



Differences in Inhibitory Effectiveness of Salam Leaf Extract (*Syzygium Polyanthtalam*) and Secang Wood Extract (*Caesalpinia sappan L.*) Against the Growth of Escherichia Coli Bacteria

Nur Patria Tjahjani^{1*}, Candra Aini Roro Rias²

^{1,2}Akademi Kesehatan 17 Agustus 1945, Semarang, Indonesia

Email: nurpatriacahyani@gmail.com

KEYWORDS

Escherichia coli, Bay Leaf Extract, Secang Wood Extract

ABSTRACT

Bay leaves (*Syzygium polyanthum*) and a second wood (*Caesalpinia sappan L.*) are widely known to the public as natural antiseptics for prevention and treatment, including treating digesting diseases, one of which is diarrhea caused by *Escherichia coli*. Bay leaves and Secang wood can be used as antiseptics because they contain the active substance tannin. This research aims to determine the difference in the antibacterial effectiveness of 96% ethanol extract of bay leaves (*Syzygium polyanthum*) and 96% ethanol extract of second wood (*Caesalpinia sappan L.*) on growth *Escherichia coli* bacteria. The type of research is experimental. The method used in this research is the Kirby-Bauer diffusion method. This method observes the clear zone produced from a blank disk that has been treated with bay leaf extract and second wood extract at a concentration of 30%, 60%, and 90% for incubation at 37°C for 24 hours. From the research results, it can be concluded that the ethanol extract of bay leaves with a concentration of 30%, 60%, and 90% with an inhibition zone diameter of 0 mm, 7,6 mm, and 9,6 mm. The second wood at a concentration of 30%, 60%, and 90% with an inhibition zone diameter of 10 mm, 11,3 mm, and 14 mm. The statistical test shows that the data is normally distributed with a parametric test ANOVA, $p = 0,00$, which means that there is no significant difference in the diameter of the inhibition zone between the ethanol extract of bay leaves and the ethanol extract of second wood.

DOI: 10.58860/ijsh.v3i10.257

Corresponding Author: Nur Patria Tjahjani*

Email: nurpatriacahyani@gmail.com

INTRODUCTION

Diarrhea is a clinical condition characterized by increased frequency and decreased stool consistency when compared to normal individuals. In patients with diarrhea, the frequency of defecation occurs more than three times within 24 hours with a mushy or liquid stool consistency (Iskandar & Mustarichie, 2019). Diarrhea can be caused by bacterial, viral, and parasitic infections. The second most common cause of diarrhea after rotavirus is viral infections that cause inflammation in the gastrointestinal tract (Abdel-Monem et al., 2014).

Along with the development of science and technology, people now prefer to utilize natural remedies rather than chemical-based remedies. The utilization of plants as medicinal plants is chosen because these materials have relatively low side effects. In addition, medicinal plants are much more affordable than chemical treatment.

Indonesia is rich in traditional medicinal plants. Traditional medicinal plants are known or believed to have properties as raw materials for traditional medicine. One of the traditional medicinal

plants that is often used by the community is bay leaf. This bay leaf can also be used as an antibacterial for diarrhea, hypertension, diabetes, and gout. Bay leaves contain substances that are efficacious as antimicrobials, namely tannins, essential oils, flavonoids, alkaloids, and saponins (Anand et al., 2020).

Sappan wood is one of the plants that is currently not fully utilized by the community. Sappan wood has properties in treating several diseases. One of them is antidiarrheal (Alvin et al., 2014). The chemical contents of second wood are tannins, gallic acid and others (Alvin et al., 2014).

Escherichia coli is a rod-shaped, gram-negative bacterium that is a normal inhabitant in the digestive tract of humans and animals. However, *Escherichia coli* is also one of the bacteria that cause diarrhea (Bouzari et al., 2018). *Escherichia coli* is the bacteria that causes diarrhea with the highest percentage (Gomes et al., 2016). Maceration is a technique used to extract or take the desired compounds from a solution or solid by soaking the material to be extracted (Manousi et al., 2019).

