Antibacterial Activity of *Cinnamomum burmannii* Extract Against *Escherichia coli*

Febrina Rosa Winda*, Suparno¹, Zuhdan Kun Prasetyo¹


**Abstract:** This study aims to determine the antibacterial activity of the cinnamon extract in inhibiting the growth of *Escherichia coli*. The research design was a laboratory experiment consisting of three phases, namely the preparation phase of cinnamon extract using the maceration method using 96% ethanol, the characterization phase consisting of qualitative analysis with phytochemical tests and quantitative analysis with UV-Vis spectrophotometry, and the application phase including antibacterial activity test by disc diffusion method (Kirby-Bauer Test). The concentration series were 20%, 40%, 60%, 80%, and 100%, chloramphenicol as the positive control and methanol as the negative control. The results showed that cinnamon extract positively contained flavonoids with a total content of 1.310 mg QE/g or 0.131%. Cinnamon extract is effective as an antibacterial in the medium category with the optimum concentration to inhibit *Escherichia coli* is 40% indicated by the diameter of the inhibition zone of 9.55 mm at 12 hours of incubation. Therefore, the cinnamon extract has potential activity against *Escherichia coli*.

**Keywords:** Antibacterial; *Cinnamon*; *E.coli*.

**Introduction**

The use of antibiotics (antibacterials) that are not according to indications can cause antimicrobial resistance (AMR) (Parisa et al., 2019; Repi et al., 2016). The risk of resistance to synthetic antibiotics can occur and cause treatment failure in patients infected with the disease for a long time. New antibacterial agents must be synthesized for the treatment of resistant bacterial diseases in response to the increasing antibiotic resistance that has continued to increase over the past few decades (Handayani et al., 2015). The use of natural-based antibiotics is one of the efforts to reduce the rate of antibiotic resistance (Isti’Azah & Zuhrotun, 2020). The use of plant-based antibacterial products is currently a research concern in an effort to overcome the widespread infectious diseases caused by bacteria and fungi (Awang et al., 2016). One of the plants that can potentially become a natural antibiotic is cinnamon (*Cinnamomum burmannii*).

*Cinnamomum burmannii* is one of the plants known by the wider community as a spice and is also widely used as traditional medicine. The benefits of consuming cinnamon include lowering cholesterol, lowering blood sugar levels, anti-fungal, anti-viral, anti-parasitic, antiseptic and as an antibacterial (Liakos et al., 2014; Repi et al., 2016). Cinnamon produces essential oils that contain phenolic compounds such as eugenol and sinamaldehyde that can fight many pathogenic bacteria and fungi (Pratiwi et al., 2015). Most of the compounds in the bark of the cinnamon plant are cinnamaldehyde, which has an anti-bacterial effect (Lewa & Gugule, 2022). In some research reports, cinnamon has shown potential activity against bacteria. Cinnamaldehyde, a major constituent of cinnamon, shows anti-inflammatory and antibacteri activity. It inhibits the production of nitric oxide and has also been shown to prevent the production of COX-2 (Julianti et al., 2017). Therefore, cinnamon has antibacterial and anti-inflammatory properties.

Cinnamon possesses excellent antimicrobial properties, anti-tumor activity, and antioxidative capacity (Ahmadi et al., 2021). It contains a wide variety of metabolites, including cinnamaldehyde, cinnamate,
eugenol, and essential oils, which exhibit antimicrobial properties (Yeong Jin et al., 2023). Phytochemical screening results show that cinnamon extract is positive for flavonoids, tannins, alkaloids, triterpenoids, phenolics, and other secondary metabolite compounds (Wen et al., 2016). From the description above, it is known that cinnamon extract contains active compounds that can have potential as antibacterial ingredients.

Antibacterial is a compound that can inhibit bacteria through the mechanism of action by damaging the cell wall, disrupting protein synthesis, changing membrane permeability, and inhibiting enzyme action (Septiani et al., 2017). Compounds that play a role in damaging cell walls include phenols, flavonoids, and alkaloids (Safratiilofa, 2016). The antibacterial ability of cinnamon is evidenced by the results of research conducted by Repi et al. (2016) which shows that cinnamon extract can inhibit the growth of Streptococcus pyogenes. Research conducted by Waty (2022) reported that ethanol extract of cinnamon bark has antibacterial activity in inhibiting the growth of Streptococcus mutans bacteria (ATCC). Cinnamon extract also showed antibacterial activity against the growth of Staphylococcus aureus and Pseudomonas aeruginosa as reported from the research results of Rollando et al. (2018). Based on the research that has been done, it can be concluded that cinnamon extract can inhibit microbial growth.

