



Activity test of antibacters combinations of avocado leaf extract (*persea americana*) and papaya leaf (*carica papaya l.*) against the growth of *eschericia coli atcc 25922*

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ABSTRACT

The purpose of this research is to find out whether extracts from avocado and papaya leaves can suppress the growth of the *Eschericia coli* ATCC 25922 bacterium. This study used a post-test-only control group design, which is typical of genuine experiments. The antibacterial activity of avocado leaf and papaya leaf extracts was tested using the well diffusion method. The concentration ratios of the extracts were 25%:75%, 50%:50%, and 75%:25%. The growth of *Eschericia coli* ATCC 25922 was used as a positive control, and aquadest as a negative control. The inhibition zone data was analyzed using the one way anova test. Both extracts tested positive for flavonoids, alkaloids, tannins, and saponins, according to this study. The average diameter of the inhibition zone was 15.99 mm, 18.44 mm, and 24.36 mm, respectively, when the concentration ratio was 25%:75%, 50%:50%, and 75%: 25%. On average, the positive control group's inhibition zone measured 27.79 mm in diameter. A one-way ANOVA test revealed a significant difference between all treatments and the positive control group ($p=0.000$, $\text{sig}.<0.05$). Findings indicate that a 75%:25% mixture of avocado and papaya leaf extracts, with a very strong category, has the most effective antibacterial action.

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1. Introduction

Microorganisms or microbes are small living organisms or single-celled organisms that can only be observed using a microscope (Rini & Rohmah, 2020). Microorganisms are evenly distributed throughout the hemisphere and are everywhere, namely in the air, water, soil, floor, table, skin, food and everywhere (Ngatirah, 2022). One of the microorganisms around us is bacteria. Bacteria are unicellular prokaryotic microbes, including the Schizomycetes class, reproducing asexually by cell division (Suryani & Taupiqurrahman, 2021). Bacteria can provide benefits to human life and can also be detrimental to human life because they can cause infections (Rini & Rohmah, 2020).

Infection is a disease caused by pathogenic microorganisms that enter and grow in or on the human host (Gerba, 2019). Bacterial infection can occur when there is interaction with microbes so that it can cause damage to the host body and this damage causes various clinical signs and symptoms (Novard et al., 2019). In general, the process of infection begins with the entry of bacteria into the host body in various ways, including the respiratory tract, digestive tract, oral cavity, nails and others. Then

the bacteria attach to the surface of the host cell, then the bacteria begin to multiply themselves and spread throughout the body resulting in damage to tissues and organs (Pratiwi, 2021).

There are various diseases that can be caused by bacterial infections, one of which is diarrhea. Diarrhea is the release of stool with increased frequency, namely three or more times in 24 hours, accompanied by changes in stool consistency to mushy or liquid, with or without blood/mucus in the stool (Wijoyo, 2020). Worldwide, 1.9 million children under the age of five die from diarrhea every year, and there are almost 2 billion bouts of diarrhea, according to WHO and UNICEF. Due to its high morbidity rate, diarrheal illness is a priority eradication initiative in Indonesia. The most recent numbers from the 2020 Indonesian Nutrition Status Survey show that 9.8% of the population suffers from diarrhea. The data from the 2020 Indonesian Health Profile indicate that diarrhea is a serious issue, accounting for 14.5% of the fatalities in the country. Diarrhea was the cause of death in 4.55 percent of the 1259 toddlers in the study (Utami et al., 2020; Ekawati et al., 2022). The most recent numbers available from the East Nusa Tenggara Central Bureau of Statistics (BPS) in 2022 indicate that there were 15,386 instances of diarrhea in the region (Situmorang et al., 2024). One of the bacteria that cause diarrhea is *Escherichia coli* bacteria (Indriani et al., 2022).

Escherichia coli is a gram-negative bacterium that lives in the large intestine of humans and animals, in soil, and in water. This bacterium is included in the Enterobacteriaceae family, which are pathogenic bacteria in the gastrointestinal tract that can cause infectious diseases in the human gastrointestinal tract. Disease transmission can occur through direct contact and usually occurs in places that have poor sanitation and environment. *Escherichia coli* infections often take the form of diarrhea with blood, abdominal spasms, fever, and can sometimes cause kidney problems (Radji, 2021). Treatment can use antibiotics and traditional medicine.

Antibiotics are compounds or substances produced by microorganisms, especially fungi, or produced synthetically that are used to inhibit or can kill other microorganisms without harming the human host. However, currently there are many improper uses of antibiotics that cause antibiotic resistance, namely, bacteria become resistant to antibiotics and can also cause unwanted side effects such as nausea, vomiting, abdominal pain and rashes on the skin. The existence of side effects caused so that people prefer medicinal plants as an alternative treatment (Purwaningdyah et al., 2021).

