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Antibacterial Activity Test Of Ethanol Extract Of Dadap Serep Leaves (Erythrina Lithosperma Miq) Against The Growth OF Escherichia Coli

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ABSTRACT
Dadap serep leaves (Erythrina lithosperma Miq) are one type of important medicinal plant that is widely used in traditional medicine. This dadap serep leaf plant can be used as a medicine for fever, to facilitate breast milk, stomach ache, diarrhea, and to prevent miscarriage. The chemical compounds contained in dadap serep leaves which function as antibacterial compounds are flavonoids. Escherichia coli bacteria are gram-negative bacteria, and are facultative anaerobes. This study aims to determine the antibacterial activity of dadap serep (Erythrina lithosperma Miq) leaf extract which was dissolved using 70% ethanol solvent. The type of research. The phytochemical screening test uses the maceration method and for the antibacterial activity test uses the agar diffusion method. The results of research conducted on phytochemical screening tests were the content of secondary metabolite compounds in dadap serep leaves, namely saponins, tannins, flavonoids and alkaloids. And the results of research carried out on testing the antibacterial activity of ethanol extract of dadap serep leaves (Erythrina lithosperma Miq) on the growth of Escherichia coli bacteria at concentrations of 5%, 10% and 20%. With an average inhibition zone at a concentration of 5% 10.36 mm, 10% 11.8 mm, and 20% 13.13 mm. Cotrimoxazole as a positive control has an inhibition zone of 20.53 mm, and distilled water as a negative control does not have an inhibition zone.

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INTRODUCTION

Indonesia is one of the world's most biodiverse countries, possessing an extraordinary variety of flora and fauna that hold significant potential for development as raw materials for medicines. According to (Hariani, 2018) approximately 30,000 plant species are found in Indonesia, with 7,000 of them identified as having medicinal benefits. Furthermore, Nugroho (2017)(Nugroho, 2017) states that 2,000 medicinal plant species are distributed across various forest types in Indonesia, 772 of which are found in tropical rainforests. Parts of these plants, such as leaves, roots, stems, fruits, seeds, and sap, are utilized for various medicinal purposes. Around 300 plant species have been used in the production of conventional and

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traditional medicines, highlighting the vast potential of Indonesia's biodiversity for the pharmaceutical industry (Noor, 2023).

One medicinal plant with significant potential but limited public awareness is dadap serep (*Erythrina lithosperma Miq.*), a member of the Papilionaceae family. According to (Revisika, 2011) the bark and leaves of dadap serep are traditionally used to thin mucus, alleviate postpartum fever, control internal bleeding, relieve stomachaches, treat diarrhea, and prevent miscarriage. The plant contains active compounds such as saponins, flavonoids, polyphenols, tannins, and alkaloids, which provide antimicrobial, anti-inflammatory, antipyretic, and antimalarial properties (Desianti, 2007). However, the limited knowledge among Indonesians about the plant's benefits has hindered its broader utilization (Sari & Wijayanti, 2020). Further research into its effectiveness for specific medical conditions is essential to support its wider application in medicine.

One condition that could potentially be treated using medicinal plants like dadap serep is diarrhea. According to (Jap & Widodo, 2021) diarrhea is characterized by loose or watery stools occurring more than three times within 24 hours. This condition is often caused by *Escherichia coli* bacterial infections, which can lead to dehydration due to excessive fluid loss. Common symptoms include vomiting, increased stool frequency, and changes in stool consistency. (Apriani et al., 2022) highlight that diarrhea remains a significant public health concern, particularly in developing countries like Indonesia.

Previous studies have identified the bioactive compounds in dadap serep as having antimicrobial activity against bacteria such as *Escherichia coli*, which causes diarrhea (Parmadi & Pratama, 2020). However, there is a research gap due to limited empirical studies linking the clinical application of dadap serep to the treatment of diarrhea compared to conventional drugs. This study aims to address this gap by providing novelty through an in-depth exploration of the mechanisms of bioactive compounds in dadap serep against *Escherichia coli*. The findings are expected to contribute to the development of phytopharmaceuticals based on local resources for diarrhea treatment, supporting the independence of the national pharmaceutical industry (Zainuddin, 2018). This study aims to determine the antibacterial activity of ethanol extract from dadap serep (*Erythrina lithosperma Miq.*) leaves.

METHODS

The research employed an experimental design conducted in a laboratory setting to assess the effect of dadap serep (*Erythrina lithosperma Miq.*) leaf extract on the growth of *Escherichia coli* bacteria using the well diffusion method. The sampling process began with collecting fresh dadap serep leaves between 6:00 and 7:00 AM WIT in Negeri Seith, Leihitu District, Central Maluku Regency, in June. The leaves were cleaned through wet sorting to remove any remaining debris, washed under running water, chopped, and air-dried. Once dried, the leaves were sorted again, ground into a fine powder, and stored in glass containers for the maceration process.

