Fractionated Ethanol Extract of Red Ginger (Zingiber officinale var. rubrum) as Anti-Inflammatory Drug: An In-Silico Study

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Abstract

The prevalence of gingivitis and periodontitis in Indonesia is still high at 74% and 74.1%, respectively. The inflammatory process cause pain that affect the children's quality of life. Ibuprofen often used has side effects on kidney function, blood pressure, and gastrointestinal. The in silico study is an experiment using a computational method to determine the potential and mechanism of fractionated ethanol extract of red ginger (Zingiber officinale var. rubrum) as an anti-inflammatory drug candidate.

PASS Online to analyze the anti-inflammatory, PyRx 0.8 to run and analyze molecular docking, STRING to analyze pathway mechanisms involved in the anti-inflammatory process. There were 3 compounds having a Pa value more than 0.7, i.e. bisabolene, curcumene, and shogaol. Red ginger compounds having more negative binding affinity score than ibuprofen were gamma-bisabolene, phenetanamine, curcumene, sesquiphellandrene, zingiberene, shogaol, and 6-gingerol.

Fractionated ethanol extract of red ginger is predicted to have potential as an anti-inflammatory drug.

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Introduction

Oral inflammatory diseases often in children are gingivitis, periodontitis, stomatitis, angular cheilitis, and cellulitis. In Indonesia, according to RISKESDAS 2018 data, the gingivitis prevalence reached 74% while the periodontitis prevalence is 74.1%[1]. The inflammatory process has a protective role by activating the immune system, both adaptive and innate immune responses. This adaptive immune response cause pain[2]. The pain caused affect the children's quality of life because the oral cavity has many functions related to daily life, such as food intake, speech, social relations, and facial appearance. Decreased food intake due to pain in the oral cavity cause growth disorders and may lead to the poor nutritional status of children. Pain might also have a negative effect on the ability to engage in social relationships and education[3].

Currently, an anti-inflammatory drug often used and prescribed in inflammation therapy is ibuprofen. Ibuprofen is the first member of a Non-Steroidal Anti-Inflammatory Drug (NSAID) propionic acid derivative that works by inhibiting the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2)[4]. Based on research, ibuprofen given to children has fewer side effects than other NSAIDs. However, this drug still affects kidney function and blood pressure. On the other hand, several studies have concluded that the use of ibuprofen has a toxic effect on the gastrointestinal tract[4].

In recent decades, public confidence in the benefits of plants in medicine has increased. Currently, herbal medicines are not only used by people in developing countries but also in a great demand in developed countries because of their usefulness, safety, and lower side effects[5,6]. One of the plants most often used as herbal medicine is red ginger (Zingiber officinale var. rubrum). The chemical content of red ginger varies, depending on the cultivation location and the form of ginger (fresh, dried, or processed)[5]. However, the red ginger rhizome usually contains

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compounds with therapeutic activity, non-volatile and volatile compounds. The non-volatile compound consists of the phenol oleoresin gives it a spicy flavor. Meanwhile, volatile compounds called essential oils that consist of sesquiterpene and monoterpene derivatives play a role in aroma[7,8].

Various studies have proven that red ginger has the potential as an anti-inflammatory. In these studies, the content of compounds in red ginger, phenol oleoresin and essential oil, is still mixed. One of them is a study conducted by Hidayati et al., with the results that the administration of red ginger extract affected decreasing the number of macrophage cells in traumatic ulcers of the oral mucosa as known that macrophage cells play a role in the inflammatory process[9]. The study used 96% ethanol extract of red ginger where the phenolic oleoresin content still mixed with the essential oil content.

The potential of fractionated ethanol extract of red ginger (Zingiber officinale var. rubrum) has been proven as antibiofilm for S. mutans[10]. The fractionation method was chosen to obtain the active compounds in red ginger essential-oils-free to determine the anti-inflammatory potential because there have been many studies on the content of red ginger compounds that are still mixed with essential oils proven to have anti-inflammatory potential. In this study, the same compound will be tested for its anti-inflammatory potential via in silico study. This study also used the anti-inflammatory drug ibuprofen as a comparison of the anti-inflammatory activity of the fractionated ethanol extract of red ginger (Zingiber officinale var. rubrum) to determine its potential as a new drug candidate.

