ANTAGONISM TESTING ON AN ETHANOL EXTRACT PREPARATION OF YELLOW ROOT STICKS (Arcangelisia flava L. Merr) TOWARD Yersinia enterocolitica AND Salmonella typhi BACTERIA

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Abstract

Yellow root (Arcangelisia flava L. Merr) contains ethanol compounds which is able to inhibit bacterial growth, both gram negative bacteria such as Stapylococcus aureus, Shigella sp., and Escherichia coli, as well as gram positive bacteria such as Yersinia enterocolitica, Streptococcus, Salmonella typhi, and Clostridium. This research is an experimental research that aims to determine the nature of antagonism (adaptability) between Yersinia enterocolitica bacteria and Salmonella typhi bacteria by administering yellow root extract at concentrations of 30%, 40%, 50%, and 60% within 24 hours, 48 hours, and 72 hours. The results showed that the positive control and negative control treatmens were not able to inhibit the growth of Yersinia enterocolitica bacteria and Salmonella typhi bacteria. Yellow root extract has acidity that effectively inhibits Yersinia enterocolitica bacteria at a concentration of 50%, while the most effective concentration for inhibiting the growth of Salmonella typhi bacteria is a concentration of 30%. Besides that, there are also differences in adaptability between Yersinia enterocolitica bacteria and Salmonella typhi bacteria. Salmonella typhi bacteria is more adaptable then Yersinia enterocolitica bacteria, this is evidenced by the inhibition zone formed which is smaller than Yersinia enterocolitica bacteria.

Keywords: Adaptation, Salmonella typhi, Yellow root, Yersinia enterocolitica.

INTRODUCTION

The eight countries with the highest levels of biodiversity in the world, including both flora and wildlife, include the tropical nation of Indonesia. This is evident from the numerous plant species that flourish, including some with therapeutic qualities (Friska et al., 2021; Indah et al., 2021). To prevent and treat many illnesses, including infectious diseases, the community has
traditionally used plant materials with therapeutic characteristics. Microorganisms that arise as a result of poor sanitation and damp air are what cause infectious diseases (Zulita et al., 2018). The reason why people currently prefer traditional medicine over other types of treatment is that it is generally inexpensive, simple to get, and has few adverse effects (Wahyuni & Putri, 2020).

The yellow root plant (Arcangelisia flava L. Merr) is a plant with therapeutic qualities. The Indonesian people, particularly the Dayak people in Central Kalimantan, have long recognized yellow root (Arcangelisia flava L. Merr) as a plant having natural therapeutic characteristics that can treat a variety of illnesses (Nursyam, 2013). In addition to Central Kalimantan, yellow root is also employed in South Kalimantan as a requirement for the traditional herbal medicine sector, while its availability and quantity are still adequate to suit local needs (Rinaldi et al., 2018). In this Indonesian traditional medicine, the major ingredient is yellow root stem (Arcangelisia flava L. Merr). The stems of the yellow root (Arcangelisia flava L. Merr) are used as a traditional medicine by a number of tribes in Kalimantan, including the Dayak, Banjar, and Kutai tribes, to treat jaundice, hepatitis, digestive issues, worms, fever, and canker sores. Stems are boiled.

The roots of the plant known as yellow root (Arcangelisia flava L. Merr) contain a variety of chemically active substances, including flavonoids, saponins, tannins, alkaloids, steroids, and phenolhydroquinone. Palmatine, jatrorrhizine, dihydroberberine, and berberine are among the substances found in the yellow root stem that are considered to be active (Widyastuti et al., 2021; Balitbang Palangkaraya, 2018). One of the secondary metabolites of yellow root known to exhibit a relatively potent anticancer effect on several cancer cell types is the chemical compound berberine. The antiproliferative impact of berberine on cancer cells is one of the substance's anticancer properties (Pratama, 2016; Hujjatusnaini et al., 2022). In addition, alkaloids are a class of secondary metabolite chemicals that are alkaline and have nitrogen (N) atoms in a heterocyclic or aromatic circular structure that is present in plant and animal tissues (Hanani, 2015; Hujjatusnaini et al., 2022).

Yellow root extract (Arcangelisia flava L. Merr) has been studied, and its ethanol content has the potential to inhibit the growth of Salmonella typhi, Stapylococcus aureus, Trichophyton rubrum, and Pseudomonas fluorescens bacteria at concentrations of 1%, 2%, 3%, 4%, and 5%, respectively (Nursyam, 2013). This yellow root stem ethanol extract has antimicrobial benefits, both as an antibacterial and as an antifungal (Jawetz et al., 2012; Hujjatusnaini et al., 2022). The alkaloid chemical compound protoberberine in the stem of yellow root is known to have an antibacterial effect against gram-positive and negative bacteria (Kaharap et al., 2016). Bacteria that are pathogenic and can cause disease include Yersinia enterocolitica and Salmonella typhi.
Yersinia enterocolitica causes zoonotic illnesses in both humans and animal (Liang et al., 2019). This bacteria is classified as a gram-negative bacteria which is in the form of a bacillus and is a facultative anaerobe (Reuter et al., 2015). Yersinia enterocolitica causes diarrhea accompanied by abdominal pain and fever (Ong et al., 2012). This bacterium infects humans through invasion of epithelial cells and penetration of intestinal mucosal cells, then multiplies in lymphoid tissue and the large intestine (Odyniec et al., 2020).