On sappan wood extract granules (*Caesalpinia sappan L.*) and temu mango rhizomes (*Curcuma mango Val & Zijp.*) as antibacterial shows sappan wood extract granules with concentrations of 12.5%, 25%, 50% and 100% can inhibit *Escherichia coli*, *Salmonella sp* and *Shigella sp* bacteria. Research by Andri Wahyu Saputri, et al (2022) on the Antimicrobial Test of Salam Leaf Extract (*Syzygium polyanthum*) Against the Growth of *Escherichia coli* Bacteria shows bay leaf extract with concentrations of 25%, 50%, 75% and 100% can inhibit the growth of *Escherichia coli* bacteria.

Based on the description above, the researcher is interested in conducting research on differences in the inhibitory concentration of ethanol extracts of bay leaves and second wood against the growth of *Escherichia coli* bacteria at concentrations of 30%, 60%, and 90%.

This study aims to address the gap in research by comparing the inhibitory concentrations of ethanol extracts of bay leaves and sappan wood on *Escherichia coli* at specific concentrations of 30%, 60%, and 90%. This comparative analysis provides insight into the efficacy of these traditional plants at different extract concentrations, contributing novel findings on optimal concentrations for antibacterial activity. This research could expand options for affordable, plant-based diarrhea treatments, enhancing the scientific basis for traditional Indonesian medicine.

METHOD

This study is an experimental study to see the difference in each concentration of ethanol extract of bay leaves (*Syzygium polyanthum*) and ethanol extract of second wood (*Caesalpinia sappan L.*) against the growth of *Escherichia coli* bacteria. This research was conducted at the Chemistry Laboratory and Microbiology Laboratory of the Health Academy on 17 August 1945 Semarang. Bay leaves, and sappan wood are sold at Dami Sariwana Herbal and Simplisia Store. Bay leaves, and secang wood that have become 600 grams of simplisia are sold at Toko Jamu and Simplisia Dami Sariwana.

The data collected are primary data obtained from the measurement of the inhibition zone and each concentration of bay leaf extract and secang wood with the Kirby-Bauer disc method against *Escherichia coli* bacteria in the laboratory.

Data Analysis

Data from the study of the effect of bay leaf extract and secang wood on *Escherichia coli* were analyzed using the SPSS program to see if there were significant differences in the effectiveness of each test disc containing negative control, various concentrations of bay leaf extract, secang wood and positive control in inhibiting the growth of *Escherichia coli*.

From the test results, data is obtained, which is made with a table diagram, and if the data obtained is normally distributed, then a statistical test is carried out with the ANOVA test and continued with the T-test. If the data is not normally distributed, then use a non-parametric test, namely the Mann-Whitney test.

RESULT AND DISCUSSION

The results of maceration of bay leaves (*Syzygium polyanthum*) and secang wood (*Caesalpinia sappan L.*) with 96% ethanol solution obtained yields the results can be seen in the table below:

Table 1.

Extract Weighing Results and Yield Salam Leaf and Secang Wood Using 96% Ethanol Solvent

Simplisia Name	Simplisia Weight (gram)	Extract Weight (gram)	Yield Value (%)
Bay Leaves	300,0	27,09	9,03
Secang Wood	300,0	25,33	8,44

The test results on the activity test of 96% ethanol extract of bay leaves (*Syzygium polyanthum*) and 96% ethanol extract of secang wood (*Caesalpinia sappan L.*) against the growth of *Escherichia coli* bacteria that have been grown in Petri dishes using Mueller Hinton Agar media apparently show different abilities in each concentration given can be seen in the table below:

Table 2.

Results of Measurement of Inhibition Zone Diameter of 96% Ethanol Extract of Salam Leaf Against *Escherichia coli* Bacteria

Extract Type	Concentration (%)	Inhibition Zone Diameter (mm)
Bay Leaves	30	0,00
	60	7,60
	90	9,60
	Control +	25,00
	Control -	0

Table 3.

