Formulation and Evaluation of Edible Film Combination Gingerol and Curcumin As An Antibacterial *Streptococcus pyogenes* Causes Inflammation The Throat

Lusi Nurdianti¹, Tresna Lestari, Anisa Nurmalasari, Winda Trisna Wulandari, Keni Ida Cahyati, Fajar Setiawan, Ardianes Firmansya

Department of Pharmacy, Bakti Tunas Husada University, Tasikmalaya, West Java Indonesia

*corresponding author: e-mail : lusinurdianti@universitas-bth.ac.id

ARTICLE INFO

**ABSTRACT**

Ginger (*Zingiber officinale*) and turmeric (*Curcuma longa* L) are well-known herbal plants appreciated for their therapeutic advantages. These botanicals consist of bioactive elements referred to as gingerol and curcumin, respectively, which display potential antibacterial qualities. The purpose of this study was to obtain an edible film combination of gingerol and curcumin that met the evaluation requirements and had antibacterial activity against *Streptococcus pyogenes*. The study employed the solvent casting method, evaluating the edible film through sensory analysis, thickness measurement, weight uniformity, pH, folding resistance, friability, water content, elongation and tensile strength, disintegration and dissolution time analysis, stability examination, hedonic testing, antibacterial activity evaluation against *Streptococcus pyogenes* bacteria, and functional group analysis. The outcomes of the research revealed that edible films had rectangular shapes measuring 2x3 cm with different colors for each formulation, sweet and spicy taste, distinctive smell, and the evaluation results meet the appropriate requirements of Japanese Industrial Standards (JIS) and existing theories, but the percent elongation has poor results. Then, edible film single and combination formulations were able to inhibit bacteria with diameters of inhibition zones F2 6.56 mm ± 0.0920, F3 6.36 mm ± 0.0748, and F4 6.92 mm ± 0.0632 against *Streptococcus pyogenes* bacteria with respectively medium category.

This is an open access article under the CC-BY-SA license.

1. Introduction

Today's society is increasingly aware of how important it is to pay attention to health issues. However, it often ignores mild symptoms, such as sore throat, which should be anticipated so they do not develop into more severe chronic conditions (Agustiawan, 2015). Sore throat is a condition with pain in the throat due to inflammation that occurs at the back of the throat (pharynx). Based on data from basic health research, the incidence of respiratory tract infections, commonly known as upper respiratory tract infections, in West Java province reached 11.2%. Meanwhile, the province with the highest number of upper respiratory tract infections sufferers is located in East Nusa Tenggara (NTT), with a percentage of 13.1%. In contrast, the province with the lowest number of upper respiratory tract infections sufferers is recorded in Jambi province, with a percentage of 5.5% (Kemenkes RI, 2018). The *Streptococcus pyogenes*, better known as *Streptococcus group A*, is responsible for the occurrence of sore throat. *Streptococcus pyogenes* is one variant of pathogenic bacteria that infects humans. About 5-15% of the healthy population contains these bacteria, which can generally be found in the respiratory tract. Bacteria *Streptococcus pyogenes* can cause infection when the immune system is weakened, allowing bacteria to spread to vulnerable tissue and cause infection (Clinic, 2018). In society, efforts to use traditional medicinal plants as natural solutions are widely known. Several types
of plants can be used to treat problems such as sore throats, dry coughs, flu, itching, vomiting, and diarrhea (Awanis, 2016).

Natural medicinal plants that are useful in relieving sore throats include ginger and turmeric. Ginger (Zingiber officinale) is a spice plant that originates from the South Asian region and has spread to various corners of the world, including Indonesia. The part used by this plant for medicinal purposes is the rhizome. Ginger rhizomes contain various active chemical compounds such as essential oils, flavonoids, phenols, and terpenoids (Debora et al., 2021). Several compounds in ginger, such as terpenoids, gingerol, and shogaol, have anti-inflammatory, antioxidant, and antibacterial properties (Kandungan Bahan Aktif Jahe Dan Pemanfaatannya Dalam Bidang Kesehatan, 2014).

