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(Research/Review) Article

Antimicrobial Activity of Ethanol Extract of Walang Leaves against Salmonella typhi and Candida tropicalis

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Abstract: Leaves of Etlingera walang (Blume) RMSm have been empirically used in traditional medicine to relieve stomach disorders and are known to contain secondary metabolites with potential antimicrobial properties. Salmonella typhi infection can cause typhoid fever with symptoms such as diarrhea and abdominal pain, while Candida tropicalis is a fungus that can infect the gastrointestinal tract and lead to digestive disturbances. This study aimed to evaluate the antibacterial and antifungal activities of the ethanolic extract of E. walang leaves against S. typhi and C. tropicalis. The antimicrobial assay was carried out using the disk diffusion method at extract concentrations of 25%, 50%, and 100%, with chloramphenicol and ketoconazole as positive controls, and DMSO as the negative control. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, and tannins. However, the results showed that the extract did not produce inhibition zones against either test microorganism. Extract evaluation indicated a moisture content of 12.23%, total ash 13.39% (above the standard), acid-insoluble ash 1.25%, total plate count 1.8×10^{4} , and mold and yeast count 1.0×10^{3}. The findings suggest that although the ethanolic extract of E. walang leaves contains secondary metabolites and is traditionally used for stomach ailments, it does not exhibit antimicrobial activity against S. typhi or C. tropicalis.

Keywords: Antimicrobial; Candida tropicalis; Etlingera walang; Ethanolic Extract; Salmonella typhi

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1. Introduction

Indonesia as a tropical country has very high biodiversity, including the Zingiberaceae family which grows abundantly in various regions and has long been used as a traditional medicine ingredient (Ramdhan et al., 2015). One member of this family is the walang plant (Etlingera walang (Blume) RMSm), whose leaves are known for their distinctive aroma and have been used more as a food flavoring. However, empirically, walang leaves are also used in traditional medicine, for example as a rice pest repellent by the Baduy people (Alwi, 2019) and as a concoction to relieve stomach aches through leaf decoction (Ramdhan et al., 2015).

Digestive disorders, particularly diarrhea, remain a common health problem in Indonesia. Diarrhea is a clinical manifestation of gastrointestinal infections that can be triggered by various microorganisms, including bacteria and fungi (Simadibrata et al., 2024). One bacteria often associated with typhoid is Salmonella typhi , which can cause symptoms such as fever, diarrhea, and abdominal pain. This bacterium can survive in the environment, including water, soil, and food, and reproduce more rapidly in foods rich in protein, fat, and sugar (Kaunang, 2022). Furthermore, the fungus Candida tropicalis can also infect the digestive tract and cause stomach upset, especially in conditions of microflora imbalance (Zhai et al., 2021).

Given the traditional use of walang leaves as a remedy for stomach aches and the presence of secondary metabolites, scientific research is needed to test the antimicrobial activity of ethanol extract of walang leaves against S. typhi and C. tropicalis. Furthermore, standardization of the extract's quality is also important to ensure its safety and effectiveness as a candidate herbal medicine (Saifudin, 2011).

2. Preliminaries or Related Work or Literature Review

Walang Plant

The rice vine (Etlingera walang) is a plant in the Zingiberaceae family that grows wild around the regencies of Lebak, Pandeglang, and Serang, Banten Province. This plant is known as a cooking spice by the people of Banten and has characteristics similar to pseudo-stem galangal (Banki et al., 2021). Extracts from the Etlingera genus contain active compounds with pharmacological potential, such as antioxidant, anti-inflammatory, and anticancer properties (Yallac, Novi, & Abdilah, 2023). However, there has been little research to test the potential toxicity of rice vine leaves, particularly those belonging to the Etlingera genus.

Salmonella typhi

Salmonella typhi (S. typhi) is also called Salmonella choleraezae serovar typhi, Salmonella serovar typhi, Salmonella enterica serovar typhi (Holt, et al., 1994 and Anonymous, 2001). S. typhi is a strain of bacteria that causes typhoid fever.