Materials and methods

The tools used are software such as PyRx 0.8, Discovery Studio, PyMol and webserver PubChem, PASS Online, Protein Data Bank (PDB), STRING, and NetInfer. The materials used are target protein (COX-2) and ligands i.e. ibuprofen and active compounds of red ginger fractionated ethanol extract obtained from a previous study by Nuraini, 2021 using Gas Chromatography-Mass Spectroscopy (GC-MS) method. The research obtained 22 compounds i.e. Cyclohexane, Cyclotetracosane, Gamma-bisabolene, Gamma-sitosterol, Methylisonicotinic (Isonicotinic acid), Phenethanamine, Propionate (Ethyl 3-(4-hydroxy-3-methoxyphenyl) propionate), Bisabolene (Beta-bisabolene), Curcumene, Cyclooctacosane, Methoxyphenol (4-ethyl-2-methoxy phenol), Methylbenzyl (3-amino -4-methyl benzyl alcohol), Methylmandelate, Methylnespiro (3R, 4S-2 ethyl), Sesquiphellandrene, Tolban, Zingiberene, Zingiberanol, Nonacosene, Tetradecylcyclohexane, Shogaol (8-Shogaol), and 6-gingerol.

Preparation of ligands and protein

For each active compound of fractionated ethanol extract in red ginger and ibuprofen, the Simplified Molecular Input Line Entry System (SMILES) structure was searched in the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The COX-2 3D structure coded 1CX2 was downloaded from the PDB database (www.rcsb.org).

Biological function analysis

The SMILES structure of each compound was analyzed for its biological function as an anti-inflammatory using the PASS Online webserver (http://way2drug.com/PassOnline/). The analysis result was the value of probability to be active (Pa).

Molecular docking analysis

Molecular docking between the ligands and protein after preparation, was performed using PyRx 0.8 software to obtain the binding affinity value. Discovery Studio software was used to identify the position and type of chemical bond interactions, formed in the protein-ligand complex. The docked 3D structures were visualized using PyMol software with coloring and structural selection.

Pathway mechanisms analysis

All 22 compounds in red ginger's fractionated ethanol extract were analyzed to predict several candidate protein targets from each compound using SMILE notation data on the NetInfer webserver. The analysis result is a collection of the top 5 target proteins based on their probability values. Furthermore, candidate protein targets from 22 compounds were further analyzed to determine several pathway mechanisms involved in the anti-inflammatory process using the STRING webserver. Then the validity was analyzed based on the false discovery rate (FDR) score.

Results

Biological function analysis

The biological function analysis of each active compound of red ginger fractionated ethanol extract showed that each compound had various Pa values related to its anti-inflammatory function. However, there were 3 compounds having a Pa value more than 0.7, i.e. bisabolene, curcumene, and shogaol and there were 2 compounds, methylnespiro and tolban, having no biological function related to anti-inflammatory (Figure 1). Comparison between Pa and Pi values showing the Pa value greater than the Pi value means that all compounds contained in red ginger were active compounds.

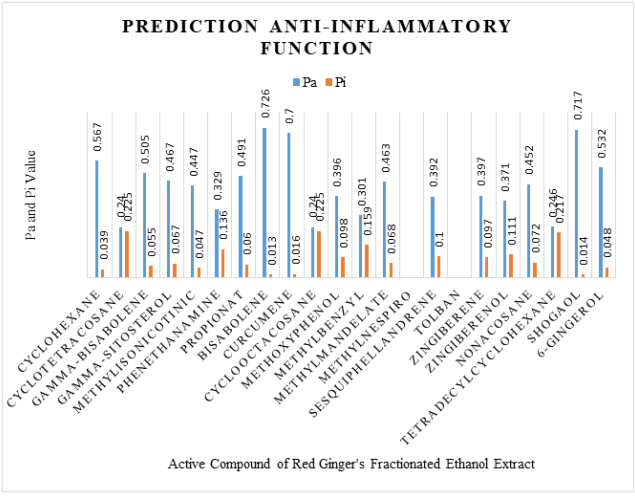

Figure 1. Graph of Pa and Pi values of each anti-inflammatory related compound.

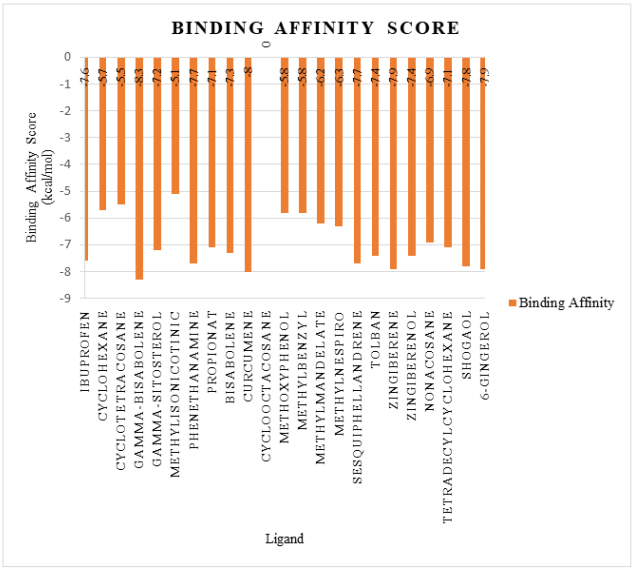

Figure 2. Binding affinity score in ibuprofen as a comparison and active compunds of fractionated ethanol extract in red ginger.