Measurement Result of Inhibition Zone Diameter of 96% Ethanol Extract of Secang Wood Against *Escherichia coli* Bacteria

Extract Type	Concentration (%)	Inhibition Zone Diameter (mm)
Secang Wood	30	10,00
	60	11,30
	90	14,00
	Control +	25,00
	Control -	0

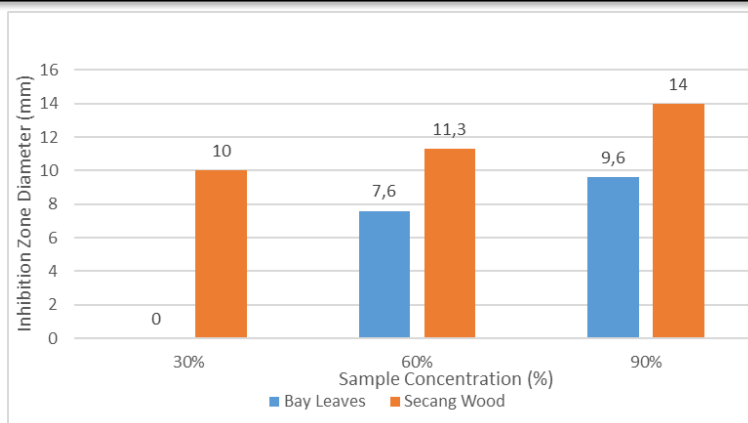


Diagram of Measurement Results of Inhibition Zone Diameter of Salam Leaf and Secang Wood

Data Analysis

Research data in the form of concentrations and differences in bay leaves and secang wood against *Escherichia coli* growth were analyzed descriptively using average values, then presented in tables. Furthermore, it was analyzed statistically, and it was shown that the data was normally distributed using the Saphiro-Wilk test, which was continued with the ANOVA test. The significance value in this study uses the variables analyzed at a value of $p > 0.05$.

The results of the ANOVA test on the three concentration groups of bay leaf ethanol extract and second wood ethanol extract against *Escherichia coli* bacterial growth showed no difference in the inhibition zone between bay leaf ethanol extract and second wood ethanol extract against *Escherichia coli* bacterial growth with the analyzed significance value of $p = 0.00 < 0.05$.

Hypothesis

H₀: There is no difference in the zone of inhibition between the ethanol extract of bay leaves and ethanol extract of secang wood against the growth of *Escherichia coli* bacteria.

H₁: There is a difference in the inhibition zone between the ethanol extract of bay leaves and ethanol extract of secang wood against the growth of *Escherichia coli* bacteria.

The results of the study using the Kirby-Bauer Diffusion method showed that 96% ethanol extract of secang wood proved to be stronger in inhibiting the growth of *Escherichia coli* bacteria than 96% ethanol extract of bay leaves in various concentrations, which can be seen in Figure 3 Diagram of the Inhibition Zone Diameter Measurement Results of Bay Leaves and Secang Wood.

The greater the concentration, the greater the inhibition against the growth of *Escherichia coli* bacteria. Bay leaf extracts with concentrations of 30%, 60%, and 90% with inhibition zone diameters of 0 mm, 7.6 mm, and 9.6 mm, while sappan wood at concentrations of 30%, 60% and 90% with inhibition zone diameters of 10 mm; 11.3 mm and 14 mm indicate that both bay leaf extracts and sappan wood have antibacterial abilities but there are differences in the inhibition zones produced in bay leaf extracts with a concentration of 30% cannot inhibit *Escherichia coli* bacteria because there is no clear zone around the paper disk.

There is a difference in the ability to inhibit the growth of *Escherichia coli* bacteria between young bay leaves and old bay leaves. The results of research by Mufti Kharismawati, et al. (Hidayati et al., 2023) stated that the levels of tannin phytochemical compounds contained in young bay leaves and old bay leaves are 1.51% and 0.29%.

Research conducted by Andri Wahyu Saputri et al. (2022) using bay leaves was able to inhibit *Escherichia coli* bacteria at concentrations of 25% and 50% with inhibition zone diameters of 4 mm & 7 mm, while in this study, at a concentration of 30%, no inhibition zone was formed around the disk.

Bay leaf samples taken randomly may affect the results of this study. Internal and external factors can influence the phytochemical content of a plant. Internal factors such as genes and external factors such as light, temperature, humidity, pH, nutrient content in the soil and altitude (Permatasari et al., 2015).

