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ANTIBACTERIAL ACTIVITY AND ANTIOXIDANT CAPACITY OF SELECTED LOCAL BANANA PEEL (Musa sp.) METHANOL EXTRACTS CULTIVATED IN BALI

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ABSTRACT

This study aims to reveal the antibacterial activity and antioxidant capacity of selected local banana peels cultivated in Bali. In addition, total flavonoid and phenolic contents determination was done to investigate the correlation to their activity. Seven kinds of selected banana peels were used for this study. Extraction was conducted by maceration methods. Antibacterial activity assay was carried out by well difusion agar, while antioxidant capacity was conducted by DPPH reduction using galic acid as a standard. The content of total flavonoid and phenolic compounds was determined by Ultra Violet-visible (Uv-vis) Spectrophotometry.Antibacterial assay on selected banana peels against Staphylococcus aureusvaried between 11.00 and 14.77 and that of Escherichia colibetween 9.00 and 13.37 at concentration of 20% (b/v). