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Exploring the Ellagic Acid Potential Mechanism Againts Inflammation and Bone Development Using Network Analysis

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Abstract

Ellagic acid (EA) is very beneficial for human health, especially for its therapeutic potential in inflammation and osteoblastogenesis. This study aimed to determine the mechanism of EA intervention on target proteins SMAD3 and AKT1. The EA bioactive compound (PubChem ID 5281855) was downloaded as canonical SMILES. Protein targets and interactions were predicted using the Similarity Ensemble Approach target prediction web server, Swiss Target Prediction, STRING webserver, and Cytoscape version 3.9.0. As a result, EA target proteins play roles in inflammation, osteoblast development and differentiation, and bone. EA is predicted to interact with SMAD3, and this interaction with SMAD3 is thought to help the role of EA in tooth repair. Moreover, betweenness centrality results from Cytoscape, Akt is a node that has a major role in tissue pathways. In conclusion, EA can help in tooth repair, which is predicted to possess anti-inflammatory and support bone development via SMAD3 and AKT1.

***Keywords**: Akt1, Cytoscape, ellagic acid, inflammatory, Smad3.*

Abstrak

Abstrak ditulis dalam bahasa Indonesia dan bahasa Inggris dengan jenis huruf Arial, ukuran 10, italic, spasi tunggal. Abstrak mencakup latar belakang, tujuan, metode, hasil, serta kesimpulan dari penelitian. Abstrak terdiri dari satu paragraf dengan jumlah kata paling banyak 200 kata dalam bahasa Indonesia dan 150 kata dalam bahasa Inggris. Jika artikel ditulis dalam bahasa Inggris maka abstrak pertama dalam bahasa Indonesia dan sebaliknya. Asam ellagic (EA) sangat bermanfaat bagi kesehatan manusia, terutama potensi terapeutiknya terhadap inflamasi dan osteoblastogenesis. Penelitian ini bertujuan untuk mengetahui mekanisme intervensi EA terhadap protein target SMAD3 dan AKT1. Senyawa bioaktif EA (PubChem ID 5281855) diunduh sebagai SMILES kanonik. Target dan interaksi protein diprediksi menggunakan server web prediksi target Similarity Ensemble Approach, Swiss Target Prediksi, server web STRING, dan Cytoscape versi 3.9.0. Hasilnya, protein target EA berperan dalam inflamasi, perkembangan dan diferensiasi osteoblas, dan tulang. EA diprediksi berinteraksi dengan SMAD3, dan interaksi dengan SMAD3 ini diperkirakan membantu peran EA dalam perbaikan gigi. Selain itu, hasil sentralitas antara dari Cytoscape, Akt merupakan simpul yang memiliki peran utama dalam jalur jaringan. Kesimpulannya, EA dapat membantu dalam perbaikan gigi, yang diprediksi memiliki anti-inflamasi dan mendukung perkembangan tulang melalui SMAD3 dan AKT1.

***Kata kunci**: Akt1, Cytoscape, ellagic acid, inflammatory, Smad3.*

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Introduction

Tooth extraction is the most commonly performed surgical procedure in dentistry (Sjuhada et al., 2020). Tooth extraction causes a wound in the socket that undergoes physiological healing either acutely or chronically (Sharif et al., 2020). The wound-healing process is the process of repairing or changing tissue and functional capacity damaged by injury (Brasilino et al 2018, Mozzati et al., 2022). It is physiologically divided into four overlapping sequential phases: hemostasis, inflammatory, proliferation, and maturation or remodeling (Sjuhada et al., 2020).

The inflammatory response after tissue injury plays an important role in both normal and pathological healing. Immediately after injury, the innate immune system is activated, setting in motion a local inflammatory response that includes the recruitment of inflammatory cells from the circulation (Wilkinson et ., 2020). This rapid response begins with the degranulation of both platelets that arrive at the site and mast cells that are induced when injured (Raziyeva et al 2021, Krzyszczyk et al., 2018).

