Canrea Journal: Food Technology, Nutritions, and Culinary, 2023; 6 (2): 86–99 OPEN ACCESS

e-ISSN : 2621-9468

Type of the Paper (Article)

The testing of photochemical compounds, antioxidant activities, and antibacterial activities of "Sambel Matah" composition from Bali

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Abstract

Sambel matah is a traditional Balinese spice that can be found throughout in Bali. The constituent ingredients of *sambel matah*, such as onions, limes, lemongrass, and kaffir lime leaves. *Sambel matah* is served fresh without any heating process. This study aimed to test the content of phytochemical compounds, antibacterial activity and antioxidant in the onions, limes, lemongrass, and kaffir lime leaves. The results were then compared with the extracts of red onion, limes, lemongrass, and kaffir lime leaves. The bacteria used in this study were *Salmonnela typhimurium and Escherichia coli.* Antibacterial activity was tested by disc diffusion method (KirbyBauer Test). The result of the research, onions, kaffir lime leaves, limes, and lemongrass contain flavonoid compounds, tannins, and phenols in both slurry and ethanol extract. The ethanolic extracts of red onion, limes, kaffir lime leaves, and lemongrass are classified as strong antioxidants. The ethanolic extracts of red onion, limes, kaffir lime leaves, and lemongrass have antibacterial properties against *S. typhimurium* and *E. coli* bacteria. Meanwhile, in fresh material (slurry), only limes have antibacterial activity against *S. typhimurium* and *E. coli* bacteria with inhibition zone diameters of 14.58±0.767 mm and 8.30±0.483 mm, respectively. Thus, the components of onions, limes, kaffir lime leaves, and lemongrass in *sambel matah* can inhibit the growth of bacteria, so that the *sambel matah* becomes more durable even though it is processed without going through the heating process.

Article History
Received January 8, 2023
Accepted November 7, 2023
Published December 28, 2023

Keywords
Antioxidant, Antibacterial Activity, *Sambel Matah*.

1. Introduction

Sambel matah is a traditional Balinese condiment found throughout Bali. *Sambel matah* is made from raw ingredients without being crushed (pulverized). The ingredients of *sambel matah* are onions, garlic, red chilies, shrimp paste, salt, lemongrass, limes, and coconut oil. In the process of making *sambel matah*, the ingredients are chopped, the shrimp paste is soaked and crushed in coconut oil, and after the shrimp paste is mixed with oil, it sprinkles on the ingredients that have been chopped. *Sambel matah* is used as a complementary food, especially when consuming Balinese foods such as shredded chicken and grilled fish. Some of

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ISSN 2621-9468 online, DOI 10.20956/canrea.v6i2.956

the raw ingredients used in making *sambel matah*, such as onions, oranges, limes, lemongrass, and kaffir lime leaves, are believed to have health benefits.

Onions (*Allium cepa L. var. aggregatum*) are the main components of *sambel matah* and have various benefits in treating various diseases, ranging from common ailments such as coughs, dyspepsia, and flatulence, to degenerative diseases such as heart problems, cholesterol, hypertension, and diabetes. The content of routine and quercetin compounds in onion can be used as anti-inflammatory and antioxidant agents (1,2). According to Utami (3), the flavonoids contained in onions can be used to protect the cell structure, increasing the effectiveness of vitamin C, as an anti-inflammatory and antioxidant, to prevent porous bones, and as a natural antibiotic. Onions contain sulfur compounds, such as allyl propyl disulfide (APDS), and flavonoids, such as quercetin, which are believed to reduce the risk of cancer, heart disease, and diabetes. The outer skin of the onion, which is dry and often brownish, is rich in fiber and flavonoids, as well as an antibacterial agent against *Stapylococcus aureus* and *E. coli* (4).

Lime (*Citrus × amblycarpa*) is one of the ingredients used to make *sambel matah*. Lime contains the main components of beta-pinene, citronellal, limonene, and terpinene-4-ol (5). β-Pinene compounds have been shown to exert antibacterial effects by inhibiting DNA synthesis (6). Citronellol, stronella, isopulegol, and linolol have antibacterial activity against *E. coli, P. aeruginosa, P. mirrabilis, K. peneumoniae, A. baumanni, S. aureus, E. faecalis, B. subtilis, C. albicans*, and *C. parapsilosis* (5). Based on research by Yuliani et al. (7), the essential oil of lime leaves has antibacterial activity against *S. aureus*. Naringin is the main flavonoid found in the skin and fruit and is a strong antioxidant (8).