Stated that bay leaves contain tannin, alkaloid, flavonoid, and saponin compounds that can inhibit *Escherichia coli* bacteria. Research conducted by Indrias Cahyayuliani, et al. (2019) states that bay leaf decoction can inhibit diarrhea.

The phytochemical screening of bay leaves conducted by Arum Samudra (2014) contains tannins, flavonoids, alkaloids, saponins, and triterpenoids. Phytochemical screening research of secang wood conducted contains tannins, flavonoids, phenolics, and triterpenoids.

Tannins are active secondary metabolite compounds that are known to have several properties, including antidiarrheal, astringent, antibacterial, and antioxidant (Ucella-Filho et al., 2022). Bay leaves and secang wood contain tannins that can inhibit the growth of Gram-negative bacteria such as *Escherichia coli*.

This research is in line with research conducted, which proves that secang wood extract (*Caesalpinia sappan* L.) is able to inhibit the growth of *Escherichia coli* bacteria at a concentration of 12.5%; 25%; 50% and 100% with an inhibition zone diameter of 8 mm; 8 mm; 13.3 mm and 16.2 mm. The results of Susanto's research, stated that the content of tannin phytochemical compounds in ethanol extract of secang wood was 10.42%, and in water extract of secang wood was 5.95%.

Sappan wood plants have the chemical content of tannins, brazilin, alkaloids, saponins, flavonoids, phenyl propane, and terpenoids. Sappan wood contains phenolic compounds such as flavonoids, which have free radical-catching antioxidant activity. Anthocyanins are one of the flavonoid groups in secang wood. Anthocyanin compounds are compounds that are good for health because they have antioxidant activity (Trinovani et al., 2020).

Escherichia coli is an opportunistic germ that is widely found in the human colon, and as a normal colon flora, the nature of *Escherichia coli* can cause primary infection in the colon so that it can cause diarrheal disease (Halijah et al., 2021). In the cell wall of gram-negative bacteria (*Escherichia coli*), the peptide chain is arranged irregularly and not tightly between one glycan chain with another so that the ethanol extract of bay leaves and secang wood will more easily penetrate the cell wall of *Escherichia coli* bacteria. The difference in osmotic pressure inside and outside the cell causes nutrients that should be needed by the cell to come out. Lack of nutrients blocks cell wall formation, which causes cell death.

The active compounds in bay leaves, besides tannins, also have flavonoids as antibacterial active compounds with the mechanism of action of forming complex compounds with extracellular and soluble proteins so as to damage the bacterial cell membrane, followed by the release of intracellular compounds. The structure of the cell wall and bacterial cytoplasmic membrane becomes unstable because the structure of the bacterial cell protein is damaged. As a result, the cell permeability function is disrupted, and the bacterial cell will experience lysis, which results in the death of the bacterial cell.

Sappan wood contains saponins that increase membrane permeability, which causes cell hemolysis; if saponins interact with bacterial cells, the bacteria will be lysed (Ardiana et al., 2015).

This study uses a simple extraction method, namely maceration using simplisia made by soaking because this process does not use the heating stage, which causes the active substance content to be damaged, simple equipment, costs are not too expensive and easy to work on. In principle, extraction is to dissolve and withdraw compounds using the right solvent (Sumarni et al., 2022). The solvent used is 96% ethanol, the purpose of using ethanol is to remove the active substances in the simplisia and prevent the simplisia from being overgrown with other bacteria during the soaking process.

Maceration of bay leaves and secang wood using 96% ethanol. Ethanol 96% has a boiling point of 78.4°C; ethanol has the ability to distill with a wide polarity ranging from non-polar to polar

compounds. The selection of 96% ethanol solvent is based on the compounds to be taken. Ethanol is also non-toxic and harmless, and it has a high polarity, so it is easy to dissolve resin compounds, fats, oils, fatty acids, carbohydrates and other organic compounds (Cañadas et al., 2020).

The extract obtained is concentrated with a water bath until a thick extract is obtained, which is greenish brown in bay leaves and reddish brown in secang wood. Then, the yield calculation is carried out so that the average percent yield is obtained, namely for bay leaves at 9.03% and the average percent yield of secang wood is 8.44%, it can be seen in Table 4 that the percentage of yield indicates the maximization of the solvent used to extract. The higher yield value produced indicates the value of the extract produced more and more (2018). The yield of an extract can be influenced by several factors, one of which is the extraction method used.