Macrophages are activated and initiate the innate immune response of the body. Polarisasi makrofag menjadi fenotip M1 pro-inflamasi atau M2 anti-inflamasi depending on the signal (Yang et al., 2023). The activation of the PI3K/Akt pathway is an important modulator of macrophage survival, migration, proliferation, metabolic responses, and inflammatory signaling and induces the expression of anti-inflammatory cytokines, leading to the emergence of the M2 phenotype to repair and resolve inflammation (Basu et al., 2021). Akt is a family of three serine–threonine kinases: Akt1, Akt2, and Akt3 (Basu et al., 2021). Each Akt has a specific task related to its pathway in the inflammatory response. Akt modulates and activates transforming growth factor-β (TGF-β), which activates the Smad3 pathway at the wound site. Supported by the presence of proinflammatory cytokines or TLR ligands, TGF-β inhibits the synthesis of macrophage-derived inflammatory genes. Smad3 has been confirmed to be the signaling molecule pathway of TGF-β1 in inflammatory conditions and organ fibrosis (Wu et al., 2022, Chen et al., 2019).

Prolonged inflammation exacerbates the effects of infection. Infection can affect the developing replacement tooth, causing bone loss, hypomineralization, hypoplasia, arrest of tooth development, regional odontodysplasia, delayed eruption, etc. (Latyushinta et al., 2020). Tooth resorption involves active inflammatory pathways with diverse signaling pathways. Blockade of these pathways can be prevented by administering anti-inflammatory agents with antioxidant properties.

Ellagic acid (EA) is a polyphenol class compound found in pomegranate fruit, berries, and green tea (Son et al., 2019). This compound has antioxidant, anti-inflammatory, and anti-cancer properties and plays a role in osteoblastogenesis (Son et al., 2019, Wardhana et al., 2021). Previous research also revealed that EA protects against bone loss in mice through osteoclast differentiation and bone resorption inhibition (Lin et al., 2020). As a bioactive molecule, EA has many benefits for human health, especially therapeutic potential that can be used as a model for natural product-based drug discovery and development approaches that could be based in the future (Torre et al., 2017).

However, to make an active compound work effectively, a precise target of action is required. Therefore, research on the molecular mechanism of EA is crucial for both new drug design and clinical treatment. Although it is not yet known exactly how the mechanism of action pathway of EA works, it is necessary to trace the exact target action protein in studying the

molecular level.

Proteins are the main building blocks of basic tissues in the body. Functional proteins do not run independently but are interconnected with other proteins to form a network called protein–protein interaction (PPI) (Soleymani et al 2022). The structure of a PPI is like that of a graph or network that has vertices and edges. The vertices of the graph represent the protein itself, and the edges connecting the two vertices represent the functional relationships of the proteins (Cook et al 2018). To perform large protein networks, the role of network profiling using computational algorithms is very helpful in simplifying and speeding up the analysis. This network profiling integrated with knowledge extraction will help to better understand the biochemical mechanism of biological systems (sornsiri et al 2018). Therefore, an *in silico* network analysis was conducted to predict the potential of EA with specific protein targets so that the mechanism of the pathways involved can be known to support its role as anti-inflammatory and support bone development.

Materials and Methods

EA Bioactive Compound Collection

EA bioactive compounds (PubChem ID 5281855) were downloaded in the form of canonical SMILES using the PubChem online database (https://Pubchem.ncbi.nlm.nih.gov). The collected canonical SMILES were used for bioactivity and target protein prediction.

EA Target Protein Prediction

Target protein prediction using the Similarity Ensemble Approach (https://sea.bkslab.org/) and Swiss Target Prediction (http://www.swisstargetprediction.ch/). This tool determines the target protein of a compound based on the similarity of its structure. The target protein is predicted by entering the canonical SMILES of EA bioactive compounds.

Prediction of the Target Protein Bioactivity

The obtained target proteins were imported into the STRING DB V.11 database (https://string-db.org/) using KEGG Pathway enrichment with an input high confidence score of 0.7 on *Homo sapiens* organs. The STRING DB sources used were text mining, experiments, and databases. This aims to determine the bioactivity of the target protein.

Network Analysis Using Cytoscape

Network analysis was performed using Cytoscape version 3.9.0 to determine the roles of proteins in the pathway. The GOlorize plug-in and the network analysis with Cytoscape were used in this study. GOlorize is used to determine the roles of genes/proteins in the pathway using the BinGO (Gene Ontology [GO]) approach and visualized with certain colors. BinGO–GOlorize used a significant value of 0.05, and the statistical test used was the hypergeometric test with multiple testing correction Benjamini–Hochberg false discovery rate (FDR) correction with a biological process ontology file in *Homo sapiens*. FDR is a statistical approach used in multiple hypothesis testing to correct for multiple comparisons.

