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Maja Fruit Extracts Inhibit *Escherichia coli*, Reduce Fly Larvae Population, and Ammonia Emission of Chicken Excreta

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ABSTRACT

The characteristic of chicken excreta has a very potential as a breeding media for flies and is known for causing odorous pollutants (NH_3 emission) from undigested protein and the activities of urease microorganisms. This study is focused on extracting Maja fruit, to quantify marmelosin from different fruit conditions using HPLC, and to determine the biological activity for handling the chicken excreta problems. In this study, the Kirby-Bauer Test was used to observe the antibacterial activity of marmelosin, the NH_3 trapping method was used to determine ammonia emission, and the larvae population was determined by the Fly-Grill method. Marmelosin contents in MFE from immature, mature, and fermented fruit condition were 108.65 μg/g; 65.83 μg/g, and 23.02 μg/g, respectively. The increasing level of marmelosin addition to 50, 100, 150, and 200 μg/mL caused the higher diameter of inhibition zone against *E. coli* ($p<0.05$), which were 2.50, 2.90, 5.06, and 7.27 mm, respectively. The increasing level of MFE addition at 5, 7.5, and 10% (v/v) showed a higher inhibition effect on the NH_3 emission from the excreta. The addition of MFE up to 10% (v/v) had no significant effect on the total larvae population of flies that existed in the excreta. It can be concluded that the highest marmelosin content was confirmed in the immature fruit condition. The highest antibacterial activity of marmelosin from MFE was shown at the concentration of 200 μg/mL. The application of 10% (v/v) MFE to the excreta gave the highest inhibition of NH_3 emission and minimized the average larvae population of flies.

Keywords: maja fruit extract; marmelosin; antibacterial activity; ammonia emission; chicken excreta

INTRODUCTION

Ammonia as an odorous air pollution and the explosion of house fly populations at the poultry farms become the severe problems that often occur in a tropical country such as Indonesia. This condition stimulates negative impacts in various communities. The negative impact of this condition must be resolved to prevent the worse conditions. Odorous mitigation treatment can be performed by inhibiting the formation of ammonia in the excreta. At the same time, the handling of flies can be done by damaging the life cycle and ecosystem of the flies itself. Fitriyanto *et al*. (2017) state that microbiological activity will always have a correlation to the occurrence of the ammonia gas emission from the excreta. Ammonia emission at poultry farms is a consequence of microbial activity and decomposition of nitrogenous compounds in the excreta. Chicken excreta generally has a low C/N ratio value with the high moisture and high nutrient content (Singh *et al*., 2018). That condition stimulates the increasing activity and populations of decomposer microorganisms, which is important as the feed source for the growth and development of fly larvae (Putra *et al*., 2013).

Nitrogen compounds such as uric acid, undigested proteins, and urea are the primary sources for the production of ammonia emission from the excreta (Maliselo & Mwaanga, 2016). The partial breakdown of uric acid produces urea, and in a short time after the excretion, urease bacterial activity converted urea compounds into ammonia (Wood & Van Heyst, 2016). Several types of bacteria are found to have urease enzymes, such as *Helicobacter pylori*, *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Proteus* spp., *Yersinia* spp., *Klebsiella* spp., and *Escherichia coli* (Konieczna *et al*., 2012). Urease inhibitors can block these processes, thereby preventing ammonia formation and reducing ammonia emissions from the excreta. The application of natural antibacterial from plant-derived oils as urease inhibitors has been explored, such as limonene, thymol, carvacrol, linalool, carvone, and geranyl acetate. These compounds have the potency to inhibit the population of pathogenic microorganisms in the dairy rumen, reducing the volatilization of ammonia, and methane and may be applied in livestock waste (Caroprese *et al*., 2020).

