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Antibacterial Activity Test of Tempuyung Leaf Extract (*Sonchus arvensis* L.) Against *Streptococcus pyogenes* and Its Formulation in an *Edible Film*

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ABSTRACT

Pharyngitis, an upper respiratory tract infection commonly caused by *Streptococcus pyogenes*, remains a significant health concern. The increasing prevalence of antibiotic resistance has encouraged the exploration of natural products as alternative antibacterial agents. Tempuyung (*Sonchus arvensis* L.) leaves contain various secondary metabolites with antibacterial potential. This study aimed to evaluate the antibacterial activity of the ethanol extract of *S. arvensis* leaves against *S. pyogenes* and to assess its potential application in an edible film formulation. The leaves were extracted by maceration using 96% ethanol, yielding an extraction yield of 13.85%. Phytochemical screening confirmed the presence of flavonoids, polyphenols, tannins, quinones, and steroids. Antibacterial activity was evaluated using the disk diffusion method at extract concentrations of 2%, 4%, 6%, 8%, and 10%. The extract was subsequently formulated into edible films containing 3%, 4%, and 5% extract and evaluated for organoleptic properties, pH, thickness, dissolution time, moisture content, and weight uniformity. Evaluation parameters were analyzed descriptively, whereas antibacterial activity data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test ($p < 0.05$). The 10% extract produced the largest inhibition zone (65.53 mm), while the edible film containing 5% extract exhibited the greatest antibacterial activity with an inhibition zone of 27.12 mm, categorized as very strong. These findings indicate that the ethanol extract of *S. arvensis* leaves possesses promising antibacterial potential and may serve as a natural active ingredient in edible film formulations for managing pharyngitis caused by *S. pyogenes*.

Keywords: Tempuyung leaves, Sonchus arvensis, antibacterial activity, Streptococcus pyogenes, edible, film

Introduction

Pharyngitis is a common upper respiratory tract infection in adults and children, characterized by pharyngeal inflammation causing sore throat, fever, and dysphagia. In the context of bacterial pharyngitis etiology, *Streptococcus pyogenes* (Group A Streptococcus) is the dominant pathogen and is consistently associated with a significant proportion of cases, reaching about 15–30 percent in the pediatric age group and 5–10 percent in the adult age group based on reported data (Muliawan & Yuliyani, 2023).

Globally, *Streptococcus pyogenes* causes more than 600 million cases of pharyngitis each year and is responsible for various infectious complications. A study by Meles *et al*. (2024) reported a prevalence of *Streptococcus pyogenes* of 8.3% among elementary school children in Ethiopia, with more than 50% of isolates showing resistance to antibiotics, indicating a growing threat of resistance and challenges in treating infections.

Synthetic antibiotics such as amoxicillin, penicillin, erythromycin, and clindamycin are commonly used to treat *Streptococcus pyogenes* infections. Increasing resistance to macrolide and lincosamide antibiotics, on the other hand, has reduced the effectiveness of currently available treatments. Furthermore, long-term antibiotic use can lead to side effects such as common disruptions of the microbiota, allergic reactions, and secondary infections resulting from dysbiosis. These side effects can make managing the infection more difficult (Ferreira *et al*., 2021).

Antibiotic resistance has spurred the exploration of natural compounds as more viable and safer treatment alternatives. *Sonchus arvensis*, also known as tempuyung leaf, is a medicinal plant widely distributed in Indonesia that contains numerous bioactive compounds, including tannins, polyphenols, and flavonoids (luteolin and apigenin) (Wahyuni *et al*., 2022; Zulkefle *et al*., 2025). The plant's antibacterial potential is supported by its secondary metabolites. Flavonoids, including luteolin, apigenin, and kaempferol, are known to have the ability to inhibit enzymes responsible for bacterial metabolism, inactivate structural proteins, and prevent biofilm formation. Additionally, phenolic and polyphenolic compounds inhibit bacterial growth by inducing oxidative stress, which damages bacterial DNA, proteins, and cell membranes (Wahyuni *et al*., 2023).

Through complimentary processes, the presence of alkaloids, tannins, and terpenoids enhances the antibacterial action of tempuyung leaves, perhaps creating a synergistic impact in preventing bacterial development. Furthermore, tempuyung leaves have been shown to have anti-inflammatory properties that can support the overall efficacy of treatment and lessen the inflammatory response brought on by infection (Parisa *et al*., 2025). According to the study, tempuyung leaf ethanol extract can stop Gram-positive bacteria like *Staphylococcus aureus* and *Staphylococcus epidermidis* from growing. This implies that *Streptococcus pyogenes* may be susceptible to the antibacterial action of this extract. Because ethanol is semi-polar and can dissolve different bioactive components found in tempuyung leaves, it is also thought to be a useful solvent for extracting active chemicals (Wahyuni *et al*., 2022).

