



# Formulation and Antibacterial Activity Test of *Salmonella thypimurium* and *Lactobacillus acidophilus* in Microencapsulation of Mangosteen Peel Extract

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**Abstract:** Xanthone compounds in the ethanol extract of mangosteen peel have antibacterial activity against both pathogenic and beneficial bacteria. The purpose of this study was to examine the effect of various formulations of ethanol extract of mangosteen peel and maltodextrin from palm starch on *Salmonella thypimurium* and *Lactobacillus acidophilus*. This research was conducted by culturing the bacterial cultures of *S. thypimurium* and *L. acidophilus* on nutrient agar (NA) media in petri dishes according to the Kirby-Bauer method. The NA media that had been made in the petri dish was then made wells to test each treatment as much as 10  $\mu$ L/disk at various concentrations (100, 75 and 50%). The formulations tested in this study consisted of: T1 (30% ethanol extract of mangosteen peel + 70% maltodextrin), T2 (40% ethanol extract of mangosteen peel + 60% maltodextrin), T3 (50% ethanol extract of mangosteen peel + 50% maltodextrin), T4 (60% ethanol extract of mangosteen peel + 40% maltodextrin), T5 (70% ethanol extract of mangosteen peel + 30% maltodextrin), T6 (ethanol extract of mangosteen peel only), T7 (maltodextrin only), T8 (positive control containing 100 ppm bacitracin) and T9 (negative control/no treatment). The results of each sample were observed by measuring the sample inhibition zone on the growth medium that had been prepared. Data from the antibacterial activity test results were then analyzed using one-way ANOVA followed by Duncan's test. The results showed that the ethanol extract of mangosteen peel and maltodextrin had a significant effect ( $P < 0.05$ ) on the antibacterial activity of *Salmonella thypimurium* and *Lactobacillus acidophilus*. The results of this study indicated that the formulation of ethanol extract of mangosteen peel and maltodextrin had the ability as an antibacterial against *Salmonella thypimurium* and *Lactobacillus acidophilus* at a medium level.

**Keywords:** Antibacterial; *Lactobacillus acidophilus*; Maltodextrin; Mangosteen peel; *Salmonella thypimurium*.

## Introduction

Mangosteen peel contains secondary metabolites in the form of polyphenols which are useful as antibacterials (Valmai et al, 2019). To produce bioactive compounds in mangosteen peel, an appropriate extraction method is needed, both the type of solvent and the time of maceration (Wijayanti et al, 2016).

Extraction using ethanol with an extraction time of 24 hours has proven to be more effective in extracting bioactive compounds in mangosteen peel (Kusmayadi et al, 2018). The ethanol extract of mangosteen peel contains chemical compounds in the form of alkaloids, flavonoids, glycosides, saponins, tannins, and steroids. These chemical compounds have been shown to be able to inhibit bacterial growth (Melkianus et al, 2019).

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Bioactive compounds in mangosteen peel extract have weaknesses, namely poor water solubility, easily degraded and oxidized during storage (Sriwidodo et al, 2022).

The bioactive compounds possessed by mangosteen peel extract need to be carried out by microencapsulation techniques so that they remain stable and safe when the extract product is to be used. The microencapsulation method is a process of protecting the core material, namely mangosteen peel extract which is encapsulated or protected using an encapsulant material. Encapsulant materials have been widely studied in the microencapsulation process, one of which is the best, namely maltodextrin derived from palm starch (Kusmayadi et al., 2019). The choice of encapsulant material greatly influences encapsulation efficiency because it relates to the amount of active ingredient encapsulated (Jayanudin and Rochmadi, 2017). Maltodextrin is reported to be one of the most widely used encapsulating materials in the microencapsulation process because it has excellent ability to form emulsions, low viscosity, easy to find, disperses quickly, high solubility, is able to form a good matrix, inhibits the crystallization process, has strong binding power and stable (Laohasongkram et al., 2011), and able to prevent oxidation so that the resulting microcapsule products have a better shelf life (Gharsallaoui et al., 2007; Djafar and Supardan, 2019).

