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RESEARCH ARTICLE

Functional Characteristics Improvement of Edible Film through Addition of Gambier and Bay Leaf Extract

Budi Santoso[1,*], Reni Dwiyanti[1], Agus Wijaya[1], Gatot Priyanto[1], Hermanto[1] and Friska Syaiful[1]

[1]*Study Program of Agricultural Product Technology, Agricultural Technology Department, Faculty of Agriculture, Sriwijaya University, Ogan Ilir, South Sumatera, Indonesia*

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Abstract: ***Background*:** In this study, the composition of the functional edible film focused on local materials was examined. However, the production of the edible film with strong capability as an antioxidant and antimicrobe has not been successful. Therefore, the incorporation of one or more functional compounds such as gambier and bay leaf extract into canna starch is a possible solution.

***Objective*:** These compounds work in synergy to improve the functional characteristics of edible film. Furthermore, the film should have mechanical characteristics which satisfy the Japanese Industrial Standard (JIS) (1975), *i.e*. strong category.

***Methods*:** This study examined the effects of gambier and bay leaf extract addition on edible film characteristics, by implementing a completely randomized design method. The two factors examined were gambier (1.0, 2.0 and 3.0 percent, w/v) and bay leaf extract (0.0, 3.0 and 6.0 percent, w/v) addition.

***Results*:** The parameters observed include mechanical (thickness, elongation percentage, water vapor transmission rate) and functional characteristics (antioxidant and antibacterial activity). The results showed that thickness, elongation percentage and water vapor transmission rate of the functional edible film were 0.18-0.27 mm, 7.33-9.00% and 30.43-46.07 $g.m^{-2}.d^{-1}$, respectively. While antioxidant and antibacterial activities (value of inhibition diameter) were 23.24-40.58 $mg.mL^{-1}$ and 1.33-1.83 mm, respectively.

***Conclusion*:** The edible film produced in this study had a strong category of antioxidant activity with a thickness that satisfied the JIS 1975 standard.

Keywords: Antioxidant activity, antibacterial activity, canna starch, bay leaf extract, gambier extract, edible film.

1. INTRODUCTION

Edible film composes of biopolymer, consisting of polysaccaharide, lipid, plasticizer, emulsifier and natural functional compounds. The combination of these biopolymers produces complex bonding as an edible film matrix [1]. Lee *et al*., stated that edible film prevents physicochemical and biological deteriorations on food products such as weight loss, flavor change, oxidation process and microbial growth [2]. The application of natural and synthetic compounds with functional characteristics to produce edible film having antimicrobial and antioxidant characteristics had extensively been carried out.

According to Nguyen *et al*., the addition of *rambai* (*Sonneratia caseolaris* (L.) leaves extract in the matrix of chitosan-based edible film improves antimicrobial characteristics and water vapor transmission rate [3]. Furthermore, the shelf life of bananas was significantly increased by this edible film. Mahcene *et al*. [4] incorporated rosemary essential oil in sodium alginate-based edible film. This produced edible film having antibacterial characteristics with inhibition diameter of 18.5 to 38.67 mm and an antioxidant capacity of 4.57 to 23,09%. Dinika *et al*. stated that cheese whey containing lactoferrin can be used to form a composite edible film having antibacterial characteristic. From several studies that focused on the development of edible film, the following can be concluded: 1) exploration of forming materials, 2) production process, and 3) application as food product packaging [5].

The exploration of forming raw materials of the edible film has been developed, especially those focused on local materials such as canna tubers [6], red palm oil [7] and gambier extract [8]. However, the use of local materials, especially natural functional compounds and gambier extract, has not been able to produce edible film with strong capability as an antioxidant and antimirobia [6]. Furthermore, the hydrox-

*Address correspondence to this author at the Study Program of Agricultural Product Technology, Agricultural Technology Department, Faculty of Agriculture, Sriwijaya University, Ogan Ilir, South Sumatera, Indonesia;
E-mail: budisantoso@fp.unsri.ac.id

yl group (-OH) in catechin compound of gambier extract is a determinant of strength for antioxidant and antimicrobial characteristics. That is, the higher the free OH group in the edible film matrix, the higher the functional characteristics and *vice versa*. The addition of natural materials containing more than one functional compound is one of the solution alternatives that can be implemented. These compounds should have synergy attributes to increasing the functional characteristics of edible film.