Lemongrass (*Cymbopogon citratus*) is another ingredient in making *sambel matah* and has various health benefits. Lemongrass contains Alkaloids, Flavonoids and monoterpenes. These substances have antiprotozoal, anti-inflammatory, antimicrobial, antibacterial, antidiabetic, anticholinesterase, molluscicidal, and antifungal (9). Lemongrass also contains some essential oils composed of monoterpene compounds such as citral and geraniol (10). These oils have antibacterial and antifungal properties; therefore, they are used in the treatment of bacteria, such as *S. aureus* and *S. Typhimurium* with an MIC of 0.5 L/mL.

Kaffir lime leaves (*Citrus hystrix*), which are used to make *sambel matah* also have antibacterial properties. Kaffir lime leaves contain chemical compounds which are secondary metabolites such as essential oils, flavonoids, tannins, saponins, steroids, phenols, and polyphenols (11). In addition, Maimunah et al. (12), kaffir lime leaf extract showed antibacterial activity against *Staphylococcus aureus* at concentrations of 5% (6.7 mm), 10% (7.2 mm), 15% (7.3 mm), and 20% (8.3 mm), and the average diameter of the inhibition zone was categorized as medium. According to Siregar et al. (13), the antibacterial test of the infusion from kaffir lime leaves against *E. coli* bacteria at a concentration of 100% had an inhibition zone diameter of 14.3 mm and was classified as strong. The kaffir lime leaf extract (*C. hystrix*) at concentrations of 30%, 25%, 20%, 15%, 10%, and 5% had antibacterial activity against *Shigella dysenteriae* bacteria (14). The use of raw ingredients helps retain their natural flavors and nutrients, which can contribute to the overall health benefits of the condiment. Rich in antioxidants: Many ingredients in *sambel matah*, such as chilies, shallots, and garlic, are rich in antioxidants (15). Antioxidants protect the body against damage caused by free radicals, which can contribute to the development of chronic diseases. Anti-inflammatory properties: Some of the ingredients in *sambel matah*, such as lemongrass and kaffir lime leaves, have anti-inflammatory properties. Inflammation is a natural response in the body;

however, chronic inflammation can contribute to the development of various diseases. Consumption of foods with anti-inflammatory properties can help reduce inflammation in the body.

The ingredients for making *sambel matah*, such as onions, limes, lemongrass, and kaffir lime leaves, have been shown to have antibacterial and antioxidant activities; therefore, they are very good for use in making *sambel matah* because *sambel matah* is served fresh without any heating process. The heating process of foodstuffs can cause decomposition of their bioactive compounds contained in them (16). Fresh ingredients tend to be spoiled quickly during the storage process; therefore, the presence of ingredients containing antibacterial compounds will inhibit the growth of bacteria that cause the foods to spoil. Several studies have tested antibacterial extracts of onions, limes, lemongrass, and kaffir lime leaves. No research has been conducted to test the antibacterial and antioxidant activities of onions, limes, lemongrass, and kaffir lime leaves in fresh form. Therefore, in this study, the antibacterial and antioxidant activities of onions, limes, lemongrass, and kaffir lime leaves were tested in fresh form, and the results were compared with those of ethanolic extracts of onions, limes, lemongrass, and kaffir lime leaves. *Escherichia coli* and *Salmonella typhimurium* were used in this study were *E. coli* and *S. typhimurium*.

2. Materials and Methods

2.1. Materials

The materials used in this study were onions, limes, lemongrass, kaffir lime leaves, aquadest, 96% ethanol, citrate phosphate buffer, Folinciaocalteu Na_2CO_3 (Merck, Germany), $NaNO_2$ (Merck, Germany), and $AlCl_3$ (Merck, Germany). Germany), NaOH (Merck, Germany), DPPH (Merck, Germany), methanol (Merck, Germany), quercetin standard (Merck, Germany), gallic acid standard (Merck, Germany), tannic acid standard (Merck, Germany), Germany), *Escherichia coli isolates*, *Salmonella typhimurium isolates*, filter paper, paper discs, ampicillin antibiotic (Oxoid, UK), nalidici acid antibiotic (Oxoid, UK), streptomycin antibiotic (Oxoid, UK), sterile cotton swab, and Mueller-Hinton media agar (Oxoid, UK).