Avocado plants are one type of plant that is used by the people of East Nusa Tenggara as a traditional medicine to overcome constipation, cure inflammation, maintain endurance and also diabetes mellitus (Kopon et al., 2020). In the research of Indriani et al. (2022), it has been proven that the results of phytochemical screening of avocado leaves contain alkaloid, tannin, flavonoid and saponin compounds. Avocado leaves (*Persea Americana*) have efficacy as antibacterial against *Escherichia coli* bacteria at concentrations of 75%, 100%, 200% which successively produce inhibition zones of 21 mm, 24 mm, and 25 mm (Indriani et al., 2022).

Apart from avocados, papaya plants have also long been used as herbal medicine because they are believed to have properties for the treatment of malaria, fever reduction, appetite enhancers improve the digestive tract and can inhibit bacterial growth. Several studies have shown that papaya leaf extract has antibacterial activity against gram-negative and gram-positive bacteria and the results of phytochemical analysis of papaya leaves contain alkaloids, saponins, flavonoids and tannins (Nor et al., 2018; Karisma, 2019). Papaya leaves (*Carica papaya L.*) have antibacterial properties against *Escherichia coli* bacteria at concentrations of 100%, 75%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56% which successively produce inhibition zones of 16 mm, 13.33 mm, 11.66 mm, 10.66 mm, 10.33 mm, 9.66 mm, 9.33 mm, 7 mm (Nor et al., 2018).

Research on the synergistic effect between avocado leaf and papaya leaf extracts on antibacterial activity is limited. However, a study showed that a combination of ethanol extracts of avocado leaves and mint leaves (*Mentha piperita*) had an antibacterial effect against *Propionibacterium acnes*, although the activity was not higher than the single extract of mint leaves (Alta et al., 2024). In addition, another study reported that a combination of medicinal plant extracts, including papaya leaves, with the antibiotic cefotaxime showed increased antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria (Arifin et al., 2016).

The increasing resistance of *Escherichia coli* to antibiotics has heightened the urgency of researching antibacterial alternatives from natural materials. The widespread and often indiscriminate use of antibiotics has led to the emergence of multidrug-resistant (MDR) *E. coli* strains, limiting treatment options and increasing the risk of severe infections, particularly in healthcare settings. This resistance threatens public health by reducing the efficacy of standard antibiotics, leading to prolonged illness, higher medical costs, and increased mortality rates.

Researchers are interested in determining if a combination of ethanol extracts from avocado (*Persea americana*) and papaya (*Carica papaya* L.) leaves can inhibit the growth of *Escherichia coli* bacteria. The concentrations of the two extracts are 25%:75%, 50%:50%, and 75%:25%, respectively, based on the description above.

2. Methods

This research method was designed to preserve the active compounds in avocado (*Persea americana*) and papaya (*Carica papaya*) leaves using a controlled extraction process. The extraction used 70% ethanol, a widely used solvent that efficiently extracts polar and non-polar secondary metabolites while minimizing compound degradation. The authenticity of the test samples was verified through plant determination at the Plant Taxonomy Laboratory, FMIPA UNPAD. To maintain the integrity of bioactive compounds, the rotary evaporator was set at 40°C, a temperature that prevents thermal degradation of heat-sensitive phytochemicals such as flavonoids and alkaloids. The extraction process included maceration for three days, allowing optimal diffusion of secondary metabolites without excessive oxidation. Phytochemical screening was performed to confirm the presence of key bioactive compounds, including alkaloids, flavonoids, saponins, and tannins, to ensure that the extracted materials retained antibacterial potential. For the antibacterial activity test, all laboratory tools were sterilized using an autoclave, and bacterial rejuvenation was performed using NA medium. *Escherichia coli* ATCC 25922 was identified through Gram staining, and standardization was done through the preparation of McFarland solution and bacterial suspension, which were then isolated using the pour plate technique. The pitting method was used to measure the zone of bacterial inhibition, to ensure consistency in antibacterial testing. Data analysis using Analysis of Variance (ANOVA) showed that the combination of avocado and papaya leaf extracts exhibited superior antibacterial activity against *Escherichia coli* ATCC 25922 compared to the control group, confirming the effectiveness of the extraction method in preserving and utilizing active compounds for antibacterial applications. Analysis of Variance (ANOVA), ascertains significant differences between treatment and control groups by systematically comparing within- and between-group variances. In the context of this study, ANOVA helped determine whether the antibacterial activity of various concentrations of papaya (*Carica papaya*) and avocado (*Persea americana*) leaf extracts resulted in a statistically significant difference in the diameter of the inhibition zone compared to the control group.

