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Effervescent Tablet with Lime Peel and Tamarind Extract as Fruit and Vegetable Decontaminants

Zelly Salmyanti Rahman Zam[1)], Novia Elsaputri[2)], Farah Alya Amril[3)], Fathiya Rizqillah Ranza[4)], Najla Khalisha Alem[5)], Zuhrah Taufiqa[6)]*
[1,2,3,4,5,6)] Department of Medicine, Faculty of Medicine, Universitas Negeri Padang, Padang, West Sumatra, Indonesia

*Corresponding Author
Email : zuhrah.taufiqa@fk.unp.ac.id

Abstract

The safety of fruits and vegetables is important to prevent diseases caused by contamination by pathogenic microbes such as Escherichia coli and Staphylococcus aureus. This study aims to formulate and test the antimicrobial activity of effervescent effervescentes based on lime (Citrus aurantifolia) and tamarind (Tamarindus indica) peel extracts as natural decontaminants. The study was conducted experimentally with a post-test only design in the Integrated Laboratory and Biomedical Laboratory of the Faculty of Medicine, Padang State University. Effervescent tablets were made by the wet effervescentation method using three variations of extract concentrations (7%:5%), (6%:6%), and (5%:7%). The antimicrobial activity was tested against Escherichia coli and Staphylococcus aureus using the disc diffusion method on MHA media. The results showed that the extracts contain flavonoids, tannins, and organic acids that act as antimicrobial agents. The three formulations produced different inhibition zones, with formula 1 (7%:5%) effective against Escherichia coli (7.5 mm) and formula 3 (5%:7%) effective against Staphylococcus aureus (13 mm). The effervescent tablet had a dissolution time of <2 minutes.

Keywords: Antimicrobial; Tamarind; Decontaminant, Lime Peel, Effervescent Tablet.

INTRODUCTION

Food safety is a key prerequisite in efforts to improve public health and nutrition. Fresh fruits and vegetables are highly susceptible to contamination by pathogenic microorganisms such as *Staphylococcus aureus* and Escherichia coli, which can cause various health problems and increase the risk of foodborne diseases such as diarrhea, dysentery, and poisoning (Hussain and Gooneratne, 2017; Mostafidi *et al.*, 2020; Karila *et al.*, 2022). However, the consumption of fruits and vegetables plays an important role in meeting the intake of vitamins, minerals, fiber, antioxidants, and prebiotics that support immunity, prevent non-communicable diseases, and support growth and development, especially in children, pregnant women, and the elderly (Taufiqa, 2023; Yoo *et al.*, 2024).

Contamination of fruits and vegetables can occur during cultivation and consumption, mainly due to the use of contaminated irrigation water, raw manure, or unhygienic equipment. Although routine decontamination is carried out, clean water alone is not effective in removing pathogens and non-natural contaminants (Kaushik *et al.*, 2020). Chemical-based disinfectants such as hypochlorite, surfactants, and hydrogen peroxide are effective but carry the risk of leaving residues that impair taste and pose health hazards. Therefore, there is a need for safe, residue-free, affordable, and user-friendly natural innovations (Silviani and Saktiningsih, 2020).

Lime (Citrus aurantiifolia) is known as an herbal plant rich in active compounds such as alkaloids, saponins, flavonoids, and ascorbic acid, which function as antimicrobials and are capable of reducing pesticide residues (Nisa', 2020; Sari and Asri, 2022). Lime peel extract has been shown to inhibit the growth of various pathogenic bacteria such as *Staphylococcus aureus* and Klebsiella pneumoniae and reduce pesticide residue levels (Nisa', 2020; Monitria and Indirawati, 2021). Similarly, tamarind (*Tamarindus indica*) is a plant that is widely used in the daily lives of Indonesians, both as food, traditional medicine, and for other needs. Its pod-shaped, sour-tasting fruit contains flavonoids and ascorbic acid, which act as antibacterial agents against both gram-positive and gram-negative bacteria (Silalahi, 2020). The ascorbic acid content in tamarind can reduce pesticide residues in fruit by up to 80% (Fitriadi and Putri, 2016).

Based on the antimicrobial potential of these two natural ingredients, researchers want to develop an effervescent tablet formulation as a fruit and vegetable decontaminant based on natural extracts from lime peel and tamarind. This formulation is expected to kill pathogens that adhere to the surface of fresh fruits and vegetables, reduce pesticide residues, while maintaining the natural taste and aroma of fruits and vegetables. Previous studies have found that the application of ascorbic acid to foodstuffs can be an alternative method to inhibit the growth of *Staphylococcus aureus* and Escherichia coli bacteria as an anti-quorum sensing (AQS) agent and inhibitor of extracellular polymer production (Przekwas *et al.*, 2020). However, further research is needed to determine the effective dose for killing pathogens on fruits and vegetables that are safe to consume and do not affect their quality. Compared to synthetic decontaminants, this innovation is more practical, safe, and effective because the tablets dissolve completely and provide uniform cleaning.

RESEARCH METHODS

The study was conducted from July to November 2025 at the Integrated Laboratory of Padang State University, the Biomedical Laboratory of the Faculty of Medicine, Padang State University, and the Laboratory of the Imam Bonjol Pharmacy Academy in Bukittinggi. This study used a posttest-only design to evaluate the antimicrobial activity of effervescent tablet formulations of lime peel extract and tamarind against Escherichia coli and *Staphylococcus aureus*. This study obtained ethical approval from the UNP Research Ethics Committee with research number No.052/KEPKUNP/9/2025.

The research samples used consisted of two types, namely test material samples in the form of lime peel and tamarind, as well as test microorganism samples taken from water used to wash tomatoes, pears, and lettuce. The independent variable in this study was the variation in the concentration of lime peel and tamarind extract in each formulation (F1, F2, and F3), while the dependent variable was the zone of bacterial growth inhibition formed on Mueller-Hinton Agar (MHA) media. The positive control used was 500 mg of Amoxicillin, and the negative control was tap water. The test results from each formulation were then compared with the controls in order to determine the antimicrobial effectiveness of the formulated effervescent tablets.