Escherichia coli is a group of gram-negative bacteria that can infect the gastrointestinal tract to cause diarrhea (Taufiqurrahman & Pijaryani, 2023). Escherichia coli is a flora that generally colonizes the large intestine, but these bacteria can produce toxins, namely shiga, which can damage the mucosal cells of the small intestine (Parisa et al., 2019). Diarrhea management can be done by giving ORS and antibacterials. The use of plant-based antibacterial products is currently widely used to overcome the spread of infectious diseases caused by bacteria and fungi (Awang et al., 2016). One of the plants that can potentially become a natural antibiotic is cinnamon (Cinnamomum burmannii).

Considering the abundant availability of cinnamon in Indonesia and the content of active compounds possessed by cinnamon, this study aims to determine the antibacterial activity of cinnamon extract in inhibiting the growth of Escherichia coli so that it can be used as an alternative treatment to overcome health problems caused by *Escherichia coli* infection.

**Method**

The equipment used are test tubes, erlenmeyer, petri dishes, ose, tweezers, pipettes, micropipettes, paper discs, rotary evaporator, UV-Vis spectrophotometry, bunsen, and incubator. The materials used are cinnamon powder, 96% ethanol, Muller Hinton Agar (MHA) media, test microbes namely Escherichia coli, chloramphenicol, methanol, Mg powder, HCl, FeCl₃, H₂SO₄, 1% NaOH, and mayer reagent.

The work procedure is carried out in the following stages started off preparation phase, characterization phase, and application phase. In preparation phase, the extraction process was carried out by method maceration. Simplicia used in this study is cinnamon (Cinnamomum burmannii) in powder form. Total of 250 grams of cinnamon powder was mixed with 96% ethanol solvent in a ratio of 1: 4 and allowed to stand for 5 days. Then filtering was done to separate the solution from the sediment. The filtering results were then evaporated with a rotary evaporator at 50°C until a thick extract was obtained.

Characterization phase divided into two analyses which is qualitative and quantitative analysis. In this stage, phytochemical tests are carried out to determine the content of active compounds in cinnamon extract qualitatively. Phytochemical tests were conducted to qualitatively identify the content of active compounds in cinnamon extract. The active compounds to be identified are flavonoids, tannins, saponins, triterpenoids, alkaloids, and quinones.

Quantitative test with UV-Vis spectrophotometry is used to determine the total content of active compounds contained in cinnamon extract. The total compound content to be known in this study is the total flavonoid content. Determination of flavonoid content of the extract was done by first determining the maximum wavelength of quercetin and making a standard curve of quercetin. Furthermore, the determination of total flavonoids was carried out by adding 2 mL of 2% AlCl₃ which had been dissolved in methanol into 2 mL of extract solution added, then vortexed and read the absorbance on a UV-Vis spectrophotometer. The results were plotted against a standard curve of quercetin prepared in the same way. The total flavonoid content was expressed as mg of quercetin equivalent/g sample (Tan et al., 2018).

The last phase is the application. At this stage, activity tests were carried out using the disc diffusion method (Kirby-Bauer Test). The concentration variations used were 20%, 40%, 60%, 80%, and 100% with positive control chloramphenicol, negative control methanol solvent, and the test microbe used was Escherichia coli.
The activity test with the disc diffusion method was carried out by first leveling the bacterial suspension on the surface of 38 g/L MHA medium. Paper discs (blank disc OXOID) with a diameter of 6mm were soaked in extracts with concentrations of 20%, 40%, 60%, 80%, and 100%, chloramphenicol solution, and methanol. The discs then placed on MHA medium that had been coated with bacterial suspensions. The ability of the simplisia extract to inhibit bacterial growth is indicated by the width of the inhibition zone formed around the disc paper area.

Measurement of the diameter of the inhibition zone (mm) with a caliper was done every 3 hours for 72 hours of observation. The diameter of each inhibition zone was measured vertically, horizontally, and diagonally. The diameter of the inhibition zone was obtained after subtracting the diameter of the disc paper. The category of antibacterial ability in terms of inhibition zone diameter is categorized as weak (≤ 5 mm), moderate (6-10 mm), strong (11-20 mm), and very strong (≥ 21 mm) (Repi et al., 2016).

\[ \text{Inhibition zone diameter} = \left( \frac{D_v + D_h + D_d}{3} \right) - D_{bd} \] (1)

\( D_v \): vertikal diameter
\( D_h \): horizontal diameter
\( D_d \): diagonal diameter
\( D_{bd} \): blank disc (6mm) diameter

**Result and Discussion**

Phytochemical tests are carried out to identify compounds based on changes in the color of the sample after being given a reagent. The results of the phytochemical test in Table 1 show that cinnamon extract (Cinnamomum burmannii) contains secondary metabolite compounds. Isolation of secondary metabolite compounds, such as flavonoids, tannins, alkaloids, terpenoids, and others from plants are cytotoxic to cancer cells, inhibit histamine release, anti-inflammatory, anti-fungal and anti-bacterial (Mulyani et al., 2013).