A group of gram-positive bacteria with flagella is known as Salmonella typhi. One of the bacteria that can cause infectious disorders is Salmonella typhi, which enters the body through contaminated food and beverages. The gallbladder, bile ducts, and portions of the colon or urinary system are where the majority of patients with this bacterial infection are carriers. Typhoid fever and diarrhea are just a couple of the health issues this bacteria can cause (Jawetz et al., 2012).

Based on this background, this research needs to be carried out with the aim of knowing the comparison of the adaptability of Yersinia enterocolitica and Salmonella typhi bacteria by providing yellow root extract concentrations of 30%, 40%, 50% and 60%, taking into account the distinct growth rate and generation time of three x 24 hours of incubation.

The analytical balance, autoclave, measuring cup, beaker, petri dish, pipette pump, incubator, caliper, and cover paper were the items of equipment utilized in this study. Yellow root extract preparations, distilled water, chloramphenicol, and pure cultures of Yersinia enterocolitica and Salmonella typhi bacteria are the substances utilized.

Each prepared medium included a single unit of pure Yersinia enterocolitica and Salmonella typhi cultures. Incubate for a further six times in 24 hours. Make NA and cool it to a temperature of roughly 50°C after 6 x 24 hours. Inoculate the Yersinia enterocolitica pure culture in two doses, shake it between your palms to disseminate the germs, and then pour it aseptically into a sterile petri dish. After that, watch for the medium to set up. A 1 cm² slice of the Salmonella typhi colony should then be placed on a firm medium. Next, let the medium sit for one round of 24 hours.

The yellow root extract preparation was dissolved using distilled water to form concentrations of 30%, 40%, 50% and 60%. Next, carry out experiments using six treatments, namely positive control using chloramphenicol, negative control using distilled water, yellow root extract preparations with concentrations of 30%, 40%, 50% and 60% which have been made. After being treated, it was then incubated again for 3 x 24 hours, every 1 x 24 hours the size of the inhibition zone for each culture was calculated.

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Salmonella typhi and Yersinia enterocolitica were examined for their capacity to grow following treatment. It can be assumed that the yellow root extract has inhibitory power against these microorganisms if the zone of inhibition is significant following treatment. (Hujjatusnaini et al., 2021). The inhibition zone formed in each treatment will have different results. According to Handayani et al., (2017), the differences in results were caused by differences in the concentration of active compounds in the yellow root extract.

RESULT AND DISCUSSION

The diameter of the inhibition zone at each concentration during the course of 72 hours of incubation provides information from research on the antagonism test of Yersinia enterocolitica and Salmonella typhi against ethanol extract preparations of yellow root stem (Arcangelisia flava L. Merr). The information gained demonstrated that Yersinia enterocolitica growth might be prevented by an ethanol extract of yellow root stem (Nada et al., 2022). This is consistent with the findings of Hujjatusnaini et al., 2021, which indicate that turmeric root has the ability to act as a gram-negative antibacterial agent. The average observation findings demonstrate that Yersinia enterocolitica growth pattern is highly diverse, as illustrated in Figure 1.

![Figure 1. Adaptation ability of Yersinia enterocolitica](image)

The results of the adaptability of Yersinia enterocolitica for 72 hours shown in Figure 1 state that the bacterial inhibition zone was the highest at a concentration of 50% and the bacterial inhibition zone

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experienced a very significant decrease at a concentration of 60%. Concentrations of 30% and 40% produce quite strong inhibition zones. In the positive control, an inhibition zone was also found, but it was very small, while in the negative control no inhibition zone was found. The average of the observational data obtained shows that the ethanol extract of yellow root stems is also able to inhibit the growth of *Salmonella typhi* but not to a greater extent in *Yersinia enterocolitica*. The growth pattern of *Salmonella typhi* is shown in Figure 2 below.

