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Kecombrang leaf extract (*Etlingera elatior*) as a natural preservative alternative for tilapia (*Oreochromis niloticus*)

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ABSTRACT

Formaldehyde is commonly used by fishermen to preserve unsold fish, but it is hazardous to health and prohibited as a food additive. Kecombrang leaves serve as a natural preservative alternative due to their bioactive antimicrobial compounds. This study aimed to determine the best solvent and concentration of kecombrang leaf extract applied to tilapia. A nested design was employed with two factors: the type of solvent (ethanol and distilled water) and the extract concentration (2%, 2.5%, and 3% v/w). The research was conducted in two stages: extraction followed by phytochemical and inhibition zone tests, and subsequent application on tilapia to evaluate TPC, Aw, and pH. Phytochemical screening revealed that kecombrang leaf extract contains flavonoids, terpenoids, tannins, and saponins. The highest antibacterial activity was observed in the ethanol extract with an inhibition zone of 13 mm (strong category) against Escherichia coli. The application results on tilapia showed that 3% ethanol extract was the best treatment, yielding a TPC value TPC 2.84 x 105 CFU/g (meeting SNI 2729:2013 standards), an Aw of 0.88, and a pH of 6.23.

Keywords: freshwater fish; kecombrang leaf extract; natural food additives; tilapia; natural preservative

INTRODUCTION

Fish is a highly perishable food commodity susceptible to biochemical deterioration caused by post-mortem enzymatic activity and microbiological spoilage triggered by its high nutrient content, such as protein and fat, which serve as substrates for the growth of spoilage bacteria. Therefore, the use of preservatives in fishery products is crucial. Quality deterioration is often accelerated by lipid oxidation and protein degradation, which produce pungent volatile compounds. Therefore, preservation technology, whether natural or synthetic, is necessary to extend shelf life and maintain food safety (Olatunde & Benjakul, 2018). The use of natural antioxidants and antimicrobials is currently a major trend in the seafood industry. These substances mitigate the growth of pathogenic bacteria and inhibit enzymatic autolysis. This helps preserve the texture and nutritional value of seafood products (Pandey et al., 2026).

Tilapia is one of the most widely consumed species of freshwater fish in Indonesia. It is a popular source of animal protein due to its high protein content (17-20%), low fat content, and high levels of essential amino acids and minerals that promote health. Tilapia is also a strategic commodity in the national aquaculture sector because it grows quickly, is easy to farm, and is in high demand (Fitriyanti et al., 2023). According to data from Kementrian Kelautan dan Perikanan Republik Indonesia (2025), national tilapia production surpassed 1.4 million tons in 2025, establishing tilapia as one of the country's leading freshwater aquaculture commodities. However, the high production of tilapia requires proper post-harvest handling technology to maintain the quality of the fish during distribution and storage. However, tilapia's high water and protein content makes it prone to spoilage due to microbial activity, necessitating effective and safe preservation methods.

Adding preservatives can inhibit the growth of spoilage bacteria in fresh fish. Commonly used preservatives include sodium benzoate and formalin. Sodium benzoate is still permitted within established limits, but formalin is prohibited as a food additive due to health hazards. Nevertheless, formalin is still frequently used to preserve fish because it is readily available, relatively inexpensive, and practical for fishermen and vendors to use to extend the shelf life of fresh fish (Hasanah et al., 2021). The continuous use of formalin can cause various health effects, including respiratory tract irritation, digestive disorders, and organ damage. It may also be carcinogenic if it accumulates in the body. Therefore, there is a need for safe, readily available, natural preservative alternatives with good antibacterial activity to ensure the safety of fishery products.

One natural ingredient with potential as a preservative is kecombrang (*Etlingera elatior*). Also known as *Etlingera elatior*, kecombrang is a local spice plant renowned for its potent antibacterial and antioxidant properties. Various parts of the plant, including the flowers, leaves, stems, and rhizomes, contain bioactive compounds such as flavonoids, alkaloids, saponins, tannins, phenolic compounds, triterpenoids, steroids, glycosides, and essential oils, which act as natural antimicrobials. The phenolic compounds and flavonoids in kecombrang damage bacterial cell membranes, inhibit microbial enzyme activity, and prevent oxidation processes. This could potentially extend the shelf life of food products (Nurlaili et al., 2022). A study by Khairunnisa et al. (2025) reported that kecombrang extract contains high levels of phenolic compounds, making it a potential source of natural antioxidants and antibacterial agents for use in food products. Additionally, using plant extracts as natural preservatives is considered safer and more environmentally friendly than using synthetic preservatives (Irawan et al., 2025).