Although the antibacterial activity of *Sonchus arvensis* has been reported in several previous studies, its application in an *edible film* dosage form for the inhibition of *Streptococcus pyogenes* has received limited attention. *Edible films* offer several advantages as local drug delivery systems because they adhere directly to the oral mucosa, dissolve gradually, and enable active compounds to remain longer at the site of infection. This characteristic is expected to improve the effectiveness of antibacterial compounds while enhancing patient convenience. Therefore, the development of an *edible film* containing *S. arvensis* extract represents an alternative dosage form with potential for the local management of bacterial infections in the oral cavity and throat (Amelia *et al*., 2023).

Based on the above discussion, research on the formulation of an *edible film* containing ethanol extract of tempuyung leaves as an antibacterial agent against *Streptococcus pyogenes* is warranted. This study specifically aims to assess the antibacterial efficacy of the ethanol extract obtained from Sonchus arvensis leaves, while also evaluating the physicochemical characteristics and quality standards of the developed edible film formulation. More broadly, the outcomes of this study are expected to contribute as a basis for the development of innovative pharmaceutical formulations based on natural resources, which meet standards of safety, efficacy, and practicality of use in the context of bacterial pharyngitis therapy (Sukmayadi *et al.*, 2023).

Methodology

Research Design

This study compares the antibacterial activity of tempuyung leaf extract against Streptococcus pyogenes in an in vitro laboratory setting. The study used a experimental laboratory study with a positive control (standard antibiotic) and a negative control (solvent/vehicle).

Materials

The materials used in this study included powdered tempuyung leaves (Sonchus arvensis L.), 96% ethanol (Merck), distilled water, Nutrient Blood Agar medium, Streptococcus pyogenes bacterial culture, dilute HCl, concentrated HCl, 2N HCl, $FeCl_3$, magnesium, 1N NaOH, Dragendorf's reagent, chloroformate, toluene, chloroform, anisaldehyde-sulfuric acid, vanillin-sulfuric acid, anhydrous acetic acid, Lieberman's reagent, phosphatidylcholine, and cholesterol.

Statisticals Analysis

Each experiment's data were presented as mean ± standard deviation (SD). IBM SPSS Statistics version 27 was used for the statistical analysis. The Shapiro-Wilk test and Levene's test were used to assess the data for homogeneity of variance and normality prior to hypothesis testing. One-way analysis of variance (One-Way ANOVA) was used to assess group differences, and Tukey's Honestly Significant Difference (HSD) post hoc test was used to find significant group differences. Statistical significance was defined as a p-value of less than 0.05.

Plant identification

Plant identification was conducted at the Integrated Laboratory of Bakti Tunas Husada University in Tasikmalaya to ensure the accuracy and authenticity of the identified plants.

Extraction

Dried *Sonchus arvensis* leaf powder was extracted by maceration using 96% ethanol at a material-to-solvent ratio of 1:7.5 (w/v). The simplicia was immersed in the solvent and allowed to stand for 72 hours at room temperature with occasional stirring to facilitate the diffusion of bioactive compounds into the solvent. After the first maceration period, the mixture was filtered, and the residue was remacerated twice under the same conditions using fresh solvent to maximize the extraction of secondary metabolites. All filtrates were combined and concentrated using a rotary evaporator at a temperature not exceeding 40°C. The concentrated extract was then

further evaporated using a water bath until a thick extract with a constant consistency was obtained, which was subsequently used in phytochemical analysis, antibacterial activity testing, and the formulation of an edible film preparation. (Mulyani *et al*., 2021 ; Sari, 2020).

Non-Specific Parameters

Moisture Content by Toluene Distillation Mmethod (azeotropic distillation)

a. Toluene Saturation

A round-bottom flask is initially filled with 200 mL of toluene and 2 mL of distilled water as the solvent system in order to determine the moisture content by distillation. The flask is then continuously distilled for two hours until toluene saturation is reached. The total volume of water is measured and recorded to the closest 0.05 mL after the system has cooled for 30 minutes. (M. Sari & Mambang, 2022).

b. Determination of the Moisture Content of Herbal Materials

After the saturated toluene has been separated from the water, five grams of herbal powder are placed in a flask and then distilled until all water has been removed from the flask. The volume of the collected water is then measured. The moisture content of the herbal material, expressed as a percentage, is calculated based on this measurement (M. Sari & Mambang, 2022).