One of the natural functional materials that are capable of synergizing with gambier powder extract is bay leaf extract. This is because both materials have hydrophilic characteristics and contain antioxidant compounds. Verawati *et al*. reported that bay leaf extract had an antioxidant activity with IC_{50}value in the range of 35.05-49.98 $\mu g.mL^{-1}$ and total phenol of 69.76-103.91 $mg.g^{-1}$ [9]. According to Aditya *et al*., super gambier contained a catechin compound of 73.3% with antioxidant characteristics. In addition to both compounds, the addition of red palm oil in the edible film matrix, served as a lipid component as well as antioxidant source [10]. Furthermore, Cassiday *et al*. stated that red palm oil contained very high concentration of carotenoids in the form of β- and α-caroten with a magnitude of 48.2% and 38.9%, respectively [11]. The incorporation of these natural compounds forms complex bonds of edible film matrix. This produces an edible film with mechanical characteristics that fulfill JIS 1975 standard and contains a functional compound of strong category.

2. MATERIALS AND METHODS

2.1. Materials and Equipment

The materials used in this study include: 1) bay leaf (*Syzygium polyanthum*) from local market at KM 5 Palembang, 2) gambier extract (*Uncaria gambir* Roxb) from Babat Toman Village, Musi Banyuasin District, South Sumatra, 3) canna starch of *Mama Kamu* brand, 4) red palm oil from *Salmira* brand, 5) bacterial culture of *Staphylococcus aureus* FNCC 0047 from Food Microbiology Laboratory, Agricultural Technology Department, Sriwijaya University, 6) lecithin of *Lansida* brand, 7) solution of 2,2 diphenyl 1 picrylhydrazyl (DPPH) and 8) nutrient agar (NA) media.

Furthermore, the equipments used include: 1) haze meter (serie NDH - 200, Nipon Denshoku Kogyo Co., Ltd.), 2) micrometer (Roch, A281500504, Sisaku SHO Ltd, Japan), 3) testing machine MPY (Type: PA-104-30, Ltd. Tokyo, Japan), 4) spectrophotometer, 5) water vapor transmission rate tester (Bergerlahr, cup method), 6) vacuum pump (model, DOA-P504-BN), 7) hot plate (Torrey Pines Scientific brand), 8) analytical balance (Ohaus Corp. Pine Brook, N.J. USA), 9) magnetic stirrer, 10) vortex mixer, 11) dryer oven, 12) incubator and 13) desiccator.

2.2. Study Design

The method implemented in this study was a completely randomized design, which consisted of two treatment factors with three replications for each. The treatments consisted of gambier extract filtrate (A): $A_1 = 1$, $A_2 = 2$ and $A_3 = 3$ (percent, w/v) and bay leaf (B): $B_1 = 0$, $B_2 = 3$, and $B_3 = 6$ (percent, w/v). Furthermore, the data were processed using analysis of variance (ANOVA). The treatment with significant effect was further analyzed using honestly significant different (HSD) test at 5% level. The parameters observed, such as thickness, elongation percentage, and water vapor transmission rate were carried out using the protocol [12]. The equipment used for testing thickness, elongation percent, and water vapor transmission rate of edible film were micrometer (Roch) (A281500504, Sisaku SHO Ltd., Tokyo, Japan), Testing Machine MPY (Type: PA-104-30, Ltd. Tokyo, Japan) and the water vapor transmission rate tester Bergerlah cup method, respectively. The antioxidant activity was tested using the DPPH (2,2 dipenyl-1-picrylhidrazyl) method [13] and Testing for antibacterial activity using the well diffusion method with the test bacteria *Staphylococcus aureus* FNCC 0047 [14].

