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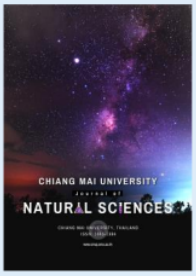

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Research article

The Role of Ellagic Acid on Inflammatory Protein NFKB1 and HSP70 with STITCH Prediction

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Abstract The objective of this study was to determine the intervention mechanism of Ellagic Acid (ES) against inflammatory proteins NFKB1 and HSP70. Target Protein Analysis using SeaTarget (http://sea.bkslab.org). The compound is then analyzed for its target protein in the human body using the STITCH database (http://stitch.embl.de/). Then analyzed the interactions between proteins using the STRINGdb database (https://string-db.org/cgi/input.pl). In the analysis results with SEA, Ellagic Acid can target HSP70. Based on the results of interactions using STITCH, ellagic acid can target the Alpha Protein Kinase Catalytic Sub-unit (PRKCA). Protein Kinase Catalytic Sub-unit Alpha facilitates ellagic acid interactions with Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1) and Heat Shock 60kDa Protein 1 (HSPD1). Where NFKB1 is a pleiotropic transcription factor in various processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis, and apoptosis. Ellagic acid can help reduce inflammation. It is predicted that the active compound in ellagic acid acts as an anti-inflammatory. The NFKB1 and HSP70 pathways play a role in inflammation and have activity for inflammation regulation.

Keywords: Ellagic acid, HSP70, Human & Health; Inflammation, NFKB1, STITCH

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INTRODUCTION

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation (Medzhitov, 2010), and acts by eliminating adverse stimuli and initiating the healing process (Nielsen, 2006). Therefore, inflammation is an important defense mechanism for health (Nathan and Ding, 2010). Usually, during acute inflammation, cellular and molecular events and interactions efficiently minimize impending injury or infection (Chen et al., 2017).

One of the biological molecules found in pomegranate is ellagic acid, and this molecule has anti-inflammatory potential. Ellagic acid is detected not only in pomegranate but also in a wide variety of fruits and nuts. Ellagic acid showed the anti-inflammatory activity, free radical scavenging (Mari Kannan and Darlin Quine, 2011), anti-oxidative stress (Kannan and Quine, 2011) and anti-apoptotic (Mari Kannan and Darlin Quine, 2012). Other potential, it also showed that the ellagic acid, is considered to have osteo-inductive capability and have anti-inflammatory properties (Usta et al., 2013). The anti-inflammatory properties works by inhibit the pro-inflammatory cytokines that affected on inhibition of bone formation or osteogenesis (Al-obaidi et al., 2014). The osteogenesis stimulation by ellagic acid involve the increased the osteocalcin (OCN), osteoprotegerin (OPG) and stimulate the osteoblast during bone regeneration (Wardhana et al., 2020).

Nuclear factor kappa B (NFKB) consists of a family of transcription factors that play important roles in inflammation, immunity, cell proliferation, differentiation, and survival. The transcription factor family NFKB in mammals consists of five proteins, p65 (RelA), RelB, c-Rel, p105 / p50 (NFKB1), and p100 / 52 (NFKB2). The gene encoding human nfkb1 has 24 exons covering 156 kb (Oeckinghaus and Ghosh, 2009). The nfkb1 gene encodes a protein consisting of 968 amino acids with a molecular weight of about 105 kDa, which is considered a precursor to the p50 subunit of the NFKB complex. In the N-terminal NFKB1 region, there is a Rel homology domain (RHD) consisting of ~ 300 amino acids which are responsible for DNA binding, dimerization with other members of the Rel family, and interactions with the IkB protein (Chen, 2011).

Heat shock protein 70 (Hsp70) is a stress-inducible 72-kDa intracellular protein with functions in the prevention of protein aggregation, facilitation of protein refolding, and chaperoning of proteins. Its expression is up-regulated in various states of physiological and environmental stresses, such as infections, inflammation, cellular injury, or heat stress (Pockley, Muthana and Calderwood, 2008). Extracellular Hsp70 (eHsp70) can act as a damage-associated molecular pattern (DAMP) via Toll-like receptors TLR2 and TLR4, and stimulate immune and inflammatory responses leading to sterile inflammation and propagation of already existing inflammation (Hulina et al., 2017). Hsp70 has been shown to activate the host innate immune and inflammatory responses by engaging TLR2 and TLR4 receptors, via signaling pathways dependent on myeloid differentiation factor 88 (MyD88), which in turn activate nuclear factor kappa B (NFKB) and mitogen-activated protein kinases (MAPK), causing a release of pro-inflammatory cytokines (Piccinini and Midwood, 2010).