Total Ash Content

Two grams of crude drug powder are added to a porcelain crucible that has been pre-fired and tared. The crucible is then heated to 600°C for three hours until a consistent weight is achieved. The initial weight of the crude medication utilised is then compared to the total ash content (R. P. Sari & Laoli, 2019).

Drying Loss

A covered porcelain crucible, which has been conditioned and calibrated, is filled with 2 grams of crude drug. In the oven, the sample is then heated at a temperature of 100–105 °C until it reaches a constant weight. The difference in the weight of the crude drug before and after drying is used to calculate the percentage of drying loss (R. P. Sari & Laoli, 2019).

Water-Soluble Extract Content

Over a 24-hour period, five grams of crude drug powder were macerated with 100 mL of chloroform-saturated water P at regular intervals. The resulting 20 mL of filtrate was then evaporated to dryness and heated to a constant weight at 105 degrees Celsius. Next, the chloroform-soluble extract content was calculated by comparing the initial weight of the crude drug used (R. P. Sari & Laoli, 2019).

Ethanol Soluble Extract Content

The extraction process begins by soaking 5 g of crude drug powder in 100 mL of ethanol for 24 hours, with occasional agitation to maximize contact between the sample and the solvent. Twenty milliliters of the resulting maceration filtrate is then separated and slowly evaporated until all the solvent has evaporated and a dry residue is formed, which is then weighed once the residue weight has stabilized. The percentage of ethanol-soluble extract is then calculated based on the weight of the crude drug measured at the start of the procedure (R. P. Sari & Laoli, 2019).

Specific Parameters

Organoleptic Test

Tempuyung leaf crude drug was examined macroscopically using the five senses (sight, smell, touch, and taste, if necessary) to observe its color, odor, taste, and texture (Evifania *et al*., 2020).

Microscopic Examination

Powder preparations were made and examined using a light microscope (magnification 400× to 1000×). Diagnostic structures such as cell walls, stomata, nuclei, and vascular bundles were recorded to ensure the purity and authenticity of the crude drug (Utami *et al*., 2020).

Identification of Chemical Compounds

Flavonoids

For ten minutes, two grams of crude drug powder were boiled in 100 mL of hot water. Then, five milliliters of the resulting filtrate were mixed with magnesium powder, one milliliter of concentrated HCl, and two milliliters of amyl alcohol in sequence. The yellow to orange color that appeared in the amyl alcohol layer indicated a positive reaction to flavonoid compounds (Cahyono *et al*., 2020).

Tannins and Polyphenols

After adding water and boiling one gram of crude drug powder, the filtrate was obtained by filtering. The resultant filtrate was split into two parts for two distinct treatments. The first part was reacted with a $FeCl_3$ solution, and the creation of a blue-black colour signified the presence of tannins and polyphenols. When a 1% gelatin solution was added to the second part, a white precipitate formed, which was considered positive indication that the sample contained tannins (Mulyani *et al*., 2021).

Quinone

The filtrate was obtained by filtering 2 g of crude drug powder that had been cooked in 100 mL of hot distilled water for ten minutes. A few drops of 1 N NaOH were added to 5 mL of the filtrate. It was determined that the presence of quinone compounds in the sample was indicated by the production of a yellow colour in the solution (Harahap, 2020).

Triterpenoids and Steroids

2 g of crude drug powder was macerated using 20 mL of petroleum ether to extract nonpolar compounds. The resulting 5 mL filtrate was evaporated to a dry residue. The Liebermann-Burchard reagent, which consists of one drop of concentrated sulphuric acid and two drops of acetic anhydride, was then used to react with the residue. Triterpenoids are shown by a red or purple colour, whereas steroids are indicated by a blue colour in the sample (Cahyono *et al*., 2020).

Antibacterial Activity Test

Sterilization

Before the equipment is used for testing, a thorough preparation process is carried out, including washing, drying, and sterilization. For glassware, sterilization is performed in an autoclave for fifteen minutes at 121°C or in an oven for one hour

at 170°C. Rubber-based instruments are decontaminated by soaking in 70% alcohol; inoculation loops are cleaned by flaming over a Bunsen burner; and the Laminar Air Flow (LAF) system is sanitized across all working surfaces before and after use via exposure to ultraviolet light and 70% alcohol (Hainil *et al*., 2021).

Preparation of Blood Agar (BA) Medium

Dissolve 5.6 g of nutrient agar in 200 mL of distilled water, heat until homogeneous, and sterilize in an autoclave for 15 minutes at 121°C. Once the medium has cooled to 45–50°C, add 5–10% sterile human blood aseptically. Next, the medium is poured into Petri dishes and allowed to solidify (Hainil *et al*., 2022).

