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Differences effect of red and big white ginger extract as anti-inflammatory agents by *In vitro*

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ABSTRACT

This research was conducted to evaluate the possibility of different anti-inflammatory effects from the extract of red ginger and big white ginger by measuring their protein (bovine serum albumin) denaturation inhibitory capability. This study is a quasi experimental, using a model reaction between 0.2 % BSA were heated to form protein denaturation by five groups: red ginger water extract group, red ginger ethanol extract group, big white ginger water extract group, and big white ginger ethanol extract group as the test group (200 ppm, 100 ppm, 10 ppm, and 1 ppm) and diclofenac sodium as the standard group (40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, and 1.3 ppm). Potential inhibition of protein denaturation is known to determine the value of IC50. The results of this study indicate that the red ginger water extract has the IC50 value of 107.4513 ppm (r=0.967), the red ginger ethanol extract has the IC50 value of 193.4338 ppm (r=0.959), the big white ginger water extract has the IC50 value 160 ppm (r=0.969), the big white ginger ethanol extract has the IC50 value 160.9536 ppm (r=0.994), of whereas for diclofenac sodium by 27.1133 ppm (r=0.874). These results indicate that the red ginger and big white ginger as a potential inhibitor of protein denaturation for inflammatory processes *in vitro*.

Key words: *Anti-inflammatory, Ginger, Bovine serum albumin, Denaturation, Protein*

Introduction

Indonesia is a country rich in natural resources including resources for traditional medicine as out of 30,000 plant species from a total of 40,000 plant species in the world, 940 of them are medicinal plants (Rahayu and Andini, 2019). Red ginger and big white ginger of Zingiberaceae are widely known as medicinal plants in Indonesia. In Asia, varieties of ginger are spread across wet tropical regions. Ginger is abundant in North Sumatera, Bengkulu, West Java, Central Java and East Java. Ginger is divided

broadly into three kinds, red ginger, big white ginger and small white ginger. The difference between the three is based on their essential oil quantity. The red ginger contains the most essential oil and small white ginger contains the least essential oil.

Red ginger (*Zingiber officinale var. rubrum*) has 42-43 mm diameter, 52-104 mm in height and 123-126 mm in length while the big white ginger (*Zingiber Officinale var.* officinarum) has 48-85 mm, 62-113 mm in height and 158 –327 mm in length. The red ginger is very spicy with a strong aroma which contains 2.6-3.9% essential oil. Big white ginger on the other hand has less spicy taste and less strong compared to the red ginger and small white ginger. It also contains 0.82–2.8 % essential oil so both of them are used as medicinal plants. Fresh ginger is categorized into two kinds, volatile and non-volatile. The volatile ginger contains sesquiterpene hydrocarbons and monoterpenoids which give a distinctive aroma and taste. In contrast, the compounds which are not easily evaporated include gingerol, shogaol, paradol, andzingerone and they have anti-inflammatory, anti-oxidant, anti-bacterial and anti-thrombocytopenic properties (Jolad *et al.*, 2004; Williams and Lamprecht, 2008; Mashadi *et al.*, 2013; Chandra *et al.*, 2012).

Inflammation is a body response from an injury or infection marked by fever, reddened skin, pain, swell and physiological function disorder. It is a normal protection response against tissue injury caused by physical trauma, chemical substance or dangerous microbes (Perumal *et al.*, 2008). In inflammation, denatured protein has a role as autoantigen. It is commonly found in some illnesses such as rheumatism. Various therapies and treatments have been developed to treat the inflammation process, such as NSAIDs (Non Steroidal Anti Inflammatory Drugs) with diclofenac drug group. Diclofenac is an anti-inflammatory drug which works by inhibiting COX, the COX-2 inhibitor (Perumal *et al.*, 2008; Williams *et al.*, 2008). Other than NSAIDs, medicinal plants can also be utilized. The natural materials are expected to contain a certain compound with significant anti-inflammatory activity. The advantages from the natural material (herbal medicine) are for its accessibility, inexpensiveness and lesser side-effects. One of the natural resources with bioactive compounds and potential for development is red or big white ginger (Perumal *et al.*, 2008).