Results and Discussion

On the basis of the analysis using KEGG Pathway enrichment, the predicted EA target proteins are involved in several pathways, including the chemokine signaling pathway related to immunity

and the estrogen signaling pathway related to bone remodeling and resorption (Figure 1).

On the basis of the analysis of BinGO–GOlorize, EA target proteins play roles in inflammation, osteoblast development and differentiation, and bone. EA is predicted to interact with SMAD3, and this interaction with SMAD3 is thought to help the role of EA in tooth repair.

On the basis of the network analysis with the betweenness centrality (BC) value, AKT1 is the node that plays the biggest role in the pathway. BC is an analysis that shows which nodes play the greatest role in the communication pathway. The higher the BC value, the more important the protein. A protein with high closeness centrality is easily accessible. It will easily become the center of regulation for other proteins, and degree is the number of proteins that can target the node (Long et al 2019).

Then, 12 proteins (Table 3) from the enrichment analysis process were obtained and sorted on the basis of the BC results from Cytoscape. PPI analysis is an effective method to demonstrate the biochemical mechanism of anti-inflammation for EA. On the basis of network analysis by observing the BC value, AKT1 is the node that plays the greatest role in the pathway (Table 3).

EA has potential in bone disease treatment (0.354) and as an anti-inflammatory (0.749), a TNF expression inhibitor (0.412), an MMP9 expression inhibitor (0.546), and a calcium channel activator (0.529). This score is predicted on the basis of the similarity of the structure and features of the EA compound with the compounds in the database. The more similar the structure and features, the higher the prediction score.

The selected target proteins (41 proteins) (Table 1) were downloaded from STRING and analyzed using Cytoscape ver. 3.9.0 (Cytoscape Consortium) and the GOlorize plug-in (Makiyah et al 2023). First, the protein network was analyzed using NetworkAnalyzer to obtain the relatedness and closeness centrality and degree data. Moreover, the network was analyzed with GO using the GOlorize plug-in, which generated GO data GOlorize plug-in, which generated GO data associated with the proteins in the network and colored the protein nodes in the network according to their bioactivity.

In this study, we reported the potential bioactivity of target proteins positively involved in bone development (GO-ID 60348), regulation of osteoblast differentiation (GO-ID 45667), immune system development (GO-ID 2520), osteoblast differentiation (GO-ID 1649), osteoblast development (GO-ID 2076), and inflammatory response (GO-ID 6954) targeted by EA compounds (Table 2). These bioactivities are closely related to inflammation, development, osteoblast differentiation, bone remodeling, and bone resorption. Furthermore, these bioactivities will be selected for further study. EA is predicted to interact with SMAD3 (Figure 2), which is thought to aid the role of EA in tooth repair. Mechanistically, SMAD3 transcriptionally regulates many downstream pathways of target genes, including micro-RNAs and long noncoding RNAs, which cause cell death, inflammation, and fibrosis (Wu et al 2022).

In addition to playing a role in immune regulation and inflammation, SMAD3 plays an important role in osteoblast differentiation and bone formation in a time-dependent manner (Lin et al 2020). Numerous studies have confirmed that the Smad signaling pathway is essential for regulating osteoblast and osteoclastic differentiation during bone development,

formation, and homeostasis, indicating a close relationship between Smad signaling and bone remodeling. Smad proteins are essential intracellular effectors for TGF-β and bone morphogenetic protein (BMP) members, which act as transcription factors (Zou et al 2021).

SMAD mediates signal transduction in TGF-β and BMP signaling pathways that affect osteoblast and osteoclast functions and thus plays an important role in the regulation of bone remodeling (Zou et al 2021). TGF-β is a pathway that is closely related to Akt (Zhang et al 2019), This agrees with the BC results from Cytoscape, which show that Akt is a node that plays a major role in the network pathway (Table 3). BC is the control amount of one human protein exerted over other protein interactions in the human PPI network, and the higher the BC score, the more important its role in the network (Khorsand et al 2020).