Bacterial Replication

The initial stage of the procedure begins with the preparation of an agar culture, which is done by streaking 1 loop of Streptococcus pyogenes bacterial suspension onto the surface of fresh agar medium; the culture is then incubated for 24 hours at 37°C until an active culture is obtained (Hainil *et al*., 2022).

Preparation of the McFarland Standard

In the procedure for standardizing bacterial inocula, a McFarland 0.5 turbidity standard solution is prepared by mixing 0.05 mL of a 1.75% $BaCl_2 \cdot 2H_2O$ with 9.95 mL of H_2SO_4 solution in a measured manner until a homogeneous turbid suspension is produced, which is subsequently used as a visual reference in standardizing the turbidity of the test bacterial suspension to ensure the uniformity of inoculum density before testing is conducted (Abubakar *et al*., 2019).

Preparation of a Bacterial Suspension

Bacterial colonies that had been incubated for 24 hours were suspended in 10 milliliters of sterile 0.9% sodium chloride solution, and the mixture was thoroughly shaken. In addition, the turbidity of the resulting suspension was adjusted to the McFarland standard to indicate the inoculum concentration (Muljono *et al*., 2016).

Testing the Antibacterial Activity of Ethanol Extracts from Tempuyung Leaves

Antibacterial activity testing against *Streptococcus pyogenes* was conducted using the Kirby-Bauer disk diffusion method, with ethanol extracts from tempuyung leaves (*Sonchus arvensis* L.) and *edible film* formulations as test samples. Standardized bacterial suspensions were evenly inoculated onto the surface of Blood Agar medium; then, sterile discs containing the respective extract treatments, *edible film* solution, amoxicillin positive control, and DMSO negative control were placed on the medium surface aseptically. For the *edible film* test specifically, the formulation was first dissolved in DMSO, and the sterile discs were soaked in the solution until fully absorbed before being placed on the test medium. Afterward, all plates were incubated for 24 hours at 37°C. The antibacterial potential of each treatment was assessed by measuring the diameter of the inhibition zone formed around the discs. Three edible film formulations containing different concentrations of *Sonchus arvensis* leaf extract were prepared. The extract was incorporated into the film-forming solution at concentrations of 3% (FI), 4% (FII), and 5% (FIII) (w/v), while the formulation without extract served as the blank control (F0). The different extract concentrations were selected to evaluate their effect on the antibacterial activity and physicochemical characteristics of the *edible films* (Misna & Diana, 2016).

Formulation and Preparation of *Edible Film* Formulations

Edible film formulations of ethanol extract from tempuyung leaves (*Sonchus arvensis* L.) were formulated into four formulations: a formulation without extract (F0) as a control, and formulations containing extract at concentrations of 3% (F1), 4% (F2), and 5% (F3). The *edible film* preparations were prepared by dissolving Na-CMC in hot water until homogeneous, then adding PEG 400, menthol, and sucralose while stirring continuously. Next, the ethanol extract of tempuyung leaves was added according to the concentration of each formulation and homogenized. The resulting solution was poured into a mold, then dried at 40°C for 24 hours until a film layer formed. After drying, the films were carefully removed from the mould and stored in a desiccator prior to further evaluation (Nurdianti *et al*., 2022).

Result and Discussion

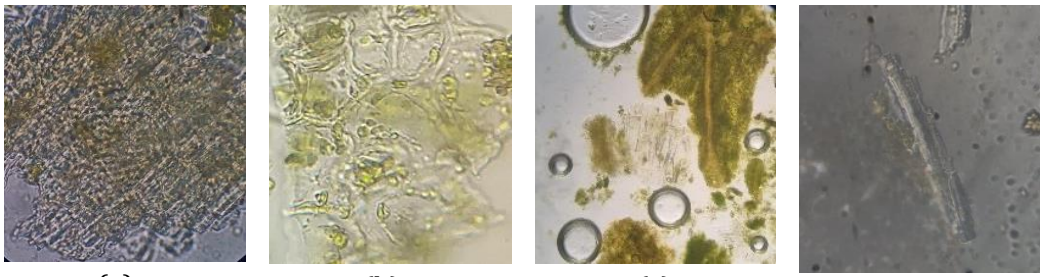
Extraction of Tempuyung Leaves

The extraction process yielded 13.85% ethanol extract of *Sonchus arvensis* leaves. This result is slightly higher but comparable to the extraction yield of 12.38% reported by (Aldeeb *et al*., 2025) using the same plant material. The similarity in extraction yield indicates that the maceration method using 96% ethanol is effective in extracting bioactive constituents from *S. arvensis* leaves. The slight difference in yield may be attributed to variations in the geographical origin of the plant material, harvesting time, drying conditions, particle size of the dried leaves, solvent-to-material ratio, extraction duration, and other extraction conditions. These factors are known to influence the amount of secondary metabolites extracted from medicinal plants (BPOM RI, 2023).