Aegle marmelos, or in Indonesia known as Maja fruit, has various active compounds contained in all parts of the tree that have potentials as natural antimicrobial and insecticidal agents (Neeraj & Johar, 2017). Maja fruit could be found in part of the Indonesian region (mostly in Java, Madura, and Bali), Indian subcontinent, and the other Southeast Asia countries, which have a suitable climate for Maja fruit production. Due to the rich content with bioactive compounds, Maja fruit has a high potency as an odor reducing agent at the layer industry. One of the bioactive compounds which have the potential to inhibit the growth of bacteria is marmelosin. Marmelosin ($C_{16}H_{14}O_4$), also called imperatorin, is a major chemical constituent of the fruit and has been proved to have antibacterial activity. Marmelosin has antibacterial activity against *E. coli* and *B. subtillis,* each with growth inhibition of 64.6 and 74.9%, respectively (Chinchansure *et al*., 2015).

By keeping this view, the further investigation of the bioactive compound from Maja Fruit Extract (MFE) as a natural antimicrobial additive in biological approaches for handling the poultry waste problems that are safe for the environment, inexpensive, and easy to use. In the previous study, the essential oils including thymol, geraniol, glydox, linalool, pine oil, plinol, and terpineol can be used as the natural antimicrobial additives for urease inhibitor, reducing zoonotic pathogens bacteria, and reducing odorous emission in livestock manure handling (Wells *et al*., 2014). This study was conducted to quantify the bioactive compound of marmelosin from Maja fruits, to observe the potency as a natural antibacterial additive to inhibit pathogen bacteria, and the application potency as biological approaches for handling the chicken excreta problems, especially for reducing ammonia emission and flies.

MATERIALS AND METHODS

Materials

Maja fruits were obtained from Maja plants in Girikerto Village, Turi District, Sleman Regency, Yogyakarta, Indonesia (latitude -7°37′52″, and longitude 110°23′29″). Maja fruit was prepared in fresh (immature and mature) and also fermented conditions. The defined criteria of immature fruit condition used in this study were a soft skin texture with green color, and fruit flesh tended to be slightly soft in white color, had an approximately pH value of 5, and less than 10 months old. The defined criteria of mature fruit condition used in this study had the hard skin texture, yellowish-brown color, fruit flesh tended to have the grayish color, with the texture to be softer and more watery compared with immature fruit, had an approximately pH value of 4, and more than 10-month-old (Figure. 1).

Furthermore, the fermented fruit condition in this study was made by 3 kg of immature fruits chopped, crushed, and then inserted in the chamber. Then, as much as 300 g of molasses were dissolved in 3 liters of water and poured into the chamber. The mixture of ingredients was fermented for 10 days.

Maja Fruits Extract (MFE). The immature Maja fruits were extracted using modified the maceration method (Koziol, 2016) with methanol as a solvent. The flesh of the fruit was weighed and added with the methanol 1:10 (weight/volume). Stirring was carried out with magnetic stirrer for 30 min, left to stand for 48 hours, and then filtered using filter paper (Whatman No. 1). The filtrate was evaporated with Vaccum Rotary Evaporator and water bath heater at 60°C and then poured into heated porcelain on the water bath to get the concentrated extract. The concentrated extract of the fruit was kept in a closed container glass in the refrigerator until further observation.

Excreta medium. The excreta were obtained from a local layer poultry farm in Yogyakarta, Indonesia. The excreta were collected using plastic for covering the ground under the cages. There were two types of excreta medium, the medium for determining the potency of mitigating ammonia emission (Medium A) and the medium for observation of the number of live larvae population (Medium B). Medium A was made by mixing the fresh excreta with distilled water with a ratio of 1:10 (there were 20 g excreta and 200 mL distilled water) and stirred until homogeneous in the 500 mL Erlenmeyer. Medium B was made by fresh excreta heated at 46.5°C for 15 min and placed in the plastic plate (200 g/plate). The excreta were steamed, aimed to kill all fly eggs without changing the composition of the excreta nutrient so that the fly larvae calculation would be more precise. In this study, there were 5 treatments with triplicates applied in the medium A and B. The excreta medium was used as a control treatment (control 1), the excreta medium with the addition of 10% (v/v) methanol (control 2), and the treatment of addition of MFE applied in three different concentrations (5%, 7.5%, and 10% (v/v)). The concentration of marmelosin was equal to 5.13 μg/ml, 7.58 μg/ml, and 9.9 μg/ml, respectively. The observation was performed for 39 hours of cultivation period.