3. Results and Discussion

Plant Determination

The findings from the analysis performed at Herbarium Jatinagor, Plant Taxonomy Laboratory, Department of Biology FMIPA UNPAD with letter No. 46/HB/09/2024 showed that the plants used in this study were avocados (*Persea americana*). The findings from the analysis performed at Herbarium Jatinagor, Plant Taxonomy Laboratory, Department of Biology FMIPA UNPAD with letter No. 46/HB/09/2024 showed that the plants the papaya (*Carica papaya* L.) fruit was utilized in this research.

Extract Yield

First, avocado leaf yield calculation the results of the calculation of the yield of ethanol extract of avocado leaves (*Persea americana*) can be seen in Table 1.

Table 1.
Yield Calculation Result of Leaf Extract Avocado

| Powder weight (g) | Weight of condensed extract (g) | Yield (%) |
|-------------------|---------------------------------|-----------|
| 100 | 37,1 | 37,1% |

Based on Table 1, the extraction of 100 grams of avocado leaf powder produced 37.1 grams of thick extract, with a yield of 37.1%. Additionally, Table 2 displays the outcomes of the yield calculation for the ethanol extract of papaya leaves (*Carica papaya* L.).

Table 2.
Yield Calculation Result of Leaf Extract Papaya

| Powder weight (g) | Weight of condensed extract (g) | Yield (%) |
|-------------------|---------------------------------|-----------|
| 100 | 22,1 | 22,1% |

Based on Table 2, the extraction of 100 grams of papaya leaf powder produced 22.1 grams of thick extract, with a yield of 22.1%.

Phytochemical Test Results

Both the papaya and avocado leaf extracts were positive for alkaloid, flavonoid, tannin, and saponin components in the phytochemical analysis. The results can be seen in table 3 below:

Table 3.
Phytochemical Content Identification Results Avocado Leaf Extract

| No. | Identification | Results | Conclusion |
|-----|----------------|---|---|
| 1 | Alkaloid | Formation of a white to yellowish precipitate. Formation of brown precipitate. | Alkaloid positive Positive for alkaloids |
| 2 | Saponin | Formation of a fairly permanent foam. | Positive for saponins |
| 3 | Tanin | Formation of greenish black color. | Positive for tannins |
| 4 | Flavonoid | Formation of orange color. | Flavonoids positive |

The results of the phytochemical test of avocado leaf extract showed that this extract contains alkaloids, saponins, tannins, and flavonoids. The presence of alkaloids was characterized by the formation of a white to yellowish precipitate and a brown precipitate, while saponins were identified through the appearance of a fairly permanent foam. The tannin test produced a greenish black color, and flavonoids were detected by changing the color to orange. The content of these active compounds indicates that avocado leaf extract has pharmacological potential that can be further developed in the health sector.

Table 4.
Phytochemical Content Identification Results Papaya Leaf Extract

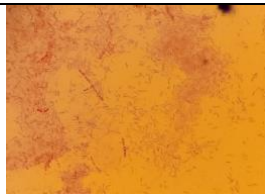
| No. | Identification | Results | Conclusion |
|-----|----------------|---|---|
| 1 | Alkaloid | Formed a white to yellowish precipitate. Reddish brown precipitate formed. | Alkaloid positive Positive for alkaloids |
| 2 | Saponin | Formed a fairly permanent foam. | Positive for saponins |
| 3 | Tanin | Formed blackish green color. | Positive for tannins |
| 4 | Flavonoid | Formed red color. | Flavonoids positive |

The results of the phytochemical test of papaya leaf extract show that this extract contains alkaloids, saponins, tannins, and flavonoids. The alkaloid content was identified through the formation of a white to yellowish precipitate and a reddish brown precipitate. The presence of saponins is indicated by the appearance of a fairly permanent foam, while tannins produce a blackish green color. In addition, flavonoids were detected by a change in color to red. These findings indicate that papaya leaf extract has active compounds that have potential for various pharmacological and health applications.

Antibacterial Activity Test Results

First, the identification of bacteria by Gram stain is shown in Table 5.