The use of mangosteen peel extract as a core ingredient with maltodextrin from palm starch as an encapsulant has never been studied on its antibacterial activity. The formulation has been tested for characterization of particle size, zeta potential, encapsulation efficiency, and microcapsule stability. In this study, various formulations of ethanol extract of mangosteen peel and maltodextrin from Arenga starch very urgent were examined for antibacterial activity tests on *Salmonella thypimurium* and *Lactobacillus acidophilus* bacteria.

## Method

### *Antibacterial Activity Test*

The microencapsulation process and the preparation of samples based on Kusmayadi et al. (2019). Antibacterial activity testing was carried out using the Brooks et al. (2007) namely in the following way: Bacterial inoculums were incubated on Mueller Hinton Agar (MHA) media at 37°C for 18 hours, then diluted with 0.85% sterile NaCl solution so as to achieve turbidity equivalent to McFarland standard no. 0.5 (10<sup>6</sup>-8 CFU/ml). Then each bacterial inoculum was spread slowly on a petri dish containing solid MHA media and wells were made with a diameter of 5 mm. As much as

500 microliters of each formulation of ethanol extract of mangosteen peel and maltodextrin was filled in each well with the following formulation: 30% ethanol extract of mangosteen peel + 70% maltodextrin (T1), 40% ethanol extract of mangosteen peel + 60% maltodextrin (T2), 50% ethanol extract of mangosteen peel + 50% maltodextrin (T3), 60% ethanol extract of mangosteen peel + 40% maltodextrin (T4), 70% ethanol extract of mangosteen peel + 30% maltodextrin (T5), ethanol extract of mangosteen peel mangosteen alone (T6), maltodextrin only (T7), positive control containing 100 ppm bacitracin (T8) and negative control (T9). Testing of each formulation was repeated four times. After that, the media was incubated at 37°C for 24 hours, then the zone of inhibition of bacterial growth around the wells was measured (Kusmayadi et al., 2023).

### *Data Analysis*

The data on the diameter of the inhibitory growth zone of *Salmonella thypimurium* and *Lactobacillus acidophilus* were analyzed using Kruskal Wallis Test with SPSS 25.0.

## Result and Discussion

Based on the data in Table 1 it shows that the formulation of mangosteen peel extract combined with maltodextrin had a significant effect ( $P < 0.05$ ) on antibacterial activity both on *Salmonella thypimurium* and on *Lactobacillus acidophilus*. This shows that the ethanol extract of mangosteen peel and maltodextrin treatment at various ratios had an effect on the growth of *Salmonella thypimurium* and *Lactobacillus acidophilus* bacteria. Antibacterial activity on *Salmonella thypimurium* as presented in Table 1. showed a significant effect ( $P < 0.05$ ). The mean value of the diameter of the inhibition zone of the mangosteen peel extract treatment on *Salmonella thypimurium* ranged from 6.68 – 7.79 mm. This condition indicates that mangosteen peel extract has moderate level of antibacterial activity against *Salmonella thypimurium*. This is in accordance with the research of Ibrahim et al. (2016) that  $\alpha$ -mangostin and its derivatives have antibacterial activity against *S. aureus*, *P. aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis* with a moderate level of susceptibility. The xanthone bioactive compound owned by EKM shows effective antibacterial activity in inhibiting *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* because it contains  $\alpha$ -mangostin with the strongest antibacterial activity compared to other derivatives such as  $\beta$ - and  $\gamma$ -mangostin, gartinin, 1- and 3-isomangostin (Poeloengan and Praptiwi, 2010).

**Table 1.** Inhibitory zone diameter of antibacterial activity

Treatments	Inhibitory zone diameter (mm)	
	<i>Salmonella thypimurium</i>	<i>Lactobacillus acidophilus</i>
T1	7.60±0.09 <sup>bc</sup>	6.51±0.27 <sup>b</sup>
T2	7.73±0.41 <sup>c</sup>	6.60±0.33 <sup>bc</sup>
T3	7.79±0.36 <sup>c</sup>	6.73±0.39 <sup>bc</sup>
T4	7.51±0.23 <sup>bc</sup>	6.77±0.52 <sup>bc</sup>
T5	7.23±0.70 <sup>bc</sup>	6.75±0.40 <sup>bc</sup>
T6	7.65±0.43 <sup>bc</sup>	7.13±0.18 <sup>bc</sup>
T7	6.68±0.39 <sup>b</sup>	7.25±0.27 <sup>c</sup>
T8	17.95±1.52 <sup>d</sup>	27.85±0.83 <sup>d</sup>
T9	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

Description: \*Superscript different within the same column shows a significant difference ( $P < 0.05$ ).

