 2001;85:115-21.
6. Brunelle JK, Chandel NS. Oxygen deprivation induced cell death: An update. Apoptosis 2002;7:475-82.
7. Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene 2006;25:4798-811.
8. Alves IA, Miranda HM, Soares LA, Randau KP. Simaroubaceae family: Botany, chemical composition and biological activities. Rev Bras Farmacogn 2014;24:481-501.
9. Kuo PC, Damu AG, Lee KH, Wu TS. Cytotoxic and antimalarial constituents from roots of Eurycoma longifolia. Bioorg Med Chem 2004;12:537-44.
10. Ueda JY, Tezuka Y, Banskota AH, Le Tran Q, Tran QK, Harimaya Y, *et al.* Antiproliferative activity of Vietnamese medicinal plants. Biol Pharm Bull 2002;25:753-60.
11. Trott O, Olson AJ. Auto dock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 2010;31:455-61.
12. Laskowski RA, Swindells MB. LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011;51:2778-86.
13. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, *et al*. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem 2004;25:1605-12.
14. Goel RK, Singh D, Lagunin A, Poroikov V. PASS-assisted exploration of new therapeutic potential of natural products. Med Chem Res 2011;20:509-1514.
15. Fernández-Medarde A, Santos E. Ras in cancer and developmental diseases. Genes Cancer 2011;2:344-58.
16. Suzuki A, Nakano T, Mak TW, Sasaki T. Portrait of PTEN: Messages from mutant mice. Cancer Sci 2008;99:209-13.
17. Mulholland DJ, Kobayashi N, Ruscetti M, Zhi A, Tran LM, Huang J, Gleave M, *et al*. Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells. Cancer Res 2012;72:1878-89.
18. Paramashivam SK, Elayaperumal K, Natarajan BP, Ramamoorthy MD, Balasubramanian S, Dhiraviam KN. *In silico* pharmacokinetic and molecular docking studies of small molecules derived from *Indigofera aspalathoides* Vahl targeting receptor tyrosine kinases. Bioinformation 2015;11:73-84.

Source of support: Nil; Conflict of interest: None Declared