Several studies have shown that kecombrang extract exhibits strong antibacterial activity against both pathogenic and food-spoiling bacteria. Nasution et al. (2023) reported that the n-hexane and ethyl acetate fractions of

kecombrang leaf extract inhibited the growth of Staphylococcus aureus and *Escherichia coli* (*E. coli*), achieving the highest inhibition zones at a concentration of 70%. Another study by Dasi & Leliqia (2023) showed that nearly all parts of the kecombrang plant, such as the flowers, leaves, stems, and fruits, possess strong antimicrobial activity against various foodborne pathogenic bacteria. These parts exhibit antimicrobial activity against *Staphylococcus aureus, E. coli, Salmonella typhimurium*, and several other pathogenic bacteria.

The antimicrobial activity of the plant is caused by the content of secondary metabolites, such as flavonoids, tannins, alkaloids, saponins, and essential oils. These compounds theoretically act by disrupting cell wall integrity and denaturing bacterial membrane proteins, ultimately leading to cell lysis. Using the contact method, the minimum inhibitory concentration (MIC) values obtained were 90% against *E. coli* and 15% against S. aureus. However, extracting these bioactive compounds effectively depends on the "like dissolves like" principle, where the polarity of the solvent must match that of the target compound to achieve optimal inhibitory activity. While various studies have demonstrated the antibacterial potential of kecombrang leaves, research on the effectiveness of different extraction solvents and concentrations of kecombrang leaf extract as a natural preservative for tilapia is limited. The authors argue that optimizing solvents is crucial because different solvents produce different metabolite profiles, which directly determine their ability to suppress the rate of biological deterioration in fish meat, which has a high water and protein content.

The urgency of this study is also based on efforts to meet food quality standards. According to SNI 2729:2013 on fresh fish, fishery products suitable for consumption must meet minimum sensory criteria (a score of 7) and have a maximum Total Plate Count (TPC) of 5.0 x 10^5 CFU/g. Without effective preservation, tilapia deteriorates rapidly, exceeding the SNI standard thresholds. Thus, determining the optimal type and concentration of kecombrang leaf extract solvent is crucial for using it as a natural preservative for tilapia. The study is expected to provide a technical solution for maintaining tilapia quality in compliance with SNI standards while serving as a safe, effective, and easily applicable alternative to synthetic preservatives for fishery products.

METHODOLOGY

Materials

The materials used in this study were kecombrang leaves obtained from Bumiaji in Batu City and tilapia purchased from the Landungsari Market in the Malang Regency. The chemical materials used were distilled water, ethanol, sodium benzoate, MHA (Mueller-Hinton Agar) medium, NA (Nutrient Agar) medium, 0.9% NaCl solution, magnesium sulfate, ferric chloride, concentrated hydrochloric acid, sulfuric acid, 70% alcohol, and *Escherichia coli* bacterial cultures.

Equipment

The following equipment was used to prepare and analyze kecombrang leaf extracts and apply them to tilapia included an Ohaus analytical balance, a FOMAC DHY-24H food dehydrator with a 600-liter capacity, an electric blender, a BUCHI R-100 rotary evaporator, a Rocker 300F vacuum filter, a Krisbow laminar airflow hood, an HVA-85/110 electric autoclave, a Thermo Scientific vortex mixer, an SI Analytics pH meter, an MMM Group Incucell incubator, an Aqualab 4TE Aw meter, and a Galaxy 230 colony counter.

Data analysis

The collected data was statistically analyzed using Analysis of Variance (ANOVA) at a 5% significance level. When significant differences among treatments were identified, Duncan's Multiple Range Test (DMRT) at a 5% significance level was subsequently performed to determine differences between treatments. All statistical analyses were conducted using SPSS software version 25.