Determining and quantifying of marmelosin using HPLC. The procedure for determining, quantifying, and analysis of marmelosin compound from Maja fruit was performed according to Bhattacherjee *et al*. (2013) using the HPLC, and the research was conducted in the Laboratory of Biopharmaceutics, Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada. The HPLC instrument (Hitachi series 2000 Model, Shimadzu, Japan) with μBondapak™ C-18 column (300×3.9 mm i.d., 125 Å, 10 μm film thickness) was used for marmelosin analysis. It is coupled with a photodiode array detector (PDA) and a rheodyne injector (20 μL loop). Furthermore, methanol: water (66: 34, v/v) was used as eluent with a flow rate of 1 mL/min. Marmelosin standard was obtained from Sigma Aldrich, Singapore.

Amount of 10 g samples from fresh fruit (immature and mature) and the pulp of fermented fruit were taken in conical flasks, and 25 mL benzene (AR grade) was added, and the mixture was kept overnight (24 hours) at room temperature. After overnight, marmelosin was

extracted from the samples homogenized for 2 min. Benzene was filtered through filter paper (Whatman No. 1). This process was repeated twice by using 25 mL for every repetition. The benzene extract was then evaporated in a rotary vacuum evaporator at 50°C. The residue was dissolved in 10 mL of HPLC grade methanol and 20 μL volume of sample solution was taken for injection to the HPLC instrument. The analysis was performed in triplicate. The quantification of marmelosin concentrations of the samples was done using a calibration standard curve obtained after HPLC analysis.

Marmelosin antibacterial assay. To determine the ability of marmelosin as an antibacterial compound, a research was conducted by measuring the inhibition zone around the bacterial growth on nutrients agar medium using the Kirby-Bauer disc diffusion method (Hudzicki, 2016). Nutrients Agar (NA) as a medium, was prepared at 100 mL of 1 g meat extract, 1 g peptone, 0.5 NaCl, 1 g agar powder, and distilled water. The solution of the NA medium was then sterilized by autoclave at 121°C for 15 min. The bacterial culture of *Escherichia coli* (obtained from the Laboratory of Microbiology, Biotechnology Study Center, the Graduate School of Universitas Gadjah Mada) was grown on the NA medium and then incubated at room temperature for 24 hours. The research was continued by taking four or five colonies and transferred into 10 mL of distilled water in a glass tube. Furthermore, 0.1 mL of pure inoculum solution was spread on a solidify NA medium.

Disc paper (5 mm diameter) was dripped with 20 μL at 4 different levels concentrations (P1= 50 μg/mL, P2= 100 μg/mL, P3= 150 μg/mL, and P4= 200 μg/mL) of marmelosin standard, dripped with sterile distilled water as control-1, and with methanol as control-2. Disc paper is placed on the surface of bacterial media and slightly pressed to ensure complete contact between the disk and the agar surface. The incubation of inoculated plates was done in an inverted position at 30°C. Observation for the inhibition zone was done after 24 hours of incubation.

Analysis of MFE ability to reduce ammonia emission from chicken excreta. The ability to reduce ammonia emission from chicken excreta was determined by the total value of ammonia trapped in the boric acid by following Pastawan *et al*. (2017). The medium was conducted in triplicate using Erlenmeyer 500 mL and placed in a rotary shaker 120 rpm. The ammonia emitted from the excreta (Medium A) was driven by air from the constant speed aerator through the plastic hose into the other Erlenmeyer, which contained boric acid (200 mL of 0.02 N boric acids) to trap the NH_3 formed. Periodically, the ammonium concentration in the boric acid solution was measured by spectrophotometry using the Nesslerization method at the wavelength of 425 nm.