The tools and materials used in this study were lime peel, tamarind, oven, blender, sieve, basin, 96% ethanol, scales, glass jars, filter paper, rotary evaporator, water bath, glass bottles, Liquid Chromatography -Mass Spectrometry (LC-MS/MS) for analysis of active compounds in the extract, refrigerator, digital scale, lettuce, tomatoes, pears, water, dropper pipettes, micropipettes, sterile cotton swabs, test tubes, syringes, measuring cups, Bunsen burners, beakers, Erlenmeyer flasks, distilled water, 0.9% NaCl solution, autoclave, incubator, spectrophotometer, aluminum foil, plastic wrap, Petri dishes, MacConkey Agar, Mannitol Salt Agar (MSA), Nutrient Agar (NA), Mueller Hinton Agar (MHA), disposable loop, object glass, hot plate, magnetic stirrer, iodine solution, lugol, safranin, 70% alcohol, laminar safety cabinet, microscope, ruler, disc paper, tweezers, lime peel extract, tamarind extract, lactose, sodium bicarbonate, PEG 6000, CMC -Na, citric acid, tartaric acid, No. 18 mesh sieve, mortar and pestle, spatula, tablet press, pH meter, vernier caliper, friability tester, synthetic decontamiant, and 500 mg amoxicillin tablets.

This research was conducted in two stages. The first stage involved the preparation of lime peel and tamarind simplicia, extraction of lime peel and tamarind, analysis of active compounds using LC-MS/MS, formulation of effervescent tablets, and evaluation of tablet quality. The second stage involved antimicrobial activity testing and organoleptic evaluation of fruits and vegetables after decontamination using the effervescent tablets as shown in **Figure 1**.

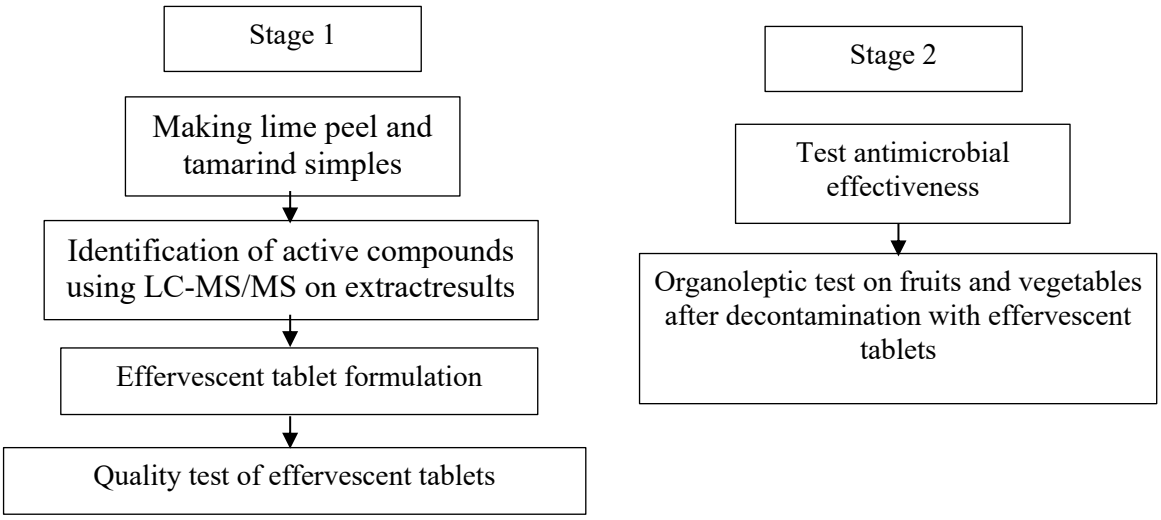

Figure 1. Research Stages

Simplisia Production

The lime peel and tamarind samples were wet sorted, then washed using running water and drained to remove any remaining water. The lime peel and tamarind were then chopped into specific sizes to speed up the drying process. A total of 6.4 kg of wet lime peel and 4 kg of tamarind were dried at room temperature for 3 days. Then, drying was carried out using an oven at a temperature of 40°C for ±5 days. The dry simplisia obtained was ground separately with a blender and sieved to obtain a powder with a uniform size (Dewangga *et al.*, 2017).

Extract Preparation

The powdered simplisia of lime peel and tamarind, obtained in quantities of 500 g each, were macerated using 96% ethanol three times at ratios of 1:3, 1:2, and 1:2 in glass jars for 3x24 hours. During maceration, stirring was performed every 2 hours for 5 minutes. Each filtrate was then separated and evaporated using a rotary evaporator at 40°C, followed by a water bath for 2 days to obtain a thick extract (Dewangga *et al.*, 2017; Sari and Asri, 2022).

The dry extract was prepared by adding lactose to the thick extract of lime peel and tamarind at a ratio of 1:3. The mixture was ground until compact and dried in an oven for 1 hour at a temperature of 50°C. Then, the dried mixture was sieved with a 18-mesh sieve to form granules of dried lime peel and tamarind extract (Mopuri *et al.*, 2018; Mayefis and Bidriah, 2022).

Identification of Active Compounds in Extracts

The identification of active compounds in lime and tamarind extracts was carried out using Liquid Chromatography Mass Spectrometry (LC-MS/MS). This method combines the physical separation capabilities of liquid chromatography with the analytical capabilities of MS. The analysis results are in the form of chromatograms that display the peaks of each compound based on their molecular weight and intensity, so that the type and amount of compounds in the sample can be determined (Indriyati, Andayani and Sunarwidhi, 2023; Kaliawan and Danardono, 2023).

Effervescent Tablet Formulation

Effervescent tablets were formulated with three variations in concentration between lime peel extract (*Citrus aurantifolia*) and tamarind extract (*Tamarindus indica*) with a total extract of 10%. The detailed formulation can be seen in Table 1.