Knowledge of the interactions between proteins and small molecules is essential for an understanding of molecular and cellular functions. However, information about such interactions is widespread in various databases and literature. To facilitate access to this data, the Search Tool for Interacting Chemicals (STITCH) integrates information on interactions of metabolic pathways, crystal structure, experimental binding and drug-target relationships. (Kuhn et al., 2008). The chemicals are the basis of STITCH and are currently imported from PubChem. All stereoisomers and form charges of a compound are combined into a single record via the canonical SMILES string (Eriksson et al., 1995).

In pharmacology and biochemistry, the interplay of chemicals and proteins has been extensively studied, but much of the existing data on chemicals is either hidden in a large number of scattered literature or is locked in commercial databases such as the Chemical Abstracts Service Registry. Recently, however, several projects have begun to provide easy public access to chemical information. Resources such as PubChem (Wheeler et al., 2007), ChEBI (Brooksbank, Cameron and Thornton, 2005) and ChemDB (Chen et al., 2007) provides a continuously growing inventory of chemical spaces that can be used as a basis for the integration of knowledge about the chemicals themselves, their biological

interactions and, their typical phenotypic effects. (Kuhn et al., 2008). STITCH is a database and tool for analyzing interactions between bioactive compounds and their target proteins (Kuhn et al., 2008). Therefore, this study aims to determine the content of ellagic acid and its target predictions and interactions in reducing inflammation.

MATERIALS AND METHODS

Laboratorium

The research was conducted at the Inbio Indonesia Biocomputing Laboratory Malang in January-February 2020 (IB325 Project) http://inbio-indonesia.org/.

Target protein analysis using Hitpick

The SEA Target Web Server (http://sea.bksl.org) is used to predict which proteins can be targeted by Ellagic Acid (Pubchem ID 5281855). Techniques for linking receptors to each other quantitatively based on the chemical similarities between ligands. In this method, called the Similarity Ensemble Approach (SEA), two sets of ligands are often judged to be similar even though no single identical ligand is shared between them.

Ellagic acid-protein interaction analysis

To predict the interaction between ellagic acid and protein NFKB1 (ID NCBI 4790) and HSP70 (ID NCBI 9606) in humans, the STITCH web server (http://stitch.embl.de/) is used. This program can predict protein interactions and can be displayed using a specific color visualization with a certain score. The tools used in the analysis of target proteins and their interactions are a computer, the STITCH database (http://stitch.embl.de/), and the STRINGdb database (https://stringdb.org/cgi/input.pl). Molecular docking uses autodock4 algorithm software using PyRx-0.8. The bonds between ligands and receptors were visualized using Discovery studio software.

Analysis of target proteins and their interactions

The compounds in EA are analyzed for their target proteins in the human body using the STITCH database (http://stitch.embl.de/). The way to use STITCH is by entering the STITCH website address in the browser then selecting the 'search' menu. Then select the "multiple names" menu to input more than one compound at the same time. In the item name column, enter the names of the compounds that you want to know the target. Homo sapiens is selected in the organism column. Then click 'search' and a list of compounds recognized by the system will appear, then select 'continue' so that interactions will appear between chemical compounds and their target proteins. The interaction between the active compound and the protein was then analyzed for affinity using the molecular docking method. The proteins that have been predicted to be targets of the active EA compound are then analyzed for interactions between proteins using the STRINGdb database (https://string-db.org/cgi/input.pl). The way to use STRINGdb is by entering the STRINGdb website address in the browser then selecting the 'search' menu, then selecting the "multiple protein" menu, and entering the protein names in the "List of Protein" column. Homo sapiens is selected in the organism column. Then click 'search' and a list of proteins recognized by the system will appear, then select 'continue' so that interactions between proteins appear.