**Table 1. Phytochemical Test Results of Cinnamomum burmannii Extracts**

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Indication</th>
<th>Color change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>Greenish brown</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
<td>Blackish red</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>No foam formed</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>White sediment formed</td>
</tr>
<tr>
<td>Quinon</td>
<td>+</td>
<td>Red-brown</td>
</tr>
</tbody>
</table>

The positive results of the secondary metabolite content of cinnamon extract are the basis for the inhibitory ability of the extract against bacterial growth. The inhibition zone formed from cinnamon extract is due to the presence of metabolite compounds. Cinnamon contains antibacterial compounds such as alkaloids, flavonoids, terpenoids, tannins, and quinones. Products from plants containing alkaloids, flavonoids, and tannins can be used as antimicrobial agents (Waty, 2022).

Quantitative test to determine the total flavonoids of cinnamon extract was done first by making a calibration curve of quercetin. Quercetin was used as a standard solution because quercetin is a flavonoid of the flavonol group that has a keto group at C-4 and has a hydroxyl group at C-3 or C-5 atoms that are neighbors of flavones and flavonols (Aminah et al., 2017). The concentration series used to obtain the linear regression equation are 0, 6, 8, 10, 12, and 14ppm. After running from a wavelength of 400nm to 450nm, the maximum wavelength of quercetin standard was obtained at a wavelength of 435nm.

**Table 2. Absorbance Measurement Results of Quercetin**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Absorbance at 435 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>0.278</td>
</tr>
<tr>
<td>8</td>
<td>0.378</td>
</tr>
<tr>
<td>10</td>
<td>0.442</td>
</tr>
<tr>
<td>12</td>
<td>0.555</td>
</tr>
<tr>
<td>14</td>
<td>0.628</td>
</tr>
</tbody>
</table>
Based on the above results, it can be concluded that the concentration is directly proportional to the absorbance, namely the higher the concentration used, the higher the absorbance obtained. From the measurement results, a linear regression equation "y=0.0423x+0.034" was obtained with a value of R²=0.9974. Determination of total flavonoid content of cinnamon extract can be done with the equation of quercetin calibration curve that has been obtained. Using a sample of Cinnamomum burmannii with a mass of 0.00075 mg, the following results were obtained.

Table 3. Total Flavonoid Content

<table>
<thead>
<tr>
<th>Absorbance (Total flavonoid (%))</th>
<th>Total flavonoid (ug QE/g)</th>
<th>mg QE/g</th>
<th>Average total flavonoids (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>0.137</td>
<td>1373.71</td>
<td>1.37</td>
</tr>
<tr>
<td>0.118</td>
<td>0.134</td>
<td>134.77</td>
<td>1.34</td>
</tr>
<tr>
<td>0.11</td>
<td>0.121</td>
<td>1213.98</td>
<td>1.21</td>
</tr>
</tbody>
</table>

The measurement of flavonoid content of cinnamon extract (Cinnamomum burmannii) was obtained at 1.310 mg QE/g or 0.131%. Flavonoids are the largest class of phenol compounds synthesized by plants in response to microbial infection, so they are effective against microorganisms (Safratiolofa, 2016). The mechanism of flavonoid compounds as antibacterial is by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane and damage the cell membrane without repairing it again. Flavonoids have hydrox groups that can bind to peptidoglycan in the cell wall, so that the bacterial cell membrane is damaged due to lipopolysaccharide binding (Suhendar & Fathurrahman, 2019).

From qualitative and quantitative analysis, it can be seen that cinnamon contains active compounds that have potential as antibacterials. To determine the level of bacterial susceptibility to cinnamon extract, an activity test was conducted by disc diffusion method.

![Figure 3. Inoculation of bacteria on medium](image)

The antibacterial activity test of cinnamon extract in inhibiting the growth of E.coli was observed for 72 hours. The results of the inhibition zone diameter listed in Table 4 are the average of replicate one and replicate two which are displayed every 12 hours of measurement. The results of inhibition zone measurements taken every 3 hours can be seen in the graph.