Meanwhile, Turmeric (Curcuma longa L) is a tropical plant originally from Southeast Asia and is now grown for trade purposes in various countries, including China, India, and Indonesia. Besides functioning as a spice that gives food a bright yellow color, turmeric has a long history of use as a coloring agent and medicine since 600 BC (Before Christ) (Shan & Iskandar, 2018). The central part of the turmeric plant that is used is the rhizome. Turmeric rhizomes contain various active compounds such as alkaloids, glycosides, flavonoids, steroids, tannins, and essential oils (Meilina & Mukhtar, 2019). The curcumin compound in turmeric rhizomes has beneficial antibacterial properties (Diansyah et al., 2021).

The use of natural products produced through unique designs for treatment in a health context, such as extracts administered directly through oral medication, faces several limitations, including an unpleasant taste and difficulties in application, which can ultimately cause discomfort for the user. However, to overcome this obstacle, one option that can be considered is the creation of new dosage forms, such as edible films, which can make product use more accessible and provide a more convenient solution. Edible films are thin layers of polysaccharides derived from starch, cellulose ether, alginate, carrageenan, pectin, and chitosan. These materials provide density, crackability, hardness, viscosity, gel-forming, thickening, and adhesion to consumable films (Grumezescu & Holban, 2018). The purpose of this study was to obtain an edible film combination of gingerol and curcumin that met the evaluation requirements and had antibacterial activity against Streptococcus pyogenes.

2. Materials and Methods

Tool

The equipment used includes a stirring rod (Pyrex), funnel (Pyrex), petri dish (Pyrex), test tube (Pyrex), incubator (B- One), autoclave (Hirayama HVE-50), oven (B-One), caliper (Mitutoyo), pH meter (Mettler Toledo), friability (Benchtop), and FTIR (Shimadzu).

Material

The materials used in this investigation include gingerol from Wellgreen Biotech.Ltd, curcumin from Merck, ethanol with 70% and 96% concentrations, Mayer's reagent, Dragendorf's reagent, and distilled water (aquadest). Then gelatin, PEG 400, maltodextrin, sucralose, and sodium benzoate were obtained from Brataco, Nutrient Agar from OXOID, MHA (Mueller-Hinton Agar) from OXOID, FG Troches from PT. Meiji Indonesian Pharmaceutical Industries, and Streptococcus pyogenes bacteria with code 19615. This research also refers to the ethical code SK No.050/E.01/KEPK-BTH/IV/2023.

2.1. Preparation of samples

2.1.1 Testing the characteristics of active ingredients

Testing of active ingredient characteristics includes shape, color, odor, and solubility.

2.1.2 Phytochemical screening

The samples were analyzed phytochemically to identify the types of chemical compounds they contained. This procedure includes checking for alkaloids, flavonoids, tannins, saponins, and steroids/terpenoids.
2.1.3 Gingerol and Curcumin Edible Film Formulation

<table>
<thead>
<tr>
<th>Material</th>
<th>Formulas % (b/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Gingerol</td>
<td>-</td>
</tr>
<tr>
<td>Curcumin</td>
<td>-</td>
</tr>
<tr>
<td>Gelatin</td>
<td>3</td>
</tr>
<tr>
<td>PEG 400</td>
<td>0.75</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>1</td>
</tr>
<tr>
<td>Sucralose</td>
<td>0.2</td>
</tr>
<tr>
<td>Peppermint oil</td>
<td>q.s</td>
</tr>
<tr>
<td>Sodium Benzoate</td>
<td>0.1</td>
</tr>
<tr>
<td>Aquadest add</td>
<td>100mL</td>
</tr>
</tbody>
</table>

Information: F1 = Basis
F2 = Active substance gingerol
F3 = Active substance curcumin
F4 = Combination of active substances gingerol and curcumin

2.1.4 Making Edible Film

Make edible film by dissolving gelatin in aquadest using a magnetic stirrer at a speed of 500 rpm, temperature ±60°C, and add PEG 400 as much as 0.75 mL stirred until homogeneous. Additionally, combine 1 gram of maltodextrin, 0.2 grams of sucralose, and 0.1 gram of sodium benzoate in 20 mL of aquadest. Then, add gingerol, curcumin, and 15 drops of peppermint oil to the mixture, stirring until thoroughly mixed. After that, leave it at room temperature first to remove air bubbles. Subsequently, pour 15 grams of the mixture into a petri dish and place it in an oven at 30°C for 48 hours for drying. Once dried, remove the edible film and cut it into rectangles measuring 3x2 cm (Dewi & Mulya, 2019).