The extraction process involved macerating 200 grams of powdered dadap serep leaves in 2 liters of 70% ethanol. The mixture was placed in a glass container, sealed, and left

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for three days, with occasional stirring, in a place protected from direct sunlight. After three days, the mixture was filtered using filter paper to separate the residue. The filtrate was then concentrated by evaporation using a water bath to obtain a thick extract. Phytochemical screening was performed to identify the bioactive compounds. The saponin test involved dissolving 1 gram of the extract in distilled water, shaking vertically for one minute, letting it sit for ten minutes, and adding 2N HCl. The presence of foam indicated positive saponin content. For flavonoid testing, 1 gram of extract was mixed with 2 mg of magnesium powder and three drops of concentrated HCl. A yellow coloration indicated the presence of flavonoids. The tannin test used 1 ml of extract mixed with three drops of 1% FeCl3 solution. A dark green color indicated positive tannin content. Alkaloid testing involved mixing 1 gram of extract with three drops of Dragendorff's reagent and 11 drops of H2SO4. The presence of precipitate indicated positive alkaloid content.

The antibacterial activity test used the well diffusion method to evaluate the inhibitory effect of the ethanol extract on *Escherichia coli* growth. Nutrient Agar (NA) media was sterilized and poured into five petri dishes, each containing 20 ml of NA. Once the media solidified, it was inoculated with a bacterial suspension using a pipette. Wells of 6 mm diameter were created in the solidified agar, and 50 µl of the extract at concentrations of 5%, 10%, and 20% were added to the wells. Positive control (cotrimoxazole) and negative control (distilled water) were also added to separate wells. The petri dishes were incubated at 37°C for 24 hours to observe the inhibition zones formed by the extract against *Escherichia coli*.

RESULTS AND DISCUSSION

Phytochemical Screening Test

Table 1. Results of Phytochemical Screening Test on Dadap Serep Leaves (*Erythrina lithosperma Miq.*)

No	Compound	Reagent	Observation	Result
1	Saponins	Aquadest + 2N HCl	Formation of foam	+
2	Flavonoids	Mg powder + HCl	Formation of orange color	+
3	Tannins	1% FeCl3	Formation of dark green color	+
4	Alkaloids	Dragendorff + H2SO4	Formation of brown precipitate	+

Note:

(+) : Presence of chemical compound

(-) : Absence of chemical compound

Based on the phytochemical screening results in Table 1 above, it is confirmed that the ethanol extract of dadap serep (*Erythrina lithosperma Miq.*) contains saponins, flavonoids, tannins, and alkaloids.

Antibacterial Activity Test Results

Table 2. Results of Antibacterial Activity of Ethanol Extract from Dadap Serep Leaves (*Erythrina lithosperma Miq.*) Against *Escherichia coli* Growth

Extract Concentration (%)	Test Results (mm)	Average
	P1	P2

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Extract Concentration (%)	Test Results (mm)	Average
5%	9.8	10.5
10%	11.5	11.9
20%	12.8	13
Positive Control	20.5	20.5
Negative Control	0	0

Note:

Weak: < 5 mm

Moderate: 5-10 mm

Strong: 10-20 mm

Very Strong: > 20 mm

K(+): Positive Control (Cotrimoxazole)

K(-): Negative Control (Distilled Water)

From the results of the antibacterial activity test in Table 2 above, it is observed that the ethanol extract of dadap serep (*Erythrina lithosperma Miq.*) against *Escherichia coli* showed an average inhibition zone of 10.36 mm at a 5% concentration, 11.8 mm at a 10% concentration, and 13.13 mm at a 20% concentration. The positive control, cotrimoxazole, exhibited an inhibition zone of 20.53 mm, while the negative control, distilled water, showed no inhibition zone.

Preparation of Dadap Serep Leaf Extract (Erythrina lithosperma Miq)

This research began with the sample collection process, followed by processing according to the stages of making simplicia. First, after the raw materials were collected, wet sorting was performed to separate undesirable parts of the leaves. Next, the leaves were washed with running water to remove dirt and contaminants. After washing, the leaves were shredded to facilitate the drying process for quicker results. Once shredded, the sample was air-dried to prevent damage. After drying, the sample was blended into a coarse powder to increase the surface area, which accelerates the extraction process. By increasing the surface area, more contact is made between the powder and solvent, facilitating the extraction. After blending, the simplicia was stored in a glass container for the maceration process. The maceration process began with weighing 200 grams of simplicia and adding 2 liters of 70% ethanol solvent to fully submerge the simplicia. The use of 70% ethanol was chosen because it has high polarity, allowing it to extract more substances and kill microbes, while being volatile and inexpensive (Syamsul et al., 2020). The maceration process was carried out for 3×24 hours, with occasional stirring. The purpose of the 3×24-hour maceration was to ensure that the compounds in the herbal material were completely extracted (Fauzana D.L., 2022). The stirring was done to maintain the concentration balance of the materials for quicker extraction in the solvent (Syamsul et al., 2020). After maceration, the mixture was filtered to obtain the filtrate and residue. The filtrate was then evaporated to obtain a concentrated extract. Evaporation was done to ensure that no solvent remained, as it could affect the sample's effectiveness (Faisal, 2018). The resulting concentrated extract from the Dadap Serep leaves (Erythrina lithosperma Miq) weighed 77.70 grams.