2.3. Gambier Extract Filtrate

The gambier extract was made using the method [15]. Dry gambier extract was ground with a mortar and sieved with an 80 mesh sieve. Furthermore, 30g of gambier powder and 100mL of aquadest were added. The suspension was heated at 40ºC and stirred with a magnetic stirrer for 10 minutes. The gambier powder suspension was filtrated using Whatman no. 1 filter paper. The filtrate was dried using a rotary evaporator to produce the gambier extract.

2.4. Bay Leaf Extract Filtrate

The bay leaf extract was prepared using [16] method. Using an oven drier, it was dried at a temperature of 40ºC for 24 hours. The dried bay leaf was mashed with a blender to produce leaf powder. Furthermore, 50g of bay leaf powder and 200 mL of aquadest were added into Beaker glass (250 mL in volume). The suspension was heated at 70°C using a water heater for 2 hours. The bay leaf powder suspension was sieved and concentrated using Whatman no. 1 filter paper and rotary evaporator, respectively, until its extract was produced.

2.5. Edible film

The method was implemented to prepare the edible film. Canna starch (6 g) was added into a volumetric flask (100 ml in volume) [17]. Aquadest was added till mark to suspend the starch. Furthermore, the suspension was transferred into a 250mL Beaker glass and stirred at 700 rpm at 65°C. By the time the suspension was gelatinized, which was indicated by the color change from white to transparent, the bay leaf extract was added according to treatments (0, 3 and 6 percent, w/v) and subsequently stirred until the suspension was homogenized. Afterward, the gambier extract was added according to treatments (1, 2 and 3 percent, w/v) and stirred for 5 min. Gradually, 3 mL of lecithin was added and stirred for another 5 min. Finally, a percentage of red palm oil was added into the suspension and stirred again for 5 min. The suspension was degassed using a vacuum pump for 1 hour. Furthermore, 25 mL was poured into a 15 cm diameter-petri

dish and dried at 50°C for 24 h in an oven drier. The resulted dried edible film was added into a desiccator for 24 h.

3. RESULTS AND DISCUSSION

3.1. Thickness

The thickness of the produced edible film ranged from 0.18 to 0.27 mm. The treatment of 3% (w/v) gambier extract filtrate and bay leaf extract had the highest thickness of 0.27 mm. Meanwhile, that of 1% (w/v) gambier extract filtrate and 0% (w/v) bay leaf extract produced the lowest thickness of 0.18 mm (Fig. **1**).

The treatment of gambier extract filtrate and bay leaf extract with their interaction had a significant effect on edible film thickness. The HSD test (5%) for the effect of gambier extract filtrate treatment on thickness, water vapor transmission rate, antioxidant activity and antibacterial activity is shown in Table **1**.

The thickness was in direct proportion with the addition of gambier extract (Table **1**). This was influenced by the magnitude of the total solid within gambier extract filtrate in the form of a catechin compound. According to Yunarto *et al*., gambier extract contained catechin compound with a magnitude of 92.69% [18]. Furthermore, this compound has semi-polar characteristics and contains total solids. Therefore, the edible film thickness is in direct proportion with the addition of gambier extract filtrate concentration.

The HSD test (5%) results for the effect of bay leaf extract filtrate treatment on thickness, water vapor transmission rate and antioxidant activity of edible film are shown in Table **2**.