Furthermore, the extraction yield obtained in this study exceeded the minimum requirement of 7.5% specified in the Indonesian Herbal Pharmacopoeia, indicating that the extraction process was adequate for obtaining the extract from *S. arvensis* leaves.

Determination of Specific and Non-Specific Parameters

Microscopic observation was conducted to verify the identity and ensure the quality of the herbal materials used. Analysis of the powdered crude drug of tempuyung leaves (*Sonchus arvensis* L.) revealed the presence of characteristic diagnostic fragments, including the epidermis, stomata, mesophyll, and trichomes, all of which can be used as identification parameters as well as quality control indicators for the crude drug (Firawati *et al*., 2024).



(a) (b) (c) (d)

Figure 1. Microscopic Examination of Tempuyung Leaf Powder at 400x Magnification

(a) Lower epidermis with anomositic stomata (b) upper epidermis (c) mesophyll with epidermis and palisade cells (d) trichomes

Test results for the quality parameters of tempuyung leaf crude drug show that the moisture content, total ash content, and loss on drying are 4.00 ± 0.00%, 6.80 ± 0.09%, and 11.77 ± 0.13%, respectively. The moisture content value, which meets the requirements of the Indonesian Herbal Pharmacopoeia, indicates that the crude drug has good stability during storage, while the low total ash content indicates that inorganic contamination remains within acceptable limits. The loss on drying value indicates the presence of volatile components during the heating process (FHI, 2022).

Based on the research results, the water-soluble extract content was recorded at 27.40 ± 0.55%, while the ethanol-soluble extract content was only 23.07 ± 0.88%. This difference suggests that the main constituents in the tempuyung leaf crude extract tend to be polar compounds that are more soluble in water. The high water-soluble extract content indicates that the crude extract contains a sufficient amount of bioactive compounds, making it a potential source of raw material for the production of traditional medicines (Febrianti *et al*., 2019).

Table 1. **Results of Specific and Nonspecific Parameters**

Test Parameters	Mean ± SD (%)	Requirements (FHI, 2017)
Yield	13,85	>7,5%
Moisture Content	4,00 ± 0,00	<12,5%
Total Ash Content	6,80 ± 0,09	<15,4%
Loss on Drying	11,77 ± 0.13	<10%
Water-Soluble Extract Content	27,40 ± 0,55	>12,1%
Ethanol-Soluble Extract Content	23,07 ± 0,88	>7,4%

Table 2. Formula for an *Edible Film* Made From Ethanol Extract ff Tempuyung Leaves

Ingredients	Formulation (% b/v)				Function
	F0	FI	FII	FIII	
Tempuyung leaf extract	-	3%	4%	5%	Active ingredient
Sodium carboxymethylcellulose	2	2	2	2	Film-forming polymer
PEG 400	0,8	0,8	0,8	0,8	Plasticizer
Sucralose	0,1	0,1	0,1	0,1	Sweetener
Menthol	0,2	0,2	0,2	0,2	Flavoring
Sodium benzoate	0,1	0,1	0,1	0,1	Preservative

Green food coloring	-	q.s	q.s	q.s	Colorant
Purified water	100	100	100	100	Solvent

Evaluation of Edible Film Formulations

Edible film formulations are evaluated to determine their physical characteristics. The tests conducted include organoleptic testing, pH measurement, thickness measurement, dissolution time, and moisture content.

Identification of Chemical Compounds

Phytochemical analysis revealed the presence of flavonoids, polyphenols, tannins, quinones, and steroids in both the crude drug and the tempuyung leaf extract. The presence of secondary metabolites was confirmed by positive responses, as indicated by color changes and/or the formation of specific precipitates in each testing method. These compounds are known to contribute to antibacterial activity through mechanisms that inhibit bacterial growth and damage bacterial cell structure, thereby potentially supporting the antibacterial activity of tempuyung leaf extract. In contrast, alkaloids and saponins tested negative. The absence of these secondary metabolites may be related to differences in plant origin, environmental growing conditions, harvest period, or extraction conditions. In addition, th polarity of the extraction solvent may influence the types of compounds extracted from the plant material. Although alkaloids and saponins were absent, the presence of flavonoids, tannins, phenolic compounds, quinones, and steroids is considered sufficient to contribute to the antibacterial activity observed in this study through their complementary mechanisms against bacterial cells (Tiranakwit et al., 2023).