Molecular docking analysis

The molecular docking results proved the binding affinity of 7 active compunds of fractionated ethanol extract in red ginger have more negative scores than ibuprofen i.e. gamma-bisabolene, phenetanamine, curcumene, sesquiphellandrene, zingiberene, shogaol, and 6-gingerol (Figure 2). The 3D structure of molecular docking visualized with PyMol software can be seen in (Figure 3 and Figure 4).

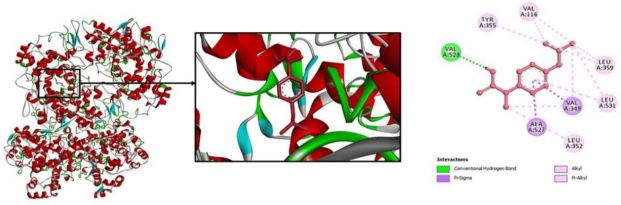

Figure 3. Interaction between 1CX2 and ibuprofen as comparison.

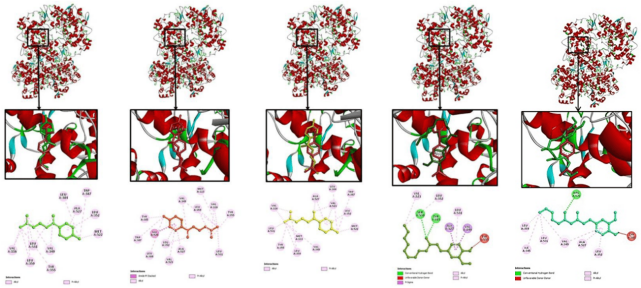

Figure 4. Interaction between 1CX2 and 5 compounds having the highest binding affinity. Ibuprofen (pink) shown to proves 5 compounds have the same binding site as ibuprofen.

Pathway mechanisms analysis

The analysis results are collection of the top 5 from each target proteins candidate list. Prediction of the target protein was measured based on the probability score where the closer to 1, the higher the predicted target protein. The top 5 target proteins of each active compound of fractionated ethanol extract in red ginger, were analyzed for the mechanism pathway involved in the inflammatory process using the STRING webserver, then the validity score was analyzed based on False Discovery Rate (FDR). Results in (Table 1) proved that there were 2 pathway mechanisms related to the anti-inflammatory function, direct regulation of the inflammatory response and blood circulation regulation. The

visualized figure of these 2 mechanisms can be seen in (Figure 5).

GO term	Biological process	Protein	False discovery
GO:0050727	Regulation of inflammatory response	NR1D1, CYP19A1, ESR1, CNR1, CNR2	0.0346
GO:0008015	Blood circulation	CNR1, SLC6A4, CYP11B1, CYP11B2, HMGCR	0.0263

Table 1. Results of target protein pathway analysis

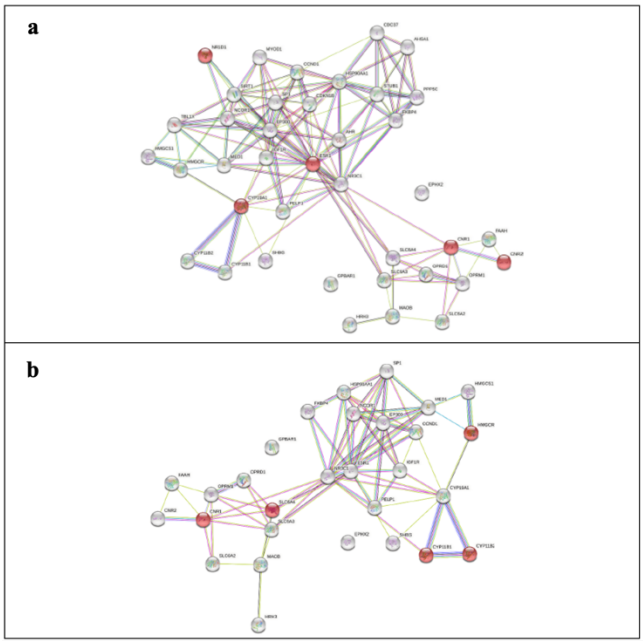

Figure 5. Visualization Overview (a) direct regulation of the inflammatory response visualized in red, (b) blood circulation visualized in red.