Akt is a family of three serine–threonine kinases: Akt1, Akt2, and Akt3. Akt signaling pathway components have distinct and isoform-specific roles in macrophage biology and inflammatory disease regulation by controlling inflammatory cytokines, miRNAs, and functions, including phagocytosis, autophagy, and cellular metabolism (Yang et al 2023). Akt activation also affects FoxO3, Runx2, and Osx and activates transcription factor-4, which is directly implicated in bone development and bone cell function (Torre et al 2017).

EA has the greatest potential as an anti-inflammatory agent that can support bone development (Figure 3). This is supported by Lin et al. (2020), who demonstrated that EA blockade IL-1β induces inflammation and extracellular matrix degradation in human chondrocytes (15). Moreover, the link with bone development involving Akt was confirmed by Laha's (2022) study, which showed that the addition of polyphenolic components, including EA, can reduce the number of autophagy vesicles and levels of amino acid molecules through Akt inactivation in osteoclast differentiation (Laha et al 2022). Thus, Akt and Smad3 regulation can be said to determine the progression of bone cell differentiation that contributes to bone development.

Conclusion

In conclusion, EA is predicted to act on Akt1 target protein via the Smad3 mechanism pathway, which plays an important role in anti-inflammatory regulation and bone development. This potential can be further developed toward research to study its relationship with the mechanism of tooth repair.

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Conflict of Interest

The authors declare that they hold no competing interests.

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Lampiran Gambar Dan Tabel

Table 1: Target Protein Prediction.

Swiss Target Prediction			**SEA Target**		
Target	Common name	Score probability	Target	Common name	MaxTC
G protein-coupled receptor 35	GPR35	1	DNA polymerase iota	POLI	1
Receptor protein–tyrosine kinase erbB-2	ERBB2	1	ELAV-like protein 3	ELAVL3	1
Aldose reductase	AKR1B1	1	DNA polymerase eta	POLH	1
Cyclin-dependent kinase 4/cyclin D1	CCND1 CDK4	1	Mothers against decapentaplegic homolog 3	SMAD3	1
Platelet-derived growth factor receptor beta	PDGFRB	1	Heat shock 70 kDa protein 1A	HSPA1A	1
Vascular endothelial growth factor receptor 3	FLT4	1	Solute carrier family 22 member 6	SLC22A6	1
Insulin-like growth factor I receptor	IGF1R	1	NUAK family SNF1-like kinase 1	NUAK1	1
Insulin receptor	INSR	1	G protein–coupled receptor 35	GPR35	1
Epidermal growth factor receptor erbB1	EGFR	1	Carbonic anhydrase 13	CA13	1
Carbonic anhydrase II	CA2	1	Cysteine protease ATG4B	ATG4B	1
Cyclin-dependent kinase 2/cyclin A	CDK2 CCNA1 CCNA2	1	Carbonic anhydrase 6	CA6	1
Serine–threonine–protein kinase Aurora-B	AURKB	1	Macrophage migration inhibitory factor	MIF	1

