Anti inflammatory Activity of the Ethanol Extract of Cinnamon (Cinnamomum burmannii) Bark using Membrane Stabilization Method and Protein Denaturation

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Abstract
Inflammation is the body's reaction to infection, irritation, or foreign substances, as the body's defenses. Inflammatory mediators such as bradykinin, prostaglandins, fluid extravasation, and tissue damage are essential to protect our body as an inflammatory response. One of the plants in Indonesia that can develop is Cinnamomum burmannii. These plants are identified to contain some substances such as flavonoid, tannin, and saponin. The purpose is to determine the anti-inflammatory activity of the Cinnamomum burmannii extract using in vitro method compared with diclofenac sodium. Cinnamon bark was extracted using 96% ethanol, and phytochemical screening was determined. Anti-inflammatory test in vitro using two methods, membrane stabilization and protein denaturation inhibition. Anti-inflammatory activity using the membrane stabilization method, the IC50 of the extract was 84.45 ± 3.55 µg/mL, while in the protein denaturation method, the IC50 of the extract was 57.412 ± 0.718 µg/mL. Even though the IC50 of the extract was lower, it is still lower than diclofenac sodium as drug control. Our result showed that cinnamon is potential as an antiinflammation and needs to be developed further.

Keywords: Antiinflammatory; Cinnamomum burmannii; Membrane stabilization; Protein denaturation

Aktivitas Anti Inflamasi Ekstrak Etanol Kulit Kayu Manis (Cinnamomum Burmannii) dengan Metode Stabilisasi Membran dan Denaturasi Protein

Abstrak

Kata Kunci: Antiinflamasi; Kayu manis; Denaturasi protein, Stabilisasi membran
1. Introduction

Inflammation involves various physiological processes in the body, such as activating mediators, enzyme activation, cell activation, tissue repair, and the movement of white blood cells through capillaries to areas of inflammation. Inflammation is often painful and involves increased vascular permeability, protein denaturation, and membrane changes. Injured tissue cells release kinins, prostaglandins, and histamine, which collectively cause vasodilation and increased capillary permeability. Changes in cell membranes and protein denaturation with the occurrence of inflammatory mechanisms. The red blood cell membrane is similar to the lysosomal membrane, which plays a role in the inflammatory process. Stabilizing the lysosomal membrane is vital in limiting the inflammatory response. Protein denaturation is the process of protein loss of secondary and tertiary structures caused by external compounds, such as strong acids, strong bases, inorganic salts, heating, and organic solvents. The autoantigens produced in inflammatory disease may be due to the protein denaturation process.

In general, to overcome inflammation, drugs from the non-steroid anti-inflammatory drugs (NSAID) group and steroid groups help reduce pain and swelling due to inflammation. However, in reality, long-term use of these drugs poses a risk of gastrointestinal, cardiac, and other toxicity. Therefore, research to find active compounds from plants that have the potential as new therapies that are more effective and safe is needed.

One of the plants from Indonesia that can have an anti-inflammatory effect is Cinnamomum burmannii, also known as cinnamon. Reported that four species of Cinnamomum, i.e., C. burmannii, C. zeylanicum, C. aromaticum, and C. loureiroi, have the same health benefits used for various conditions, including digestive disorders, inflammation, diabetes mellitus, and infections. Previous research conducted showed that aqueous and ethanol extracts from the red ginger rhizome and cinnamon bark inhibit cyclooxygenase (COX)-2 and xanthine oxidase (XO) enzymes in vitro. Based on ethnopharmacological data, C. zeylanicum, known as Ceylon, provides an anti-inflammatory effect in vivo. Chemical screening shows that cinnamon bark contains flavonoid compounds, saponins, and tannins. Flavonoids and saponins have been reported to stabilize lysosomal membranes in vivo and in vitro assays. Meanwhile, tannins and saponins can bind cations, thereby stabilizing the erythrocyte membrane.

Sufficient scientific evidence has not followed the widespread use of cinnamon bark in the community. The study of ethanol extract from cinnamon bark as an anti-inflammatory in vitro using stabilization membrane and protein stabilization method has never been done before. Based on approach chemotaxonomy and research that has been done previously.

2. Materials and methods
2.1. Tools

Analytical balance (Ohaus PA214), oven (Hettich, EBA 20), centrifuge (Hettich), pH meter (Eutech), rotary evaporator (Heidolph), water bath (Thermostat Water Bath HH-6), and UV-Vis spectrophotometer (Hitachi U-2910).

