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Revealing of Antiinflamatory Agent from Zingiber officinale var. Roscoe via IKK-B Inhibitor Mechanism through In Silico Simulation

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ABSTRACT
Inflammation is a response to the immune system from attack by infectious agents. Its condition triggered by a phase of dilatation in the blood flow and increased membrane permeability in the area of infection. The results of in vitro research showed that compounds from *Zingiber officinale* var. Roscoe extract could trigger a decrease in NF-κB protein activity and pro-inflammatory cytokine production. NF-κB activity is influenced by the IKK-B enzyme for the phosphorylation of IκBα and NF-κB complexes, phosphorylation triggers IκBα dissociation and releases NF-κB to trigger proinflammatory gene expression. Extract from the rhizome of *Zingiber officinale* var. Roscoe can prevent and treat inflammation in the human body, this treatment is classified as an alternative according to previous research. This study aims to identify the anti-inflammatory potential of *Zingiber officinale* var Roscoe through the mechanism of inhibition of IKKB enzyme activity through bioinformatics simulation. *Zingiber officinale* var. Roscoe is predicted to act as an anti-inflammatory agent through 6-shogaol with a mechanism of IKK-B phosphorylation activity inhibition at Ser177 and Ser181 residues, 6-shogaol is predicted to act as a drug-like molecule, the anti-inflammatory potential of *Zingiber officinale* var. Roscoe must undergo further analysis to provide strong scientific evidence.

Keywords: Anti-inflammatory, Bioinformatics, IKK-B, NF-κB, *Zingiber officinale*

INTRODUCTION

Human Inflammation is a response to the immune system from attack by infectious agents such as bacteria, fungi, protozoa, viruses and triggers the activation of immunocompetent cell responses such as the release of antibodies, activation of immune cells, and phagocytosis[1]. Swelling and

† Footnotes relating to the title and/or authors should appear here.

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redness of the skin are signs of an inflammatory process, in such cases triggered by a phase of dilatation in the blood flow and increased membrane permeability in the area of infection[2]. Inflammation is caused by cancer, tumors, and infection with microorganisms[3,4]. The results of in vitro analysis showed that compounds from *Zingiber officinale* var Roscoe extract could trigger a decrease in NF-κB protein activity and pro-inflammatory cytokine production.

NF-κB is a complex protein molecule for the regulation of DNA transcription, cell survival, and cytokine production[5,6]. NF-κB is found in almost all multicellular organisms because it has an important role in cellular responses through the secretion of specific cytokines[7]. Abnormalities of NF-κB regulation are often associated with cancer, autoimmune, inflammatory, and infectious microorganisms[8]. NF-κB activity is influenced by the IKK-B enzyme for the phosphorylation of IκBα and NF-κB complexes, phosphorylation triggers IκBα dissociation and releases NF-κB to trigger proinflammatory gene expression[9].

The use of ginger has become a tradition in various parts of the world, besides being used for cooking spices, ginger can be used as a mixture in herbal ingredients. Extract from the rhizome of *Zingiber officinale* var[10]. Roscoe can prevent and treat inflammation in the human body, this treatment is classified as an alternative according to previous research[11]. This study aims to identify the anti-inflammatory potential of *Zingiber officinale* var. Roscoe through the mechanism of inhibition of IKKB enzyme activity through bioinformatics simulation. The solution proposed through this research is useful to help the public in knowing alternative anti-inflammatory drugs that are easily available in the future.

METHODS

Ligand-protein Retrieval

The chemical compounds of *Zingiber officinale* var. Roscoe consist of 6-shogaol, 4-gingerol, 10-gingerol, 6-gingerol, and 12-gingediol[10,11]. 3D structures with Canonical *.sdf*, ID, and SMILE formats on candidate compounds were obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov) IKK-B was used as the target in this study, the 3D structure of the protein with *.pdb* format was obtained from the Protein Databank database (https://www.rcsb.org/).

Molecular Docking Simulation

Docking simulation aims to determine the level of activity of the ligand binding to the target. Molecular docking plays a role in knowing the interaction pattern and screening potential of compounds drug candidate[12]. The compound from *Zingiber officinale* var. Roscoe extract acts as a ligand and the target protein is IKK-B. The docking simulation was carried out using PyRx 0.8.8 software. ver. The 3D structure and molecular interaction of the ligand-protein complex is shown with the structure of cartoons, transparent surfaces, and sticks using PyMol 2.5 ver software[13].