Analysis of MFE ability to decrease fly larvae population in the excreta. In this study, observation of the potential application of MFE to decrease the number of live larvae populations that appear in the excreta was conducted by using the Fly-Grill method (Wolfe *et al*., 2017). The experiment was carried out in two stages for 6 days. The first stage was to provide the opportunity for flies to lay eggs in the excreta (Medium B). The medium B was placed in the ground area around the cage in chicken farm for 3 days. The average number of flies in the landed area on the surface of medium B was determined with the modified Fly-Grill method by placing white paper A4 (21 cm x 29.7 cm) surrounding the excreta medium. To estimate the fly's number around the medium contaminating, it was calculated by the excreta stains attached to the paper each day. Stage two was conducted for incubation of the fly eggs contained in the Medium B to hatch into larvae. The medium B was placed in a closed styrofoam box and incubated for 3 days at room temperature in a room that was no insect detected at the laboratory room. On the 7th day, the number of fly larvae that exist on the medium was calculated.

Data analysis. The data of marmelosin characteristics from different fruit conditions were shown as a chromatogram for HPLC analysis. The data of reduction of ammonia emission from chicken excreta were shown as graphical figures using Ms. Excel. The data concentration of marmelosin, antibacterial assay, and total larvae population data was arranged in a Completely Randomized Design with triplicates for each treatment, and the results were analyzed using ANOVA and Tukey test for the significant difference. Statistical analysis and graphical work were carried out by using IBM SPSS statistics 23 and Excel. The results were considered significant at probability $p<0.05$ and presented as means ± SD (standard deviation of means).

RESULTS

Marmelosin Content in Maja Fruits Extract

The appearance of Maja fruits from Girikerto, Turi, Sleman, Yogyakarta, Indonesia, is shown in Figure 1. The immature fruit had a bright white color with less than 10 months old, while the mature one had a darker dominant color with more than 10 months old. The

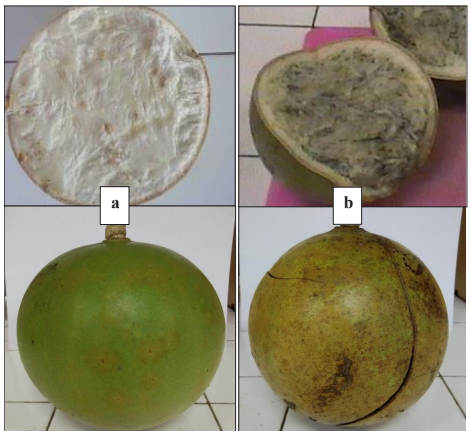

Figure 1. Maja fruit at different conditions obtained from Girikerto Village, Turi District, Sleman Regency, Yogyakarta Indonesia; (a) immature and (b) mature.

bioactive compounds of marmelosin from the fresh MFE (immature and mature) and also from the fermented one, were successfully determined and quantified using High-Performance Liquid Chromatography (HPLC). Marmelosin from Maja fruit was detected by their respective retention times, which can be seen in Figure 2. From the linearity test, the correlation coefficient value of standard marmelosin was accounted for 0.9967 with a linear regression equation of y = 58243x ± 1110.1. The retention time was 8.25±0.07 minutes in the range of