Method

Extraction of kecombrang leaves using the maceration method following the method of Arifin, et al. (2025) with modifications. Preparation of the kecombrang leaf extract began with weighing 3.300 g of kecombrang leaves. The leaves were then washed, sized, and dried at 60 °C for 24 hours using a food dehydrator. After drying, the leaves were weighed 435 g and ground into a powder using a blender. The extract was obtained by maceration of powder in aquadest and ethanol solution (1:5 w/v) for two days at room temperature (25 – 30 °C) with three daily stirrings, then concentrated using a rotary evaporator to yield concentrated kecombrang leaf extract.

Following the method of Sulistijowati et al. (2020) with modifications, we began by cleaning the tilapia and removing their heads and tails. Then, the fish deboned and cut into piece. Next, the sample was weighed as much as 5 g. The tilapia samples were then placed in zip-top bags and the extract was added using ethanol and distilled water as solvents at concentrations of 2%, 2.5%, and 3% v/v, respectively. The samples were stored at room temperature for 12 hours (Figure 1) following this, the tests were conducted.

Figure 1. Application of kecombrang leaf extract to tilapia

Research Design

This study employed a nested design based on the research design by Wahdah *et al.* (2023). There were two factors: extract concentration (2%, 2.5%, and 3% v/v) and solvent type (ethanol and distilled water). Controls consisted of an untreated control and a sodium benzoate control. Each treatment was repeated three times.

$$Concentration\frac{v}{b} = \frac{Extract\ Volume\ (mL)}{Sample\ Weight\ (g)} x\ 100\%$$

Note:
Extract (mL) = Desired Percentage x Sample Weight (g)

Data Analysis

The data were analyzed using ANOVA, and treatments showing a significant effect were tested further using the Duncan Multiple Range Test (DMRT). Data analysis was performed using Microsoft Office Excel 2019, and statistical data processing was conducted using SPSS software (version 2021). Tests conducted on kecombrang leaf extract included bioactive compound tests for flavonoids, terpenoids, steroids, tannins, and saponins (Lestari *et al.*, 2025), as well as inhibition zone tests (Pratiwi & Lumowa, 2025). Tests conducted on tilapia included total polar compounds (TPC) and pH tests (Nurfilawati *et al.*, 2025), as well as activity water (A_w) test (Dalus *et al.*, 2025).

RESULTS AND DISCUSSION

Bioactive Compounds in Kecombrang Leaf Extract

This study's qualitative analysis aimed to identify bioactive compounds in kecombrang leaf extract with potential as natural preservatives. Table 1. presents the results of the analysis using ethanol and distilled water as solvents.

Table 1.
Bioactive compounds in kecombrang leaf extract

Analysis	Aquades Extract	Ethanol Extract
Flavonoid	(+) red	(+) red-purple
Terpenoid	(+) red	(+) red-purple
Steroid	(-) red	(-) red-purple
Tanin	(+) dark blue	(+) green-brown
Saponin	(+) foam	(+) foam

According to the data in Table 1, kecombrang leaf extract contains four classes of bioactive compounds: flavonoids, terpenoids, tannins, and saponins. In the flavonoid and terpenoid tests, which used two solvents (distilled water and ethanol), a color change to red or purplish red was observed. This phenomenon occurs because the two solvents have similar polarity characteristics, meaning they are both polar. These results are consistent with those of a study conducted by Inaku, et al. (2025). That study found that testing the flavonoids in extracts from the stems and leaves of patchouli (*Pogostemon cablin Benth*) using magnesium powder and concentrated hydrochloric acid

produced a positive reaction, indicated by the appearance of purple and yellow colors. The addition of magnesium powder and concentrated HCl chemically reduces the benzopyrone ring in the flavonoid structure. This reduction process triggers the formation of flavilium salts, which are compounds that produce characteristic color responses red, yellow, or orange indicating the presence of flavonoids (Lestari et al., 2025; Mulyani et al., 2026; Tarigan et al., 2026). Meanwhile, the results of the terpenoid test are consistent with a study by Handayani et al. (2022). In that study, testing for bioactive compounds using an ethanol extract showed that terpenoids were only detected in the leaves, as indicated by red to purple coloration. The terpenoid compound identification test was performed by adding a few drops of anhydrous acetic acid, followed by drops of concentrated sulfuric acid (H_2SO_4), to the sample. This produced a golden yellow color. A positive result in this test is indicated by the appearance of a purple, orange, or golden yellow color. Mechanistically, the anhydrous acetic acid forms acetyl derivatives of the target compound, and the concentrated sulfuric acid hydrolyzes the water. The water then reacts with the acetyl derivatives to produce a colored solution. This color change is the result of an oxidation process in the terpenoid structure that triggers the formation of a conjugated double bond system (Biadi et al., 2026; Lestari et al., 2025; Ramadhan et al., 2023).