Druglikeness and Bioactivity Prediction

Prediction of drug-like molecules on candidate ligands of anti-inflammatory agents from *Zingiber officinale* var. Roscoe was carried out in this study via the server http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp by following at least one of the five Lipinski rules[14]. The parameters used in the Lipinski Rule of Five are molecular mass, LogP, hydrogen acceptors, donors, and molar refractivity[15]. Prediction of bioactivity as anti-inflammatory was performed via PASSOnline (http://way2drug.com/PassOnline/)[16,17].

RESULT AND DISCUSSION

Zingiber officinale var Roscoe. compounds binding affinity on the IKK-B

The active compound of *Zingiber officinale* var. Roscoe was obtained from Pubchem and then carried out a docking simulation to determine the ability of the chemical bond activity produced in the IKKB domain[18]. The IKKB domain that is the target for binding is the phosphate-binding domain, it aims to inhibit the release of NF-κB from the IκBα complex[19]. This simulation aims to identify the chemical bonding activity of ligands in protein-specific domains. The docking results show that 6-

shogaol compound can bind with more negative binding-affinity and is predicted to trigger inhibition of IKK-B (Table 1). The inhibitory activity of 6-shogaol on NF-κB has been tested by an in vitro approach by previous studies, 6-shogaol is predicted to inhibit NF-κB activation through IKK-B. The 3D structure of protein-ligand with more negative binding affinity was visualized with transparent surfaces and cartoons (Figure 1).

Table 1. The binding affinity from the docking simulation

Compound	PubChem ID	Target	RCSB ID	*Binding affinity* (kcal/mol)
6-shogaol	5281794	IKKB	4KIK	-7,2
12-gingediol	86196540	IKKB	4KIK	-6,2
10-gingerol	86196540	IKKB	4KIK	-6,0
6-gingerol	442793	IKKB	4KIK	-6,7
4-gingerol	46901319	IKKB	4KIK	-6,8

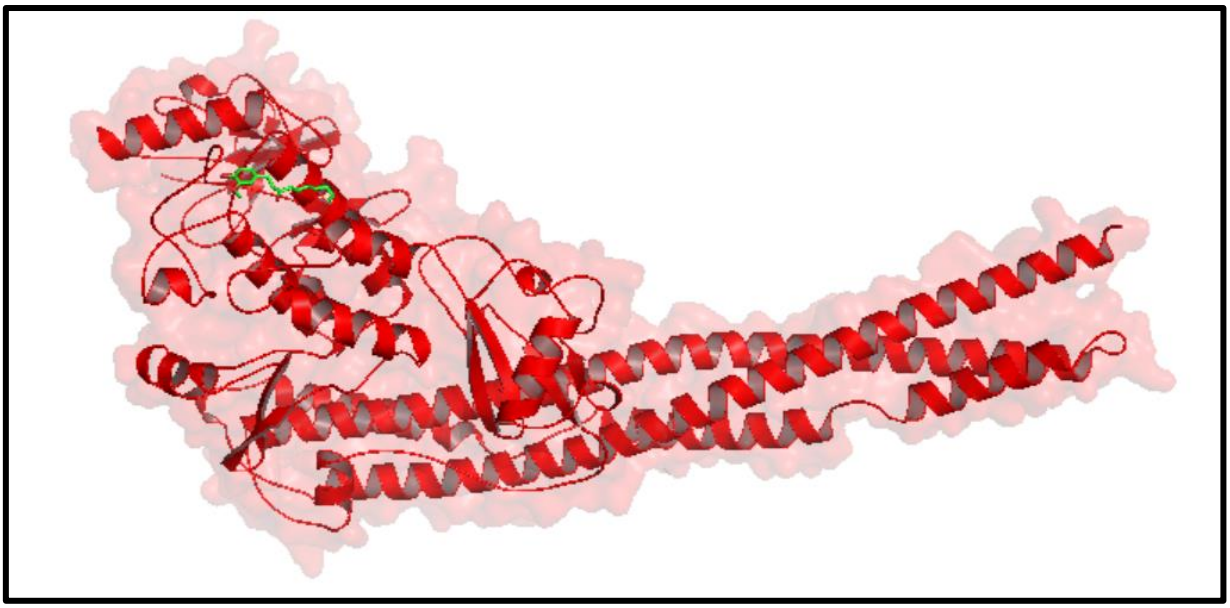

Figure 1. 3D structure of molecular docking 6-shogaol_NFKB through PyMol software visualization. The cartoons structure in red is IKK-B and 6-shogaol is a stick in green.

Molecular interaction of ligan-protein and strategic binding positions

The position of the phosphate binding domain was displayed in the PyMol software, and the IKK-B protein (Figure 2) in the cartoons-transparent surface structure was selected for staining based on the protein chain. The position of the 6-shogaol binding domain on the target protein was identified through the PyMol software. The analysis of the bond position plays an important role in determining the accuracy of the interaction probability in the target protein domain to inhibit phosphate binding in IKKB. The residues in the IKK-B domain that are responsible for the phosphate binding domain are Ser-177 and Ser-181[20,21]. Inhibition of interaction between IKK-B and phosphate aims to inhibit IKK-B activation and regulatory activity of NFκB for transcription of pro-inflammatory proteins. 6-shogaol may act as an anti-inflammatory agent by inhibiting IKK-B activity

by interacting with strategic domains such as Ser-177 and Ser181.

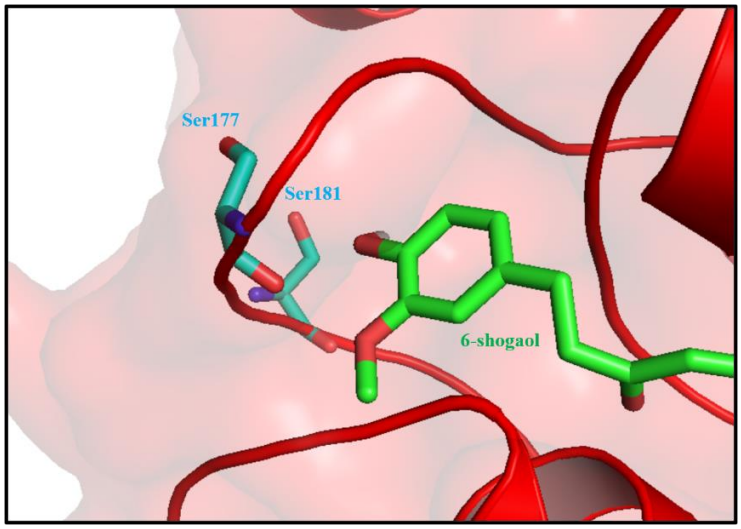

Figure 2. Molecular visualization of phosphate binding domain with PyMol. The phosphate binding domain is shown by the blue sticks structure.

The potency of drug-like molecule

Prediction of 6-shogaol bioactivity was carried out through PASS Online to validate its potential as an anti-inflammatory in general[22]. The prediction process is done by entering SMILE Canonical on the web server. Prediction results are categorized as proven to be potential in computational and wet labs if they have an activation probability value[23]. Prediction with probability (Pa) > 0.7 is accuracy > 80%, prediction result shows 6-shogaol has Pa>0.7 as anti-inflammatory through inhibition of IKK-B phosphorylation. Lipinski's rule can be used to identify drug-like molecules in candidate compounds[24]. This method can predict the properties of drug candidate compounds with parameters of molecular weight, LOGP, hydrogen bond acceptor, donor, and molar refractivity[25]. Lipinski's analysis results show that 6-shogaol compounds with more negative binding affinity values are predicted to have potential as drugs because they meet Lipinski's five rules, so 6-shogaol from Zingiber officinale var. Roscoe can act as a drug like molecule for anti-inflammatory agents.

CONCLUSION

Zingiber officinale var. Roscoe is predicted to act as an anti-inflammatory agent through 6-shogaol with a mechanism of IKK-B phosphorylation activity inhibition at Ser177 and Ser181 residues, 6-shogaol is predicted to act as a drug-like molecule, the anti-inflammatory potential of *Zingiber officinale* var. Roscoe must undergo further analysis to provide strong scientific evidence.

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