The bay leaf concentration in the edible film matrix was in direct proportion with the thickness (Table **2**). This is related to the addition of total solids in the edible film matrix due to the addition of bay leaf extract. The study result by Fahrullah *et al*. [19] showed that the addition of konjac ex-

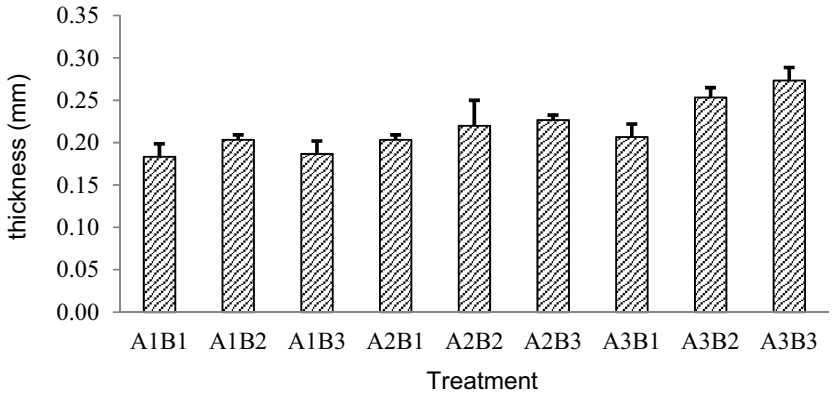

Fig. (1). The average value of edible film thickness (mm). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Significant difference (HSD) ($\alpha = 0.05$) test for effect of gambier extract addition on thickness, water vapor transmission rate, antioxidant and antibacterial activity.

Treatment	Thickness (mm)	Water Vapor Transmission Rate ($g.m^{-2}.d^{-2}$)	Antioxidant Activity (IC_{50})	Antibacterial Activity (mm)
A_3(3%)	0.24 ± 0.03^a	32.63 ± 2.03^a	26.77 ± 4.58^a	1.79 ± 0.05^a
A_2(2%)	0.22 ± 0.02^b	35.68 ± 1.18^a	30.50 ± 4.98^{ab}	1.64 ± 0.08^b
A_1(1%)	0.19 ± 0.01^c	41.05 ± 5.86^b	31.96 ± 7.95^b	1.44 ± 0.12^b

Remarks: Numbers followed by the same letter at the same column meant no significant difference and *vice versa.*

Table 2. Significant difference (HSD) ($\alpha = 0.05$) test for the effect of bay leaf extract addition on thickness, water vapor transmission rate, and antioxidant activity.

Treatment	Thickness (mm)	Water Vapor Transmission rate ($g.m^{-2}.d^{-2}$)	Antioxidant Activity (IC_{50})
B_1(0%)	0.20 ± 0.02^a	33.12 ± 2.33^a	24.43 ± 1.03^a
B_2(3%)	0.22 ± 0.03^b	37.80 ± 4.17^{ab}	28.84 ± 3.23^b
B_3(6%)	0.23 ± 0.04^c	38.43 ± 6.81^b	35.91 ± 4.36^c

Remarks: Numbers followed by the same letter at the same column meant no significant difference, and *vice versa.*

tract results increases the total solids in the edible film matrix. Subsequently, this has an effect on its increasing thickness.

Furthermore, the concentration combination of gambier extract filtrate and bay leaf extract is in direct proportion with the thickness of the edible film produced (Fig. **1**). It was previously mentioned that gambier extract filtrate and bay leaf extract contain total solids. This has an effect on the increase of total solids within complex bonds of canna starch-bay leaf extract-gambier extract filtrate-lecithin-red palm oil as matrix former of edible film. The produced edible film thickness was in the range of 0.18 to 0.27 mm. Furthermore, the average value of edible film thickness was 0.22 mm. This satisfies the JIS 1975 standard with the maximum edible film thickness of 0.25 mm. This result was lower compared to the edible film thickness obtained from the study by Santoso [17] with an average value of 0.26 mm. Also, it was higher compared to the thickness of catfish surimi-based edible film with a magnitude of 0.049 mm [20].

3.2. Elongation Percentage

The elongation percentage of the produced edible film ranged from 7.33 to 9.00%, in which A_3B_3 treatment had the highest elongation percentage. Meanwhile, A_1B_2 and A_3B_2 treatments had the lowest elongation percentage. The average value of edible film elongation percentage is shown in Fig (**2**).