2.2. Edible Film Evaluation

2.2.1 Organoleptic Test

Organoleptic tests observe each edible film's physical properties, such as shape, aroma, color, and taste (Harmely et al., 2015).

2.2.2 Thickness Test

The thickness of the edible film was measured using a caliper at three different locations, namely at the left edge, the middle, and the right edge of the film. After that, the measurement results were taken as an average (Santoso et al., 2013). By Japanese industrial standards (JIS), the thickness requirements that must be met by edible film are less than 0.25 mm.

2.2.3 Weight Uniformity Test

This study randomly took ten layers of edible film, and the average weight was measured using an analytical balance. One of the film layers is chosen randomly, weighed, and compared with the average weight calculated previously. This calculates the deviation value (Dewi & Mulya, 2019).

2.2.4 pH Test

The edible film was arranged on a petri dish, soaked with 10 mL of distilled water, and left for 5 minutes. After soaking, the pH value is measured with a pH meter device. The expected pH criteria for edible film is between 5.5 to 7.9 (Dewi & Mulya, 2019).

2.2.5 Britleness Test

Twenty pieces of edible film free from dust were weighed simultaneously with the initial weight (W1). Then, the sheets were put into the Friabilator and run for 4 minutes at a rotation speed of 25
rpm. After that, the 20 pieces of edible film were cleaned of dust and reweighed to obtain the final weight (W2) (Harmely et al., 2015).

2.2.6 Fold Resistance Test

Fold tenacity is measured by continuously folding the film in the exact location and observing the number of folds until the film is damaged (Dewi & Mulya, 2019).

2.2.7 Disintegration Time and Dissolution Time Test

A 10 cm diameter petri dish is filled with 10 mL of pH 6.8 phosphate buffer solution. The outer side of the petri dish contains distilled water, whose temperature is 37 ± 0.5 °C. Then, a piece of film is placed in the center of the petri dish, and the time required to dissolve is calculated. The expected time requirement for the film to dissolve is less than 30 seconds, following research by Sari et al. in 2019. Additionally, to achieve the desired solution level, the film dissolution time should not exceed 60 seconds, as noted by (Nurdianti et al., 2021).

2.2.8 Water Content Test

A total of 2 grams of edible film was placed in an aluminum cup on the moisture analyzer and spread evenly across the bottom of the aluminum cup. Next, the tool is set at a temperature of 105°C, and the test time is set for 5 minutes. After the test is complete, the value of the water content released during the process can be generated (Nurhidayati & Warmiati, 2021).

2.2.9 Elongation

Elongation measurements are carried out similarly to tensile strength measurements. The elongation value is obtained by comparing the gap distance at the time of fracture with the sample's initial length, then expressed as a percentage (%) (Hasdar & Rahmawati, 2017).

2.2.10 Tensile Strength

Tensile strength is the result of testing the maximum strength of a film after a tensile force is applied so that it stretches until it breaks. This parameter reflects the maximum power point that occurs on the film during the testing process. Tensile tests are carried out to understand the elasticity of a material and the material's ability to withstand pressure at the bending point (Safitri et al., 2016).

2.2.11 Stability Test

The consumable film preparation is coated with aluminum foil and placed in a tight, airtight container. It was stored at temperatures between 4°C and 40°C for six cycles. During this period, observations were made daily by evaluating the organoleptic and morphological characteristics, pH, disintegration time, and dissolution time.

2.2.12 Hedonic Test

The parameters observed were panelists' preferences for the color, shape, aroma, and taste of each edible film formula combination of gingerol and curcumin (Harmely et al., 2015). Then, the assessment data was obtained by comparing samples and assessment forms to the panelists. Assessment is in the form of a score based on color, shape, aroma, and taste. Panelists used as many as 15 people. The assessment criteria can be seen in Table 2.