RESULTS

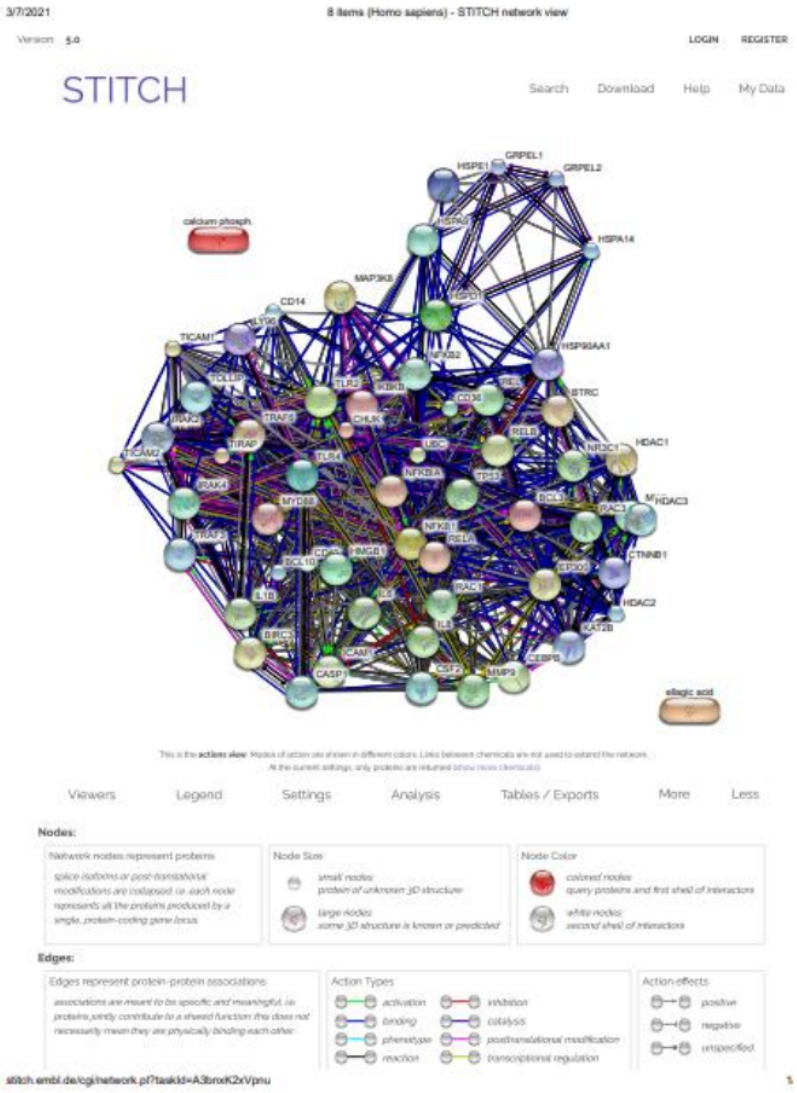


Figure 1. STITCH Network View.
Project results from http://stitch.embl.de/cgi/network.pl?taskId=A3bnxK2xVpnu

Table 1. STITCH with this chemical (Input).

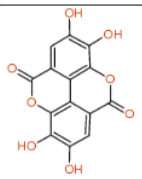
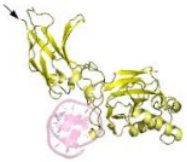
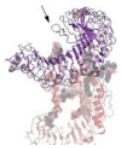
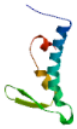
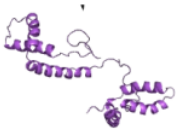
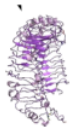
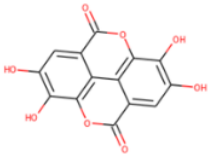
No	Compound	Structure	Information
1	Ellagic Acid		Ellagic acid is a natural phenol antioxidant found in numerous fruits and vegetables. The antiproliferative and antioxidant properties of ellagic acid have prompted research into its potential health benefits.
2	NFKB1		Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; NF-kappa-B is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events.
3	TLR2		Toll-like receptor 2; Acts via MYD88 and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response.
4	HSPD1		Heat shock 60kDa protein 1 (chaperonin); Implicated in mitochondrial protein import, and macromolecular assembly. May facilitate the correct folding of imported proteins.
5	HMGB1		High mobility group box 1; DNA binding proteins that associate with chromatin and have the ability to bind DNA .Binds preferentially single-stranded DNA.
6	TLR4		Toll-like receptor 4
7	HSPA14		Heat shock 70kDa protein 14; Component of the ribosome-associated complex (RAC)

Table 2. Prediction of ellagic acid with SEA.