*Salmonella* is a non-sporing gram-negative bacterium (Hidayati and Wardani, 2015) which has a complex cell wall consisting of auto-membrane proteins, lipoproteins and peptidoglycan. The presence of tannins in the ethanol extract of mangosteen peel has the ability to shrink the cell walls of the *Salmonella thypimurium* bacteria so that they can interfere with cell permeability. By disrupting the permeability of the bacterial cells, the bacterial cells cannot carry out life activities so that the growth of *Salmonella* bacteria becomes inhibited (Ajizah, 2004; Hidayati and Wardani, 2015). *Salmonella* bacteria have complex antigens, namely the H antigen which is thermostable and can be damaged by acids, alcohol and heating, the Vi antigen is a surface antigen which is thermolabile and can be damaged by phenolic compounds, while the O antigen is resistant to boiling, alcohol and acids (Hidayati and Wardani, 2015). Some of the chemical compounds contained in mangosteen peel can inhibit and even kill *Salmonella thypimurium* bacteria.

According to Reveny (2011) the presence of a combination of several bioactive compounds such as tannins and flavonoids in the ethanol extract of mangosteen peel can reinforce each other and have antimicrobial effectiveness. The mechanism of action of tannins as an antibacterial is related to the ability of tannins to deactivate adhesion to microbial cells (molecules attached to host cells) found on the cell surface (Noorhamdani et al., 2013; Romas et al., 2015). Tannins have antimicrobial potential because they can inactivate bacterial cell adhesins (molecules attached to the host) found on the cell surface, and are able to inhibit protein transport enzymes through the cell membrane. This compound also has a complex form with polysaccharides in the bacterial cell wall (Hayati et al., 2009; Romas et al., 2015). Meanwhile, flavonoids are polar compounds which include phenolic compounds which have very active properties in slowing the growth of viruses, bacteria and fungi. Flavonoid chemical compounds are generally antioxidants and many have

been used as components of raw materials in the manufacture of medicines (Naim, 2005; Romas et al., 2015).

Apart from mangosteen peel extract, maltodextrin also acts as an antibacterial agent in the research results. It was proven that the T1 - T7 treatments containing maltodextrin at different levels had moderate levels of inhibition zone diameters. Maltodextrin has been shown to increase the persistence of intracellular *Salmonella* in various cell types involved in intestinal defense (Nickerson et al., 2014). *Salmonella* is a pathogenic bacterium that is carried through food, especially in spices which can cause fever, diarrhea and vomiting (Wenwen et al., 2015). Maltodextrin in the digestive tract is able to clean pathogenic bacteria such as *Salmonella thypimurium* in mice infected in vivo and in vitro (Nickerson et al., 2014). This is due to maltodesktrin playing a role in reducing pathogenic microbes by performing the body's defense as an antimicrobial agent by producing a new niche for the survival of *Salmonella* in macrophages and intestinal mucosa.

Based on the statistical data in Table 1. it shows that the ethanol extract of mangosteen peel and maltodextrin had a significant effect ( $P < 0.05$ ) on the antibacterial activity of *Lactobacillus acidophilus*. This shows that the formulation of ethanol extract of mangosteen peel and maltodextrin at various levels affect the growth of *Lactobacillus acidophilus*. Mangosteen peel extract showed an effective zone of inhibition against *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus oralis* and *Lactobacillus acidophilus* (Janardhanan et al., 2017). Mangosteen peel extract has broad-spectrum antibacterial activity against several Gram-positive and Gram-negative bacteria (Vishnu Priya, 2010).