<table>
<thead>
<tr>
<th>Assessment Criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dislike (score 1)</td>
</tr>
<tr>
<td>Color/shape/aroma/taste</td>
<td></td>
</tr>
</tbody>
</table>

2.2.13 Antibacterial Activity Test

The antibacterial activity of edible film combining gingerol and curcumin was tested using the well method. 0.2 mL of bacterial suspension *Streptococcus pyogenes* was inoculated into MHA media, then spread evenly and left to thicken. Wells were made using a perforator, and film sheets were
inserted into the holes formed. The following process involved incubation for 24 hours at 37°C. The area around the well was observed to have a clear zone (Nurhidayati & Warmiati, 2021).

2.3. Data Analysis

The observations obtained were processed using SPSS (Statistical Package for the Social Sciences) software by applying the non-parametric Kruskal Wallis Test statistical test, followed by the Post Hoc Mann Whitney test.

3. Results and Discussion

Characterization tests include organoleptic analysis, solubility, and identification of functional groups. Based on the results of organoleptic testing, gingerol and curcumin have a yellow and orange powder form and emit a distinctive aroma of gingerol and turmeric, as depicted in Figure 1. In addition, these two extracts are soluble in ethanol.

![Fig. 1. Organoleptic of Gingerol (A), Curcumin (B)](image)

Phytochemical research was carried out on gingerol to identify and understand the role of secondary metabolites in its biological activity. Details of the phytochemical results are listed in Table 3.

<table>
<thead>
<tr>
<th>Phytochemical Test</th>
<th>Reactor</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mayer</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>FeCl₃</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Gelatin 1%</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>Shake vigorously</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Liebermann-Burchard</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Mg powder and Amyl alcohol</td>
<td></td>
</tr>
<tr>
<td>Monoterpenes/Sesquiterpenes</td>
<td>Anisaldehyde</td>
<td>-</td>
</tr>
</tbody>
</table>

Information (+) = Indicates the presence of the identified compound
(-) = Does not indicate the presence of the identified compound

The results of the phytochemical screening test on the gingerol showed that it positively contained triterpenoid compounds, which resulted in a blackish-brown color change on the surface. The terpenoid compounds in gingerol are volatile and give gingerol its aroma. Then, the antibacterial way triterpenoids work is by interacting with porins (transmembrane proteins) found in the outer membrane of bacterial cell walls, forming strong polymer bonds and causing damage to the porins (Dohude et al., 2023).

The method used to make edible film is solvent casting. In this method, a large number of solvents and the principle of gelatinization are used. By adding a certain amount of hot water, gelatin is formed at a high temperature, so the amylose bonds tend to close together due to hydrogen bonding. This mixture is then poured into a petri dish. The drying process causes shrinkage because the water evaporates, forming a thin layer on the gel surface (Nofiandi et al., 2021).
The results of sensory evaluation on F1, F2, F3, and F4 showed that edible film has the shape of a thin rectangular layer with a size of 3x2 cm, a distinctive aroma, sweet taste on F1, spicy on F2, slightly sweet on F3, and sweet and slightly spicy on F4, transparent on F1, transparent yellow on F2, and orange in F3 and F4 (Figure 2). This is because each formulation has a different ratio of active ingredients added, where the taste is slightly spicy due to the presence of the active substance gingerol in the edible film. Meanwhile, the orange color appears due to the presence of active substances curcumin and gingerol in the edible film.

![Fig. 2. Edible Film (F1) Base (F2) Gingerol (F3) Curcumin (F4) Combination](image)

Edible film thickness measurements were carried out using digital calipers, with thickness measurements at three locations. Formulations F1, F2, F3, and F4 meet JIS (Japanese Industrial Standard) standards with a thickness of less than 0.25 mm. PEG is used as a plasticizer, and it is an organic material with a low molecular weight that can reduce the stiffness of polymers while increasing their flexibility in making edible films (Nofiandi et al., 2021).

Weight consistency testing shows weight variations for each different sheet of film. More information can be found in Table 4, which records body weight consistency test results ranging from 60 mg – 68.87 mg. The difference in weight and thickness of edible film can be caused by variations in the amount of active material, where the higher the amount of active substance used, the greater the weight and thickness of edible film. In addition, uneven side factors of the container while in the oven can cause the thickness and weight of the edible film to be unequal (Kalaka et al., 2022). Then, a pH check is carried out to determine the edible film and compare it with the pH of the human mucosa. In this study, the pH of edible film by human mucosa ranged from 5.6 to 7 (Anisa, 2021). Therefore, it can be concluded that the edible film has met the requirements, is considered safe to apply in the mouth, and does not react with other ingredients after the active substance is added Nofiandi et al., 2021.