2.2. Materials

96% ethanol (Merck), distilled water (Otsuka), NaCl (Sigma), Na2HPO4 (Merck), Sodium diclofenac (Sigma), 1N HCl (Merck), bovine serum albumin (BSA, Sigma), fresh human blood (Indonesian Red Cross Society), normal saline (Widatra Bhakti). The cinnamon powder was obtained and determined by Materia Medica, Batu District, Malang, East Java (No. 074/356A/102.7/220).

2.3. Methods
2.3.1. The Extraction of Cinnamon Bark Extract

Cinnamon bark powder was extracted using the maceration method for three days using 96% ethanol solvent with a ratio of 1:10. The filtrate obtained was then concentrated with a rotary evaporator, and the extract yield was calculated.
2.3.2. Phytochemical Screening of Ethanol Extract of Cinnamon Bark
Analyzed to detect flavonoid compounds, alkaloids, saponins, triterpenoids, steroids, phenols, and tannins.\textsuperscript{13}

2.3.3. Anti-Inflammatory Activity using Membrane Stabilization Method
Fresh human blood (5 mL) was centrifuged at 3000 rpm for 10 min. The top layer (supernatant) was separated so that what remains in the tube is the bottom layer (pellet). The pellets that had been obtained were then rinsed using 5 mL of normal saline. Each rinse was followed by centrifugation. The rinsing process was carried out three times. The part used was the pellet containing red blood cells from the first rinse to the last rinse. The next step was to make a 10\% v/v suspension of red blood cells in saline phosphate buffer pH 7.4 (0.2 g NaH2PO4, 1.15 g Na2HPO4, and 9 g NaCl dissolved in 1 L of distilled water) by pipetting 9 mL of saline phosphate buffer and then mixed in a tube. The reaction mixture containing 1 mL of the pellet was then mixed until homogeneous.\textsuperscript{14} Sodium diclofenac solution as a positive control was made with concentrations of 20, 40, 60, 80, 100, and 120 µg/mL in 96\% ethanol. The ethanol extract of cinnamon bark was prepared at 20, 40, 60, 80, 100, and 120 µg/mL in 96\% ethanol. The IC50 value was determined through the linear regression equation of each test compound.

2.3.4. Anti-Inflammatory Activity using Protein Denaturation Method
A total of 0.5 mL of the solution added 0.2\% bovine serum albumin to 5 mL and incubated at room temperature for 30 minutes. The solution was then boiled at 100\°C for 45 minutes and allowed to cool at room temperature for 25 minutes. The solution was vortexed for 1 minute and measured at 660 nm. The test was carried out in three replications.\textsuperscript{15} Sodium diclofenac solution as a positive control was made with concentrations of 20, 40, 60, 80, 100, and 120 µg/mL in 96\% ethanol. The ethanol extract of cinnamon bark solution was prepared with 20, 40, 60, 80, 100, and 120 µg/mL in 96\% ethanol. The IC50 value was determined through the linear regression equation of each test compound.

2.4. Data Analysis
All IC50 data were displayed in mean ± SD. IC50 was compared in each group using independent t-test analysis with a 95\% confidence level. Significant differences between groups were indicated by the p-value < 0.05.

3. Result
The percentage of cinnamon bark yield obtained was 32.63\% w/w. Phytochemical screening of the ethanolic extract from Cinnamon bark found flavonoids, saponins, triterpenoids, steroids, and tannins, as shown in Table 1.

Table 1. Phytochemical Screening Result from Ethanol Extract of Cinnamon Bark

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>no brown or red-orange precipitate occurs</td>
<td>Negative</td>
</tr>
<tr>
<td>Saponin</td>
<td>Stable foam is formed</td>
<td>Positive</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>Formation of purplish-red color</td>
<td>Positive</td>
</tr>
<tr>
<td>Steroid</td>
<td>A red ring is formed</td>
<td>Positive</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Purple color</td>
<td>Positive</td>
</tr>
<tr>
<td>Tannin</td>
<td>Formed blackish-green color</td>
<td>Positive</td>
</tr>
<tr>
<td>Polyphenol</td>
<td>no blackish-green color change after adding FeCl3</td>
<td>Negative</td>
</tr>
</tbody>
</table>
and membrane stabilization methods, the percentage of inhibition of extract in both method were lower than diclofenac sodium as seen in Table 2. The IC50 of the ethanol extract of cinnamon bark was below 100 µg/mL (Figure 1).

4. Discussion

The yielded result obtained in this study was slightly different from other research, which obtained 29.25% and 29.59%. The difference in yields obtained may be due to the extraction method used and the difference in the place of plant sampling.