![Figure 2. Adaptation ability of *Salmonella typhi*](image)

The bacterial inhibition zone had the largest concentration at a concentration of 30% and saw a very significant decline at a concentration of 60%, according to the results of *Salmonella*'s adaptability for 72 hours displayed in Figure 2. Although not high enough, concentrations of 40% and 50% create a rather potent inhibitory zone. A very small inhibitory zone was identified in the positive control after 72 hours of incubation, demonstrating the lesser efficiency. This is consistent with the findings of Abdulkareem et al. (2022) that the well-diffusion technique was used to evaluate the antimicrobial property of tannin extract through various concentrations with the highest zone of inhibition for the bacteria. Thus, the concentrations used in this study meet the criteria for evaluating antimicrobial properties. The findings of the Duncan 1% follow-up test can be used to compare the efficacy of yellow root's antibacterial potency against *Yersinia enterocolitica* and *Salmonella typhi* (Table 1).

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Table 1. Comparison of the significance of the effectiveness of the antibacterial power of yellow root against *Yersinia enterocolitica* and *Salmonella typhi*

<table>
<thead>
<tr>
<th></th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yersinia enterocolitica</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Hour</td>
<td>135.350</td>
<td>34.618</td>
<td>.000*</td>
</tr>
<tr>
<td>48 Hour</td>
<td>169.005</td>
<td>8.661</td>
<td>.000*</td>
</tr>
<tr>
<td>72 Hour</td>
<td>184.847</td>
<td>11.430</td>
<td>.000*</td>
</tr>
<tr>
<td><strong>Salmonella typhi</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Hour</td>
<td>7.170</td>
<td>9.198</td>
<td>.000*</td>
</tr>
<tr>
<td>48 Hour</td>
<td>10.684</td>
<td>11.673</td>
<td>.000*</td>
</tr>
<tr>
<td>72 Hour</td>
<td>11.519</td>
<td>3.686</td>
<td>.018*</td>
</tr>
</tbody>
</table>

Based on Table 1, which indicates a significant value of p 0.01, it is possible to infer that secondary metabolite components in yellow root extract (*Arcangelisia flava* L. Merr.) have a substantial impact on the metabolites' ability to adapt to both *Yersinia enterocolitica* and *Salmonella typhi*.

Figure 3 illustrates a comparison of *Yersinia enterocolitica* and *Salmonella typhi*'s ability to effectively adapt to the antibacterial properties of yellow root (*Arcangelisia flava* L. Merr), which reveals their antagonistic ability.

Figure 3. Effectiveness of the antibacterial power of yellow root (*Arcangelisia flava* L. Merr) against *Yersinia enterocolitica* and *Salmonella typhi*

The data are interpreted in Figure 3 to show that yellow root ethanol extract has extremely excellent antibacterial action at a concentration of 50% against *Yersinia enterocolitica*. This concentration is recognized as the optimal concentration for *Yersinia enterocolitica* because it can adjust over a period of 24 to 72 hours. On the other hand, *Salmonella typhi* can only...
remain adaptable for up to 48 hours at a concentration of 30%. Pada masa inkubasi 72 hours, it was claimed that there was no discernible difference between the 30% concentration of yellow root ethanol extract and greater concentrations. Since *Salmonella typhi* is able to adapt to its environment and yellow root's antibacterial strength has significantly dropped at a concentration of 30%, it can be said that the metabolite chemicals in yellow root have a maximum 48-hour shelf life.

Bacteria's flexibility and hostility toward other similar microbes are indicators of their capacity to thrive in their cellular environment. Table 2 compares the ability of *Yersinia enterocolitica* and *Salmonella typhi* to compete with one another.

Table 2. Comparison of the adaptation abilities of *Yersinia enterocolitica* and *Salmonella typhi*

<table>
<thead>
<tr>
<th>Pair</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Y. enterocolitica</em></td>
<td>7.729</td>
<td>6.7722</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>1.660</td>
<td>1.8820</td>
</tr>
<tr>
<td><em>Y. enterocolitica</em> – <em>S. typhi</em></td>
<td>6.0694</td>
<td>5.9370</td>
</tr>
</tbody>
</table>

| Correlation | 0.555 |
| Sig.        | 0.000 |
| N           | 72    |
| t           | 8.675 |
| df          | 71    |
| Sig. (2-tailed) | 0.000 |

Table 2 compare the adaptability of the bacteria *Yersinia enterocolitica* and *Salmonella typhi* during incubation durations of 24 hours, 48 hours, and 72. The calculation results are displayed in the table, and the positive mean value indicates that there is a tendency for differences between the two, specifically a difference of 6.0694. The correlation significance value of 0.000, which reinforces the trend for statistical differences in sig. 5%, also supports the existence of disparities in adaptation. The correlation value between the adaptability of *Yersinia enterocolitica* bacteria and *Salmonella typhi* bacteria shows a result of 0.555 and this shows a strong and positive relationship.