Table 1. Effervescent Tablet Formulation

Material	Function	Formula (%)		
		F1	F2	F3
Lime peel extract	Active Substance	7	6	5
Tamarind extract	Active Substance	5	6	7
Sodium bicarbonate	Base Source	30	30	30
PEG 6000	Lubricant	2	2	2
CMC-Na	Binding agent	4,5	4,5	4,5
Sitric acid	Source of Acid	7	7	7
Tartaric acid	Source of Acid	23	23	23
Lactose	Filler Substance	Ad 100	Ad 100	Ad 100
Total weight of effervescent tablets		500 mg	500 mg	500 mg

* One formula (500 mg) produces 1 effervescent tablet

The formulation uses the wet granulation method. mass-1 consists of effervescent lime peel extract, effervescent tamarind extract, lactose, sodium bicarbonate, PEG 6000, and CMC-Na. The ingredients are mixed and sieved using an 18-mesh sieve. Mass-1 is dried in an oven for 15 minutes at 60°C. Mass-2 consists of a mixture of tartaric acid and citric acid. Mass-2 mixture is homogenized and then sieved using an 18 mesh sieve. After mass-1 is dry, it is mixed with mass-2 and stirred until homogeneous. Sieving is carried out using an 18 mesh sieve so that the mixture has a uniform size, resulting in a granule weight of 500 g. Then, effervescent tablets are pressed (Yulianti *et al.*, 2021; Mayefis and Bidriah, 2022).

Effervescent Tablet Quality Test

Organoleptic Tests

Organoleptic testing includes color, shape, aroma, and taste visually (BPOM RI, 2019).

Weight Uniformity Test

Weight uniformity testing by weighing 20 tablets, there should be no 2 tablets deviating larger than column A and there should not be 1 tablet deviating larger than that specified by column B (BPOM RI, 2019). The weight uniformity test can be seen on **Table 2**.

Table 2. Weight Uniformity Requirements

Average weight	Deviation from average weight	
	A	B
25 mg or less	15%	30%
26 mg to 150 mg	10%	20%
151 mg to 300 mg	7,5%	15%
More than 300 mg	5%	10%

Size Uniformity Test

Measurement uniformity testing was performed on 20 tablets by observing the diameter and thickness of the tablets using a caliper (BPOM RI, 2019). Requirements for uniformity of size unless otherwise stated, the diameter of the tablet should not be more than 3 times the thickness of the tablet and should not be less than one-third the thickness of the tablet (Kemenkes RI, 2020).

Fragility Test

The test of the fragility or frivolity of the tablets is carried out by means of 20 tablets being released and then weighed and then inserted into the device friability tester. The tool runs at 25 rpm for 4 minutes with 100 revolutions. Next the tablets are weighed again. Good tablets have a fragility value of less than 1% (Kemenkes RI, 2020).

With the formula:

$$\%\text{Penyimpanan} = \frac{W_0 - W_1}{W_0} X100\%$$

W0 = initial weight
W1 = Final Weight

Dissolved Time Test

Dissolution time testing is carried out by means, one tablet effervescent put in a glass containing 200 mL *Aquadest*. Prepared *Stopwatch* Then it starts to count when the tablet is dipped and the time is stopped when the tablet dissolves perfectly in water. Tablets are said to be good if they dissolve within 1-2 minutes (Kemenkes RI, 2020).

pH Test

Testing of the degree of acidity or pH by means, a tablet effervescent dissolved in 200 mL aquadest. Then the solution is measured in pH using a pH meter (Mayefis and Bidriah, 2022).

Foam Height Test

The test is carried out by measuring the height of the foam during the acid and alkaline reactions, measured from the surface of the water, at the maximum height during the reaction (Puspitasari and Suharsanti, 2022).

Antimicrobial Effectiveness Tests

Sterilization of Tools and Materials

The glass tools and media are wrapped in aluminum foil then put into the autoclave at 121°C for 2 hours.

Sample Dilution

Sample laundry immersion water tomatoes, pears, and lettuce taken as much as 1 mL, then diluted using a 0.9% NaCl solution with a ratio of 1:9 dilution was carried out three times in a row (dilution 10^{-1} up to 10^{-3}). This aims to reduce the amount of microbial content contained in the sample (Ardiana, Augustin and Advinda, 2021; Putri, Kasasiah and Saula, 2023; Cahyaningtyas, Gaina and Tangkonda, 2024).

Selective Media Creation

The selective media used in this study were *MacConkey Agar* for the isolation of Gram-negative bacteria such as *Escherichia coli* and *Manitol Salt Agar* (MSA) for the isolation of Gram-positive bacteria such as *Staphylococcus aureus*.

Each medium was dissolved in 250 mL of aquadest in erlenmeyer, Medja *MacConkey Agar* used as much as 12.8 g, while MSA was 27.75 g. Then, homogenized and heated with *a magnetic stirrer* until it dissolved completely. After that, the media is sterilized by autoclave at 121°C for 2 hours, cooled to ±50°C, and then poured into a sterilized petri dish. The sampling technique was carried out by inoculating the dilution results on selective media using ***sterile*** disposable ose. Next, it was incubated with an incubator at a temperature of 37 °C for 24 hours, then the colonies were observed and counted using the direct observation method.

Creation of Agar Nutrient Media (NA)

As many as 7 g NA powder dissolved in 250 mL aquadest inside erlenmeyer homogenized and heated using *Magnetic Stirrer* until it dissolves completely. Then, it is sterilized with an autoclave at 121°C for 2 hours. NA media is used as a colony purification media the result of isolation from selective media. Media culture isolation *MacConkey Agar* identified as *Escherichia coli*, characterized by pink colonies because they are able to ferment lactose (Dewi, 2013; Jung and Hoilat, 2024). Meanwhile, MSA culture isolates were identified as *Staphylococcus aureus*, characterized by colonies that form yellow zones because they can ferment mannitol. The obtained pure colonies were then incubated with an incubator at 37 °C for 24 hours before Gram staining and antimicrobial activity tests (Retnoningrum *et al.*, 2019; Lasmini and Margaretta, 2022).

Gram Staining

Single colony *Escherichia coli* and *Staphylococcus aureus* obtained in the NA media was then identified with crystal violet dye, iodine, 96% alcohol, and saffron. Then the bacteria were observed

using a microscope with a 100x magnification of the objective lens. The positive results of the observations show that *Escherichia coli* constitute Gram-negative bacteria colored ones pink with stem shape, while *Staphylococcus aureus* constitute Gram-positive bacteria colored ones purple with shape of the coccus (Lasmini and Margaretta, 2022; Putri, Kasasiah and Saula, 2023).

Manufacturing of Mc Farland 0.5 Solution

Solution McFarland 0.5 made by mixing 0.05 mL $BaCl_2$ 1% solution and 9.95 mL H_2SO_4 1% solution, then homogenized until it is formed turbid suspension. Standard solution is stored in t to avoid direct light exposure to room temperature (±25°C) and divortex before use to restore its homogeneity (Chandra, Putri and Restapaty, 2024).

Manufacture of Bacterial Suspension

Bacterial colonies were taken from NA culture stock with sterile ose needles and suspended into a test tube containing 10 mL of 0.9% NaCl. Then, measurements are made of the turbidity of the solution with a comparison of turbidity *McFarland* on a scale of 0.5 or equivalent to 1x108 CFU/mL (Rizki, Latief and Rahman, 2021).

Measurements are carried out with measure in advance the absorbance of McFarland's standard solution of 0.5, followed by measurement of bacterial suspension test using a spectrophotometer at a wavelength of 600-625 nm**.** When higher absorption value of bacterial test suspension rather than the standard (0.08–0.1), then the suspension diluted with a 0.9% physiological NaCl solution until an absorbance value is obtained equivalent to McFarland's standard solution of 0.5 (Rosmania and Yanti, 2020)**.**

Creation of Mueller-Hinton Agar Media (MHA)

A total of 9.5 g of *Mueller-Hinton Agar* (MHA) powder was dissolved in 250 mL of aquadest in erlenmeyer, then homogenized and heated using a magnetic stirrer until it was completely dissolved. The MHA solution is sterilized using an autoclave at 121°C for 2 hours, then cooled to 45–50°C before being poured into a sterile petri dish. After hardening, the media is ready for use for testing.

Antimicrobial Effectiveness Test Using Disc Diffusion Method

Test bacterial suspension that has been customized with McFarland standard 0.5 taken using sterile cotton swab, then spread evenly over the entire surface of the MHA media with 60° rotation movement between smear directions to ensure a homogeneous distribution of inocuum. After the surface of the medium has dried for ±15 minutes at room temperature, it is carried out disc paper placement (disc) that has been soaked for 15 minutes in a control solution, three tablet formulations effervescent (F1, F2, and F3), and synthetic decontaminants (Kurniawan, Zuhdi and Nasution, 2023).

The testing technique is carried out using Kirby–Bauer agar diffusion method with disc paper (*disc diffusion method)* to find out the antimicrobial activity of tablets effervescent formulated. Positive control using Amoxicillin 500 mg**,** negative control in the form of tap water**,** Tablet formulation effervescent (F1, F2, and F3) and synthetic decontaminants, each of which is dissolved in 200 mL aquadest. Sterile disc paper dipped into each test solution, then placed on the surface of the MHA media that has been inoculated with a test bacterial suspension and marked with a label (Aviany and Pujiyanto, 2020). Then, incubated at 37ºC for 24 hours. The ONA of the inhibition formed around the disc was measured using calipers three times (triplo). After obtaining results The diameter of the barrier zone formed is then compared to the standard listed in the *Clinical and Laboratory Standards Institute* 2020 (Putri, Kasasiah and Saula, 2023). The classification of the inhibition zone can be seen in the **Table 3.**

Table 3. Classification of Buffer Zones

Jamming Zone Diameter (Light Zone)	Growth Inhibition Response
>20 mm	Very Powerful
10-20 mm	Strong
5-10 mm	Medium
<5 mm	Weak

RESULTS AND DISCUSSION

Extraction

In simplicia, 500 g were used each which produced 2500 mL of lime peel filtrate and 3000 mL of tamarind. A thick extract of orange peel as much as 50 g and tamarind as much as 175 g was obtained.

Calculation of the percentage of yield = x 100% $\frac{Berat\ hasil\ ekstrak\ kental}{Berat\ serbuk\ simplisia}$ (Chandra, Putri and Restapaty, 2024). Can be seen on **Table 4**.

Table 4. Extraction Results

Sample	Weight of Simplisia Powder	Weight of Condensed Extract Yield	Present Returns
Lime Peel	500 g	50 g	10%
Tamarind	500 g	175 g	35%

LC-MS/MS Test

The results of the analysis of the active compounds showed the chromatogram profile of LC–MS/MS lime peel extract (*Citrus aurantifolia*). The detected peak pattern illustrates the presence of secondary metabolites including alkaloids, carboxylic acids, and bronopoles that can be seen in **Figure 2**.

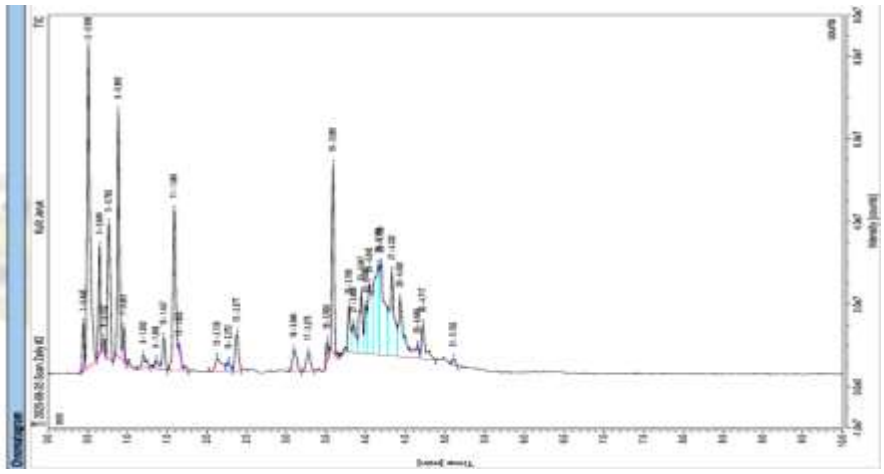

Figure 2. Lime Peel LC-MS/MS Test Results

The results of the analysis of the active compounds showed the chromatogram profile of LC–MS/MS of tamarind extract (Citrus aurantifolia). The detected peak pattern illustrates the presence of secondary metabolites including alkaloids, flavonoids, carboxylic acids, and bronopoles that can be seen in **Figure 3**.