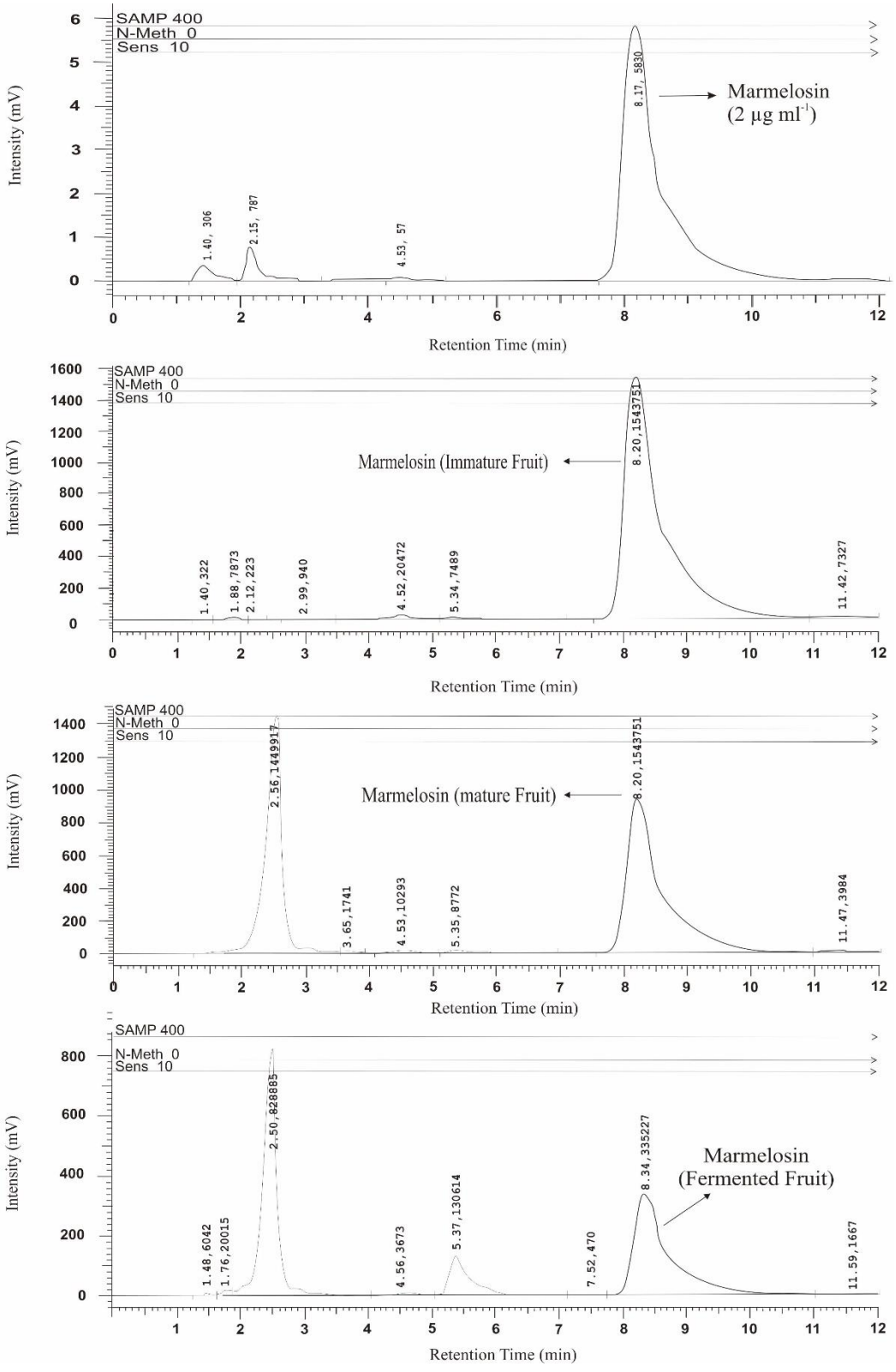

Figure 2. HPLC chromatograms of a marmelosin: Standard marmelosin (a), immature fruit (b), mature fruit (c), and fermented fruit (d).

2–10 μg/mL. The quantification of marmelosin in MFE at different conditions had various marmelosin contents. The highest content of marmelosin was observed in the immature MFE (Table 1), followed by the mature MFE and fermented MFE.

Antibacterial Activity of Marmelosin

The potency of marmelosin as an antibacterial compound against *E. coli* is shown in Table 2. The highest concentration of marmelosin added into the medium grown with *E. coli* showed the widest diameter of the inhibiting zone ($p<0.05$). The widest inhibition zone on the application of marmelosin at the level of 200 μg/mL was 7.27±0.50 mm.