Table 5.
Escherichia coli Bacteria Identification Results

| Color | Arrangement | Shape | Image |
|-------|-------------|------------|--|
| Pink | Single | Short Stem |  |

Based on the results of research using microscopy with gram staining techniques, it shows that Escherichia coli bacteria are gram-negative bacteria with characteristics of pink color, single arrangement, and short stem (Trisno et al., 2019). Second, the antibacterial test as seen in figure 1.

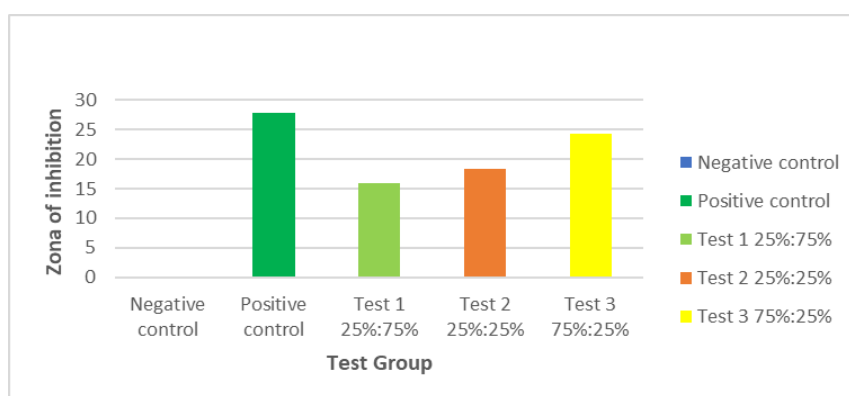


Figure 1. Bar Chart of Average Zone Diameter Inhibition

The following is an analysis of the inhibition zone diameters at different concentrations, as shown in the figure above: at a 25%:75% mixture of avocado leaf extract and papaya leaves, the average is 15.99 mm, which falls into the strong category; at a 50%:50% mixture, the average is 18.44 mm, also fitting the strong category; and at a 75%:25% mixture, the average is 24.36 mm, placing it in the very strong category.

The study's findings show that the biggest inhibitory zone diameter, 24.36 mm, is produced by a combination of 75% papaya leaf extract and 25% avocado leaf extract. Since the diameter of the inhibition zone widened with increasing concentrations of avocado leaf extract in the combination, this indicates that avocado leaf extract is more dominant in suppressing the growth of Escherichia coli bacteria. Because the diameter of the inhibitory zone formed by a single extract of papaya leaf is smaller than that of avocado leaf, increasing the concentration of papaya leaf extract enhances its efficacy in inhibiting the growth of Escherichia coli bacteria.

Two subgroups, one receiving no treatment and one receiving treatment, make up the control group. As a solution, a positive control compares the efficacy of typical antibacterial medications with that of test extract solutions. The existence of an inhibition zone is a telltale sign that the procedure is accurate; this is the goal of positive control (Fitriana et al., 2018). A very significant inhibition zone diameter of 27.79 mm was observed in the positive control group, which consists of ciprofloxacin antibiotics. Aquadest without extract is utilized as a negative control in order to ascertain whether or not it possesses antibacterial action. The results demonstrated that there was no inhibition zone provided by the negative control.

It is well-known that the test group using a mixture of ethanol extracts from papaya and avocado leaves had the largest inhibition zone, in comparison to the positive control group. It is believed that alkaloid, saponin, tannin, and flavonoid chemicals are responsible for the formation of the inhibitory

zone around the growth area of *Escherichia coli* bacteria (Nor et al., 2018). Phytochemical analysis has revealed that extracts from papaya and avocado leaves contain alkaloids, flavonoids, tannins, and saponins. When it comes to classes of secondary metabolite chemicals, antibacterial drugs work in different ways. As antibacterials, alkaloids work by blocking DNA replication and the bacterial topoisomerase enzyme. The inability of bacteria to replicate their DNA is the result of DNA replication inhibition (Gayatri et al., 2021).

According to Nor et al. (2018), flavonoids work as antibacterials by denaturing proteins. This disrupts the metabolic process of bacteria, leading to lysis and ultimately their death. Flavonoids have the ability to hinder bacterial metabolism by blocking the enzyme cytochrome C reductase, which in turn prevents the creation of metabolism (Hasanah & Ayu, 2020, Huda et al., 2019).

As an antimicrobial, tannins can bind to bacterial cells, trigger enzymes, and obstruct protein transport in the cell membrane (Rifal et al., 2024). The imperfection of cell wall construction is further brought about by tannins' assault on cell wall polypeptides. Bacterial cells are killed when they lyse as a result of physical and osmotic pressures (Hridhya & Kulandhaivel, 2018).