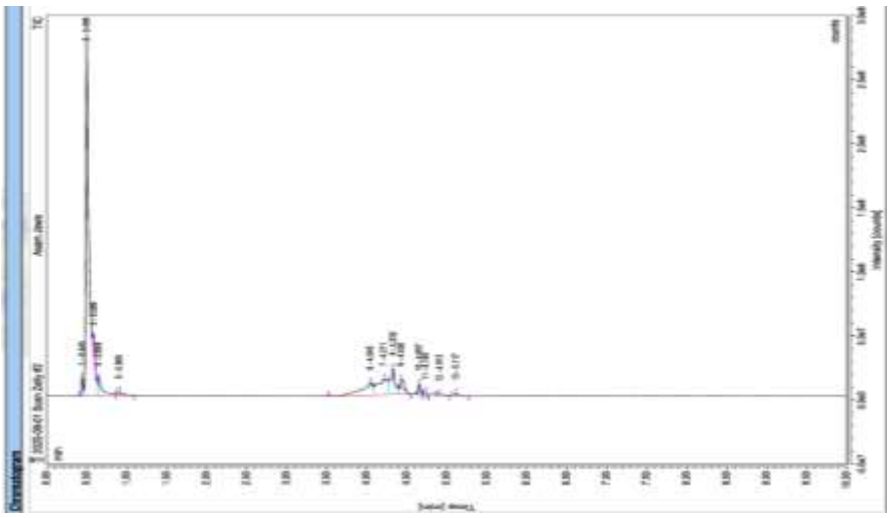

Figure 3. Tamarind LC-MS/MS Test Results

Results of analysis of active compounds in lime peel extract (*Citrus aurantifolia*) and tamarind extract (*Tamarind indica*) has antimicrobial activity such as inhibiting protein synthesis, damaging cell membranes, and interfering with microbial metabolism (Nisa', 2020; Silalahi, 2020; Sari and Asri, 2022).

The findings of this study indicate that lime peel extract (Citrus aurantifolia) and tamarind extract (*Tamarindus indica*) contain various active compounds as identified through LCMS/MS analysis. Both extracts contain alkaloids, carboxylic acids, and bronopol, while the main difference lies in the presence of flavonoids in tamarind extract. In previous studies, alkaloids have been identified as active compounds in lime peel. The alkaloid content in lime peel is known to be higher than that in lime fruit pulp (Indriyani *et al.*, 2023). This compound has also been identified in tamarind extract in previous studies (Rohama *et al.*, 2025). Alkaloids are known to have antibacterial properties with a mechanism of destroying cell walls. These compounds inhibit the enzymatic processes of bacterial cells, which ultimately leads to bacterial death (Sari and Asri, 2022).

In another study, carboxylic acid content was also detected using another method, namely Fourier Transform Infrared (FTIR). In this study, the FTIR spectrum of microbeads without extract showed clear amide peaks at 3417.97 cm^{-1} and 1622.32 cm^{-1}, confirming the bond between the carboxylate group of alginate and the amine group of gelatin. When C. aurantifolia peel extract was added, the signal intensity in that range increased, which was interpreted as an indication of the presence of functional groups similar to those found in lime peel ethanol extract. The absorption pattern that remained stable between the two conditions also indicated that there was no significant chemical interaction between the matrix and the extract components, reinforcing that the presence of carboxylic acid in the extract was indeed consistent with the functional group characteristics detected through FTIR (Julaeha *et al.*, 2024). Carboxylic acids have been identified in tamarind pulp (Mira *et al.*, 2024).

Carboxylic acids have antimicrobial activity because their undissociated form (RCOOH) is lipophilic and can penetrate the plasma membrane of microbes through passive diffusion. This mechanism makes carboxylic acids more effective than strong inorganic acids, which are generally only toxic to the outside of the cell. The level of toxicity depends on the pKa, the pH of the environment that determines the proportion of the RCOOH form and lipophilicity, because the more lipophilic an acid is, the easier it is to diffuse and the greater its toxic effect (Mira *et al.*, 2024).

The presence of flavonoids in tamarind is consistent with previous studies. Although most studies focus on their content in leaves, flavonoids are found in almost every part of the plant, making tamarind an increasingly researched source of antioxidants (Sookying *et al.*, 2022). Flavonoids are a group of polyphenols known to have the ability to inhibit bacterial growth through various action

pathways. Several studies report that these compounds can disrupt important processes within microbial cells, including genetic material synthesis, cytoplasmic membrane stability and function, and energy supply pathways. Additionally, flavonoids are reported to be able to reduce the ability of bacteria to attach and form biofilms, affect porin function, and alter membrane permeability. Disruption of these factors ultimately reduces the virulence and ability of bacteria to proliferate (Shamsudin *et al.*, 2022). The presence of bronopol in the LC-MS/MS analysis results has not been previously described in this plant. Thus, these findings still require further clarification to determine whether the compound is a natural component or the result of chemical transformation during the sample processing.