The tools used in this study were a blender (Philips), an autoclave, a stirring rod, a 1000 mL beaker (Pyrex), a petri cup (Pyrex), a 200 mL Erlenmeyer (Pyrex), a UV-Vis spectrophotometer (Libra S60, USA), cuvette, vortex, 100 mL measuring cup (Pyrex), incubator (Mammert), laminar air flow (Eyela), caliper, Bunsen lamp, tweezers, dropper pipette, micropipette, test tube, maceration container, rotary evaporator (Buchi), receiver flask (Buchi), and vials.

2.2. Sample Preparation

In this study, fresh samples of onions, limes, lemongrass, and kaffir lime leaves were tested for antibacterial activity. The sample was prepared as follows.

2.2.1. Fresh Material Preparation (Slurry)

The ingredients, onions, limes, lemongrass, and kaffir lime were sorted, washed, and drained. The ingredients were then mashed using a blender to produce slurry. The sample slurry was stored in a vial to be tested for antibacterial activity.

2.2.2. Material Extraction Preparation

The onions, limes, lemongrass, and kaffir limes were sorted, cleaned, and sliced. Samples were cut into small pieces and dried in an oven at 40 °C. The dry ingredients were then mashed using a blender to obtain simplicity. The simplicia were then macerated with 96% ethanol at a 1:5 ratio. Maceration was performed for 24 hours. After that, solvent evaporation was carried out using an evaporator so that the extract was thick. The viscous extract was stored in a vial and tested for antibacterial activity.

Extraction is the process of separating a material from a mixture using a suitable solvent. The maceration method was used. Maceration is the most widely used method for this purpose. This method was performed by placing the plant powder and a suitable solvent in an inert container that was tightly closed at room temperature. The extraction process was stopped when equilibrium was reached between the concentration of the compound in the solvent and that in the plant cell. After the extraction process, the solvent was separated from the sample by filtration and evaporated using a rotary evaporator (17). The effectiveness of the extraction of a compound using a solvent is highly dependent on the solubility of the compound in the solvent, according to the principle of like dissolves like, where a compound will dissolve in a solvent with the same properties. Active compounds such as flavonoids, tannins, and phenols have polar properties; therefore, a polar solvent is required (18). Polar solvents include ethanol, methanol, acetone and water (19). Therefore, in this study, ethanol was used as the solvent to optimally extract flavonoid compounds, tannins, and phenols.

2.3. Phytochemical Compound Test

2.3.1. Total Phenol Content

Total phenol content was determined using the Folin–Ciocalteu method (20). The extract (0.01 g) was diluted in 5 mL citrate phosphate buffer according to the manufacturer's instructions. 0.1mL of the sample was pipetted and 0.3 ml of 70% ethanol was added. 0.4 mL of folinciaocalteau was then added and incubated for 6 min. After the incubation process, 4.2 mL of 5% Na_2CO_3 was added, vortexed, and incubated for 90 min. The absorbance was measured at a wavelength of 760 nm. The total phenol content in the sample was expressed as the equivalent of gallic acid in mg GAE/g extract. The total phenol content was calculated using the following formula:

$$\text{Total phenol (mg GAE/g extract)} = \frac{CxVxFp}{w} \quad (1)$$

Where:
C = Sample concentration from the linear regression results (mg/L)
FP = Dilution factor
V = Sample volume (L)
W= Sample weight (g)

2.3.2. Total Flavonoids Content

The total flavonoid content was determined using a spectrophotometer using the $AlCl_3$ method (21). Extract 0.01 g was diluted in 5 ml of citrate phosphate buffer. One milliliter of the sample was mixed with 4 mL of distilled water and 0.3 mL of $NaNO_2$ (10%). After that, it was incubated for 5 minutes and added 0.3 mL of $AlCl_3$ solution (10%) and 2 mL of NaOH solution (1%), then immediately tested with a spectrophotometer at a wavelength of 510 nm.