*Lactobacillus acidophilus* belongs to gram-positive bacteria which have better inhibition than gram-negative bacteria (Lim et al., 2013).  $\alpha$ -mangostin and its derivatives in mangosteen peel extract have been shown to have a very good ability to inhibit gram-positive bacteria which are then followed by inhibition of gram-negative bacteria (Narasimhan et al., 2017). Antibacterial activity is reflected in the diameter of the inhibition zone where the diameter of the inhibition zone from the research results ranged from 6.51 - 7.25 mm with a positive control value of 27.85 mm. The different antibacterial activities between treatments were influenced by several factors, namely the type of bacteria being inhibited, the content of antibacterial compounds, the extract concentration and the diffusivity of an herbal extract (Brooks, 2007). *Lactobacillus acidophilus* bacteria are a group of gram-positive bacteria that have a simpler bacterial cell wall structure because they have a single layer with a low lipid content of around 1-4%, making it



easier for extracts of herbal ingredients to enter the bacterial cells more quickly (Nimah et al., 2012).

The results of the study in Table 1 show that the higher the concentration of mangosteen peel extract (T1 - T4) the higher the activity of inhibiting bacterial growth. These results are consistent with the research of Melkianus et al, (2019) that the higher the concentration of mangosteen peel extract, the higher the activity of inhibiting bacterial growth. Fatmala (2015) reported that the ethanol extract of mangosteen peel contains xanthenes, flavonoids, tannins, alkaloids, saponins and triterpenoids. Each of these compounds is proven to have good pharmacological activity, especially as an antibacterial. The antibacterial activity of xanthone compounds in mangosteen peel extract is related to the reaction of the xanthone carbonyl group which interacts with non-ionized amino acid groups such as the  $\epsilon$ -amino group of lysine residues or the terminal  $\alpha$ -amino group of a bacterial cell membrane protein, causing the function of the bacterial cell membrane protein to be lost (Putra, 2010; Fatmala, 2015).

The tannins in the ethanol extract of mangosteen peel inactivate bacterial cell adhesin, inactivate enzymes and interfere with protein transport in the inner layer of bacterial cells. Tannins also target the polypeptide part of the bacterial cell wall so that the formation of the bacterial cell wall becomes less than perfect. This causes the bacterial cells to lyse due to osmotic and physical pressure (Ngajow et al., 2013; Fatmala, 2015). Furthermore, flavonoids will damage the permeability of bacterial cell walls, bacterial microsomes and bacterial lysosomes. As a result of the interaction between flavonoids and bacterial DNA, flavonoids are also able to release transduction energy to the bacterial cytoplasmic membrane. In addition, flavonoids can also inhibit bacterial motility. The hydroxyl groups present in the structure of flavonoid compounds cause changes in organic components and nutrient transport which will eventually result in toxic effects on bacteria (Sabir, 2005; Fatmala, 2015). Saponin compounds in the ethanol extract of mangosteen cake skin can damage the cytoplasmic membrane of bacterial cells, resulting in reduced permeability of the bacterial cell membrane so that the transport of substances into the bacterial cell and out of the bacterial cell becomes uncontrolled. Alkaloids have antibacterial activity by interfering with the peptidoglycan composition of bacterial cells, so that the bacterial cell wall layer is not formed completely and causes the death of the bacterial cell (Amalia et al., 2014).

Maltodextrin has a role as an antibacterial in inhibiting the growth of *Lactobacillus acidophilus* bacteria although it is not significant (Table 1). Maltodextrin combined with *L. plantarum* probiotics had no effect on inhibiting and increasing total *Lactobacillus* in the small intestine. In the small intestine, maltodextrin will be

hydrolyzed and absorbed by pancreatic  $\alpha$ -amylase to lower the level of saccharides and  $\alpha$ -dextrans so they do not reach the large intestine (Nemcová et al., 2007). Maltodextrin acts as a prebiotic which has a transportation and metabolic system in *Lactobacillus acidophilus* by producing  $\alpha$ -glucoside through reverse phospholysis by maltose phosphorylase (Nakai et al., 2009).

## Conclusion

The formulation of ethanol extract of mangosteen peel and maltodextrin affected the antibacterial activity of *Salmonella typhimurium* and *Lactobacillus acidophilus* with moderate inhibition zone diameters for each of the tested bacteria. Mangosteen peel extract contains bioactive compounds in a broad spectrum category that can inhibit the growth of gram-negative bacteria.

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