Steroid tests in both solvents showed no presence of steroid compounds, as indicated by the absence of a color change. These results are consistent with those of a study by Ernawati et al. (2025) in which a steroid test using Liebermann-Burchard reagent (anhydrous acetic acid and concentrated sulfuric acid) revealed that the ethanol extract of Chinese ketepeng leaves (*Cassia alata L.*) tested negative for steroid compounds due to the absence of a blue-green color in the sample. A color change from green to blue actually indicates the presence of steroid compounds, which react with sulfuric acid in an anhydrous acetic acid medium to form a colored complex. When strong acids are added, these compounds can undergo a dehydration process, producing salts that are characterized by blue-to-green colors (Manullang et al., 2026; Muniaha et al., 2024; Nuraini et al., 2026).

This negative result is due to the fact that both solvents are polar, whereas steroids are generally nonpolar. Therefore, steroids can only be optimally extracted using nonpolar solvents. This finding aligns with the phytochemical screening results of Bintaro leaf extract by Sangkal et al. (2020). Their study revealed that the steroid test produced a positive reaction only in the n-hexane fraction. In contrast, 70% ethanol and acetone did not yield the same results. This phenomenon is explained by the principle of "like dissolves like" meaning steroid compounds with nonpolar characteristics dissolve optimally in nonpolar solvents, such as n-hexane (Fransiska et al., 2021; Lestari et al., 2025; Sihotang et al., 2026).

In this study, tannin tests using two solvents, distilled water and ethanol, yielded positive results, as indicated by a color change to bluish-black or greenish-brown. These results are consistent with those of a study by Ernawati et al. (2025), in which a phytochemical test for tannin compounds was performed by dissolving a concentrated extract in distilled water. Then, 2 mL of the resulting diluted solution was taken and 2 drops of $FeCl_3$ reagent were added. The test results were positive, indicating the presence of tannins in the ethanol extract of Chinese ketepeng leaves (Cassia alata L.). This was characterized by the appearance of a blackish-green color. This finding is consistent with the characteristics of tannins, which are polyphenols. Tannins are secondary plant metabolites that are characterized by a bitter taste and musty aroma. Tannins are classified into two main types based on their molecular structure: hydrolyzable and condensed. Hydrolyzable tannins form from ester bonds between simple sugars and one or more polyphenolic acids. These compounds are easily broken down through hydrolysis triggered by acids, bases, or enzymes and can be split into gallic acid when dissolved in water. Meanwhile, condensed tannins are a type of tannin that can produce derivatives, such as hydrochloric acid, when subjected to certain chemical reactions. The color change observed in the tannin test results from the formation of a complex between the tannin and Fe^{3+} ions from $FeCl_3$. In this reaction, the Fe^{3+} ions act as central atoms (electron acceptors) that coordinate with the oxygen atoms in the tannin functional groups with free electron pairs. Thus, tannins act as ligands in forming stable coordination complexes (Manullang et al., 2026; Nurwulan et al., 2026; Syukur et al., 2026).

In this study, saponin tests using two solvents, distilled water and ethanol, yielded positive results, as indicated by foam formation. These results align with those previously reported by Fitriani et al. (2025), who observed that saponin tests on Pacing Tawar leaf extracts using ethanol produced stable foam up to 2 cm high that persisted for about 10 minutes. This foam formation is related to the nature of saponins, which are steroid or triterpene glycosides containing an aglycone in the form of sapogenin. Saponin molecules are amphiphilic, containing both hydrophilic and hydrophobic groups, which reduces the surface tension of water and produces foam when shaken.