The total flavonoids in the sample were expressed as the equivalent of quercetin acid in mg QE/g extract. The total flavonoid content was calculated using the following formula:

$$\text{Total flavonoids (mg GAE/g extract)} = \frac{CxVxFp}{w} \quad (2)$$

Where:
C = Sample concentration from the linear regression results (mg/L)
FP = Dilution factor
V = Sample volume (L)
W= Sample weight (g)

2.3.3. Total Tannin Content

The total tannin content of the extract was determined using the Folin-Denis method (22). The extract (0.01 g) was diluted with 5 mL citrate phosphate. The sample that has been diluted in a pipette is 0.25 mL then added with 0.25 mL of Folin-Denis, after that it vortexed and 2 mL of 5% Na_2CO_3 is added. The solvent was vortexed and the mixture was incubated for 30 min. The absorbance was measured at 725 nm using a spectrophotometer. The readings were compared to a standard curve obtained using tannic acid. The total tannin in the sample was expressed as the equivalent of tannic acid in mg TAE/g extract. The total tannin content was calculated using the following formula:

$$\text{Total tannin (mg GAE/g extract)} = \frac{CxVxFp}{w} \quad (3)$$

Where:
C = Sample concentration from the linear regression results (mg/L)
FP = Dilution factor
V = Sample volume (L)
W= Sample weight (g)

2.4. Antioxidant Activity Test

Preparation of the main liquor for each 100 ppm sample by dissolving 10 mg of the sample in 100 mL of methanol Pro Analysis. Furthermore, dilution was performed using methanol PA solvent by varying the concentrations of 20, 60, 80, and 100 ppm for each sample. A comparison solvent was then prepared, namely, a control solution containing 2 ml of methanol PA and 1 ml of 50 ppm DPPH solution. For the test samples, 2 ml of each sample solvent and 2 ml of the DPPH solvent were prepared. Then, they were incubated for 30 min at a temperature of 27℃ until there was a change in color from DPPH activity. All samples were prepared in triplicates. All samples, namely the extract samples that had been incubated, were tested for absorbance values using a UV-Vis spectrophotometer at a wavelength of 517 nm. Antioxidant activity was analyzed using the DPPH method by examining the color changes of each sample after incubation with DPPH. If all the DPPH electrons are paired with electrons in the extracted sample, there will be a change in the color of the sample from dark purple to bright yellow. The absorbance of the sample was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

$$\%\text{inhibition} = \frac{A0-A1}{A0} x100\% \quad (4)$$

Where:
A1= Absorbance treatment
A0= Absorbance control

2.5. Antibacterial Test

Preparation and testing of antibacterial activity by the disc diffusion method (Kirby-Bauer Test). The preparation stages included bacterial rejuvenation, bacterial suspension preparation, paper disc preparation, negative control preparation, positive control preparation, and test sample preparation. Bacterial rejuvenation was carried out through a colony of bacteria taken using one sterile eye, and then implanted on nutrient agar (NA) media tilted by scratching, after which it was incubated for 18-24 hours at 37°C. Furthermore, the manufacture of bacterial suspension was carried out through the stock of bacterial culture that was grown using a sterile Ose needle and then suspended in a tube containing 10 ml of Lactose Broth (LB) media, and the turbidity of the solution was measured at a wavelength of 580 nm until a transmittance of 25% was obtained. The antibacterial activity test was performed using the Disc Diffusion method (Kirby-Bauer Test). A suspension of the test bacteria (20 L) was added to the media in a Petri dish and spread with sterile cotton swabs on the test media (23). The sterile cotton swab was rotated several times. This procedure was repeated twice. Paper discs with a diameter of 6 mm were immersed in a positive control in the form of antibiotics ampicillin, nalidixic acid, and streptomycin 10 g, a negative control with distilled water, and the test sample for 30 seconds. The disc was then placed on the surface of the media in the desired position. The medium was then incubated at 37 °C for 24 h, and the diameter of the inhibition zone was measured with a caliper three times the measurements declared in millimeters.

3. Results and Discussion

3.1. Results

3.1.1. Phytochemical Compound Content

Onions, kaffir lime leaves, limes, and lemongrass contain many bioactive compounds, such as flavonoids, tannins, and phenols. Based on the results of the study, it was observed that the levels of flavonoids, phenols, and tannins in shallots, kaffir lime leaves, limes, and lemongrass in the form of slurry were lower than those in ethanol extracts. Flavonoid levels are shown in Table 1.

Table 1. Flavonoid content of onions, limes, kaffir lime leaves, and lemongrass.

Sample Type	Level of flavonoid (mg/100g QE)	
	Slurry	Ethanol extract
Onions	36.65±1.329	23976.11±110.929
Limes	16.70±1.750	26475.19±175.374
Kaffir Lime Leaves	9.53±0.435	21051.19±36.825
Lemongrass	46.83±1.266	31414.13±139.267

Tannins are polyphenolic compounds derived from plants that have a bitter and chelating taste (24). In this study, the tannin content of fresh ingredients and ethanol extract of onions, limes, kaffir lime leaves, and lemongrass were tested can be seen in Table 2.