Table 3. Results of Phytochemical Screening of Tempuyung Leaf Crude Extracts and Extracts

Secondary metabolites	Leaf powder	Leaf extract
Alkaloids	-	-
Flavonoids	+	+
Polyphenols	+	+
Saponins	-	-
Tannins	+	+
Quinones	+	+
Steroids	+	+

Notes:
(+) : contains the tested compound
(-) : does not contain the tested compound

Results of Antibacterial Activity Testing

The antibacterial activity of the ethanol extract of tempuyung leaves (*Sonchus arvensis* L.) against *Streptococcus pyogenes* was tested using the disk diffusion method. To do this, the inhibition zones formed around the test disks were measured. The flavonoid, polyphenol, tannin, quinone, and steroid content found in the extract is believed to be responsible for the resulting antibacterial activity. These compounds are known to possess antibacterial properties through various mechanisms. These include destroying bacterial cell walls and membranes, reducing membrane

permeability, inhibiting enzymes and biomolecule synthesis, and preventing the formation of biofilms responsible for bacterial infection processes (Nguyen & Bhattacharya, 2022).

In this study, a 25 µg amoxicillin disc was used as a positive control. The mechanism of action of amoxicillin, as a β-lactam antibiotic, is to inhibit bacterial cell wall synthesis. This activity makes amoxicillin effective against various Gram-positive bacteria, including *Streptococcus pyogenes* (Maida & Lestari, 2019). Overall, the antibacterial activity of the ethanol extract of tempuyung leaves is thought to result from the synergistic action of various secondary metabolites contained within it. This combination of mechanisms disrupts the integrity and metabolism of bacterial cells, thereby inhibiting the growth of *Streptococcus pyogenes* (Maida & Lestari, 2019).

Table 4. Results of the Antibacterial Activity Test of Ethanol Extracts of Tempuyung Leaves

Concentration (%)	Average Diameter of the Inhibited Zone ± SD (mm)	Category
2	13,61±1,34	strong
4	26,37±1,53	Very strong
6	42,77±0,64	Very strong
8	63,97±1,63	Very strong
10	65,53±1,15	Very strong
Amoxicillin	25,08 ± 3,24	Very strong
DMSO	0 ± 0,00	No activity antibacterial

The antibacterial activity of tempuyung leaf extract against *Streptococcus pyogenes* was evaluated using the agar diffusion method with graduated extract concentrations of 2%, 4%, 6%, 8%, and 10%. The use of multiple extract concentrations was intended to determine at which concentration inhibitory activity against the growth of the test bacteria first became apparent. The results showed that tempuyung leaf extract was able to form inhibition zones starting at a concentration of 2%, classified as strong inhibitory activity, and at the highest concentration of 10%, it produced a very strong inhibition zone. indicating that antibacterial activity increased with increasing extract concentration. This finding may be attributed to the higher amount of bioactive compounds present in the extract, resulting in greater diffusion of antibacterial constituents into the agar medium. As the concentration of the extract increased, the amounts of flavonoids, phenolic compounds, tannins, quinones, and steroids also increased, thereby enhancing their antibacterial effects. These phytochemicals are known to inhibit bacterial growth through multiple mechanisms, including disruption of cell wall and cell membrane integrity, inhibition of essential enzymes, interference with protein and nucleic acid synthesis, and induction of leakage of intracellular components. Consequently, the greater availability of these bioactive compounds at the 10% concentration likely contributed to the formation of the largest inhibition zone observed in this study. Although the 10% extract produced the largest mean inhibition zone, statistical analysis indicated that it was not significantly different from the 8% concentration ($p > 0.05$). This suggests that increasing the extract concentration beyond 8% did not result in a statistically significant enhancement of antibacterial activity, possibly because the

diffusion of active compounds in the agar medium had approached its maximum under the experimental conditions.

Further testing in the form of an edible film formulation was conducted using a 4% extract concentration. This concentration was selected based on its ability to produce an inhibition zone diameter of 26.37 mm, which is classified as very strong according to bacterial inhibition criteria. The *edible film* formulation of tempuyung leaf ethanol extract demonstrated antibacterial activity against Streptococcus pyogenes, and increasing the extract concentration increased the diameter of the resulting inhibition zone. Meanwhile, formulations FI, FII, and FIII produced average inhibition zones of 11.07 ± 1.80 mm, 17.57 ± 1.90 mm, and 27.12 ± 2.60 mm, respectively, falling into the strong to very strong categories. Formulation FIII exhibited the highest antibacterial activity and an inhibitory effect exceeding that of the positive control. This finding may be attributed to the higher concentration of bioactive compounds incorporated into the edible film, resulting in a greater amount of antibacterial constituents being released into the surrounding medium during the diffusion process. Furthermore, the edible film matrix may have facilitated a more sustained release of the active compounds, thereby enhancing their interaction with Streptococcus pyogenes.