This research is conducted to evaluate the possibility of different effects between extract of red ginger and big white gingers to protein denaturation in their function as anti-inflammatory agents.

Materials and Methods

Media Preparation

The samples used in this study were red ginger (*Zingiber officinale var. Rubrum*) and big white ginger (*Zingiber officinale var. Officinarum*) from traditional markets in Surabaya-East Java. To create tris buffered saline solution or TBS, mix 1.21 g tris base and 8.7 g sodium chloride (NaCl) and dissolve into sterile aquadest until it weighs 900 ml. Adjust the solution pH level to 6.2-6.5 with glacial acetic acid and then add more sterile aquadest until the solution volume at 1000 ml (Erianti *et al.*, 2015). 0.2% Bovine Serum Albumin (BSA) is made by mixing 0.2 gram BSA crystal with TBS solution until the volume reaches 100 ml and then vortex the solution to completely dissolve the BSA crystal (Aditya *et al.*, 2015).

Treatment group

The negative control used for this research is 1 ml 0.2% BSA solution in a microtube and labeled. The positive control used for this research is diclofenac sodium solution made by mixing 4 mg diclofenac sodium with TBS solution until the volume reaches 1 ml and dissolving it with a vortex for several minutes. The process is done with a microtube to produce a stock solution of positive control with 4,000 ppm. From the stock solution, some other solutions are created with different concentrations; 2,000 ppm, 1,000 ppm, 500 ppm, 250 ppm and 130 ppm. Each solution is made in microtubes (Aditya *et al.*, 2015). The testing solution in this research is made by mixing 20 mg dry extract with TBS solution until the volume is at 1 ml in the microtube then dissolving it with the vortex machine. From this procedure, the stock testing solution with 20,000 ppm is acquired. From this stock solution, some serial solutions are made with different concentrations; 10,000 ppm, 1,000 ppm, and 100 ppm (Erianti *et al.*, 2015).

Protein Anti Denaturation Effect Analysis

After all series of treatment groups and positive control solutions are prepared, the samples from all concentrated solutions are taken about 10 ìLeach. Mix each sample with 0.2% BSA solution until the volume reaches 1 ml and then shake it slowly to make sure that it dissolves well. From this procedure, se-

ries of concentrated solutions are made for testing solutions (200 ppm, 100 ppm, 10 ppm, and 1 ppm) and for the diclofenac sodium (positive control) solutions (40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, and 1.3 ppm). With the negative control prepared beforehand, incubate all the samples of solutions mixed with BSA for 30 minutes at 25 °C temperature. After the incubation procedure, heat all the samples of testing solutions, positive control solutions and negative control using the heatblock for 5 minutes at 72 °C temperature. After the heating procedure, incubate them again at 23 °C for 25 minutes. After the second incubation, let them cool down and then use the vortex machine to make the mixture in the microtubes homogenous. After that, they are analyzed with a microplate spectrophotometer. Move all the samples of solutions to well microplates. Each well is filled with 250 µl solution from the series of testing solutions, positive control solutions and negative control solutions. There are three readings conducted (1 microtube = 3 wells). The readings by this spectrophotometer are conducted with 655 nanometer wavelengths (Erianti *et al*, 2015). The percentage of protein denaturation inhibition is calculated with a formula as follows:

$$\text{Inhibition (\%)} = \frac{\text{Negative Control Absorbance} - \text{Sample Absorbance}}{\text{Negative Control Absorbance}} \times 100\%$$

In vitro inflammatory activity is expressed by determining the amount of IC50 (inhibition Concentration 50) by making a linear graph y=a+bx with y=absorbance and x=concentration of red ginger (*Zingiber officinale var. Rubrum*) and big white ginger (*Zingiber officinale var. Officinarum*) (Erianti *et al*, 2015). A compound which is able to inhibit protein denaturation more than 20%, it is predicted to have strong anti-denaturation effect and the compound can be developed as an alternative anti-inflammatory drug. (Aditya *et al.*, 2015)

Results

Anti-Inflammatory Effect of Red Ginger Water Extract

The effect of red ginger water extract as anti inflammatory agent can be seen from the Figure 1 and 5. At 1-200 ppm concentration, the extract has shown 23.7%-65.48% inhibition percentage. It is higher than the diclofenac sodium as positive control which only showed 16.33-57.34% from 1-40 ppm concentration.

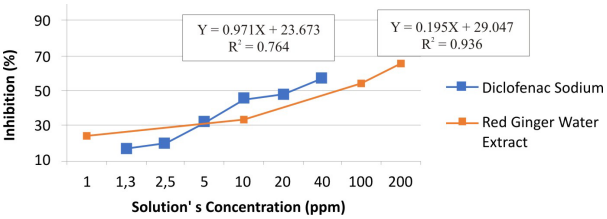

Fig. 1. Differences percentage inhibition of bovine serum albumin in vitro due to diclofenac sodium (positive control) and red ginger water extract

From the calculation of IC50 red ginger water extract and diclofenac sodium are 107.4513 ppm and 27.1133 ppm.

Effect of Red Ginger Ethanol Extract

The effect of red ginger ethanol extract as anti inflammatory agent can be seen from the Figure 2 and 5. At 1-200 ppm concentration, the extract has shown 21.31%-52.40% inhibition percentage while the diclofenac sodium as positive control only showed 16.33-57.34% from 1-40 ppm concentration.

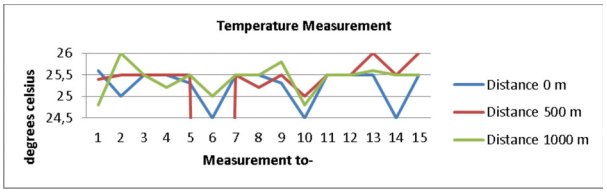

Fig. 2. Differences percentage inhibition of bovine serum albumin in vitro due to diclofenac sodium (positive control) and red ginger ethanol extract

From the IC50 calculation, red ginger ethanol extract and diclofenac sodium are 19.4338 ppm and 27.1133 ppm.

Anti-Inflammatory Effect of Big White Ginger Water Extract

The effect of big white ginger water extract as anti

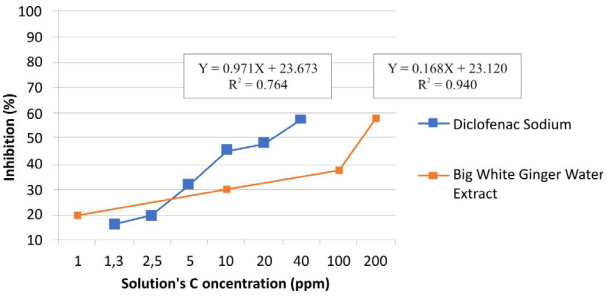

Fig. 3. Differences percentage inhibition of bovine serum albumin in vitro due to diclofenac sodium (positive control) and big white ginger water extract

inflammatory agent can be seen from the Figure 3 and 5. At 1-200 ppm concentration, the extract has shown 19.58%-57.78% inhibition percentage while the diclofenac sodium as positive control only showed 16.33-57.34% from 1-40 ppm concentration. From the IC50 calculation, big white ginger water extract and diclofenac sodium are 160 ppm and 27.1133 ppm.