Table 4. Antibacterial activity of Cinnamomum burmannii

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Inhibition zone diameter (mm) / hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12H</td>
</tr>
<tr>
<td>20%</td>
<td>5.40</td>
</tr>
<tr>
<td>40%</td>
<td>9.55</td>
</tr>
<tr>
<td>60%</td>
<td>7.38</td>
</tr>
<tr>
<td>80%</td>
<td>6.80</td>
</tr>
<tr>
<td>100%</td>
<td>5.92</td>
</tr>
<tr>
<td>Control +</td>
<td>12.62</td>
</tr>
<tr>
<td>Control -</td>
<td>0.00</td>
</tr>
</tbody>
</table>

![Figure 4. Blank disc placed on medium](image)

![Figure 5. Inhibition zone diameter graph](image)

Based on the antibacterial activity test, cinnamon extract produces a servant zone which indicates that the extract has antibacterial potential. The extract is considered to have antibacterial activity if a clear zone is
formed around the extract grown on media that has been inoculated by pathogenic microbes (Suhendar & Fathurrahman, 2019). The inhibition zone formed around the disk indicates the inhibitory activity of cinnamon extract against E.coli. The ability of the extract to inhibit bacteria is judged by the size of the diameter of the inhibition zone formed. The ability of the extract to maximally inhibit bacterial growth is known through the peak of the highest inhibition zone. Look at the clear zone diameter graph in Figure 5.

The peak inhibition zone of cinnamon extract against E.coli occurred at 12 hours incubation. The average diameter of the inhibition zone produced by each concentration series is 20% concentration produces an average diameter of 5.4 mm, 40% concentration produces an average diameter of 9.55 mm, 60% concentration produces an average diameter of 7.38 mm, 80% concentration produces an average diameter of 6.80 mm, and 100% concentration produces an average diameter of 5.92 mm. Based on these results, it can be categorized that 40%, 60%, and 80% concentrations are in the medium category in inhibiting E.coli, while 20% and 100% concentrations are in the low category. From these results it can be seen that the high and low concentration of cinnamon ethanol extract is not directly proportional to the diameter of the inhibition zone produced. The chloramphenicol as a positive control had a larger inhibition zone diameter compared to the diameter of the extract inhibition zone, which was 12.62 mm at 12 hours after incubation. This can occur because chloramphenicol has pure active ingredients that are more effective in inhibiting bacteria (Hudaya et al., 2014). Methanol as a negative control did not show any activity to inhibit the growth of E.coli which was characterized by the absence of inhibition zone.

Antibacterial activity in inhibiting microbes is determined by the length of incubation (contact time) and the accuracy of the dose given (Meila et al., 2020). Based on the graph, it can be seen that the peak of the inhibition zone is at 12 hours incubation. The activity of the extract in inhibiting E.coli works optimally at 12 hours incubation which then experiences a decrease in activity until 72 hours incubation. It can also be seen that cinnamon extract with concentrations of 20%, 40%, 60%, and 80% can still inhibit the growth of E.coli after 72 hours of incubation. The length of incubation affects the ability of antibacterial activity of the extract. This is as explained by Septiani et al. (2017) that a short contact time allows bacteria not to be killed, while a long contact time will kill more bacteria but if it is too long it will also allow bacteria to multiply because antibacterial compounds have decomposed.
In this study the 40% concentrated extract worked better than the larger concentration series. In some cases, greater concentrations do not provide a greater inhibitory effect but have a smaller inhibitory ability than other concentrations (Zeniusa et al., 2019). The diameter of the inhibition zone does not always increase in proportion to the increase in antibacterial concentration, because there are differences in the diffusion speed of antibacterial compounds in the media and different types of antibacterial compound concentrations also give different inhibition zone diameters at a certain length of time (Septiani et al., 2017). There are several factors that can cause this to happen, such as the lack of diffusion of the extract into the media. The process of extract diffusion can be influenced by dilution factors. The higher the concentration of the extract, the lower the solubility (thickens like a gel), so this can slow down the diffusion of the active ingredients of the extract into the media and ultimately reduce the ability of high concentrations of extracts to inhibit bacterial growth (Handajani & Tjahjadi, 2008). In addition, the amount of antibacterial dose given will affect the ability of the extract to kill bacteria. When the dose of antibiotic given is too much, the bacteria will experience resistance (Meila et al., 2020). In the case of low-concentration extracts having more optimum performance than larger concentrations, it can be caused because at that concentration the content of antibacterial compounds is sufficient to kill bacteria (Septiani et al., 2017).