Effervescent Tablet Formulation

Tablet formula *effervescent* from lime peel extract (*Citrus aurantifolia*) and tamarind (*Tamarind indica*) successfully made by wet granulation method. Three variations in extract concentration (lime peel: tamarind), namely F1 (7%:5%) **Figure 4.a**, F2 (6%:6%) **Figure 4.b**, and F3 (5%:7%) **Figure 4.c**. The resulting granules are molded into tablets *effervescent* 500 mg (Yulianti *et al.*, 2021; Mayefis and Bidriah, 2022).

Figure 4. *Effervescent* Tablets a. F1, b. F2, c. F3

The effervescent tablets developed in this study were formulated in three variations of active ingredient concentration, namely combinations of lime peel extract and tamarind extract totaling 10% in each formula (F1–F3). The main difference between the formulas lies in the relative composition of the two extracts, namely F1 (7%:5%), F2 (6%:6%), and F3 (5%:7%). This variation aims to obtain the most optimal antimicrobial potential (Yulianti *et al.*, 2021; Mayefis and Bidriah, 2022)

Sodium bicarbonate, citric acid, and tartaric acid were used as an effervescent acid–base system with a fixed composition in all three formulas. Sodium bicarbonate acts as a high-concentration base source (30%), while citric acid and tartaric acid each act as acid sources in proportional ratios to produce a rapid effervescent reaction and maintain the pH of the solution when the tablet is dissolved. The combination of these two acids is commonly used in effervescent formulations to optimize the dissolution rate and stability of the granules (Mayefis and Bidriah, 2022; Julianti et al., 2024).

The addition of excipients such as PEG 6000, CMC-Na, and lactose supports the formation of tablets that have good physical properties. PEG 6000 acts as a lubricant that improves the flow of granules during the compression process, thereby preventing excessive friction during tablet printing. CMC-Na functions as an agent that maintains the integrity of the granules during the printing and storage processes. Lactose is added as a filler to meet the tablet weight of 500 mg and to improve the ease of granulation and compressibility of the powder.

The third formula of effervescent tablets developed with varying concentrations of lime peel extract and tamarind acid showed relatively similar physical characteristics. This uniformity indicates that the wet granulation method used is capable of producing granules with good flow and compressibility so that the resulting tablets have a uniform shape, size, and texture (Gulab, Sampat and Ingle, 2024).

The main component of the tablet, namely the effervescent acid-base system, provides equivalent tablet stability in each formula even though the extract proportions differ. The bioactive compound content in lime and tamarind extracts does not affect the tablet's ability to compress and maintain its physical structure, so that physical characteristics do not differentiate between formulas (Mayefis and Bidriah, 2022).

With no significant differences in physical parameters, variations in extract proportions more dominantly affect antimicrobial activity compared to formulation technology aspects. Therefore, bacterial inhibition efficacy becomes the primary indicator in determining the best formula in this study.

Effervescent Tablet Quality Test

The effervescent tablet quality test results show that most of the physical parameters have met the requirements by the entire formula, including weight uniformity, size uniformity, as well as dissolving time that is within the standard limit with a disintegration time of <2 minutes. However, the results of the fragility test show that the tablets do not yet meet the requirements. This condition indicates that the mechanical strength of the tablet still needs to be improved, especially in the printing process and the composition of the excipient. An overview of effervescent tablet quality test results on F1, F2, and F3 can be seen at **Table 5**.

Table 5. Effervescent *Tablet Quality Test*

Quality Test		Requirements	Formula		
			F1 (7%:5%)	F2 (6%:6%)	F3 (5%:7%)
Organoleptic Tablets	Color	-	Yellowish white	Yellowish white	Yellowish white
	Aroma	-	Lime	Lime	Lime
	Shape	-	Tablets	Tablets	Tablets
	Taste	-	Slightly acidic	Slightly acidic	Slightly acidic
Weight Uniformity		There should be no 2 Tablets deviate greater than 5% and there should be no 1 tablet deviate greater than 10%	490 mg (Meets the requirements)	497 mg (Meets requirements)	492 mg (Eligible)
Uniformity of Size		D: > 3x thick tablet <1 1/3 thick tablet	0.92 cm<1.02 cm< 2.06 cm (Meet requirements)	0.9 cm<1.01 cm< 2.03 cm (Meet requirements)	0.89 cm<1.01 cm< 2.01 cm (Meets requirements)
Fragility		<1%	8,62%	8,32%	6,20%
Dissolution Time		1-2 minutes	1.14 minutes	1.44 minutes	1.15 minutes
pH		-	2.7 (acid)	2.7 (acid)	2.7 (acid)
Foam Height		-	6 mm	6 mm	6 mm

The quality of effervescent tablets is highly dependent on the combination of formulation, manufacturing process, and the stability of each hygroscopic component (Ambarwati, Islamiyah and Nur, 2025). The quality evaluation of effervescent tablets included organoleptic tests consisting of color, aroma, shape, and taste. Physical parameters such as weight uniformity, size uniformity, friability, dissolution time, solution pH, and foam height were also evaluated to ensure the physical stability and effectiveness of the dosage form.

In the organoleptic evaluation, all formulations exhibited a yellowish-white color, uniform tablet shape, a lime aroma, and a slightly acidic taste. In the weight uniformity test, none of the three formulations had more than two tablets deviating by more than 5%, and no tablet deviated by more than 10%. The average tablet weights were 490 mg for F1, 497 mg for F2, and 492 mg for F3. These results indicate that the granulation and tablet compression processes were stable and consistent.

All three formulations also met the size uniformity requirements, with tablet diameters more than three times the thickness and thickness less than one-third of the tablet diameter. F1 had a

diameter of 0.92–1.02 cm, F2 ranged from 0.90–1.01 cm, and F3 ranged from 0.89–1.01 cm. This finding is consistent with the Indonesian Ministry of Health regulation (2014), which stipulates that tablet diameter must not exceed three times the tablet thickness and must not be less than one-third of the tablet thickness (Syamsia, Pratiwi, and Susana, 2017).