Anti-Inflammatory Effect of Big White Ginger Ethanol Extract

The effect of big white ginger ethanol extract as anti inflammatory agent can be seen from the figure 4 and 5. At 1-200 ppm concentration, the extract has shown 18.58%-58.89% inhibition percentage while the diclofenac sodium as positive control only showed 16.33-57.34% from 1-40 ppm concentration. From the IC50 calculation, big white ginger ethanol extract and diclofenac sodium are 160.9536 ppm and 27.1133 ppm

Discussion

To produce herbal medicine, preclinical tests to animals are needed. However, there are some problems on using animals for testing, such as ethical problem, the lack of reasons using it when there are still other methods, and the cross-species extrapolation is difficult to perform due to the lack of isomorphisms between the animals and humans, especially at cellular and molecular level where the illness started (Erianti *et al.*, 2015). Previous researches have shown that some natural products are able to stabilize bovine serum albumin at pathological pH. This finding was then used to detect anti-inflammatory activity by *in vitro* method. Williams *et al*. (2008) have studied the properties of some natural resources to stabilize bovine serum albumin at pathological pH (pH level 6.5-6.8). This finding was then used to detect anti-inflammatory activity by *in vitro* method.

Higher absorbance found from the samples compared to the control solution showed the protein denaturation inhibition which was induced by heat (albumin) of ginger extract and positive control of diclofenac sodium. In this study red ginger and big white ginger extracts are more active than diclofenac sodium, which is an active lower concentration. This

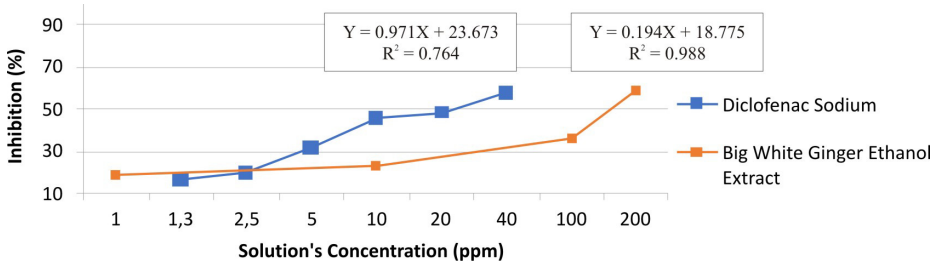

Fig. 4. Differences percentage inhibition of bovine serum albumin in vitro due to diclofenac sodium (positive control) and big white ginger ethanol extract

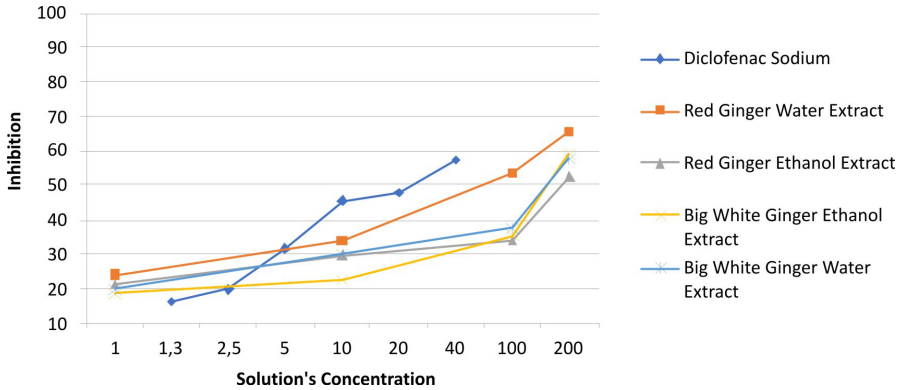

Fig. 5. Differences percentage inhibition of bovine serum albumin in vitro due to diclofenac sodium (positive control), red ginger water extract, red ginger ethanol extract, big white ginger water extract, and big white ginger ethanol extract

result showed that there was a change in turbidity due to viscosity change. This result was supported by (Chandra *et al.*, 2012) where the viscosity changes because protein solution viscosity is increasing at denaturation.