The principle of measuring anti-inflammatory activity in membrane stabilization testing is a decrease in absorbance in the test solution mixture. The smaller the absorbance value produced, the smaller the hemolysis that occurs, so the more significant the anti-inflammatory activity possessed by the sample. The IC50 value is an inhibitor concentration that can inhibit hemolysis activity by 50%. The smaller the IC50 value, the greater the inhibitory ability. The membrane stabilization activity test showed that cinnamon extract had an IC50 of 84.45µg/mL±3.55. This IC50 value is higher than the IC50 value of diclofenac sodium, which is 64.505µg/mL±1.272. Based on the results, diclofenac sodium has a stronger hemolytic activity than cinnamon extract.

Another test of anti-inflammatory activity is used protein denaturation method. The denaturation process in this study is stimulated by heat.4 When the temperature rises, it can increase the kinetic energy and cause the molecules that make up the protein to move or vibrate very quickly, damaging the molecule. The protein denaturation process takes place permanently and does not change. A denatured protein will decrease its liquid solubility to settle.17 Proteins in the body are susceptible to denaturation caused by the formation of free radicals that cause an inflammatory mechanism by stimulating the release of inflammatory mediators.18 The principle of inhibition of protein denaturation can be seen from the interaction of the sample solution with Bovine Serum Albumin. Samples that inhibit protein denaturation > 20% are considered anti-inflammatory activity and can be used as reference values for drug development.18

The cinnamon extract has protein denaturation inhibitory activity, which can

Table 2. in vitro anti-inflammatory testing results

<table>
<thead>
<tr>
<th>Group</th>
<th>Membrane stabilization % inhibition in concentration (ppm)</th>
<th>Protein denaturation % inhibition in concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Diclofenac Sodium</td>
<td>37.5</td>
<td>43.1</td>
</tr>
<tr>
<td>Ethanol extract of Cinnamon bark</td>
<td>18.9</td>
<td>24.0</td>
</tr>
</tbody>
</table>

Figure 1. IC50 Result using Membrane Stabilization (A) and Protein Denaturation (B) Test. The analysis was performed using an independent t-test at a 95% confidence level. Different superscript letters showed significant differences (p < 0.05)
be seen from the IC50 value of 57.412µg/mL±0.718. This value is still higher than the IC50 of diclofenac sodium, which is 14.655µg/mL±1.131. The IC50 value is interpreted as the sample concentration in inhibiting protein denaturing activity by 50%. The smaller the IC50 value, the higher the potential of the extract in inhibiting protein denaturation. IC50 of sodium diclofenac in inhibiting protein denaturation is classified as very strong with IC50 value < 50, however, the cinnamon extract was classified as strong because the IC50 was between 50-100µg/mL.15 Diclofenac sodium showed a more potent protein denaturing activity than cinnamon extract. Diclofenac sodium is a non-steroidal anti-inflammatory agent that can prevent inflammatory mediators' release.19

The ethanolic extract of cinnamon bark contained some constituents such as flavonoids, saponins, triterpenoids, steroids, and tannins. Flavonoids are known to have anti-inflammatory activity with the mechanism of action of inhibiting the formation of prostaglandins through the cyclooxygenase (COX) and lipoxygenase-5 (LOX-5) pathways.20 Flavonoid and saponins compounds also have anti-inflammatory activity because they inhibit inflammatory mediators and free radicals,21 while tannin and saponin compounds stabilize membranes by binding to cations.22 Free radical inhibitors could stabilize erythrocyte membranes from hypotonic solutions.23 Compounds that are also thought to have the ability to stabilize membranes are triterpenoids. The same thing was also reported24 that flavonoid and triterpenoid compounds from Centella Asiatica extract were responsible for anti-inflammatory activity in stabilizing red blood cell membranes.

In another species of cinnamon, such as C. zeylanicum and C. cassia were found E-cinnamaldehyde and o-methoxycinnamaldehyde. This compound was potent as antiinflammation after being tested in RAW.267 and J7774A.1.25 Our result about the activity of the cinnamon extract in the in-vitro study has completed other results previously. The latest study revealed that cinnamon bark oil showed anti-inflammatory activity in the in-vivo study using male Wistar rats.26 The relationship between the in vitro and in vivo study explained that if the cinnamon could reach the effective concentration in systemic, cinnamon and its component might be necessary for inflammation disease.

5. Conclusion

Based on the research, the cinnamon extract has potent anti-inflammatory activity, even it is still lower than diclofenac sodium. The exact mechanism needs to explore how the cinnamon extract can inhibit protein denaturation and stabilize the membrane.

References
8. Wongrakpanich S, Wongrakpanich A. A Comprehensive Review of Non-Steroidal AntiInflammatory Drug Use in