The friability values of all formulations were above 1% (F1 = 8.62%, F2 = 8.32%, and F3 = 6.20%). Tablets of good quality should have a friability value of less than 1% (Puspitasari and Suharsanti, 2022). The results of this study indicate that the tablets were able to dissolve relatively quickly upon contact with water. All three formulations dissolved within the required time range (1–2 minutes), with dissolution times of 1.14 minutes for F1, 1.44 minutes for F2, and 1.15 minutes for F3, respectively. The rapid release of carbon dioxide gas indicates that the acid–base reaction within the formulation occurred optimally (Kholidah, Yuliet and Khumaidi, 2015; Yulianti *et al.*, 2021; Sari and Asri, 2022).

All three formulations produced a pH of approximately 2.7, indicating acidic properties consistent with the characteristics of effervescent tablets based on citric and tartaric acids. The preparation was not excessively acidic; therefore, the effervescent tablets are considered safe for consumption. Overall, the quality evaluation indicates that the investigated effervescent tablets possessed physical characteristics and performance consistent with quality standards, supporting the principle that the quality of pharmaceutical products is determined by their physical and chemical properties (Meisner *et al.*, 2023).

Bacterial Culture in Selective Growing Media

Growth on selective media *MacConkey Agar* aims for the isolation of Gram-negative bacteria. This medium contains a pH indicator that turns pink in acidic conditions. On Gram-negative bacteria that ferment lactose will form pink colonies and those that do not ferment lactose will form white colonies (Jung and Hoilat, 2024). On **Figure 5.a** shows positive sample *Escherichia coli*. Growth on selective media *Mannitol Salt Agar* (MSA) for bacterial isolation *Staphylococcus* Based on the ability to ferment mannitol which will form a yellow zone (Cahyaningtyas, Gaina and Tangkonda, 2024). On **Figure 5.b** shows positive sample *Staphylococcus aureus.*

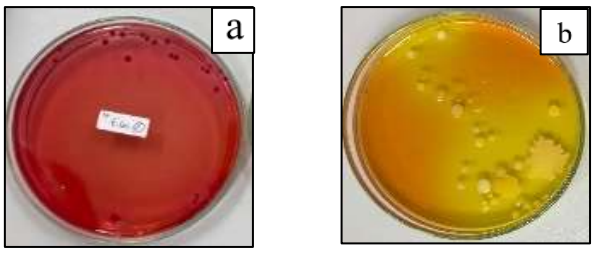

Figure 5. Bacterial Culture a. *Escherichia coli*, b. *Staphylococcus aureus*

The bacterial isolates resulting from early growth in the selective medium were transferred to *the Nutrient Agar* (NA) media. The transplant aims to obtain a single colony used for Gram staining. The results of microscope observations **(Figure 6.a)** show that the isolate of *MacConkey Agar* in the form of a bacillus (stem) is pink, indicating Gram-negative bacteria (*Escherichia coli*). **Figure 6.b** shows the isolates of the MSA in the shape of a cocus (round) in purple, indicating Gram-positive bacteria (*Staphylococcus aureus*).

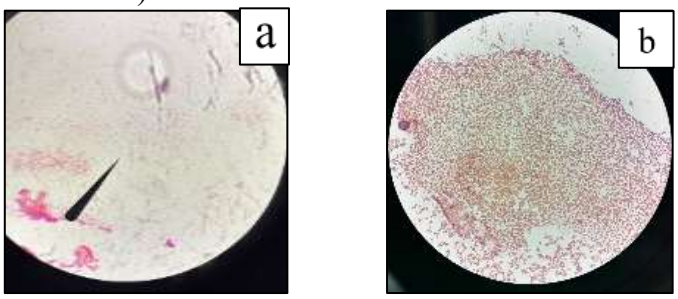

Figure 6. Coloring Gram a. *Escherichia coli*, b. *Staphylococcus aureus*

Bacterial isolates obtained from fruit and vegetable samples were cultured on MacConkey Agar to identify the presence of Gram-negative lactose-fermenting bacteria. Based on macroscopic observation of the isolate photographs, the colonies appeared pink in color, convex, smooth, and with entire margins, which are consistent with the characteristics of *Escherichia coli* as a rapid lactose fermenter. The pink coloration resulted from lactose fermentation, which produces acid and lowers the pH, leading to the absorption of the neutral red indicator by the colonies. MacConkey Agar is selective due to the presence of bile salts and crystal violet, which inhibit the growth of Gram-positive bacteria, thereby allowing the predominance of Gram-negative bacteria such as *Escherichia coli* (Widianingsih and De Jesus, 2018; Toruan *et al.*, 2019).

Gram staining of the isolates grown on MacConkey Agar revealed pink-colored, rod-shaped (bacilli) bacterial cells, consistent with the characteristics of *Escherichia coli*. The pink coloration occurs because the lipid-rich cell wall of Gram-negative bacteria undergoes dissolution during decolorization, causing enlargement of cell wall pores and release of the crystal violet–iodine complex. Consequently, the cells are unable to retain the primary stain and subsequently absorb safranin as the counterstain. These findings are consistent with the results observed on MacConkey Agar, where pink colonies indicate the presence of lactose-fermenting bacteria such as *Escherichia coli*, which was further confirmed by the Gram-negative bacillary morphology observed in Gram-stained preparations (Toruan *et al.*, 2019; Langgar, Sanam and Detha, 2021).

The results of this study also demonstrated that Mannitol Salt Agar (MSA) was able to isolate *Staphylococcus aureus* from fruit and vegetable samples, as indicated by the formation of yellow colonies resulting from mannitol fermentation. This fermentation produces an acidic environment that causes the phenol red indicator to change from red to yellow. This mechanism is consistent with the characteristics of *Staphylococcus aureus*, which is halotolerant and capable of growing in 7.5% NaCl. Therefore, MSA acts as a selective medium by inhibiting the growth of non-halophilic bacteria. Morphological observations on both alternative and control media showed colony patterns consistent with *Staphylococcus aureus*, characterized by round, smooth colonies surrounded by a yellow zone (Rafika *et al.*, 2024).