S. typhi is a bacterial strain belonging to the Enterobacteriaceae family. According to the Kauffman-White Scheme, S. typhi can be grouped into Salmonella typhi serovar Genetic Diversity based on differences in antigen formulas, namely based on the O antigen (somatic), Vi antigen (capsule) and H antigen (flagellate), while the specifications of the O antigen formula are determined from the composition and structure of the polysaccharide in addition to the O antigen formula can experience changes due to lysogenic by phage. Subdivision of S. typhi serovars can be done based on biovars, namely based on the ability to ferment xylose, so that xylose-positive S. typhi and xylose-negative S. typhi can be found, this can be used as an epidemiological marker (Holt, et al., 1994; Brenner. Et al., 1984). In addition, subdivision of serovars can be based on resistance to antibiotics.

Candida Tropicalis

Candida tropicalis is an opportunistic fungal species of the genus Candida that is a common cause of fungal infections (candidiasis), especially in patients with weakened immune systems. This fungus can cause infections of the skin, gastrointestinal tract, and urinary tract, and can spread to internal organs through the bloodstream. C. tropicalis is considered one of the most significant pathogens of the non-albicans Candida group and shows resistance to several antifungal drugs.

3. Proposed Method

Location and Place of Research

This research was conducted in several locations. Samples of walang leaves were collected from the Cikulur area, Lebak Regency, Banten, and then plant identification was carried out at the National Research and Innovation Agency (BRIN). The ethanol extraction process of walang leaves and phytochemical screening tests were conducted at the Integrated Laboratory of the Faculty of Science, Pharmacy, and Health, Mathla'ul Anwar University, Banten. Antibacterial and antifungal activity tests were conducted at the Central Laboratory of Padjadjaran University, Bandung, while extract standardization tests were conducted at the Tangerang Maritime Affairs and Fisheries Service.

Tools and materials

The equipment used includes dropper pipettes, measuring cups, stirring rods, loop needles, analytical scales, rotary evaporators, black cloth, plastic containers, blenders, Bunsen lamps, petri dishes, calipers, sterile cotton, 60 mesh sieves, tweezers, incubators, mortar-stampers, and filter paper. The research materials consist of Muller Hinton Agar (MHA) medium, Sabouraud Dextrose Agar (SDA), 2N hydrochloric acid, anhydrous acetic acid, 2N sulfuric acid, Mayer's reagent, Wagner's, $FeCl_3$ solution , chloramphenicol, ketoconazole, DMSO, and distilled water. The test microbes include Salmonella typhi (collection of the Regional Health Laboratory, University of Indonesia) and Candida tropicalis (collection from Samarinda).

Extract Preparation

A total of 1000 g of walang leaves were dried, ground, and then macerated using 96% ethanol for 3 days. The resulting filtrate was filtered and then evaporated using a rotary evaporator to obtain a thick extract.

Preparation of Test Solution

The resulting thick extract was prepared in three concentrations: 25%, 50%, and 100%. Each concentration was tested three times.

Microbial Culture

Growth media were prepared using MHA for bacteria and SDA for fungi. The media was poured into petri dishes, labeled, and then, after solidification, streaking was performed to cultivate S. typhi and C. tropicalis.

Antimicrobial Activity Test

The test was conducted using the disc diffusion method. Paper discs dipped in the extract solution were placed on the surface of MHA (for S. typhi) and SDA (for C. tropicalis). The test discs were arranged in a circle at regular intervals, then incubated for 48 hours. After incubation, the inhibition zone was measured using a caliper. The positive controls used were chloramphenicol (for bacteria) and ketoconazole (for fungi), while DMSO served as a negative control.

Extract Evaluation and Standardization

The ethanol extract of the walang leaves was then evaluated through tests of water content, total ash content, acid-soluble ash content, total plate count, and the number of molds and yeasts as part of quality standardization.

4. Results and Discussion

Extraction of walang leaves using 96% ethanol solvent produced a thick extract of 41 g from 1000 g of dried simplicia with a yield of 4.1% (Table 1). This yield result is relatively comparable to the study of Novi et al. (2024) who reported a yield of ethanol extract of walang leaves of 3.63%. Differences in yield figures can be influenced by the condition of the simplicia, extraction time, type of solvent, and the initial water content of the material used. Based on other studies, the extraction method also greatly influences the total yield obtained, for example, between the Microwave Assisted Extraction (MAE) and Ultrasonic Assisted Extraction (UAE) methods, the UAE method is shown to be the most effective (Fauziyah et al., 2022). In addition, variations in extraction temperature also affect the results (Mutripah & Badriyah, 2024). Other factors such as the ratio of material to solvent, the length of extraction time, and the drying and size reduction processes of the material also play an important role because they can affect solubility and cause degradation of active compounds, including antioxidants, which ultimately impacts the final quality of the simplicia (Fauzi et al., 2022; Fauziyah et al., 2022; Mulyadi & Damayanti, 2025).