The brittleness examination aims to evaluate or measure the physical strength of the resulting edible film. The strength test results can be found in Table 4, where all edible film formulations tested remained structurally intact and maintained their shape. Weight loss occurs due to frictional forces during strength testing. This finding aligns with previous research (Ningsih & Arel, 2022), which indicated that variations in strength test results between different formulations were caused by differences in the weight of each edible film and variations in the number of active ingredients used in each formulation. Then, the increased folding resistance shows that the film has a good consistency and will not be damaged or torn during storage. According to Zubaydah & Sahumena, 2021, a film with folding resistance ≥300 times shows excellent flexibility properties. The test results of each formulation show that it has a folding resistance of ≥500 times the film that is still intact and not damaged. This shows that the edible film can be used and meets the requirements.

Checking the disintegration and dissolution times aims to see how long the edible film takes to disintegrate and dissolve in the mouth. The disintegration time and dissolution time test results produced <60 seconds (Nurdianti et al., 2021). The thickness of the edible film influences the disintegration time and dissolution time because the thicker the edible film, the longer the edible film's disintegration time and dissolution time. Then, testing the water content of the four formulations produced a range of results between 8.58% to 12.05%, by JIS (Japanese Industrial Standards) standards. The observed differences in water content levels may be due to variations in each
formulation's active ingredient. According to JIS, the maximum standard for water content in edible film is 13%, and this research's results remain within the specified water content requirements.

**Table 4. Edible Film Evaluation Test**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>3x2 cm</td>
<td>3x2 cm</td>
<td>3x2 cm</td>
<td>3x2 cm</td>
</tr>
<tr>
<td>Color</td>
<td>White</td>
<td>Transparent</td>
<td>Yellow Transparent</td>
<td>orange</td>
</tr>
<tr>
<td>Smell</td>
<td>Distinctive Smell</td>
<td>Distinctive Smell</td>
<td>Distinctive Smell</td>
<td>Distinctive Smell</td>
</tr>
<tr>
<td>Flavor</td>
<td>Sweet</td>
<td>Spicy</td>
<td>A little sweet</td>
<td>A bit spicy</td>
</tr>
<tr>
<td>Thickness Test (mm)</td>
<td>0,12±0,0082</td>
<td>0,11±0,0033</td>
<td>0,11±0,0138</td>
<td>0,11±0,0054</td>
</tr>
<tr>
<td>Weight Uniformity Test (mg)</td>
<td>60,30 ± 5,7312</td>
<td>60,00 ± 1,6823</td>
<td>64,00 ± 1,7594</td>
<td>68,87 ± 2,7538</td>
</tr>
<tr>
<td>Surface pH Test</td>
<td>6,50 ± 0,0058</td>
<td>6,78 ± 0,0058</td>
<td>6,80 ± 0,0100</td>
<td>6,50 ± 0,0058</td>
</tr>
<tr>
<td>Brittleness Test (%)</td>
<td>1,14</td>
<td>1,24</td>
<td>0,68</td>
<td>1,95</td>
</tr>
<tr>
<td>Fold Resistance Test (time)</td>
<td>≥500</td>
<td>≥500</td>
<td>≥500</td>
<td>≥500</td>
</tr>
<tr>
<td>Crash Test (seconds)</td>
<td>19,26 ± 5,8313</td>
<td>19,34 ± 3,0452</td>
<td>16,02 ± 3,7123</td>
<td>7,10 ± 3,2616</td>
</tr>
<tr>
<td>Solubility Test (seconds)</td>
<td>50,16 ± 0,6584</td>
<td>53,37 ± 0,9193</td>
<td>49,81 ± 0,8871</td>
<td>50,49 ± 2,9738</td>
</tr>
<tr>
<td>Water Content Test (%)</td>
<td>9,58</td>
<td>9</td>
<td>12,05</td>
<td>8,58</td>
</tr>
</tbody>
</table>

Information: F1 = Base
F2 = Active substance gingerol
F3 = Active substance curcumin
F4 = Combination of active substances gingerol and curcumin