Discussion

This research of fractionated ethanol extract in red ginger (Zingiber officinale var. rubrum) was conducted to predict the anti-inflammatory drug potential of the active compound of fractionated ethanol extract in red ginger because existing anti-inflammatory drugs, ibuprofen, cause side effects on the kidneys, blood pressure, and gastrointestinal tract. Red ginger was chosen because this plant is found mainly in Java and is widely used as a herbal medicine for anti-inflammatory, antitumor, analgesic, antifungal, and antibacterial[6]. In addition, red ginger is often used as herbal medicine because the content of oleoresin (3%) and essential oil (2.58-2.72%) is greater than other types of ginger[11]. The content of these compounds belongs to the class of non-volatile and volatile compounds that provide a therapeutic effect on red ginger. Oleoresin is a group of non-volatile compounds that give a spicy taste. Meanwhile, essential oils are classified as volatile compounds that provide aroma[7,8]. From the 2 groups of compounds, red ginger was identified as anti-inflammatory, antibacterial, antioxidant, and analgesic effects. In addition, red ginger consumption is considered safe because it has a very low toxic effect[7].

In silico is a computational method that plays a role in drug discovery by facilitating the design and redesign of drug-like molecules having the desired bioactivity profile, predicting and validating drug discovery targets[12].The advantages of this method are the use of relatively fewer costs, the time required for drugs to reach consumers faster, and can reduce the use of experimental animals in research[13,14]. This method consists of 3 main stages. The first stage is the identification of therapeutic targets and literature search on heterogeneous small molecules to be tested with the target protein. This stage produces hits. Then in the second stage, the selected hits were tested for specificity by docking at the known binding site of the drug target. In the third stage, hits that pass the second stage of the test will be subjected to a computational ADMET profiling study and generate leads[15].

A study conducted by Hidayati et al., on the application of red ginger extract on the number of macrophage cells of traumatic oral mucosal ulcers due to chemicals in Rattus norvegicus, found that red ginger extract reduce the number of macrophage cells[9]. Similar results were also obtained from a study conducted by Andayani et al., on the number of neutrophils and macrophages after administration of red ginger to white rats with chronic periodontitis[16]. From this study, it was concluded that the administration of red ginger extract had no effect on the number of neutrophils but it caused decreasing the number of macrophages. Likewise, in a study conducted by Sadikim et al., regarding the effect of red ginger extract on the number of macrophage cells and blood vessels in clean wounds of male mice, it was found that giving red ginger extract for 3 days reduce the number of macrophage inflammatory cells but has not affected the number of blood vessels in clean male mice[11]. The anti-inflammatory effect provided by the red

ginger extract is shown through a decrease in the number of macrophage cells, considering the role of macrophages in the inflammatory process as phagocytic cells that damage pathogens causing inflammation[16].

Studies on the compounds in red ginger that have anti-inflammatory effects have been conducted by in vivo and in vitro. Mashhadi et al., stated that gingerol, shogaol, and other structurally related substances in ginger exert an anti-inflammatory effect by inhibiting the biosynthesis of prostaglandins and leukotrienes through suppression of 5-lipoxygenase or cyclooxygenase[17]. Research conducted by Hwang et al., on the effect of administration of 6-gingerol compound on inflammation-related osteoclast differentiation showed that 6-gingerol inhibited osteoclast differentiation through suppression of PGE2 synthesis[18]. As is well known, PGE2 plays an important role in the inflammatory process, so that the inhibitory action of 6-gingerol on PGE2 synthesis can lead to the conclusion that this compound has an anti-inflammatory effect. The literature study conducted by Kont & Fürst showed that several in vivo or in vitro studies of 6-shogaol proved that the compound exerts an anti-inflammatory effect by inhibiting leukocyte infiltration in inflammatory tissue and reducing inflammatory mediators produced by COX- 2 or iNOS[19].