Statistically, the treatments of gambier extract filtrate and bay leaf extract with their interaction had no significant effect on the elongation percentage of edible film. The average value of edible film elongation percentage as shown in Fig. (**2**) was lower compared to that of JIS 1975 standard which requires minimum value of 70%. Furthermore, the elongation percentage of the edible film is affected by constituent components of edible film matrix, especially hydrophilic components such as plasticizer, *i.e*, glycerol and sorbitol. According to Maruddin *et al*., plasticizer addition in edible film formulation was capable of increasing flexibility. The sorbitol plasticizer is better compared to glycerol and polyethylene glycol [21].

In addition, a portion of hydrophilic and hydrophobic components in the edible film matrix also had a profound effect on the elongation percentage value. This edible film matrix is formed through a complex bond of canna starch-bay leaf extract-gambier extract filtrate-lecithin-red palm oil. These constituent components showed that portion of hydrophilic and hydrophobic components was 50:50. In this case, the canna starch and bay leaf extract are the hydrophilic components. While lecithin and red palm oil are hydrophobic components and gambier extract filtrate having semi-polar characteristics. This composition causes the edible film matrix to become less elastic. Jouki *et al*. [22] stated that the addition of hydrophilic components into lettuce seeds-based edible film matrix, such as glycerol with a magnitude of 25-50% (w/b), significantly increases the elongation percentage of edible film.

3.3. Water Vapor Transmission Rate

The water vapor transmission rate of the produced edible film ranged from 30.43-46.07 $g.m^{-2}.d^{-1}$ with A_1B_2 treatment having the highest water vapor transmission rate. While A_3B_1 treatment had the lowest water vapor transmission rate. The water vapor transmission rate of this edible film did not satisfy JIS 1975 standard which requires maximum threshold of $10g.m^{-2}.d^{-1}$ (Fig. **3**).

The analysis of variance results showed that treatments of gambier extract filtrate and bay leaf extract had significant effects. However, their treatments interaction had no significant effect. From Table **1**, the results of HSD test (5%) showed that the higher the gambier extract filtrate, the lower the water vapor transmission rate of edible film. It was previously mentioned that gambier extract contains catechin compound above 75% [23] with semi polar characteristics. Furthermore, this compound causes the decrease of water vapor transmission rate of edible film. According to Lucida, catechin compound has yellow color and is in crystalline form in the dry condition and not easily soluble in water, however soluble in hot water [24].

The water vapor transmission rate of the edible film decreased with the increase of bay leaf extract concentration (Table **2**). This rate is closely related to edible film thickness,

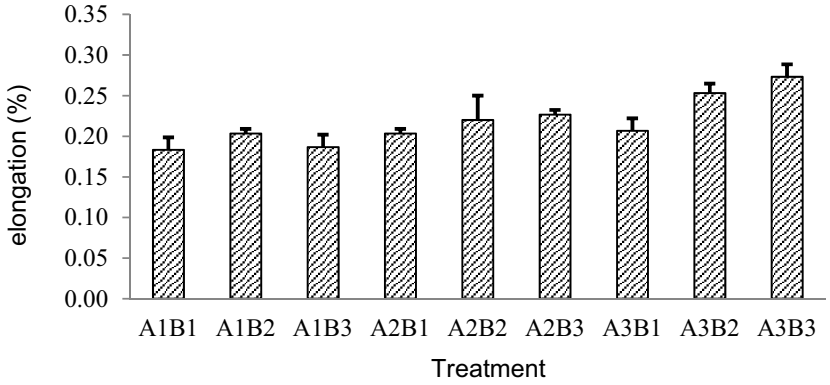

Fig. (2). The average value of edible film elongation (%). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