The results of measuring the elongation percentage describe the change in the length of the edible film when it is pulled until it breaks. This elongation percentage value is essential in determining the edible film's flexibility level. Based on the data collected, the extension of formulations F1, F2, F3, and F4 shows unsatisfactory results. JIS Standard (Japanese Industrial Standard) indicates that the elongation value considered good is >50%, while values below 50% are considered unsatisfactory. Then, the elongation percentage value is closely related to the tensile strength value of the edible film. A lack of added plasticizer can reduce the elongation percentage, which causes the edible film to become too strong and somewhat stiff (Rizkyati & Winarti, 2022). In addition, PEG 400 has a more considerable molecular weight, which produces a higher tensile effect but reduces the film's elasticity (Marpongahtun, 2016). In general, plasticizing agents can increase the elongation rate of edible film until it reaches a specific concentration. This strengthening is supported by a study conducted by Marpongahtun in 2013, where the value of the elongation level using the plasticizer PEG 400 produced a higher figure than the plasticizer xylitol and sorbitol.

The tensile strength evaluation results are the force acting on an elastic object that tends to elongate to a specific limit. This research shows that this force increases as the concentration of the active ingredient used increases. An increased tensile force indicates higher resistance to deformation due to more significant stress and pressure (Noviandi et al., 2016). Testing of tensile strength and elongation percentage shows an inverse correlation, where the lower the elongation percentage, the higher the tensile force required to produce a particular edible film.
Hedonic testing was carried out to identify the formulation most liked by participants in a test of four existing edible film formulations. This hedonic test includes assessing aspects such as shape, color, taste, and aroma of edible film. A total of 30 respondents participated in this test. The collected data was then analyzed using SPSS version 25 statistical software by applying the Friedman Test. This test aims to determine the formulation that respondents most prefer. Then, the Friedman test results for the parameters of shape, color, taste, and aroma of edible film show a significance value (Sig.) of P>0.05. Therefore, it can be concluded that there are no significant differences between the four edible film formulations in terms of shape, color, taste, and aroma.

The results obtained from the four formulations of edible film show that the most preferred one in terms of shape is F2. For the color edible film, the one that is most preferred is formulation 4. This is because it is related to the addition of curcumin, which significantly influences the color of the edible film. The attractive color of the product will attract someone to try it.

Test results Friedman, for the aroma of the four formulation edible films, the one that is most liked is formulation 3, due to adding gingerol and curcumin, which produces a more pungent, distinctive smell. Taste parameters of the four edible film formulations, the popular one is formulation 1 because no active ingredients have been added, so the sweet taste is still dominant compared to F2, with a slightly spicy taste from adding gingerol. Then, F3 is slightly sweet from adding curcumin, and F4 has a slightly sweet and spicy taste from adding gingerol and curcumin.

Stability Test

Stability tests in cycle tests on organoleptic parameters at 4℃ and 40℃ showed no changes. The organoleptic values remain consistent throughout the testing period, just as the initial conditions of the examination mean that the shape, color, aroma, and taste are stable during storage. However, the pH parameters, disintegration time, and dissolution time experienced changes where there was an
increase and decrease caused by storage for 6 cycles at both 4°C and 40°C. However, it can still be said to be good because it is still within the range of existing requirements. In addition, the effect of the concentration of active ingredients used in each formulation differs, resulting in instability during storage.

**Edible Film Antibacterial Activity Test**

The antibacterial activity of edible film was tested using an agar diffusion approach, where 6 mm film sheet fragments were cut and placed in media that had previously been planted with Streptococcus pyogenes bacteria. Information on antibacterial test results can be referred to in Table 5.