Analysis of the biological function prediction of each active compound in the fractionated ethanol extract of red ginger is based on the probability to be active (Pa) value. Determination of the Pa value was done by comparing the structure of the active compound in the fractionated ethanol extract of red ginger with drugs that have been shown to have anti-inflammatory activity. Drugs that have been shown to have anti-inflammatory activity have a Pa value of more than 0.7 so that if the compound in the fractionated ethanol extract of red ginger has a value above 0.7, the potential for the compound as an anti-inflammatory is also higher. If the Pa value is in the range of 0.5–0.7, the compound has little potential as an anti-inflammatory because of its low similarity to anti-inflammatory drugs. On the other hand, if the Pa value of the active compound in the fractionated ethanol extract of red ginger is less than 0.5, the potential as an anti-inflammatory is also smaller and may not even show anti-inflammatory activity.

In this study, the results showed that the Pa value of each active compound in the red ginger fractionated ethanol extract varied. There were 3 compounds having a Pa value of more than 0.7, bisabolene, curcumene, and shogaol. Thus, these compounds have great potential as anti-inflammatory. Others that have a Pa value less than 0.7 also have the potential of anti-inflammatory but are lower than the these 3 compounds. However, this needs to be proven by conducting a molecular docking analysis between all active compounds in the fractionated ethanol extract of red ginger and ibuprofen as a comparison to COX-2. The molecular docking result is in the form of binding affinity value, if the value is more negative than ibuprofen, the compound is predicted to have a better anti-inflammatory effect than ibuprofen.

The results of molecular docking analysis showed that 7 compounds had more negative binding affinity values than ibuprofen, i.e. gamma-bisabolene, phenetanamine, curcumene, sesquiphellandrene, zingiberene, shogaol, and 6-gingerol. This indicates that these compounds are predicted to influence the inflammatory response of the cyclooxygenase enzyme better and have fewer side effects than ibuprofen as an NSAID drug. The results in this study are following the literature study conducted by Mashhadi et al., which states gingerol, shogaol, and other structurally related substances in ginger exert an anti-inflammatory effect by inhibiting the biosynthesis of prostaglandins and leukotrienes through suppression of 5-lipoxygenase or cyclooxygenase[17].

The results of an in silico study on the anti-inflammatory mechanism in red ginger showed that there are 2 pathway mechanisms related to the anti-inflammatory function, direct regulation of the inflammatory response and the regulation of blood circulation. The direct regulatory pathway for the inflammatory response is facilitated by 5 target proteins, namely NR1D1, CYP19A1, ESR1, CNR1, and CNR2 with a false discovery rate (FDR) of 0.0346. In response to infection or injury, the body initiates a cascade of chemical signals aimed at the affected tissue healing by producing pro-inflammatory mediators either from endogenous leukocytes (macrophages, monocytes, dendritic cells, or lymphocytes) or from the tissue cells themselves[20,21].

Meanwhile, the blood circulation pathway is facilitated by 5 proteins, namely CNR1, SLC6A4,

CYP11B1, CYP11B2, and HMGCR with an FDR value of 0.0263. The vascular system plays a role in controlling tissue homeostasis. When inflammation occurs, the endothelium plays a role in controlling blood flow, vascular permeability, leukocyte infiltration, extravasation of plasma into the intercellular space, and changes that serve to eliminate the initial stimulus. If the stimulus persists, the inflammation progresses to a chronic phase. Continuous activation of the endothelium caused by persistent inflammation may promote macrophage recruitment and neotissue angiogenesis to maintain blood flow[21,22].

In this in silico study on the potential active compounds in the fractionated ethanol extract of red ginger, it was found that there were 7 compounds, i.e. gamma-bisabolene, phenetanamine, curcumene, sesquiphellandrene, zingiberene, shogaol, and 6-gingerol predicted to have the ability to inhibit the cyclooxygenase enzyme greater than ibuprofen. The mechanism of the anti-inflammatory pathway of red ginger fractionated ethanol extract is through direct regulation of the inflammatory response and blood circulation. Therefore, based on the results of this in silico study, the active compound in the fractionated ethanol extract of red ginger is predicted to be an alternative anti-inflammatory drug that has a higher therapeutic effect with minimal side effects compared to ibuprofen as an NSAID drug.

Conclusion

The active compounds in the fractionated ethanol extract of red ginger (Zingiber officinale var. rubrum) are predicted to have potential as an anti-inflammatory with 7 compounds i.e. gamma-bisabolene, phenetanamine, curcumene, sesquiphellandrene, zingiberene, shogaol, and 6-gingerol which have better potential as anti-inflammatory drugs than ibuprofen. The mechanisms of the active compounds in the fractionated ethanol extract of red ginger related to the anti-inflammatory function are direct regulation of the inflammatory response and regulation of blood circulation.

Declaration of Interest

The authors have no confilcts of interest regarding this investigation.

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