Query	Target Key	Target Name	Description	*P*-Value	MaxTC
	HS71A_HUMAN	HSPA1A	Heat shock 70kDa protein 1A	2.22×10^{-16}	1
	TLR1_MOUSE	Tlr1	Toll-like receptor 1	3.31×10^{-10}	0.31
	TLR2_MOUSE	Tlr2	Toll-like receptor 2	3.31×10^{-10}	0.31
	PCSK7_HUMAN	PCSK7	Proprotein convertase subtilisin/kexin type 7	3.41×10^{-11}	0.28
	ESR1_HUMAN	ESR1	Estrogen receptor	1.08×10^{-11}	0.44

Interactions of Ellagic acid and target protein

In the analysis, SEA Target Ellagic Acid can target HSP70 (HSPA14) with a MaxTC score of 1. MaxTC: Maximum Tanimoto is the similarity between the compound from the reference target and the compound from the query (ellagic acid) where the highest score is 1 and the lowest is 0.28. Heat shock 70 kDa protein 14 has an activity for the regulation

of inflammation intracellularly and extracellularly (Figure 2). Intracellularly it acts as an anti-inflammatory while extracellularly plays a role in the immune response (Kim et al., 2018).

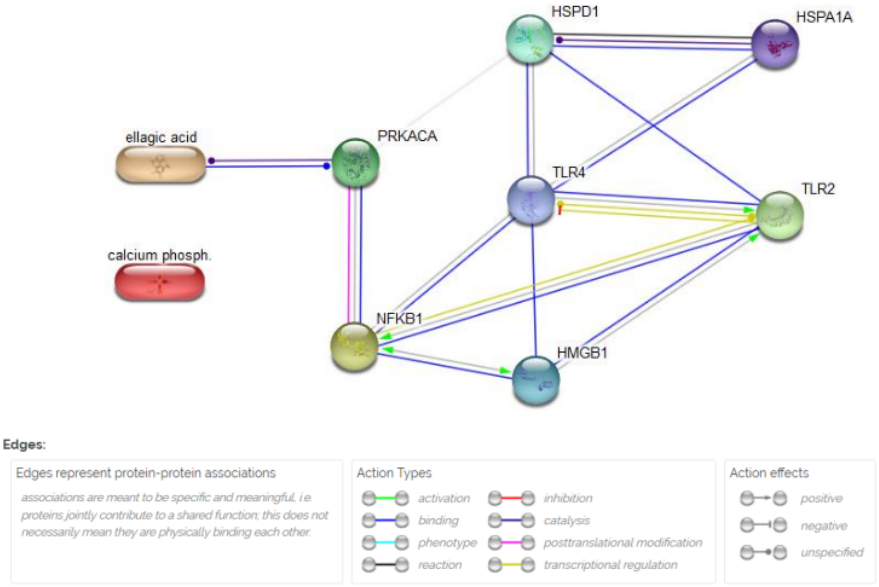


Figure 2. Interaction results using STITCH DB.

Protein kinase A (PKA) is a holoenzyme consisting of a dimer regulatory subunit and two catalytic subunits that regulate cellular functions including immune cell activity. There are two main catalytic subunits, namely PRKACA and PRKACB (Turnham and Scott, 2016). Based on the results of the interaction using STITCH, ellagic acid can target the alpha subunit catalytic protein kinase (PRKCA). PRKCA is reported to have contributed to the phosphorylation of NFKB1, CLDN3, PSMC5 / RPT6, PJA2, RYR2, RORA, and VASP (Moen, Sener and Volchenkov, 2017).

PRKACA facilitates the interaction of ellagic acid with NFKB1 and HSPD1. Where NFKB1 is a pleiotropic transcription factor in various processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis, and apoptosis. Meanwhile, HSPD1 is used to crosstalk between stress and inflammatory responses (Tiss, 2018).