**Table 5. Edible Film Test Results in Combination of Gingerol and Curcumin Against Streptococcus pyogenes Bacteria**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Average (mm) ± SD</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0±0</td>
<td>No activity antibacterial</td>
</tr>
<tr>
<td>F2</td>
<td>6,56±0,6325</td>
<td>Currently</td>
</tr>
<tr>
<td>F3</td>
<td>6,36±0,0748</td>
<td>Currently</td>
</tr>
<tr>
<td>F4</td>
<td>6,92±0,0920</td>
<td>Currently</td>
</tr>
<tr>
<td>K+</td>
<td>13,83±0,3091</td>
<td>Strong</td>
</tr>
</tbody>
</table>

Information:  
F1 = Base  
F2 = Active substance gingerol  
F3 = Active substance curcumin  
F4 = Combination of active substances gingerol and curcumin  
K+ = Positive Control (FG Troches)

Table 5 shows that in formulations F2, F3, and F4, an inhibition zone was formed on the MHA media, which experienced the growth of Streptococcus pyogenes bacteria. The average sizes of the inhibition zones formed were F2 6.56 mm, F3 6.36 mm, F4 6.92 mm, and formulation F4 produces an inhibition zone with a larger size.

The research data obtained has been tested statistically using non-parametric tests. Before running the test, the first step is to conduct a normality test to ensure that the data follows a normal distribution. This research tested the normality of the inhibitory power of an edible film, a combination of gingerol and curcumin, against Streptococcus pyogenes bacteria. Testing was carried out using SPSS version 25 software, and the normality test used the Shapiro-Wilk method because the amount of data was limited (0.05). Next, proceed with the homogeneity test. The homogeneity test results showed that the data did not meet the homogeneity assumption (p<0.05). Therefore, the conclusion that can be drawn is that the data is non-parametric. As the next step, the Kruskal-Wallis test was carried out to analyze the differences between the existing groups.

The Kruskal-Wallis test is a non-parametric statistical method used to assess whether there is a significant difference between the group of independent variables and the dependent variable as quoted in (Rozi et al., 2022). The result of the Kruskal-Wallis test 0.052 shows a significance value of p>0.05, which shows that the data are not significantly different.

The next step is to conduct a post hoc test using the Mann-Whitney method to identify which groups have significant differences. If the p-value < 0.05, this indicates a significant difference in the data. Conversely, if the p-value is > 0.05, the data has no significant difference. The results of the Mann-Whitney test show a significant value with p < 0.05, indicating significant differences in each formulation.
### Functional Group Analysis Edible Film

This study used Fourier Transform Infrared (FTIR) analysis to identify functional groups in gingerol, curcumin, and functional groups contained in edible film. Each existing functional group shows a vibration pattern in a specific wavenumber range between 4000 and 400 cm\(^{-1}\). Based on Figure 5(a), it can be seen that gingerol shows the presence of a hydroxyl group at a wavenumber of 3304 cm\(^{-1}\). Hydroxyl groups generally appear in the 3600-3200 cm\(^{-1}\) wavenumber range. In addition, the NH\(_2\) group was detected at wavenumber 2923 cm\(^{-1}\), usually present in the range 2926 cm\(^{-1}\) to 2853 cm\(^{-1}\). The amide group can also be observed at a wavenumber of 1636 cm\(^{-1}\), which often appears in the amine functional group's 1700-1600 cm\(^{-1}\) wavenumber range (Brittain, 2006). Complete information regarding the testing of gingerol and curcumin functional groups is shown in Figure 5.

![FTIR Results](image)

**Fig. 5.** FTIR Results in a) Gingerol and Curcumin, b) Edible Film

### 4. Conclusion

Based on the results of research, the edible film has a rectangular shape measuring 2x3 cm with different colors for each formulation, sweet and spicy taste, distinctive aroma, and evaluation results meet the requirements of the Japanese Industrial Standard (JIS) and existing theories, but the percent elongation has poor results. Then, the stability test on organoleptic parameters was stable during 6 storage cycles. At the same time, disintegration and dissolution time were unstable for the pH parameters during 6-cycle storage at both 4 °C and 40 °C. However, it is still within the requirement range, and the hedonic test results show no significant difference in each formulation. In addition, edible film single and combination formulations were able to inhibit bacteria with diameters of inhibition zones F2 6.56 mm ± 0.6325, F3 6.36 mm ± 0.0748, and F4 6.92 mm ± 0.0920 against *Streptococcus pyogenes* bacteria with respectively medium category.

### Author Contributions:

All authors have read and agreed to the published version of the manuscript.
Funding
No funding to report.

Competing Interests
The authors declare no conflict of interest.

Acknowledgment
Not available.

References


Nurdianti et.al (Formulation And Evaluation...