The method of determining the inhibition zone used in this study is the Kirby-Bauer disc diffusion method. This method uses filter paper discs containing antibiotics with certain levels placed on agar plates planted with bacteria to be examined and then incubated. There is a zone of inhibition around the antibiotic disk, so the bacteria examined are sensitive to the antibiotic.

From the results of direct observation, the inhibition test of ethanol extract from bay leaves and ethanol extract from secang wood showed a different inhibition zone, namely the inhibition zone of secang wood is greater than bay leaves, but both can inhibit the growth of *Escherichia coli* bacteria.

Statistical tests showed that the data were normally distributed with parametric tests, namely ANOVA Test $p = 0.00$, which means that there was no significant difference in the diameter of the inhibition zone between bay leaf ethanol extract and secang wood ethanol extract. Bay leaf ethanol extract is able to inhibit *Escherichia coli* bacteria at a concentration of 60%, while secang wood ethanol extract at a concentration of 30% is able to inhibit *Escherichia coli* bacteria.

So far, people only know diarrhea capsules derived from guava leaves. This research can be developed into a diarrhea medicine capsule preparation derived from ethanol extracts of bay leaves and secang wood made into dry granules as in the original research.

CONCLUSION

The diameter of the inhibition zone of 96% ethanol extract of bay leaves at a concentration of 30% was 0 mm; 60% was 7.60 mm, and at a concentration of 90% was 9.60 mm. The diameter of the inhibition zone of 96% ethanol extract of secang wood at 30% concentration was 10.00 mm; 60% was 11.30 mm, and at 90% concentration was 14.00 mm. There is no significant difference in the effectiveness of 96% ethanol extract of bay leaves and 96% ethanol extract of secang wood against *Escherichia coli* bacteria based on the diameter of the inhibition zone.

REFERENCES

- Abdel-Monem, M. O., Mohamed, E. A., Awad, E. T., Ramadan, A.-H. M., & Mahmoud, H. A. (2014). *Multiplex PCR as emerging technique for diagnosis of enterotoxigenic E. coli isolates from pediatric watery diarrhea*.
- Alvin, A., Miller, K. I., & Neilan, B. A. (2014). Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. *Microbiological Research*, 169(7–8), 483–495. <https://doi.org/10.1016/j.micres.2013.12.009>
- Amelia, P., & Angelina, M. (2014). *Karakterisasi ekstrak etanol daun salam (Syzygium polyanthum Wight) dari tiga tempat tumbuh di indonesia*.
- Anand, U., Nandy, S., Mundhra, A., Das, N., Pandey, D. K., & Dey, A. (2020). A review on antimicrobial botanicals, phytochemicals and natural resistance modifying agents from Apocynaceae family: Possible therapeutic approaches against multidrug resistance in pathogenic