DISCUSSION

In the analysis results with SEAT Target Ellagic Acid, it can target HSP70 that HSP70 inhibition plays an important role in the regulation of inflammation intracellularly and extracellularly (Table 2). According to research (Raghuwanshi et al., 2019), the anti-psoriatic activity of the immunomodulatory bioactive compounds contained in the ethanol extract of Woodfordia fruticosa (Wffe) flowers using a combination of bioinformatics together with an ethnopharmacological approach has been explored in this study. Myricetin (-8.024), Quercetin (-7.368), and Ellagic acid (-7.311) are the top three compounds with minimum energy levels and high therapeutic value / ADMET compared to the currently marketed anti-psoriatic drug Tretinoin (-7,195). Psoriasis is a chronic, immune-mediated, non-contagious inflammatory disease that arises due to the hyperproliferation of keratinocytes in the skin (Griffi and Barker, 2007).

The Hsp70 response is now recognized as contributing to brain damage and represents a great opportunity for further investigation and exploration of potential treatments (Kim et al., 2018). As an anti-inflammatory molecule, Hsp70 has also been

shown to interact with proinflammatory factors such as NFKB, matrix metalloproteinases (MMPs), and ROS, leading to an anti-inflammatory state. Intracellular overexpression of Hsp70 or its intracellular induction by heat stress has been shown to reduce inflammatory cell production of nitric oxide and induced nitric oxide synthase (iNOS) expression while decreasing NFKB activation (Feinstein et al., 1996). Extracellular hsp70 is also known to participate in innate immune responses by interacting with macrophages, microglia, and dendritic cells via receptors such as Tolls (TLRs). TLR binding leads to NFKB activation with subsequent upregulation of pro-inflammatory cytokines and iNOS (Srivastava, 2002).

Based on the results of the interaction using STITCH, ellagic acid can target the alpha subunit catalytic protein kinase (PRKCA). PRKACA facilitates the interaction of ellagic acid with NFKB1 and HSPD1. NFKB is a homo- or heterodimeric complex formed by proteins containing domains such as Rel RELA / p65, RELB, NFKB1 / p105, NFKB1 / p50, REL, and NFKB2 / p52, and heterodimeric complexes p65-p50 (Tiss, 2018). The inhibition of NFKB1 will inhibit the production of pro-inflammatory cytokine (Puar et al., 2018; Surboyo et al., 2019, 2021).

Research (In, 1997) create a table of stimuli that activate NFKB and proteins regulated by these transcription factors. Reviewing the role of NFKB in chronic inflammatory disease. In research (Yamamoto and Gaynor, 2001) reviewed the therapeutic potential of inhibition of the NFKB pathway in the treatment of inflammation and cancer. In research (Journal et al., 2015) the authors concluded that insulin at physiologically relevant concentrations exerted an inhibitory effect on the cardinal pro-inflammatory transcription factor NFKB and the pro-inflammatory chemokine MCP1, suggesting an anti-inflammatory effect and an antiatherogenic potential of insulin.

The results of this study indicate that ellagic acid, as natural product able to inhibit the inflammatory process through Hsp70 and NFKB in silico study. Inhibition of this inflammatory process will lead to an increase in the process of tissue regeneration. In the previous study, ellagic acid is able to increase the number of osteoblast cells, the expression of OCN and OPG in the bone regeneration process (Wardhana et al., 2020). Ellagic acid has also shown that this material is not toxic to normal cells of the body, such as fibroblast (Nirwana et al., 2021). This also confirms that this compound is safe to use and has promising pharmacological effects. The future research needs to conduct in the level of *in-vivo* and human, to confirmed the mechanism of ellagic acid inhibit the inflammatory responses.

CONCLUSION

Ellagic acid can help reduce inflammation. It is predicted that the active compound in ellagic acid acts as an anti-inflammatory by inhibit the NFKB1 and HSP70 pathways since it play a role in inflammation and have activity for inflammation regulation. A better understanding of inflammatory response pathways and molecular mechanisms no doubt contributes to improved prevention and treatment of inflammatory diseases. However, further research (*in-vitro* and *in-vivo*) needs to be done to prove the mechanism.

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AUTHOR CONTRIBUTIONS

Intan Nirwana helped to conduct experiments, analyze and visualize data and wrote scripts. Michael Josep Kridanto Kamadjaja helped conduct experiments and look for references and wrote the script. Debby Saputera designed and carried out all the

experiments and wrote the script. Meircurius Dwi Condro Surboyo wrote the script. All authors have read and approved of the final manuscript.

CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

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