Table 1. Results of 96% Ethanol Extract of Walang Leaves.

Weight of simple ingredients	Ethanol Solvent	Thick extract (g)	Yield (%)
1,000 g	7,000 ml	41 g	4.1 g

Table 2. Secondary Metabolite Content of Ethanol Extract of Walang Leaves.

Testing	Reagent	Observation result	Results Description
Flavonoid	Mg + HCl Ribbon	A red color change is formed	+
Alkaloid	Dragendorff Mayer Wagner	Orange sediment White to yellowish sediment Blackish brown sediment	+ + +
Saponin	H_2O + HCl	Foam is formed	+
Tannin	Fecl3	A blackish green color is formed	+

Table 3. Results of Antibacterial Activity Test of Walang Leaf Ethanol.

Sample	Concentration	Inhibition Diameter (d/mm)		Average Diameter (mm)	Information
Ethanol extract of *E.walang*	**100%**	**0**	**0**	**0**	**Not active**
Ethanol extract of *E.walang*	**50%**	**0**	**0**	**0**	**Not active**
Ethanol extract of *E.walang*	**25%**	**0**	**0**	**0**	**Not active**
Chloramphenicol	**100 ppm**	**10.9**	**11.31**	**11.11**	**Active**
DMSO	**2%**	**0**	**0**	**0**	**Not active**

The results of phytochemical screening of the ethanol extract of walang leaves (Table 2) showed the presence of alkaloids, flavonoids, saponins, and tannins. These compounds are consistent with research by Novi et al. (2024) , who found flavonoids, alkaloids, saponins, tannins, and steroids in walang leaf extract. These secondary metabolites play an important role in antimicrobial mechanisms. Flavonoids work by forming complex compounds with bacterial cell proteins, thereby damaging cell membranes, inhibiting nucleic acid synthesis, disrupting cell membrane function, and inhibiting energy metabolism. Alkaloids can stop the growth of gram-positive and gram-negative bacteria by triggering cell lysis and changes in bacterial shape. Tannins, as polyphenols, react with cell membranes, inhibiting vital enzymes, and destroying or halting the function of genetic material. Meanwhile, saponins protect against potential pathogens, reduce the surface tension of cell walls, and interact with cholesterol in cell membranes to prevent bacterial interaction with the membrane (Guli et al., 2024).

Although secondary metabolites were detected, the antibacterial activity test results against Salmonella typhi (Table 3) did not show any inhibition zones at concentrations of 25%, 50%, or 100%. The average diameter of the inhibition zone formed was 0 mm, indicating no activity to inhibit bacterial growth. In contrast, the positive control chloramphenicol produced an inhibition zone of 11.11 mm, which meets the criteria for strong antimicrobial activity according to Morales et al. (2003). This indicates that the method and test medium used are valid.

Similar results were found in the antifungal activity test against Candida tropicalis (Table 4), where no inhibition zone was formed at all extract concentrations. The positive control, ketoconazole, produced an inhibition zone of 24.79 mm, which is classified as very strong. Thus, the ethanol extract of walang leaves did not show any antimicrobial activity against either Gram-negative bacteria (S. typhi) or pathogenic fungi (C. tropicalis).

The results of this study are in line with the study of Nuraeni et al. (2024) , which reported that the infusion of walang leaves was only weakly bacteriostatic against Escherichia coli , without any bactericidal effect. This indicates that although the extract contains secondary metabolite compounds, the concentration is not high enough to inhibit the growth of intestinal pathogenic microbes. Novi et al. (2024a) also reported that the total flavonoid content of the ethanol extract of walang leaves was relatively low, namely 5.91 mg/g. This level is thought to be insufficient to produce a significant antimicrobial effect.

When compared with other biological activities, walang leaves have been proven to have biolarvicidal potential against Aedes aegypti larvae. (Putri et al., 2023). This indicates that the bioactivity of walang leaves is selective toward certain organisms, making it effective against insect vectors but not against bacteria or fungi that cause gastrointestinal disorders. This difference in target organisms may be due to the specific mechanisms of action of the active compounds.