Ammonia Emission from Chicken Excreta Mixed with MFE

The antibacterial and urease inhibitor activities of MFE with different concentrations in chicken excreta media are shown in Figure 3. From all treatments, the accumulation of ammonia trapped in boric acid increased during 39 hours of observation. The addition of MFE at doses of 5%, 7.5%, and 10% (v/v) showed a significant reduction in ammonia emissions from the chicken excreta medium. During the 39 hours observation, the addition of MFE at a dose of 10% (v/v) gave the highest activity to inhibit ammonia gas emission from the chicken excreta medium.

Fly Larvae Population in Excreta mixed with MFE

The observation showed that the number of flies that alighted on the surface of excreta mediums in the study for 3 days was approximately 336 flies. After 3 days of incubation, the number of live fly larvae that are existed in the excreta medium is presented in Table 3. The statistical analysis showed that the increased doses of MFE used did not significantly decrease the number of larvae population that existed in the medium. However, the number of larvae population in excreta tended to decrease with the increasing level of MFE addition.

Table 1. Concentrations of marmelosin by HPLC analysis

Maja fruit condition	The concentration of marmelosin (μg/g)
Immature	108.65 ± 0.76[a]
Mature	65.82 ± 0.68[b]
Fermented	23.01 ± 0.51[c]

Note: means in the same column with different superscripts differ significantly ($p<0.05$).

Table 2. Antibacterial activity of marmelosin compound

Treatments	Inhibition zone (mm)
Control 1 (aquadest)	-
Control 2 (methanol)	-
P1 (50 μg/mL)	2.50 ± 0.75[a]
P2 (100 μg/mL)	2.90 ± 0.75[ab]
P3 (150 μg/mL)	5.06 ± 0.40[c]
P4 (200 μg/mL)	7.27 ± 0.50[d]

Note: means in the same column with different superscripts differ significantly ($p<0.05$).

DISCUSSION

In this study, the addition of methanol treatment was intended to determine whether methanol as a marmelosin solvent in extracting Maja fruit did not affect the antibacterial activity. The concentration of ammonia emission and the number of larva populations

Table 3. The total number of living larvae in excreta medium

Treatments	The average total number of larvae
Control	175.67 ± 10.78
10% (v/v) Methanol	156.34 ± 32.75
5% (v/v) MFE	151.00 ± 31.43
7.5% (v/v) MFE	138.00 ± 10.54
10% (v/v) MFE	122.00 ± 30.51

Note: MFE= Maja Fruit Extract.

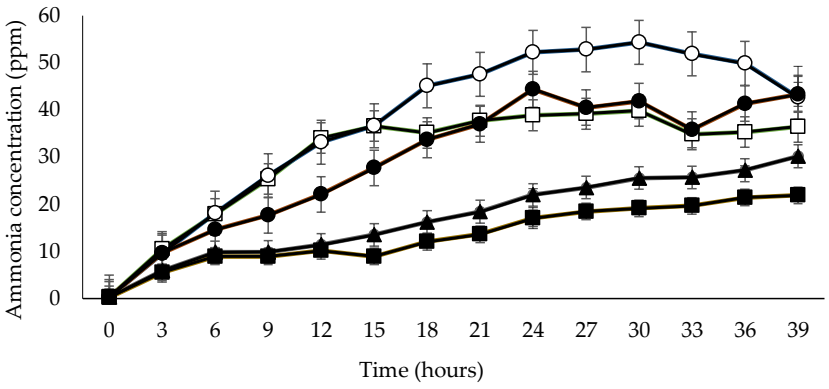

Figure 3. The accumulation of NH_3 emission from chicken excreta trapped in boric acid and analyzed spectrophotometrically. Control (□), methanol 10% (○), MFE 5% (●), MFE 7.5% (▲), and MFE 10% (■).