Table 2. The tannin content of onions, limes, kaffir limes leaves, and lemongrass.

Sample Type	Level of tannin (mg/100g TAE)	
	Slurry	Ethanol extract
Onions	11.11±0.465	45745.81±77.590
Limes	17.46±0.459	55541.54±91.999
Kaffir Lime Leaves	13.48±0.456	44747.08±75.175
Lemongrass	17.46±0.456	41437.26±72.905

Polyphenols are a group of compounds with the highest distribution among all plants. Polyphenol compounds have antioxidant activity, which is based on their glycoside structure and can be divided into groups of phenolic acids, flavonoids, polyphenol amides, and other polyphenols that have their characteristics (25). Phenol levels are shown in Table 3. Different extraction methods can lead to different phytochemical profiles, and therefore, different biological activities. Variations in the phytochemical contents of different ingredients can translate into the overall antioxidant and antibacterial activities of the combined *sambel matah*. The phytochemicals present in the ingredients contributed to the overall antioxidant and antibacterial activities of the combined *sambel matah*. The antioxidant and antibacterial activities of the combined *sambel matah* can be enhanced by using ingredients with a high phytochemical content.

Table 3. Phenol content of onions, limes, kaffir lime leaves and lemongrass.

Sample Type	Level of Phenol (mg/100g GAE)	
	Slurry	Ethanol Extract
Onions	66.73±0.978	57302.00±163.206
Limes	28.12±4.183	60372.19±838.569
Kaffir Limes Leaves	45.81±0.639	52275.74±102.235
Lemongrass	57.12±0.767	50986.77±102.235

3.1.2. Antioxidant Activity

Based on the results of this study (Table 4), it can be seen that the fresh material (slurry) contains lower antioxidant compounds than the ethanol extract of onions, limes, kaffir lime leaves, and lemongrass.

Table 4. Antioxidant activity of onions, limes, kaffir lime leaves and lemongrass.

Sample Type	IC_{50} (ppm)	
	Slurry	Ethanol Extract
Onions	2287.74±11.71 (very weak)	72.346±0.640 (strong)
Limes	1505.23±26.46 (very weak)	98.489±0.809 (strong)
Kaffir Limes Leaves	2806.88±52.67 (very weak)	97.997±2.465 (strong)
Lemongrass	3110.42±336.39 (very weak)	94.777±3.581 (strong)

3.1.3. Antibacterial Activity

The antibacterial activity of the ethanolic extracts of shallots, lime leaves, limes, and lemongrass against *S. Typhimurium* is shown in Table 5 and Figure 1.

Table 5. Diameter of slurry inhibition zone and ethanol extract of shallots, lime leaves, limes, and lemongrass against S. *Typhimurium* bacteria.

Sample	Inhibition diameter zone (mm)	
	Slurry	extract
onions	0.00±0.000	8.709±0.713
Lime leaves	0.00±0.000	9.059±0.918
Lime	14.58±0.767	19.898±1.507
Lemongrass	0.00±0.000	12.501±1.175
Negative control (-)	0.00±0.000	0.000±0.000
Positive control (+)		
Sterptomicin	18.377±0.301	17.054±0.431
Ampicilin	9.820±0.396	7.229±0.138
Nalidic acid	15.877±0.133	16.660±0.748

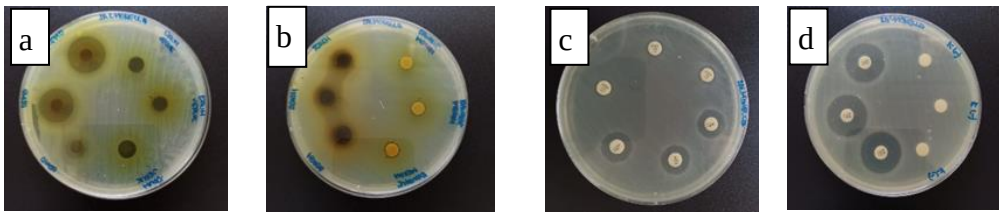

Figure 1. Antibacterial activity of ethanolic extracts of onions, lime leaves, limes, and lemongrass against *S. Typhimurium* bacteria (a. Kaffir lime and lime leaves, b. onions and lemongrass, c. Positive control of ampicillin and streptomycin and d. Positive control nalidixic acid and negative control).