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Phytochemical Screening Test

The identification of saponin compounds in this study showed a positive result, indicated by stable foam that lasted for 10 minutes. This is due to the addition of aquadest, as saponin compounds are physically soluble in aquadest and form foam when shaken. The addition of HCl increases the polarity of the saponin compound, which causes a shift in the position of its functional groups, further promoting the formation of foam as a sign of saponin in the extract (Kholidha et al., 2016). As an antibacterial, saponin alters the permeability of bacterial cell walls and damages bacterial tissue, leading to cell lysis and morphological changes (Farida, 2020). Additionally, saponin acts as an anti-diarrheal by stopping intestinal contractions or intestinal motility (Jariah et al., 2022). The identification of flavonoid compounds in this study showed a positive result, as indicated by the yellow color. This is caused by the addition of Mg powder, which bonds with the carbonyl groups of flavonoids, and the addition of concentrated HCl, which hydrolyzes flavonoids into their aglycones by hydrolyzing O-glycosides. If this mixture is reduced, a complex compound forms with yellow, orange, or red colors. The mechanism of action of flavonoids in inhibiting bacterial growth involves damaging bacterial microsomes, lysosomes, and cell walls (Arifin & Ibrahim, 2018). As an anti-diarrheal, flavonoids inhibit or reduce intestinal motility, thus decreasing fluid and electrolyte secretion (Meliala et al., 2020).

The identification of tannin compounds in this study showed a positive result, as indicated by a greenish-black color. This was caused by the addition of FeCl3, which determines if the sample contains phenolic groups. The presence of phenolic groups is indicated by a greenish-black color, confirming the presence of tannins, which are polyphenolic compounds. Tannins work as antibacterial agents by inhibiting the enzymes reverse transcriptase and DNA topoisomerase, preventing bacterial cell (Kristian, 2013). As an anti-diarrheal, tannins act as an astringent, shrinking the intestinal surface and protecting the intestinal mucosa, thus helping to stop diarrhea (Mangalik & Rusdiaman, 2022). The identification of alkaloid compounds in this study showed a positive result, indicated by a yellow to orange color and slight precipitation. This was due to the addition of H2SO4 to extract the alkaloids, which are basic compounds, using an acidic solution. The addition of Dragendorff reagent, containing bismuth nitrate and potassium iodide, resulted in the formation of a precipitate, potassium-alkaloid, and the yellow color due to the complex formed between the metal ions from Dragendorff and alkaloid compounds. Alkaloids act as antibacterial agents by inhibiting the components of peptidoglycan in bacterial cells, disrupting the formation of the cell wall and leading to cell death (Compean & Ynalvez, 2014). As anti-diarrheal agents, alkaloids suppress intestinal peristalsis. Additionally, they affect the DNA of diarrheal-causing bacteria, inhibiting bacterial growth (Juwono et al., 2017).

Antibacterial Activity Test

The antibacterial activity test was conducted using the agar diffusion method with well plates. The diffusion method was chosen because it has several advantages: it is simple (easy and practical), applicable to all rapidly growing pathogenic bacteria, and frequently used in antibiotic sensitivity testing in quality control programs (Peirce, 2019). The antibacterial

activity test began by sterilizing the equipment using an autoclave. Then, the concentrated extract was weighed according to the concentration variations and mixed with 1 ml of aquadest for each concentration, homogenized to dissolve the Dadap Serep leaf extract (Erythrina lithosperma Miq). The next step was preparing the medium and aquadest in an Erlenmeyer flask, followed by homogenization until it boiled. After boiling, the medium was poured into 5 petri dishes, with 3 petri dishes for three repetitions of each extract concentration and 2 petri dishes for the positive and negative controls.

The purpose of performing three repetitions was to increase the accuracy of the results. Next, a suspension or serial dilution was made to reduce the number of suspended microbes in the liquid. The suspension was then pipetted onto the surface of the solidified medium and spread evenly. Wells were made corresponding to each concentration, positive control, and negative control. After preparing the wells, each concentration of the extract, along with the positive and negative controls, was added to the wells and incubated at 37°C for 24 hours. In this study, controls were used for comparison: the positive control was cotrimoxazole, a trimethoprim-sulfamethoxazole combination antibiotic known to inhibit the growth of Escherichia coli. This happens because cotrimoxazole inhibits two enzymatic reactions. Sulfamethoxazole prevents PABA from entering the folic acid molecule, while trimethoprim blocks the enzyme dihydrofolate reductase, preventing the production of active tetrahydrofolate. This mechanism successfully inhibits Escherichia coli from proliferating (Putri et al., 2022). The negative control used was aquadest, which did not inhibit the growth of Escherichia coli, as it does not possess antibacterial properties (Putri et al., 2022).

CONCLUSION

The conclusions of this study are as follows the ethanol extract of Dadap Serep leaves (Erythrina lithosperma Miq) contains secondary metabolites such as saponins, flavonoids, tannins, and alkaloids. This extract also exhibits antibacterial activity against the growth of *Escherichia coli*, with inhibition zones of 10.36 mm at a 5% concentration, 11.8 mm at 10%, and 13.13 mm at 20%.

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