*Yersinia enterocolitica* is a microorganism that causes gastrointestinal infections, so the data findings in this study prove that *Arcangelisia flava* L. Merr is effective when used as a gastrointestinal antibacterial and for diarrhea. Diarrhea occurs when there is a disruption in the normal function of the digestive tract, which can be caused by various factors. Rokhmah et al. (2022) that one of the primary causes of diarrhea is bacterial or viral infection that enters the body and disrupts the normal balance of bacteria in the intestines. Some bacteria, such as *Escherichia coli* and *Yersinia enterocolitica*, can produce toxins that damage the intestinal cells and increase fluid secretion into the intestines, leading to diarrhea. Additionally, infections or irritations in the intestines can also alter the activity and sensitivity of...
receptors in the intestinal wall, triggering changes in intestinal movement and fluid secretion. As a result, the body loses important fluids and electrolytes such as sodium, potassium, and chloride, which can lead to dehydration and serious electrolyte imbalances (Guarino et al., 2014; Gawronska et al., 2022).

Furthermore, the inflammatory response that occurs in response to intestinal infections or irritations can exacerbate diarrhea symptoms and make the intestines more sensitive. These cellular mechanisms highlight the complexity of interactions among various factors that affect the normal function of the digestive tract in causing diarrhea (Viegelmann et al., 2021; Meliyya, 2023). So that it is possible to improve the digestive system and can inhibit the bacteria that cause diarrhea (Rahayu et al., 2021; Athiyyah et al., 2023).

The data obtained is also supported by the Sig value. (2-tailed): Probability value/p value Paired T test: Result = 0.000. These results can be interpreted to mean that there is a difference between the adaptability of Yersinia enterocolitica bacteria and Salmonella typhi bacteria to yellow root extract (Arcangelisia flava L. Merr) because the results obtained have a p value > 0.05.

Based on the results of experiments carried out using a positive control, namely chloramphenicol, against Yersinia enterocolitica, it was found that there was an inhibition zone. Meanwhile, in the negative control using distilled water, no inhibition zone was found. This shows that chloramphenicol has antibacterial power against Yersinia enterocolitica, while distilled water does not have antibacterial power against Yersinia enterocolitica. In contrast to the administration of yellow root extract (Arcangelisia flava L. Merr) at concentrations of 30% and 40%, it has a fairly strong inhibition zone or can be said to be in the medium group in inhibiting the growth of Yersinia enterocolitica. A large inhibitory zone or it could be said to be a strong group inhibiting the growth of Yersinia enterocolitica is found at a concentration of 50%.

Kassa et al. (2022) that included in the cellular mechanisms of diarrhea are factors such as changes in the composition of gut microbiota, allergic reactions to certain foods, consumption of certain medications like antibiotics that disrupt gut bacteria balance, and disturbances in the immune system such as irritable bowel syndrome or autoimmune diseases affecting the digestive tract. All of these factors can lead to imbalances in the digestive process and fluid secretion in the intestines, ultimately resulting in diarrhea (Markey et al., 2019). It was concluded that the tiny group prevented the growth of small bacteria in preventing the growth, of Yersinia enterocolitica because there was a shrinking of the inhibition zone at a concentration of 60%. Therefore, it may be inferred that yellow root extract (Arcangelisia flava L. Merr) at a 50% concentration is more efficient at Yersinia enterocolitica growth inhibition.

Based on the outcomes of tests performed using chloramphenicol as a positive control against Salmonella typhi bacteria, it was discovered that there was an inhibitory zone. In contrast, there was no inhibitory zone in the distilled water negative control.
This demonstrates that distilled water lacks antibacterial properties when it comes to *Salmonella typhi*, although chloramphenicol does. Contrary to yellow root extract (*Arcangelisia flava* L. Merr) given at concentrations of 40% and 50%, it has a rather significant inhibition zone or may be stated to be in the medium group in terms of preventing the development of *Salmonella typhi* bacteria. At a concentration of 30%, a sizable inhibitory zone, or what might be called a potent group, is observed that inhibits the growth of *Salmonella typhi* bacteria. It is believed that the tiny group inhibits the growth of small bacteria, reducing the growth of *Salmonella typhi* bacteria because the inhibition zone diminishes at a concentration of 60%. In light of this, it can be said that yellow root extract (*Arcangelisia flava* L. Merr) at a 30% concentration is more effective at preventing the growth of *Salmonella typhi* bacteria.

**CONCLUSIONS AND SUGGESTIONS**

Yellow root extract (*Arcangelisia flava* L. Merr) is effective at preventing the growth of *Salmonella typhi* and *Yersinia enterocolitica*. The amount of yellow root extract (*Arcangelisia flava* L. Merr) that is most successful at preventing the growth of *Yersinia enterocolitica* bacteria is 50%, whereas the amount that is most effective at preventing the growth of *Salmonella typhi* bacteria is 30%. Different levels of adaptation to yellow root extract (*Arcangelisia flava* L. Merr) were found in the two bacteria studied. *Yersinia enterocolitica* is less adaptable than the *Salmonella typhi* bacterium. The fact that the inhibitory zone created is less than that of *Yersinia enterocolitica* serves as evidence for this.

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