Table 5. Results of the Antibacterial Activity Test of an *Edible Film* Formulation of Ethanol Extract from Tempuyung Leaves

Formulation	Average inhibition zone (mm) ± SD	Category
F0	0 ± 0.00	Weak
FI	11.07 ± 1.80	Strong
FII	17.57 ± 1.90	Strong
FIII	27.12 ± 2.60	Very strong
Positive control	15.33 ± 0.42	Strong

*Positive control: commercial throat antiseptic containing 1.4% phenol.

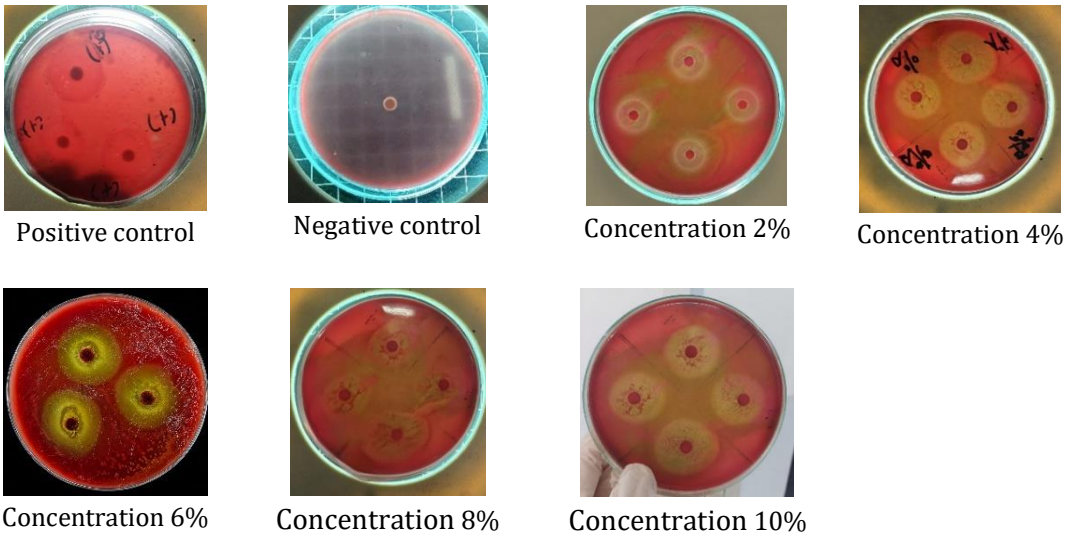


Figure 2. Antibacterial Activity Test of the Extract Against *Streptococcus pyogenes*

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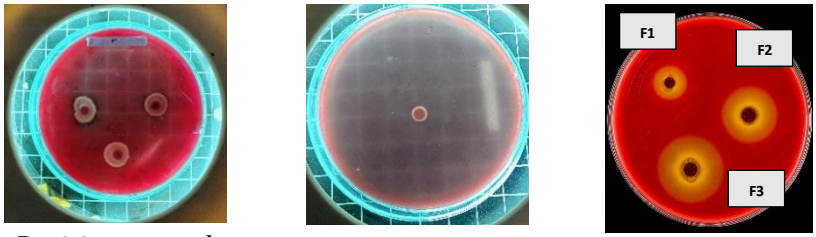

Positive control F0

Figure 3. Antibacterial Activity Test of the *Edible Film* Formulation Against *Streptococcus pyogenes*

Results of the Edible Film Formulation Evaluation

The results of the organoleptic test showed that all edible film formulations were rectangular in shape. Formulation F0 was transparent in color, had a mint aroma, and a sweet taste, while formulations FI, FII, and FIII were light green in color, had a characteristic aroma of tempuyung leaf extract, and had a bitter taste. These differences in characteristics indicate that the addition of tempuyung leaf extract affects the color, aroma, and taste of the edible film formulations.