Swiss Target Prediction			SEA Target		
Carbonic anhydrase VII	CA7	1	Carbonic anhydrase 5A, mitochondrial	CA5A	1
Carbonic anhydrase I	CA1	1	Carbonic anhydrase 7	CA7	1
Glycogen synthase kinase-3 beta	GSK3B	1	Mitogen-activated protein kinase 8	MAP3K8	1
Tyrosine–protein kinase SRC	SRC	1	Cyclin-A1	CCNA1	1
Focal adhesion kinase 1	PTK2	1	Carbonic anhydrase 4	CA4	1
Vascular endothelial growth factor receptor 2	KDR	1	G1/S-specific cyclin-D1	CCND1	1
Serine–threonine–protein kinase PLK1	PLK1	1	Carbonic anhydrase 14	CA14	1
Carbonic anhydrase VI	CA6	1	Serine–threonine–protein kinase PLK4	PLK4	1
Carbonic anhydrase XII	CA12	1	Serine–threonine–protein kinase PLK1	PLK1	1
Carbonic anhydrase XIV	CA14	1	Carbonic anhydrase 12	CA12	1
Carbonic anhydrase IX	CA9	1	Aldo-keto reductase family 1 member B1	AKR1B1	1
Casein kinase II alpha	CSNK2A1	1	Vascular endothelial growth factor receptor 3	FLT4	1
Hepatocyte growth factor receptor	MET	1	Insulin receptor	INSR	1
Carbonic anhydrase IV	CA4	1	Angiopoietin-1 receptor	TEK	1
Serine–threonine–protein kinase PLK4	PLK4	1	Ephrin type-B receptor 4	EPHB4	1
Carbonic anhydrase XIII	CA13	1	Cyclin-A2	CCNA2	1
Tyrosine–protein kinase TIE-2	TEK	1	Casein kinase II subunit alpha	CSNK2A1	1
Serine–threonine–protein kinase AKT	AKT1	1	Carbonic anhydrase 9	CA9	1
Serine–threonine–protein kinase Aurora-A	AURKA	1	Platelet-derived growth factor receptor beta	PDGFRB	1
Carbonic anhydrase VA	CA5A	1	Cyclin-dependent kinase 4	CDK4	1
Beta-secretase 1	BACE1	1	Focal adhesion kinase 1	PTK2	1
Mitogen-activated protein kinase 8	MAP3K8	1	Proto-oncogene tyrosine–protein kinase Src	SRC	1
Serine–threonine–protein kinase B-raf	BRAF	1	Aurora kinase B	AURKB	1
Ephrin receptor	EPHB4	1	Receptor tyrosine–protein kinase erbB-2	ERBB2	1
Heat shock 70 kDa protein 1	HSPA1A	1	Insulin-like growth factor 1 receptor	IGF1R	1
NUAK family SNF1-like kinase 1	NUAK1	1	Hepatocyte growth factor receptor	MET	1
Squalene monooxygenase (by homology)	SQLE	1	Carbonic anhydrase 1	CA1	1
Tyrosine–protein kinase FGR (by homology)	FGR	1	Glycogen synthase kinase-3 beta	GSK3B	1
Tyrosine–protein kinase Lyn (by homology)	LYN	1	RAC-alpha serine–threonine–protein kinase	AKT1	1
			Aurora kinase A	AURKA	1
			Cyclin-dependent kinase 2	CDK2	1
			Serine–threonine–protein kinase B-raf	BRAF	1
			Carbonic anhydrase 2	CA2	1
			Epidermal growth factor receptor	EGFR	1
			Beta-secretase 1	BACE1	1
			Vascular endothelial growth factor receptor 2	KDR	1

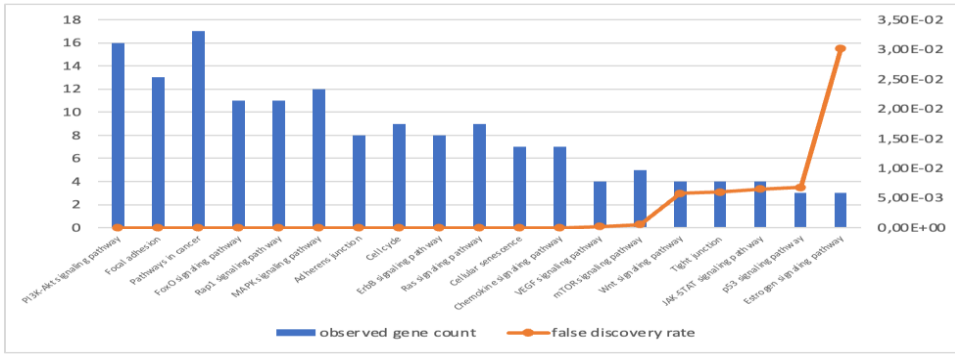


Figure 1: KEGG Enrichment Analysis.

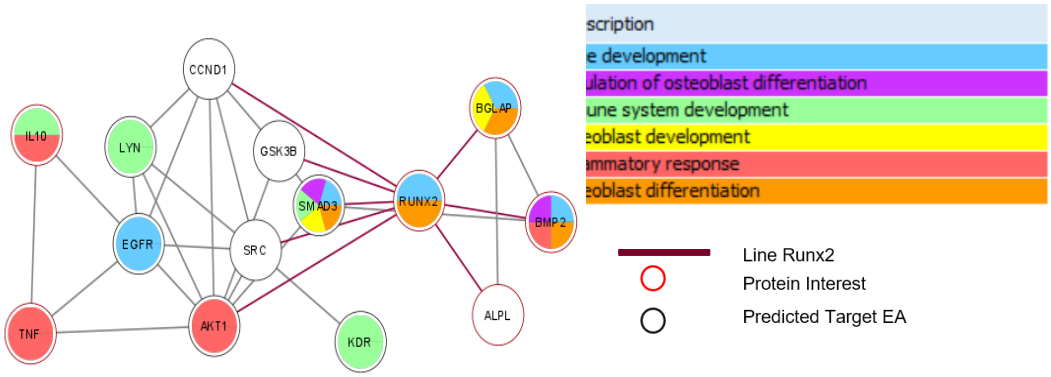


Figure 2: Protein Target Protein Interest Interaction.