The antibacterial activity of the ethanolic extracts of shallots, lime leaves, limes, and lemongrass against *E. coli* is shown in Table 6 and Figure 2.

Table 6. Diameter of slurry inhibition zone and ethanol extract of shallots, lime leaves, limes, and lemongrass against *E. coli* bacteria.

Sample	Inhibition diameter zone *E. coli*	
	Slurry	Ethanol Extract
Onions	0.00±0.000	7.179±0.226
Lime leaves	0.00±0.000	7.872±0.536
Lime	8.30±0.483	13.482±3.965
Lemongrass	0.00±0.000	9.090±0.300
Negative control (-)	0.00±0.000	0.000±0.000
Positive control (+)		
Sterptomicin	14.573±0.312	13.009±0.366
Ampicilin	9.880±0.437	8.130±0.338
Nalidic acid	13.080±0.120	18.804±0.360

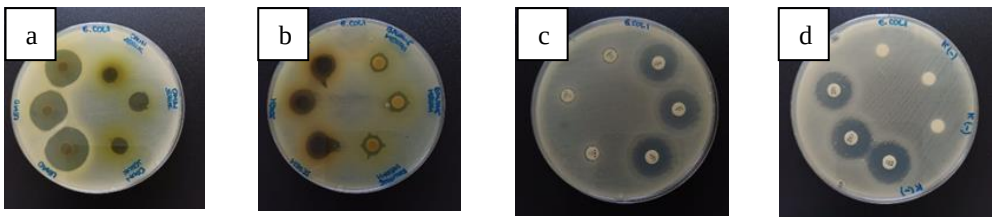

Figure 2. Antibacterial activity of ethanolic extracts of shallots, lime leaves, limes, and lemongrass against *E. coli* bacteria (a. Kaffir lime leaves and limes, b. Shallots and lemongrass, c. Positive control of ampicillin and streptomycin and d. Positive control nalidixic acid and negative control).

3.2. Discussion

The onion is the main component of *sambel matah*. Table 1 shows that the onion slurry contains flavonoids at 36.65±1.329 mg/100 g QE and that the ethanolic extract of onion contains 23976.11 mg/100 g QE. Quercetin was used as a standard compound based on previous studies, which confirmed that quercetin is one of the flavonoid group compounds present in onion peel extracts (26). According to Misna and Diana (4), onions contain a quercetin-type flavonoid that can be used as an antibacterial and antioxidant compound. Quercetin has the highest onion content and can be used to treat cataracts, heart disease, and cancer. Organosulfur compounds can reduce blood pressure and cholesterol (27). Skerget et al. (28), also stated that the main compounds in the onion extract were quercetin and glycosides (3.4'-di-dan4'-glycosides), isorhamnetin monoglycosides, and kaempferol aminoglycosides. According to Nagappan et al. (29), flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes through the interaction between flavonoids and bacterial DNA. The lipophilic nature of flavonoids causes damage to the bacterial cell membranes. Tannin compounds are thought to be related to their ability to inactivate microbial adhesion enzymes and transport proteins in cell membranes.

Other ingredients such as limes, kaffir lime leaves, and lemongrass have lower flavonoid compounds in the slurry, namely 16.70±1.750 mg/100 g QE, 9.53±0.435 mg/100 g QE, and 46.83±1.266 mg/100 g QE compared with ethanol extract of onions with higher flavonoid content, namely 26475.19±175.374 mg/100 g QE, 21051.19±36,825 mg/100 g QE, and 31414.13±139.267 mg/100 g QE. Fresh material (slurry) contains fewer flavonoid compounds due to the high water content in the ingredients, so the content of bioactive compounds in it was low. Meanwhile, in the extract, the bioactive compounds were extracted to produce higher flavonoid compounds. Extraction was performed to separate the active substances from the plant parts. Active substances are present in the cells, but plant cells have a thickness, so an extraction method with certain solvents is needed to extract them (30).