-
- microorganisms. *Drug Resistance Updates*, 51, 100695. <https://doi.org/10.1016/j.drug.2020.100695>
- Ardiana, T., Kusuma, A. R. P., & Firdausy, M. D. (2015). Efektivitas pemberian gel binahong (*Anredera cordifolia*) 5% terhadap jumlah sel fibroblast pada soket pasca pencabutan gigi marmut (*Cavia cobaya*). *ODONTO Dental Journal*, 2(1).
- A'Yun, N. Q., Sari, N. P., & Putra, R. S. (2019). The effect of Salam leaf (*Syzygium polyanthum* Wight) decoction to reduce uric acid levels in humans' blood: An attempt to globalize traditional medicine. *Proceeding Book 7th Asian Academic Society International Conference*, 253–256.
- Bouzari, S., Farhang, E., Hosseini, S. M., & Alikhani, M. Y. (2018). Prevalence and antimicrobial resistance of shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli* isolated from patients with acute diarrhea. *Iranian Journal of Microbiology*, 10(3), 151.
- Cañadas, R., González-Miquel, M., González, E. J., Díaz, I., & Rodríguez, M. (2020). Overview of neoteric solvents as extractants in food industry: A focus on phenolic compounds separation from liquid streams. *Food Research International*, 136, 109558. <https://doi.org/10.1016/j.foodres.2020.109558>
- Gomes, T. A. T., Elias, W. P., Scaletsky, I. C. A., Guth, B. E. C., Rodrigues, J. F., Piazza, R. M. F., Ferreira, L., & Martinez, M. B. (2016). Diarrheagenic *Escherichia coli*. *Brazilian Journal of Microbiology*, 47, 3–30.
- Halijah, H., Mutiah, H., & Syam, N. F. (2021). Anti-Bacterial Effectiveness Test of Basil Leaf Extract (*Ocimum basilicum* L.) Against *Escherichia coli*. *Journal of Biological Science and Education*, 3(1), 38–46.
- Herrero, M., & Ibañez, E. (2018). Green extraction processes, biorefineries and sustainability: Recovery of high added-value products from natural sources. *The Journal of Supercritical Fluids*, 134, 252–259. <https://doi.org/10.1016/j.supflu.2017.12.002>
- Hidayati, D. N., Safitri, E. I., Yumni, G. G., Salsabiila, I., & Rahayu, P. (2023). Cytotoxic Activity of *Eugenia polyantha* Wight Young Leaves Purification Extract and Fraction on T47D. *Jurnal Kefarmasian Indonesia*, 131–139.
- Iskandar, Y., & Mustarichie, R. (2019). Determination of quality standards parameters of kemangi (*Ocimum basilicum* L.) leaves extract from three locations. *Drug Invention Today*, 12(2), 374–379.
- Manousi, N., Sarakatsianos, I., & Samanidou, V. (2019). Extraction Techniques of Phenolic Compounds and Other Bioactive Compounds From Medicinal and Aromatic Plants. In *Engineering Tools in the Beverage Industry* (pp. 283–314). Elsevier. <https://doi.org/10.1016/B978-0-12-815258-4.00010-X>
- Maulana, I. T., Safira, R., Aprianti, I., Syafnir, L., & Abdul, R. (n.d.). Antibacterial Compounds Of *Streptococcus Mutans* And *Shigella Dysenteriae* In *Eucheuma Spinosum* From Tasikmalaya, West Java Screened With Tlc-Bioautography. *Welcoming Speech*, 33, 9.
- Permatasari, A., Sugiyarto, S., Marsusi, M., & Hailu, H. W. (2015). Transplantation of carica (*Vasconcellea pubescens*) at various altitudes of Mount Lawu, Central Java with treatment of shade and different types of fertilizers. *Nusantara Bioscience*, 7(1).
- Sirait, N. R., Sitohang, R., Sinaga, A. B., & Sari, M. (2022). Uji Aktivitas Ekstrak Etanol Daun Salam (*Syzygium polyanthum*) Terhadap Pertumbuhan Bakteri Methicilin-Resistant *Staphylococcus aureus*. *Jurnal Teknologi Kesehatan Dan Ilmu Sosial (TEKESNOS)*, 4(1), 425–430.
- Sumarni, W., Sudarmin, S., Sumarti, S. S., & Kadarwati, S. (2022). Indigenous knowledge of Indonesian traditional medicines in science teaching and learning using a science–technology–engineering–mathematics (STEM) approach. *Cultural Studies of Science Education*, 1–44.
-

Trinovani, E., Fauziah, R. N., & Afifah, R. R. (2020). Determination of Antosianin Total Levels and Antioxidant Activities in Black Glutinous Rice Extract and Fermented Black Glutinous Rice Extract. *International Medical Journal*, 25(5).

Ucella-Filho, J. G. M., Freire, A. da S. M., Carréra, J. C., Lucas, F. M. F., Zucolotto, S. M., Dias Júnior, A. F., & Mori, F. A. (2022). Tannin-rich bark extract of plants as a source of antimicrobial bioactive compounds: A bibliometric analysis. *South African Journal of Botany*, 150, 1038–1050. <https://doi.org/10.1016/j.sajb.2022.09.018>



© 2024 by the authors. It was submitted for possible open-access publication under the terms and conditions of the Creative Commons Attribution (CC BY SA) license (<https://creativecommons.org/licenses/by-sa/4.0/>).