The ability of cinnamon ethanol extract to inhibit E.coli is in the medium category. These results differ from previous research by Mursyida et al. (2021) with the same extraction method showing 96% ethanol extract of cinnamon bark can inhibit the growth of E.coli with an average diameter of 3.15 mm for 75% concentration and 4.85 mm for 100% concentration. This difference can be caused because there are factors that affect maceration extraction, including differences in pretreatment and the size of the material and pending treatment can extend the contact time of the material with the solvent (Khasanah et al., 2018). The research conducted by Repi et al. (2016) with cinnamon bark maceration using 80% ethanol obtained an average inhibition zone obtained of 14.3 mm. This difference can be caused by the effect of the solvent concentration used as explained by Khasanah et al. (2018) that the solvent concentration affects the quantity and quality of the extract and is also related to the solubility of the extract.

Based on phytochemical test results, Cinnamomum burmannii extracts positive contain flavonoids, alkaloid, terpenoid, saponin, and tannin. Those secondary metabolite content of cinnamon extract are the basis for the inhibitory ability of the extract against bacterial growth. Cinnamon extracts and their compounds have been reported to inhibit bacteria by damaging cell membrane; altering the lipid profile; inhibiting ATPases, cell division, membrane porins, motility, and biofilm formation; and via anti-quorum sensing effects Each compound has its own mechanism that works in inhibiting bacterial growth (Vasconcelos et al., 2018). Cinnamon could act on the cytoplasmic membrane, affecting the integrity of membrane (Zhang et al., 2016).

Flavonoids have antibacterial activity by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes or through inhibition of RNA synthesis in bacterial cell DNA (Waty, 2022). Flavonoid compounds are known to damage bacterial cell membranes by inhibiting protein synthesis (Intan et al., 2021). Terpenoid compounds also have a mechanism of action as antimicrobials by damaging and reducing the permeability of bacterial cell walls so that bacterial cells lack nutrients and the development of bacteria becomes inhibited and even dies (Wulansari et al., 2020).

Alkaloids can inhibit microbial growth due to their ability to intercalate cell walls and DNA. Alkaloids are known as DNA interchelators that can inhibit topoisomerase enzymes that play a role in replication and transcription of bacterial cell DNA (Suhendar & Fathurrahman, 2019). Alkaloid compounds work by disrupting the glycan peptide component in bacterial cells so that the cell wall layer is not formed completely and causes cell death.

The tannins have antibacterial activity because the toxicity of tannins can damage the bacterial cell membrane. Tannins attack cell walls by disrupting cell permeability which can cause cell death because they cannot carry out life activities (Kaczmarek, 2020). The antibacterial effects of tannins include reactions with cell membranes, enzyme inactivation, and destruction or inactivation of genetic material functions (Suhartati, 2018). The potential mechanisms include iron chelation, suppression of cell wall synthesis, and damage to cell membranes. Additionally, tannins can target QS systems and bacterial virulence factors, such as biofilms, enzymes, adhesion, and motility (Farha et al., 2020).

The inhibition zone resulting from the effectiveness test of cinnamon extract against E.coli bacteria is in the medium category. This can be caused by many factors that affect the size of the inhibition zone produced in the diffusion method, including the speed of diffusion, the nature of the agar medium used, the number of organisms inoculated, the speed of bacterial growth, the concentration of chemicals, and the conditions during incubation. The absence of an inhibition zone with a strong category is due to E.coli's complex cell wall structure which makes it more resistant to antibacterials.
E.coli is a gram-negative bacterium with a cell wall containing three polymers, namely the outer layer of lipoproteins, the middle layer of lipopolysaccharides, and the inner layer of peptidoglycan, and the outer membrane in the form of a bilayer that has more resistance to compounds leaving or entering the cell (Trombetta et al., 2005). The outer wall of E.coli bacteria has high permeability, contains porins that are hydrophilic, and contains a lipid layer that is nonpolar (Zeniusa et al., 2019). This is what causes the active substances in cinnamon tea extract to not be able to enter optimally into bacterial cells which results in less than optimal extract in inhibiting the growth of E.coli.

**Conclusion**

Cinnamon ethanol extract has a positive content of secondary metabolites. Antibacterial activity test shows that cinnamon extract has moderate antibacterial activity in inhibiting *Escherichia coli*. The most effective concentration to inhibit the growth of *Escherichia coli* is 40% concentration with clear zone diameter of 9.55 mm.

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**Author Contributions**
In this study, the authors made different contributions. Methodology and conceptualization, S and F.R.W.; investigation, data collecting, original draft preparation, writing and editing, F.R.W; reviewing the draft was carried out by all author, F.R.W, S, and Z.K.P.

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**Conflicts of Interest**
The authors declare no conflict of interest.

**References**