Adding HCl during testing increases the polarity of the environment. This triggers the saponin molecules to rearrange into micellar structures. The polar (hydrophilic) groups face outward toward the aqueous phase while the nonpolar (hydrophobic) groups cluster in the interior. This micellar stability maintains the foam and serves as an indicator of saponin presence in the extract. Additionally, saponins are glycoside compounds that can undergo hydrolysis to produce glucose and its derivatives (Afika et al., 2025; Fajri et al., 2023; Muliyani et al., 2025).

Inhibition Zone

We tested the antibacterial activity of kecombrang leaf extracts prepared using distilled water and ethanol against *E. coli* using the well diffusion method, which measures the inhibition zones formed on agar plates. The test evaluated the inhibitory effects of the extracts on *E. coli* growth. The treatments included extracts with 100% distilled water or 100% ethanol. Negative controls were sterile distilled water and 96% ethanol. Positive controls used sodium benzoate as a commercial preservative for comparison. Figure 2 presents the results of the inhibition zone diameter measurements.

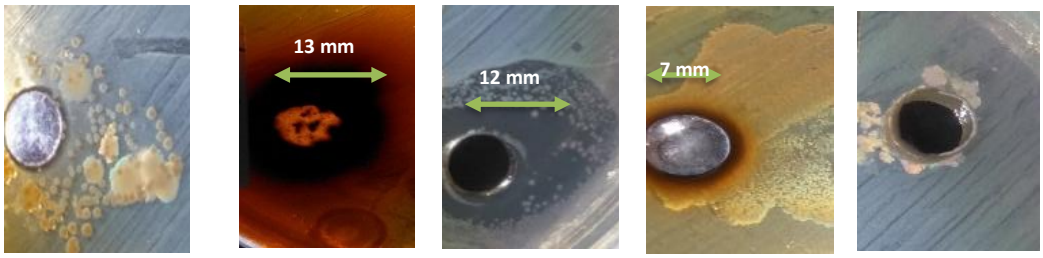

Figure 2. Results of the inhibitory zone test for kecombrang leaf extract

Figure 2 shows that the kecombrang leaf extract prepared with 100% ethanol as a solvent had the strongest antibacterial activity against Escherichia coli, with an inhibition zone diameter of 13 mm. In contrast, the extract prepared with 100% distilled water had the weakest activity, with an inhibition zone diameter of 7 mm. This difference in effectiveness relates to the cell wall structure of E. coli, a Gram-negative bacterium with a thin peptidoglycan layer and higher lipid content. These characteristics make the cell wall more difficult for polar active compounds in the distilled water extract to penetrate than for the ethanol extract, which has better penetration ability against the lipid components of the bacterial cell membrane. The diameter of the inhibition zone is a quantitative indicator of bacterial sensitivity. There is a positive correlation such that the larger the inhibition zone, the higher the antibacterial potential of the tested compound. The diameter of the inhibition zone formed is used to classify antibacterial activity into four categories: weak (less than 5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (more than 20 mm). The presence of these clear zones indicates that antibacterial compounds have diffused into the agar medium and suppressed bacterial growth in the surrounding area. According to this classification, zones with diameters between 10 and 20 mm are considered to have strong inhibitory activity. Therefore, it can be concluded that the ethanol extract tested in this study exhibits strong antibacterial activity against *E. coli* (Nurmailah et al., 2024; Pangestika et

al., 2026; Samosir et al., 2024; Taufiqurrahman & Pijaryani, 2023).

This study used an organic solvent for extraction. This solvent penetrates cell walls and diffuses into the intracellular space, where active compounds are stored. Ethanol was selected as the extraction medium because it has semipolar to polar characteristics. These properties allow ethanol to effectively penetrate the cellular structure of kecombrang leaf crude extract and extract polar compounds, such as flavonoids, saponins, and terpenoids. Kecombrang leaf extract effectively inhibits the growth of various pathogenic microorganisms. This antibacterial effect operates through several mechanisms, including damaging bacterial cell membranes, inhibiting protein synthesis, and disrupting microbial metabolic processes. Flavonoids exhibit antibacterial activity by inhibiting protein function within bacterial cells while simultaneously causing structural damage to cell walls and membranes. Meanwhile, saponins act as antibacterial agents by binding to lipid components in cell membranes. This causes changes in membrane composition, which inhibits bacteria's ability to maintain normal membrane function. Tannins act as antibacterial agents by inactivating vital enzymes, damaging cell membrane structure, and disrupting the function of bacterial genetic material. This results in the inhibition of replication and gene expression. Though these bioactive compounds have different mechanisms of action, they all aim to disrupt the structural and functional integrity of bacterial cells, thereby inhibiting growth or causing the death of microorganisms (Emelda et al., 2021; Fadillah et al., 2026; Larasati & Kristianto, 2026; Nasution et al., 2023).