Table 2: Gene Ontology BinGO Cytoscape.

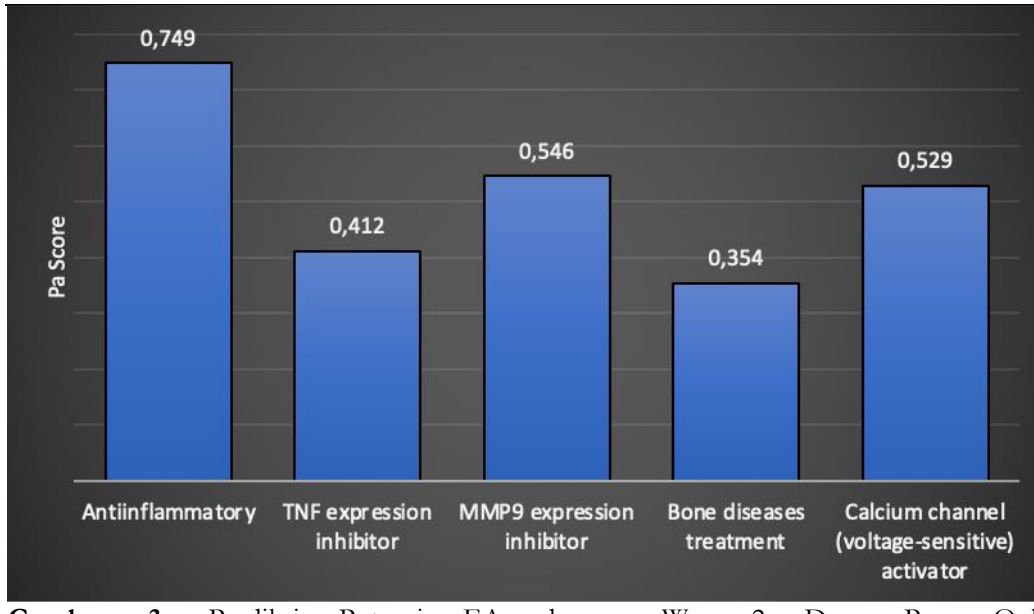
GO-ID	p-value	Corr p-value	Description	Genes in the test set
60348	1.15E−05	1.04E−04	Bone development	BMP2|SMAD3|BGLAP|EGFR|RUNX2
45667	5.45E−03	1.44E−02	Regulation of osteoblast differentiation	BMP2|SMAD3
2520	3.53E−03	1.06E−02	Immune system development	IL10|LYN|SMAD3|KDR
1649	1.31E−06	1.75E−05	Osteoblast differentiation	BMP2|SMAD3|BGLAP|RUNX2
2076	2.80E−04	1.53E−03	Osteoblast development	SMAD3|BGLAP
6954	5.71E−03	1.49E−02	Inflammatory response	IL10|BMP2|AKT1|TNF

Bold: Predicted target EA

Table 3: Network Analysis.

Name	Betweenness centrality	Closeness centrality	Degree
AKT1	0.332575685	0.653061224	15

SRC	0.21936804	0.62745098	15
RUNX2	0.159562867	0.542372881	9
EGFR	0.151720583	0.571428571	13
CCND1	0.102405027	0.603773585	12
AURKA	0.069485756	0.432432432	4
CCNA2	0.055048759	0.470588235	7
CDK4	0.042785938	0.533333333	10
GSK3B	0.033390428	0.492307692	7
CDKN1B	0.03020426	0.533333333	9
SMAD3	0.02887923	0.470588235	7
LYN	0.027079006	0.533333333	9



Gambar 3: Prediksi Potensi EA dengan Way 2 Drug Pass Online (http://way2drug.com/passonline/).