Protein denaturation is the protein's secondary, tertiary and quaternary structure change without the breaking of covalent bonds and is one of the causes of inflammation. The factors which cause protein denaturation are stress, temperature, pH, agitation, electricity, chemical substance, alcohol and reducing agents. Heat is one of the causes of protein denaturation because heat can disrupt the hydrogen bond so its capability of bonding the water decreased (Aditya *et al.*, 2015). It happens because high temperature increases the kinetic energy and causes the molecules to vibrate so rapidly and violently that the bonds are disrupted. Protein denaturation occurs steadily and unchanged. Denatured protein loses its solubility so it is easy to settle (*Nijveldt et al.*, 2001). When the protein denatures, some of its biological function might be lost. In addition, Autoantigen produced by certain illnesses related to inflammation may be caused by denaturation of protein. Protein denaturation is the loss of a structure and protein function caused by stress, chemical substance or heat. There is also loss of biological function when the protein denatures (Aditya *et al.*, 2015).

Red ginger and big white ginger extracts are capable of anti-inflammatory activity with protein denaturation inhibition percentage higher than 20%. Besides, as seen from the potential IC50, positive r values indicate the existence of a positive relation between concentration and inhibiting protein denaturation potential. A compound with anti inflammatory is flavonoid. The anti inflammatory activity mechanism can be done through some ways, Cyclooxygenase (COX) and lipoxygenase (LOX) enzyme inhibition, leukocyte accumulation inhibition, neutrophil degranulation inhibition, histamine release inhibition (Riansyah *et al.*, 2015). Anti inflammatory activity from the flavonoid with Cyclooxygenase (COX) and lipoxygenase (LOX) enzyme inhibition can cause leukotriene and prostaglandin synthesis inhibition which then inhibit mucus secretion which protect the stomach wall. Leukocyte accumulation inhibition during the inflammation process will lower the body response against inflammation. This inhibition happens because Cyclooxygenase (COX) is inhibited so thromboxane causing the leukocyte modulation is inhibited. Histamine release inhibition happens because flavonoid can inhibit mast cell release (Hidayati *et al.*, 2008).

Beside flavonoid compound, tannin compound also reported to have anti inflammatory property (Verma *et al.*, 2011; Erianti *et al.*, 2015) and saponin compound has the potential to be anti inflammatory agent by protein denaturation inhibition (Erianti *et al.*, 2015; Ahmad, 2009). Proteins of a vulnerable body can experience denaturation caused by free radical formation which causes an inflammation mechanism through inflammatory mediator release. Denaturation of protein is a process where protein loses its secondary or tertiary structure due to external compounds such as strong acid or base, inorganic salt, organic solvent and heat (Verma *et al.*, 2011). The possibility of interaction or bond between molecules in red ginger and big white ginger extracts can inhibit denaturation of protein. At high temperature, protein in BSA solution tends to form big molecules so it cannot go through the membrane and this phenomenon is known as denaturation of protein. If the BSA solution is heated, it will turn murky as the solubility of protein is decreased and protein molecules form aggregate so the molecule protein becomes bigger from the previous size (Ahmad, 2009).

One of the potentials of some non-steroid anti-inflammatory drugs is their ability to stabilize (prevent denaturation) albumin which has been given heat treatment at physiological pH (pH: 6.2-6.5) (Erianti *et al.*, 2015; Williams *et al.*, 2008). Therefore, with this initial research, we can conclude that ginger has an anti-inflammatory effect in vitro marked by protein denaturation. Further research is needed to ensure the mechanism and constituent of its anti inflammatory function

Conclusion

It could be concluded that that red ginger extract has higher anti-inflammatory activity compared to the big white ginger in protein denaturation inhibition by in vitro with the highest inhibition percentage from the red ginger water extract which is at 65.48% and also IC50 is 107.4513 ppm.

Conflict of Interest

The authors declares that there are no conflicts of interest regarding the publication of this article

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