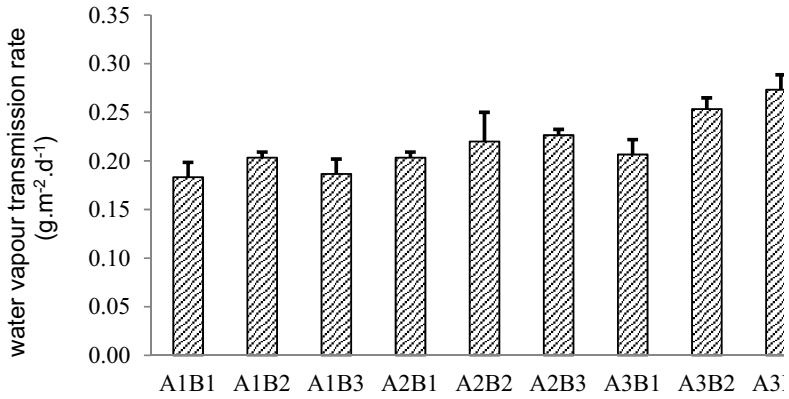

Fig. (3). Average value of edible film water vapor transmission rate ($g.m^{-2}.d^{-1}$). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

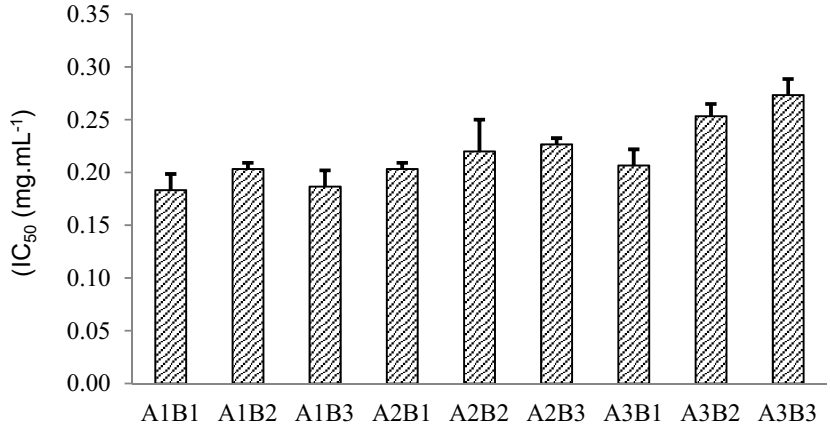

Fig. (4). Antioxidant activity of edible film, IC_{50} ($mg.mL^{-1}$). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

i.e. the thicker the edible film, the lower the water vapor transmission rate. This is due to the fact that water vapor experiences more difficult to diffuse into a thick edible film. Racmayani *et al*. reported that the thicker the natural alginate-based edible film, the lower the water vapor transmission rate [25].

3.4. Antioxidant Activity

The treatment of A_3B_3 and A_1B_1 produced the highest and lowest antioxidant compound, respectively. The average value of IC_{50} for the produced edible film was shown in Fig. (**4**).

The results of the statistical test showed that treatments of gambier extract filtrate and bay leaf extract had significant effects. Meanwhile, their treatments interaction had no significant effect on antioxidant activity. From Table **1**, the results of the HSD test (5%) showed that the gambier extract filtrate and antioxidant activity of the produced edible film were in direct proportion. This is due to the fact that gambier extract filtrate contains catechin. This compound contains a phenolic compound with antibacterial and antioxidant characteristics. Aditya and Ariyanti [10] reported that gambier extract contains catechin compound with a magnitude of 99.4-108.0 µg/mL with total phenol concentration of 13.58-13.90 g/100g. This consists of complex polyphenol with high potential as an antioxidant.

Furthermore, the antioxidant activity of edible film and bay leaf extract concentration are in direct proportion. This is due to the fact that bay leaf contains compounds that have antioxidant characteristics, which consist of flavonoid, selenium, A and E vitamins, as described by [26]. Hidayati *et al*. added that the antioxidant activity of bay leaf extract has IC_{50}value of 17.69 $\mu g.mL^{-1}$ [27].