TPC (Total Plate Count)

Total plate count (TPC) is an important indicator of fish quality deterioration. This study aims to determine the microbial population in tilapia fillets using the TPC method with kecombrang leaf extract dissolved in distilled water and ethanol. The testing principle is based on calculating bacterial colonies that grow on agar media. Testing was conducted using dilution levels of 10^3 and 10^4. Valid plates were defined as those showing colony growth in the range of 25-250 colonies. The TPC calculation results from this study are presented in Table 2.

Table 2.
Results of the TPC Test on kecombrang leaf extract in tilapia

Sample	TPC (log CFU/g)
Control 1	5.91 ± 0.01^{d}
Control 2	5.77 ± 0.35^{cd}
E1(K1)	5.45 ± 0.15^{bcd}
E1(K2)	5.92 ± 0.02^{abc}
E1(K3)	5.79 ± 0.26^{a}
E2(K1)	5.64 ± 0.21^{bcd}
E2(K2)	6.04 ± 0.01^{abcd}
E2(K3)	5.96 ± 0.01^{ab}

Note: According to Duncan's test, numbers followed by different letters indicate significant differences (α = 5%)

As shown in Table 2, the average TPC values decrease as the concentration of kecombrang leaf extract used as an antibacterial agent increases. This inverse relationship occurs because higher extract concentrations provide greater amounts of antibacterial compounds, which increases the inhibitory effect on bacterial growth and reduces the number of colonies formed. These findings are consistent with those of Mengga *et al.* (2022), who stated that the rate of bacterial death is directly proportional to the concentration of the antibacterial agent. The higher the administered concentration, the faster the inhibition or destruction of bacterial cells occurs. At the maximum tested concentration of 3%, the level of antibacterial compounds reached peak effectiveness, as indicated by the lowest number of bacterial colonies compared to other treatments.

A. *marina* leaf extract contains several bioactive compounds, including phytosterols, flavonoids, tannins, and phenols. These compounds act as antibacterial agents, inhibiting bacterial growth. These compounds control the growth of harmful bacteria. These compounds generally disrupt the cell wall by either blocking its formation or modifying its structure after synthesis is complete. Distilled water tends to extract polar compounds, such as carbohydrates and total flavonoids, at lower concentrations than other solvents because distilled water is highly polar. Consequently, extracting plant active compounds with distilled water generally yields lower antibacterial potency than using organic solvents such as ethanol or ethyl acetate (Fadillah *et al.*, 2026; Setyaningsih *et al.*, 2024; Sumartini *et al.*, 2021; Tandi *et al.*, 2026).

The TPC method can be used to perform microbiological testing of fish freshness. TPC is defined as the total number of mesophilic aerobic microorganisms (including bacteria, molds, and yeasts) in a given sample, calculated according to standard procedures and Food and Drug Administration (FDA) guidelines. TPC test results are crucial for assessing hygiene and sanitation levels during production, monitoring potential microbial contamination in the final product and processing environment, and serving as an objective quality control indicator for accepting or rejecting products based on applicable microbiological standards. Several factors influence the decline in fish freshness, including microbial contamination and the use of substandard preservatives. Physical damage resulting from the catching process, prolonged sales periods, poor sanitation at sales locations, and prolonged exposure of the product to room temperature can also accelerate quality deterioration. Bacterial populations in fish tend to increase with longer sales periods. More broadly, improper handling, inadequate packaging, incorrect storage methods, and mismatches between humidity and temperature levels and product characteristics can trigger food product spoilage (Maniani *et al.*, 2026; Puspitasari *et al.*, 2022; Rachmawati *et al.*, 2024; Rahmatang *et al.*, 2026).