on the excreta were not affected by methanol, which was used as the solvent in the extraction process of marmelosin. The highest concentration of marmelosin as one of the essential-oil derivates was found in immature Maja fruit, i.e., 108.65±0.76 μg/g. The higher marmelosin content of immature Maja fruit found in this study is similar to the result found by Jana *et al*. (2017) that the marmelosin content of the pulp will gradually decrease at the ripening condition. The decreases of the marmelosin content at mature fruit may be due to the increases in the exposure of the fruit to the ambient temperatures. It has been known that the increase in the environmental temperature causes the removal of some essential oils (Baydar *et al*., 2005). Therefore, during the fermentation process, internal temperatures in the bio fermenter may also decrease the content of marmelosin as an essential oil. The concentrations of marmelosin from Maja fruit in this study were lower than the marmelosin contents of bael (Maja) fruit reported by Bhattacherjee *et al*. (2013), i.e., 481.11 μg/g in immature fruit and 103.93 μg/g in mature fruit. The different concentrations of some bioactive compounds in fruit were assumed to be due to the variations in environmental condition of Maja plantation.

The antibacterial activity of the standard marmelosin up to the dose of 200 μg/mL in this study gave the highest inhibition. However, it gave the low-class/resistance category of antibacterial activity against *E. coli* compared to commercial antibiotics. Based on Table 2, it was confirmed that in the *in vitro* test, marmelosin as a pure compound had a potency as an antibacterial activity to inhibit the growth of *E. coli*, the strain which was confirmed to have urease activity (Friedrich *et al*., 2005) to convert urea into ammonia as a dominant odor emitted from the poultry excreta. The concentrations of marmelosin used in this experiment were assumed to be suboptimal. Therefore, the suboptimal doses of marmelosin used in the present experiment could not show an optimal antibacterial activity against *E. coli* as Gram-negative bacteria. However, this result has supported the existing idea of marmelosin utilization to inhibit Gram-negative bacteria (Chinchansure *et al*., 2015).

Marmelosin is a derivative of essential oils from the furanocoumarin group. It can be assumed that the antibacterial mechanism of marmelosin is the same as that of essential oils in general. Essential oils as secondary metabolites of the plant have different antibacterial characteristic abilities against Gram-positive and Gram-negative bacteria (Flores-Encarnacion *et al*., 2016). Generally, Gram-negative bacteria, such as *E. coli* is more resistant to natural antibacterial compounds against Gram-positive bacteria because of the differences in structures of the cell walls. The cell wall of Gram-negative bacteria is more complicated than that of Gram-positive bacteria. The cell wall of Gram-negative bacteria is composed of a double layer of phospholipids that are linked to the inner membrane by lipopolysaccharides. The peptidoglycan layer is covered by an outer membrane that contains various proteins as well as lipopolysaccharides. Lipopolysaccharides consist of lipid A, the core polysaccharide, and the O-side chain, which causes the cell wall of Gram-negative bacteria to be more resistant to the essential oils and the other natural extracts with antimicrobial function. Therefore, it is necessary to concentrate on the large essential oil and a longer time to inhibit the growth of Gram-negative bacteria. With the lipophilic essential oil properties, it can help the essential oil to pass through the cell walls of Gram-negative bacteria (Nazzaro *et al*., 2013). On the other hand, from the previous study, it was observed that the addition of MFE showed a higher inhibition zone of 14.26 mm against *E.coli* compared to the marmelosin treatment in this study with inhibition zone of 7.27 mm. It was assumed that the higher antibacterial activity of MFE against *E. coli* related to the content of phenolic compounds such as tannins, phenols, and flavonoids in the MFE (Min *et al*., 2007). The better inhibition of MFE against *E. coli* was assumed to be the combined action of various phytochemical compounds in MFE (Rajan *et al*., 2011).