This edible film contains an antioxidant compound with IC_{50}values that ranged from 23.24 to 40.58 $mg.mL^{-1}$. These IC_{50} values showed that the produced edible film contains antioxidant compounds with very strong categories such as stated by Molyneux that IC_{50} values less than 50 $\mu g.mL^{-1}$ was categorized as very strong antioxidant [28]. Furthermore, the antioxidant activity of edible film is determined by constituents' components. The developed complex bonds consisted of canna starch-bay leaf extract-gambier extract filtrate-lecithin-red palm oil. From these five constituent components, three components have compounds with antioxidant property, *i.e*. red palm oil, bay leaf extract and gambier extract filtrate.

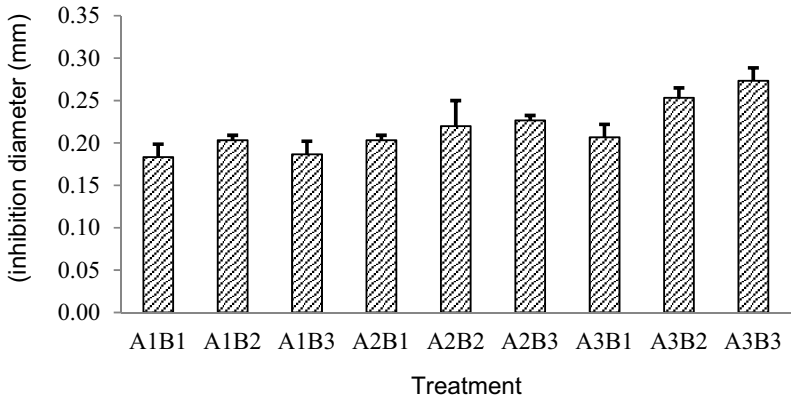

Fig. (5). Average value of edible film inhibition diameter (mm). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.5. Antibacterial Activity

The produced edible film has antibacterial characteristics specific to *Staphylococcus aureus* with inhibition diameter values which ranged from 1.33 to 1.83mm. The average value of antibacterial activity for the produced edible film is shown in Fig. (**5**).

The treatment of gambier extract filtrate had a highly significant effect on the antibacterial activity of this *edible film.* Meanwhile, the treatment of bay leaf extract and interaction treatments of gambier extract filtrate and bay leaf extract had no significant effect. Furthermore, the gambier extract filtrate concentration and antibacterial activity of edible film were in direct proportion (Table **1**). This is due to the catechin compound in gambier extract filtrate, as previously mentioned through previously carried out studies.

The inhibition diameter value of this edible film is categorized as weak according to Nazri, which categorized its values into three groups: 0-9 mm (weak activity), 10-14 mm (medium activity) and 15-20 mm (strong activity) [29]. This is due to the fact that in the complex bond of edible film matrix (canna starch-bay leaf extract-gambier extract filtrate-lecithin-red palm oil), only gambier extract is dominant as an antibacterial as mentioned by Pambayun *et al*., *i.e*. catechin compound in gambier extract is capable of inhibiting Gram-positif bacteria, one of which is *Staphylococcus aureus* [30]. It is known that the capability of catechin compound as an antibacterial is highly dependent on free OH groups in edible film matrix. Furthermore, the free OH groups and antibacterial characteristics are in direct proportion. In this case, free OH groups in the catechin compound were low because most OH groups are complex bonds. These OH groups can be bonded with the starch functional group, the active compound of bay leaf extract and lecithin.

In addition, the incorporation of the edible film with gambier and bay leaf extract resulted in better antioxidant and mechanical properties. The edible film was feasible to be commercialized. However, equipment need to be designed for the process of edible film making, from laboratory to commercial scale. The edible film containing antioxidant compounds has the advantage of reducing oxidation reactions in foods, especially fat-containing foods.

CONCLUSION

From this study, edible film as an antioxidant can be categorized as a strong category with the thickness that satisfies JIS 1975 standard and magnitude of 23.24-40.58 $mg.mL^{-1}$ and 0.18-0.27 mm, respectively. Therefore, this film is usable as a food packing material, especially for food with high-fat content, because it is able to prevent it from damage due to oxidation reactions.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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