A_w (Activity Water)

Table 3. shows the water activity (A_w) results of tilapia treated with kecombrang leaf extract using ethanol and distilled water as solvents at different concentrations.

Table 3.
Results of the water activity (A_w) test of kecombrang leaf extract on tilapia

Sample	A_w
Control 1	1.18 ± 0.05^{b}
Control 2	1.14 ± 0.14^{b}
E1(K1)	1.04 ± 0.17^{ab}
E1(K2)	0.89 ± 0.10^{a}
E1(K3)	0.88 ± 0.05^{a}
E2(K1)	1.11 ± 0.03^{b}
E2(K2)	1.05 ± 0.05^{ab}
E2(K3)	0.92 ± 0.02^{a}

Note: According to Duncan's test, numbers followed by different letters indicate significant differences (α = 5%)

According to the data in Table 3 there is an inverse relationship between the concentration of kecombrang leaf extract and the A_w value. The higher the dose of extract added, the lower the recorded A_w value. This occurs because the extract's compounds increase the food matrix's water-binding capacity, reducing the proportion of free water in the food. The extract exhibits the most optimal water-binding capacity at a 3% concentration, resulting in the lowest A_w value.

Generally, A_w represents the amount of free water in food that microorganisms can use for metabolism and growth. Therefore, a low A_w value directly correlates with increased product stability and shelf life because the limited availability of free water effectively inhibits the growth of spoilage microorganisms. This decrease in A_w is generally consistent with a decrease in total moisture content. Conversely, a high A_w value indicates an abundance of free water, which can trigger uncontrolled chemical reactions and microbial growth. This ultimately reduces product quality and edibility. An increase in A_w leads to a shorter shelf life because it provides water that supports bacterial activity and accelerates chemical and enzymatic reactions during storage (Handayani et al., 2026; Sutisna et al., 2026; Yamerbuke et al., 2020).

A_w is a key factor in estimating the shelf life of fish because this parameter directly affects the rate of chemical, physical, and microbiological reactions that occur during storage. High moisture content can accelerate spoilage through microbial growth and lipid oxidation, while low moisture content inhibits these degradative processes and extends shelf life. A_w is important for assessing product stability during storage. Moisture content represents the total water in a product, while A_w indicates the proportion of free water available to microbes and biochemical reactions. Even a slight increase in moisture content can dramatically raise the A_w value, accelerating lipid oxidation, the Maillard reaction, and microbial growth. This relationship is

particularly sensitive in low-moisture food products, where slight changes in environmental humidity can significantly alter the A_w value and potentially degrade product quality (Luo et al., 2022; Matti et al., 2026; Shaltout, 2024).

pH

Table 4 presents data on the pH values of tilapia treated with kecombrang leaf extract using various concentrations of ethanol and distilled water.

Table 4.
Results of the pH test of kecombrang leaf extract on tilapia

Sample	pH
Control 1	6.62±0.29^{b}
Control 2	6.60±0.19^{b}
E1(K1)	6.37±0.07^{ab}
E1(K2)	6.28±0.08^{a}
E1(K3)	6.23±0.07^{a}
E2(K1)	6.49±0.14^{ab}
E2(K2)	6.43±0.03^{ab}
E2(K3)	6.27±0.09^{a}

Note: According to Duncan's test, numbers followed by different letters indicate significant differences (α = 5%)

As shown in Table 4, there is a negative correlation between the concentration of kecombrang leaf extract and the pH of the fish. The higher the extract dose, the lower the recorded pH. The continued decrease in pH up to 12 hours of storage at room temperature confirms that rigor mortis was still occurring during that period. It is thought that this pH stabilization mechanism is influenced by the bioactive compounds in the kecombrang extract, particularly the flavonoids, which possess antibacterial activity. These compounds can inhibit the growth of spoilage microorganisms that break down proteins and fats into basic compounds. This prevents a rise in pH during storage (Nurlaili et al., 2022). pH is an important objective parameter for assessing the freshness of fish. Immediately after death, the fish's tissues undergo anaerobic metabolism, producing lactic acid and causing the flesh's acidity to increase and its initial pH to drop to near-neutral levels (6.8-7.0). In this study, pH meter measurements yielded a range of 6.23-6.62, indicating that the samples were fresh and of good quality. According to pH standards, fish with a pH between 6 and 7 are considered very fresh. A pH below 6 is still considered acceptable, while a pH above 7 indicates a decline in quality due to the accumulation of basic compounds, such as ammonia and trimethylamine, during decomposition. Thus, consistent monitoring of pH values can serve as an objective reference for determining the freshness and marketability of fish, as well as its safety for consumption (Asni et al., 2022; Untari et al., 2023).