Tannic acid was used as the tannin standard. Based on the results of the study, the tannin content of the fresh material was lower than that of the ethanol extract. The tannin levels in the fresh ingredients (slurry) of onions, limes, lime leaves, and lemongrass were 11.11 mg/100 g TAE, 17.46 mg/100 TAE, 13.48 mg/100 g TAE, and 17.46 mg/100 g TAE, respectively. Meanwhile, the tannin levels of the ethanolic extracts of onions, limes, lime leaves, and lemongrass were 45745.81 mg/100 g TAE, 55541.54 mg/100 g TAE, 44747 mg/100 g TAE and 41437.26 mg/100 g TAE, respectively. Tannin compounds are one of the compounds that have antibacterial properties. The mechanism of action of tannins as antibacterial agents involves lysis of *Porphyromonas gingivalis* cells. This happens because tannins target the polypeptide wall of the bacterial cell wall so that the formation of the cell wall becomes less than perfect and the bacterial cell will die (31). In addition, tannins function as secondary antioxidants because they can chelate iron ions and slow down oxidation (32).

In this study, gallic acid was used as the phenol standard. Based on the results of this research, the phenol content of the fresh ingredients (slurry) was also lower than the ethanol extract content of each ingredient. Phenol content of fresh ingredients (slurry) of onions, limes, kaffir lime leaves, and lemongrass was 66.73 mg/100 g GAE, 28.12 mg/100 g GAE, 45.81 mg/100 g GAE, and 57.12 mg/100 g GAE, respectively. The phenolic content of the ethanolic extracts of shallots, limes, lime leaves, and lemongrass was 57302 mg/100 g GAE, 60372 mg/100 g GAE, 52275 mg/100 g GAE, and 50986 mg/100 g GAE, respectively (Table 3). Phenol compounds are polar and possess antibacterial properties. The mechanism of action of

phenolic compounds in killing bacterial cells involves denaturation of bacterial cell proteins (33). Phenolic compounds constitute the largest group of compounds that act as natural antioxidants in plants. Phenolic compounds have one (phenol) or more (polyphenol) phenol rings, namely hydroxy groups attached to aromatic rings, so they are easily oxidized by donating hydrogen atoms to free radicals (34).

The slurry was classified as a very weak antioxidant (IC50>200 ppm), and the ethanol extract was classified as a strong antioxidant (IC50<100ppm) (Table 4). The high antioxidant activity of the ingredients is due to the content of flavonoids, tannins, and phenols, which have high levels in ethanol extract compared to slurry. The antioxidant activity of a plant is generally attributed to the presence of phenolic compounds, polyphenols, and simple phenols. The greater the content of phenolic compounds in a plant, the greater is its antioxidant activity. Phenolic compounds can exist in the form of polyphenols and flavonoids (35). According to Pratt and Hudson (36), flavonoids and tannins are very effective antioxidants (37,38).

Onions, lime leaves, limes, and lemongrass are ingredients that make up the Balinese *sambel matah*. *Sambal matah* is a *sambel* that does not undergo a heating process, such as frying or steaming. The spices were chopped, mixed, and served immediately in other Balinese dishes. *Sambel matah* which does not undergo heating, is often suspected to be quickly damaged because the ingredients used are fresh ingredients that are easily contaminated with microbes. However, spices that make up *sambel matah* such as onions, lime leaves, limes, and lemongrass, have been studied and proven to contain antibacterial compounds. Based on the results of this study, it was also found that onions, kaffir lime leaves, limes, and lemongrass contain flavonoid compounds, tannins, and phenols, which can act as antibacterial agents. The most common bacteria causing foodborne infections are *S. typhimurium* and *E. coli*. The antibacterial activities of shallots, lime leaves, limes, and lemongrass in the form of slurry and ethanol extracts were tested against *S. typhimurium* and *E. coli bacteria* (39).

S. Typhimurium is a movable rod-shaped gram-negative bacterium that typically ferments glucose and mannose without forming gas but does not ferment lactose and sucrose. *S. Typhimurium* is a pathogenic bacterium. In general, organisms belonging to the genus Salmonella are a source of various types of infections ranging from mild to severe gastroenteritis, such as typhoid fever and bacteremia. *Salmonella* is the causative agent of salmonellosis, an endemic disease that causes significant losses in Indonesia (40). Based on the results of this study, limes had antibacterial activity in both slurry and ethanol extracts, but ethanol extracts of limes had higher antibacterial activity against Salmonella bacteria, which was 19,898±1,507 mm compared to fresh ingredients. Meanwhile, onions, lime leaves, and lemongrass were observed to have antibacterial activity in ethanol extract samples and did not have antibacterial activity in the slurry. The antibacterial activities of the ethanolic extracts of onions, lime leaves, and lemongrass were 8.709±0.713, 9.059±0.918, and 12.501±1.175 mm, respectively (Table 5).