As an antibacterial, saponins work by lowering surface tension, which increases permeability (the capacity for cells to leak out) and allows intracellular chemicals to escape. These chemicals enter the cell through the permeable outer membrane and weak cell wall, where they bind to the inner membrane of the cell and cause its instability. The cell dies as a result of the cytoplasm leaking out (Rifal et al., 2024; Ngajow et al., 2022).

Statistical Analysis Results

In this study in analyzing the diameter of the inhibition zone using the SPSS (Statistical Product and Service Solutions) version 16 program with a confidence level of 95%. The research data will be processed with statistical tests in the form of ANOVA tests, before the ANOVA test will be carried out normality test with the aim of ensuring that the data tested is normally distributed. Normality test using the Shapiro-wilk method shows a probability value of $p > 0.05$. The normality test results show the value of positive control ciprofloxacin, negative control aquadest, test group 1 avocado leaves 25%: papaya leaves 75%, test group 2 avocado leaves 50%: papaya leaves 50%, and test group 3 avocado leaves 75%: papaya leaves 25%, namely the normality value $p > 0.05$. Based on the test data that has been carried out, it can be concluded that the data on the diameter of the inhibition zone of the test and control groups are well distributed. Normally distributed data is one of the requirements of parametrics so that homogeneity and One Way Anova analysis can be carried out (Trisia et al., 2018).

In order to ensure that the sample data set is representative of a homogenous population, the homogeneity test looks for signs that the population's variance is constant. When the probability value $p > 0.05$, we say that the data is homogeneous (Usmadi, 2020). The homogeneity test results show that the inhibition zone diameter data from the test group and control group have a variance that is not significantly different so that it is homogeneous as indicated by the value of $p = 0.092$.

The difference in mean values between two or more sample groups was also determined using the One Way Anova test. The data is considered to have a significant difference in the One Way Anova test if the probability value is less than 0.092.

If the probability value p is less than 0.05, it is not significant. A p -value of 0.00 was displayed by the One Way Anova test. In other words, the test group's *Escherichia coli* bacteria had a noticeably different average diameter of their inhibitory zone. Each test yielded a different inhibition zone, as indicated by the significant value of $p < 0.05$ in the data analysis results (Fauzi, 2023).

There was a statistically significant difference between the positive control group (consisting of 75% extract and 25% water) and the negative control group (consisting of 50% water and 25% extract) in the LSD test. A 25%:75%, 50%:50%, or 75%:25% extract from the positive control group differs significantly from the negative control group. When compared to the 50%:50%, 75%:25%, and negative control groups, the 25%:75% extract stood out significantly. In comparison to the 25%:75% and 75%:25% extract groups, as well as the negative and positive control groups, the 50%:50% extract showed a statistically significant difference. Comparing the 75%:25% extract to the 50%:50% and 25%:75% extract groups revealed a statistically significant difference. A significant value of $p < 0.05$ was observed from the data analysis.

The tukey test shows that there are substantial variances in every group. There are notable distinctions between the 25%:75%, 50%:50%, and 75%:25% extract groups, as well as the negative control and positive control groups.

3. Conclusion

The results demonstrated that the antibacterial activity of the mixture of papaya and avocado leaf extracts was evident in the establishment of inhibitory zones against *Escherichia coli* ATCC 25922. Inhibition zones with average diameters of 15.99 mm, 18.44 mm, and 24.36 mm were generated by the combination with concentrations of 25%:75%, 50%:50%, and 75%:25%, respectively. The most promising concentration as an antibacterial agent was the 75%:25% mix, which effectively inhibited the development of *Escherichia coli* with a maximum inhibition zone diameter of 24.36 mm.

The results of this study provide a strong basis for the development of natural material-based antibacterial drugs for medical and pharmaceutical applications. The demonstrated antibacterial activity of papaya (*Carica papaya*) and avocado (*Persea americana*) leaf extracts against *Escherichia coli* ATCC 25922 suggests its potential as an alternative or complementary treatment to synthetic antibiotics.

To enhance the practical application of these findings, future research should focus on developing pharmaceutical dosage forms that ensure stability and effectiveness of the extracts. Key considerations include optimizing extraction techniques to improve bioavailability, incorporating suitable excipients to enhance solubility, and selecting appropriate delivery systems such as gels, ointments, or encapsulated formulations to maintain sustained antibacterial activity. Additionally, stability studies should be conducted to evaluate the shelf life and degradation kinetics of the bioactive compounds under different storage conditions. Further investigation into synergistic interactions with conventional antibiotics could also provide insights into potential combinatory treatments for bacterial infections.

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