The average pH of fresh fish obtained from traditional and modern markets is 6.12. The activity of lactic acid bacteria promotes the formation of an alkaline environment, which increases the pH. During spoilage, changes in fish meat pH play a crucial role because they influence the rate of

autolysis and microbial growth. During glycolysis, enzymatic activity plays a key role in forming lactic acid. This indicates that lactic acid accumulation occurs more slowly, resulting in a gradual decrease in pH. Conversely, bacterial decomposition of proteins into alkaline compounds is inhibited, which results in a slower rise in pH (Rasdi et al., 2024; Suprayitno, 2020).

The Effectiveness of TPC and A_W

Table 5 and Table 6 show the efficacy of kecombrang leaf extracts obtained with ethanol and distilled water at different concentrations. The efficacy is measured by total plate count (TPC) and water activity (A_w) in tilapia.

Table 5.
Effectiveness of kecombrang leaf extract on TPC levels

Sample	Effectiveness (%)
E1(K1)	25.05
E1(K2)	46.33
E1(K3)	73.94*
E2(K1)	24.31
E2(K2)	43.30
E2(K3)	60.09

Note: * = indicates the highest effectiveness score

Table 6.
Effectiveness of kecombrang leaf extract on the A_w value

Sample	Effectiveness (%)
E1(K1)	11.78
E1(K2)	25
E1(K3)	26*
E2(K1)	6.02
E2(K2)	11.16
E2(K3)	21.64

Note: * = indicates the highest effectiveness score

As shown in Table 5 and Table 6, there is a positive correlation between an increase in the concentration of kecombrang leaf extract and its level of effectiveness. This effectiveness refers to the extract's ability to control bacterial growth and reduce the Aw value. As the concentration increases, the extract's binding capacity toward water molecules increases, reducing the availability of free water and causing the A_w value to decrease. Additionally, higher concentrations are directly proportional to increased flavonoid levels. Flavonoids are active components that function as microbial growth inhibitors. Thus, the greater the total flavonoid content, the more potent the antibacterial effect, which significantly suppresses bacterial colony formation. Increasing the extract concentration increases the amount of bioactive compounds within it (Rika et al., 2026; Saragih et al., 2026).

The maceration technique is superior for dissolving phytochemical compounds because it does not damage the natural structure of plant materials, yielding a higher extract yield. The choice of solvent is another factor that significantly influences the

success of the extraction process. Water has several advantages as a solvent: it is odorless, tasteless, colorless, and effective at extracting polar compounds. However, it is unable to dissolve nonpolar compounds. Conversely, ethanol has broader applications because it can extract bioactive compounds with a wide range of polarities. The 96% ethanol used in this study is a safe, nontoxic universal solvent. Furthermore, at this concentration, ethanol can extract both polar and semipolar compounds. It also has natural antimicrobial activity, which supports the production of optimal-quality extracts (Nurwulan et al., 2026; Purwanti, 2022; Yanuarto et al., 2026).

CONCLUSION

Using ethanol and distilled water as solvents when preparing kecombrang leaf extracts affected the results of bioactive compound tests and the zones of inhibition against *E. coli* growth. Analysis confirmed the presence of bioactive compounds, including flavonoids, terpenoids, tannins, and saponins, in both types of extracts. Of the treatments tested, the ethanol-based extract exhibited the greatest inhibitory activity against *E. coli*, with a clear zone diameter of 13 mm. Additionally, variations in solvent type and extract concentration significantly affected the chemical and microbiological properties of tilapia. The optimal treatment yielded a TPC value of 2.84×10^5, an Aw of 0.88, and a pH of 6.23 with a 3% ethanol extract.

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