E. coli are facultative anaerobic gram-negative bacteria that are rod-shaped, not encapsulated, and can move actively. *Escherichia coli*, commonly abbreviated as *E. coli*, is one of the main species of gram-negative bacteria. In general, these bacteria are known to occur normally in the digestive tract of humans and animals. Its presence outside the human body is an indicator of food and beverage sanitation and whether it has been contaminated by human waste. The presence of *E. coli* in water or food is also considered highly correlated

with the discovery of germs (pathogens) in food (41). Based on the results of the study, it was also seen in the fresh material (slurry) that only limes that had antibacterial activity against *E. coli* bacteria were 8.30±0.483 mm. Meanwhile, the ethanolic extracts of onions, lime leaves, limes, and lemongrass had antibacterial activity against *E. coli* with inhibition zone diameters of 7.179±0.226, 7.872±0.536, 13.482±3.965, and 9.090±0.300 mm, respectively (Table 6).

The results of the study showed that the ethanol extract had higher antibacterial activity because phytochemical compounds with antibacterial properties, such as flavonoids, phenols, tannins, and saponins, were extracted and found to be more effective than fresh ingredients (slurry). Lime contains the main components of beta-pinene, citronellal, limonene, and terpinen-4-ol (5). Beta-pinene compounds have been shown to have antibacterial effects by inhibiting DNA synthesis (6). Onions, lime leaves, limes, and lemongrass in fresh or slurry form have high water content, so the concentration of phytochemical compounds of antibacterial activity is smaller, so they have not been able to inhibit the growth of *Salmonella* bacteria. Onions contain sulfur compounds, such as allyl propyl disulfide (APDS) and flavonoids, such as quercetin, which have antibacterial properties (4). Lemongrass contains alkaloids, flavonoids and monoterpenes. These substances have antiprotozoal, anti-inflammatory, antimicrobial, antibacterial, antidiabetic, anticholinesterase, molluscicidal, and antifungal (9). Kaffir lime leaves contain chemical compounds with secondary metabolites, such as essential oils, flavonoids, tannins, saponins, steroids, phenols, and polyphenols, which have antibacterial properties (11). The antibacterial activity of onions, limes, kaffir lime leaves, and lemongrass can inhibit the growth of bacteria in *sambel matah* so the *sambel matah* becomes durable even though the processing is done without heating. The specific phytochemical compounds found in the ingredients of *sambel matah* may have contributed to its unique sensory profile.

4. Conclusions

The conclusions of this study are that onions, kaffir lime leaves, limes, and lemongrass contain flavonoid compounds, tannins, and phenols in both the slurry and ethanol extracts. These compounds can act as antibacterial and antioxidant compounds, and their components, such as garlic and lemongrass, have antimicrobial properties and are used primarily as preservatives. The ethanolic extracts of shallots, limes, kaffir lime leaves, and lemongrass have antibacterial properties against S. *typhimurium* and *E. coli*. Meanwhile, in fresh material (slurry), only limes had antibacterial activity against *S. typhimurium* and *E. coli* bacteria with inhibition zone diameters of 14.58±0.767 and 8.30±0.483 mm, respectively. Thus, the components of onions, limes, kaffir lime leaves, and lemongrass in *sambel matah* can inhibit the growth of bacteria, making the *sambel matah* durable even though it is processed without going through the heating process. The ethanolic extracts of shallots, limes, kaffir lime leaves, and lemongrass have been classified as strong antioxidants. The specific phytochemical compounds found in these ingredients may have implications in the culinary industry. The specific phytochemical compounds found in *sambel matah* may have implications for the health industry. The antioxidant and anti-inflammatory properties of capsaicin, flavonoids, and the essential oils found in chilies, shallots, and kaffir lime leaves have potential health benefits.

Acknowledgements

Acknowledgments were conveyed to the Food Science and Technology Department and Agricultural Analysis Laboratory Warmadewa University, which has facilitated the administration of this research, as well as to friends and students who have helped a lot in the research process until this research was published.

Author Contributions

N.M.A.S.S. and I.B.W.G. conceived and designed the experiments; N.M.A.S.S. and N.M.D.J. performed the experiments; N.M.D.J. analyzed the data; I.M.G.A.P. and P.D.W. contributed reagents, materials, and analysis tools; and N.M.A.S.S. and I.B.W.G. wrote the paper.

Funding

This research received no any external funding.

Institutional Review Board Statement

Not applicable.

Data Availability Statement

Not Applicable.

Conflicts of Interest

No conflict of interest.

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