Empirically, walang leaves have long been used as a traditional remedy to treat stomach aches (Ramdhan et al., 2015). However, based on the test results in Tables 3 and 4, which did not show any antibacterial or antifungal activity, the empirical healing effect is suspected not to be related to antimicrobial activity. More relevant mechanisms are anti-inflammatory, spasmolytic, or antioxidant effects, which can relieve symptoms of digestive disorders without having to kill the causative microorganisms (Novi, Oktavia, et al., 2024a).

Table 4. Results of Antifungal Activity Test of Ethanol Extract of Walang Leaves.

Sample	Concentration	Inhibition Diameter (d/mm)		Average Diameter (mm)	Information
Ethanol extract of *E.walang*	100%	0	0	0	Not active
Ethanol extract of *E.walang*	50%	0	0	0	Not active
Ethanol extract of *E.walang*	25%	0	0	0	Not active
Ketoconazole	1000 ppm	24.67	24.9	24.79	Active
DMSO	2%	0	0	0	Not active

Table 5. Results of Specific and Non-Specific Standardization Tests.

Parameter	Results	Standard
Walang leaf extract	Blackish green and Semisolid	
1. Microbiology Test		
a. total plate count (colony/g)	1.8x 10^4	≤ 10^7 Cfu/g (BPOM RI, 2014)
b. mold and yeast (colony/g)	1.0x 10^3	≤ 10^4 Cfu/g (BPOM RI, 2014)
2. Chemistry Test		
a. water content	12.23%	≤ 10% (BPOM RI, 2014)
b. Ash Content	13.39%	≤ 10.6% (Ministry of Health of the Republic of Indonesia, 2017)
c. Acid-Soluble Ash Content	1.25%	≤ 4.7% (Ministry of Health of the Republic of Indonesia, 2017)

Evaluation of the extract quality (Table 5) showed a water content of 12.23% and a total ash content of 13.39%, both exceeding the Indonesian Herbal Pharmacopoeia standards (Ministry of Health, 2017). High ash content may indicate inorganic mineral contamination or residue from the extraction process, which could affect the extract's quality. These results align with research by Novi et al. (2024b) , which reported that the ash content of walang leaf extract reached 24.59%. High ash content can disrupt the stability of secondary metabolites, thus reducing their biological effectiveness.

Furthermore, environmental factors also influence secondary metabolite content. Several factors, such as humidity, temperature, drying methods, and oxidation during storage, can reduce the stability of bioactive compounds (Prasetyaningrum et al., 2019). This is relevant to the differences in research results between studies, even though they all used walang leaves as samples.

Previous research also reported that the ethanol extract of walang leaves has cytotoxic activity with an LC_{50} value of 158.49 ppm in the Brine Shrimp Lethality Test (BSLT). This result indicates significant anticancer potential (Novi et al., 2024). Thus, although this study did not find antimicrobial activity, walang leaves still have other pharmacological potential that is important to study further.

In general, the results of this study indicate that the ethanol extract of walang leaves contains secondary metabolites consistent with previous reports (Novi, Oktavia, et al., 2024b)

, but lacks antimicrobial activity against S. typhi and C. tropicalis (Tables 3 and 4). This difference is thought to be due to low levels of active compounds, substandard extract quality (Table 5), and environmental factors. Nevertheless, walang leaves still have the potential to be explored further in other pharmacological fields, including antioxidant, larvicidal, and cytotoxic activities.

5. Conclusion and Suggestions

Ethanol extract of walang leaves (Etlingera walang (Blume) RMSm) with concentrations of 25%, 50%, and 100% did not show antibacterial activity against Salmonella typhi or antifungal against Candida tropicalis , even though it contains secondary metabolites such as alkaloids, flavonoids, saponins, and tannins. This low activity is thought to be influenced by the concentration of active compounds that are less than optimal and the quality of the extract that does not meet standards, especially high water and ash content. For further research, it is recommended to use more effective extraction methods, stricter quality standardization, and exploration of other pharmacological activities such as antioxidants, anti-inflammatory, larvicidal, and cytotoxic. In addition, isolation of active compounds and in vivo tests are needed to support the development of walang leaves as a candidate herbal medicine.

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