Table 6. Results of the organoleptic test of the *edible film* formulation

Test parameters	Results			
	F0	**FI**	**FII**	**FIII**
Shape	Rectangle	Rectangle	Rectangle	Rectangle
Color	Transparent	Light green	Light green	Light green
Taste	Sweet	Bitter	Bitter	Bitter
Aroma	Minty scent	Characteristic of tempuyung leaf extract	Characteristic of tempuyung leaf extract	Characteristic of tempuyung leaf extract

Table 7. Results of the evaluation test of the *edible film* formulation uji

Evaluation	Results (average)			
	F0	**F1**	**F2**	**F3**
Thickness (mm)	0,06 ± 0,01	0,07 ± 0,00	0,05 ± 0,00	0,06 ± 0,00
pH	7,45 ± 0,03	7,80 ± 0,19	7,25 ± 0,04	6,88 ± 0,09
Dissolution time (seconds)	12 ± 0,00	16,33 ± 0,58	18 ± 1,00	18,33 ± 0,58
Moisture content	10 %	14,42 %	10,28 %	16 %
Weight uniformity (g)	0,03 ± 0,004	0,03 ± 0,00	0,03 ± 0,00	0,033 ± 0,00

The thickness test results showed that all edible film formulations met the thickness requirement (<0.25 mm). Variations in thickness among the formulations were likely influenced by the printing and drying processes, while formulation FII had the lowest thickness, which could potentially result in faster dissolution times (Tobi et al., 2023).

Based on the test results, all formulations have pH values within the physiological pH range of the oral cavity (5.5–7.9), making them safe to use and unlikely to cause irritation. An increase in extract concentration tends to lower the pH of the formulation, which is thought to be influenced by the presence of flavonoids, which are slightly acidic compounds (Susanti *et al*., 2024).

The dissolution time test results showed that all formulations met the criteria for good dissolution time, which is 5–30 seconds. Formulation F0 had the fastest dissolution time, while differences in dissolution time among the formulations were likely influenced by variations in film thickness (Irfan *et al*., 2016).

The moisture content of the edible film increased with increasing extract concentration, with values of 10.00% (F0), 14.42% (F1), 10.28% (F2), and 16.00% (F3). The highest moisture content was observed in F3, which reached the maximum acceptable limit of 16% according to SNI 06-3735-1995. This increase may be attributed to the higher concentration of *Sonchus arvensis* leaf extract, which increased the total solid content and the water-binding capacity of the film matrix. In addition, Na-CMC and PEG 400 are hydrophilic materials capable of retaining water within the polymer network, thereby contributing to the increased moisture content. Nevertheless, the moisture content of F3 remained within the acceptable limit, indicating that the edible film complied with the required quality standard while maintaining its physical characteristics (Aziza *et al*., 2024).

The average weight of the edible films ranged from 0.030 g to 0.033 g. Although a slight increase in weight was observed in F3, the variation among formulations was minimal, indicating good weight uniformity. The higher weight of F3 may be attributed to the increased concentration of *Sonchus arvensis* leaf extract, which increased the total solid content of the film-forming solution. Similar to previous studies, weight uniformity is an important quality parameter because it reflects the consistency of the casting process and the uniform distribution of formulation components within the *edible film*. Differences in the absolute weight of *edible films* between studies may occur due to variations in formulation composition, casting volume, mould dimensions, and film thickness. Therefore, the slight variation observed in this study indicates that the edible films were prepared with good manufacturing consistency (Nurdianti *et al*., 2022).

Statistical testing showed that the data on the antibacterial activity of the tempuyung leaf extract and *edible film* formulations met the criteria for parametric analysis because they passed the normality and homogeneity tests ($p > 0.05$). The results of the analysis using one-way ANOVA indicated that the treatments administered produced statistically significant differences ($p < 0.05$). In the extract testing, the 8% and 10% concentrations were in the group with no significant difference, meaning both had relatively similar inhibitory capabilities against the test bacteria. In the *edible film* formulations, the formulas containing 3% and 4% extract showed efficacy equivalent to the positive control. The highest antibacterial activity was obtained in the formulation with a 5% extract concentration, which produced the largest inhibition zone based on the Davis and Stout classification, categorized as having very strong antibacterial activity. Overall, the results of the study indicate that an increase in extract concentration contributes to an increase in antibacterial activity

against *Streptococcus pyogenes*, both in the form of the extract and the edible film formulation.

Conclusion

The ethanol extract of tempuyung leaves (*Sonchus arvensis* L.), obtained through maceration, contains various secondary metabolites with potential antibacterial properties. In vitro evaluation demonstrated its inhibitory activity against *Streptococcus pyogenes*, with the strongest effect observed at a 10% concentration, producing a 65.53 mm inhibition zone categorized as very strong. The extract was successfully formulated into an *edible film* exhibiting acceptable physical characteristics, including organoleptic properties, pH, thickness, dissolution time, and moisture content. However, the formulation did not meet the weight uniformity requirement. Among the tested formulations, the 5% *edible film* showed the highest antibacterial activity and potential.

Declaration of Competing Interest

The study was carried out without any financial, commercial, or other links that would create a conflict of interest, according to the authors.

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