Furthermore, the addition of 10% (v/v) MFE gave the lowest emission of ammonia from the excreta. Another essential oil from the medicinal plant, namely, thymol, has been proven to inhibit bacterial pathogen activity and reduce the ammonia emission from livestock manure (Wells *et al*., 2014). The excreta of poultry farm contain a large and diverse population of microorganisms such as viruses, bacteria, fungi, and protozoa. Various microorganisms identified living in excreta are *Bacillus* spp., *Aerobacter* spp., *Shigella* spp., *Escherichia* spp., *Staphylococcus* spp., *Streptococcus* spp., *Kleibsiella* spp., *etc.* (Ahmed *et al*., 2012). Some of the microorganisms found in the excreta, such as *E. coli* (Friedrich *et al*., 2005), *Ureaplasma urealyticum*, *Ureaplasma parvum*, and *Helicobacter pylori* (Rothrock *et al*., 2008) have been confirmed to produce ammonia.

The application of MFE as a natural antibacterial additive had the potency to prevent the formation of ammonia, which represents the odorous pollution around the livestock farm. Wells *et al*. (2014) state that the natural antimicrobial compound from essential oils has applicable potency in livestock waste treatment because it can inhibit the fermentation producing the odor, retain the nutrients content, destroy the pathogens microorganism, and decrease the production of gaseous pollution. The antibacterial compound will slow down or stop the microbial activity to decomposing the organic matter so that the hydrolysis of urea compound in the excreta can be inhibited. The mechanism of essential oil in inhibiting bacteria growth is through its ability to change the permeability of bacterial cell walls. The dissipation of ion gradients leads to the loss of turgor pressure, inhibition of DNA synthesis, enzyme activity, and overall metabolic activities that eventually causes cell death (Patra & Baek, 2016).

Flies larvae, especially *Musca domestica*, are the insects that generally use organic waste as a food source. The livestock area is one of the industries that produce abundant organic waste. Based on the research of using various types of livestock manure shows that chicken excreta were the best medium compared to the other types of livestock manure because it contains high moisture and nutrient that are optimal for flies breeding with the success rate of fly larvae reaches 90%. In the life cy-

cle stage, the larvae of flies have a tendency to consume microorganisms (bacteria) compared to the substrate material in the living media (Putra *et al*., 2013). The observation results in the present study indicate that the doses of MFE used could not decrease 100% of larvae in the excreta. The increasing level of MFE added has a tendency to decrease the number of existed larvae populations. This result was assumed to be due to the lower concentration of the compound used. The lower concentration of active compound could not totally decrease the number of larval populations. This assumption was in line with the opinion of Ali *et al*. (2017), which stated that some botanical insecticides from a single or mixture of plant derivate oils might have a slow in action and require specific precision of concentrations for inhibiting the life-cycle of insects.

The higher bioactive compound added in the excreta will increase the possibility of inhibiting the growth of microorganisms. The bioactive compound will take a role in decreasing the microbe's population as a source of foods for the newly hatched larvae. Furthermore, the bioactive compound has a potency for inhibition of larvae growth through direct contact. The previous study proved that the phytochemical content, especially tannin and phenol, also had a potency as a larvicidal agent (Riaz *et al*., 2018), the poisonous contact to the larvae body will cause morphological abnormalities on the larvae body (Bosly, 2013).

Furthermore, the essential oil compounds extracted from plants part had the ability as a natural antibacterial as well as fumigants that can cause poisoning and morphological damage of fly during the life cycle stages of the fly (Geden, 2012). The essential oil extracted from the leaves of *Mentha piperita* (Kumar *et al*., 2011), the rhizoma of *Zingiber officinalis* (Morey & Khandagle, 2012), the flowers of *Lavandula angustifolia* (Bosly, 2013), and the fruit of *Fortunella crassifolia* (El- Sherbini & Hanykamel, 2014) have potential applications as larvicidal, pupicidal, and insecticidal by reducing the population of larvae, pupae, and adult flies. Further research is needed for more observation in the direct effect of marmelosin on the morphological changes of the microbial cells and various stages in the life cycle of the fly.

CONCLUSION

The highest concentration of marmelosin (108.65 μg/g) was observed in immature Maja fruits. The highest antibacterial activity of marmelosin against *E. coli* was shown at the concentration of 200 μg/mL. The application of 10% (v/v) MFE to the excreta medium gave the highest ammonia reduction and minimized the average larvae population of flies.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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