Subsequent Gram staining further confirmed the bacterial identity, as evidenced by Gram-positive cells appearing purple and coccoid in shape, arranged in grape-like clusters, which are characteristic of *Staphylococcus aureus*. The purple coloration occurs because Gram-positive bacteria possess a very thick peptidoglycan layer that retains the primary crystal violet stain even after exposure to alcohol during the decolorization step. These results are consistent with the appearance on MSA, where the yellow color change of the medium indicates mannitol fermentation by *Staphylococcus aureus*, and the identity was further confirmed by the presence of purple Gram-positive cocci observed under microscopic examination (Hayati *et al.*, 2019)

Antimicrobial Effectiveness Test

Antimicrobial activity testing was performed using the Kirby–Bauer agar diffusion method with Mueller-Hinton Agar (MHA) media. Test bacterial suspensions of Escherichia coli and *Staphylococcus aureus*, standardized to a McFarland 0.5 solution, were spread evenly on the surface of the MHA media. Paper discs were then dipped into each test solution, namely positive control (Amoxicillin 500 mg), negative control (tap water), synthetic decontaminant, and three effervescent tablet formulations with different ratios of lime peel extract and tamarind acid. The results of observation after incubation for 24 hours at 37°C showed an inhibition zone around the paper discs.

Based on the concentration variation (lime peel extract : tamarind extract), F1 (7%:5%) showed a moderate inhibition zone against Escherichia coli of 7.3 mm in Figure 7.a, F2 (5%:5%) showed no inhibition zone formed against both test bacteria, and F3 (5%:7%) showed a strong inhibition zone against *Staphylococcus aureus* of 13.3 mm in Figure 7.b. Meanwhile, the positive control (amoxicillin 500 mg) produced the highest inhibition zone, while the negative control (tap water) showed no antimicrobial activity.

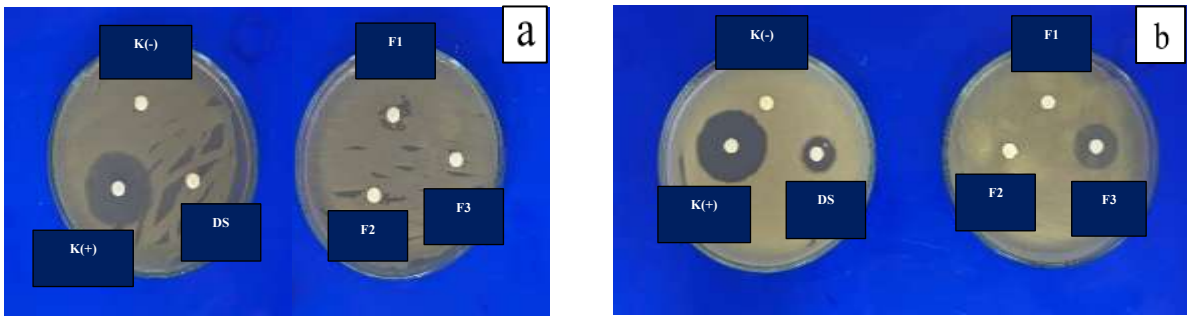

Figure 7. Inhibition Zone a. Escherichia coli, b. *Staphylococcus aureus*

The test results showed that each effervescent tablet formulation (F1, F2, and F3) was tested in three replicates against Escherichia coli and *Staphylococcus aureus*. Formulas F1 and F3 consistently produced inhibition zones, while Formula F2 showed no inhibitory activity against either bacterium. This can be seen in **Table 6.**

Table 6. Inhibition Zone Diameter

Bacteria		Inhibition Zone Formed (mm)					
		K(+)	K(-)	DS	F1	F2	F3
Esherichia coli	I	24	-	-	7,5	-	-
	II	24	-	-	7	-	-
	II	24	-	-	7,5	-	-
Staphylococcus aureus	I	24	-	8,5	-	-	13
	II	24	-	7	-	-	13,5
	II	24	-	5	-	-	13
Average		24	-	6,8	7,3	-	13,3

Description: K(+): positive control, K(-): negative control, DS: synthetic decontaminant, F1: formulation 1, F2: formulation 2, and F3: formulation 3.

Antimicrobial Effectiveness Test

The antimicrobial effectiveness test demonstrated that each effervescent tablet formulation exhibited different inhibition zone activities against *Escherichia coli* and *Staphylococcus aureus*. Variations in the concentration of lime peel and tamarind extracts showed that formulation F1 produced an inhibition zone of 7.3 mm against *Escherichia coli*, whereas formulation F3 produced an inhibition zone of 13.3 mm against *Staphylococcus aureus*. These findings indicate that variations in extract concentration influence the sensitivity of the tested bacteria (Nisa', 2020; Sari and Asri, 2022).

These results are consistent with previous studies reporting that lime peel and tamarind contain bioactive compounds such as alkaloids, flavonoids, and organic acids that are effective in inhibiting the growth of pathogenic bacteria. Other studies have also demonstrated that ascorbic acid acts as an anti–quorum sensing agent capable of reducing the virulence of *Escherichia coli* and *Staphylococcus aureus*. Therefore, the effervescent tablet formulations developed in this study further strengthen the scientific evidence regarding the effectiveness of combining bioactive compounds from both extracts (Silalahi, 2020).

CONCLUSION

This study identified three effervescent tablet formulations combining lime peel (*Citrus aurantifolia*) and tamarind (*Tamarindus indica*) extracts: F1 (7%:5%), F2 (5%:5%), and F3 (5%:7%). All formulations met acceptable physical quality characteristics, with dissolution times of less than 2 minutes, no alteration of aroma or taste in decontaminated fruits and vegetables, and demonstrated

antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* on fruit and vegetable surfaces.

The effectiveness test results showed that formulation F1 (7%:5%) was more effective in inhibiting the growth of *Escherichia coli*, whereas formulation F3 (5%:7%) was more effective against *Staphylococcus aureus*. Overall, the effervescent tablets developed in this study demonstrated strong potential as effective, safe, and environmentally friendly natural decontaminants for fruits and vegetables.

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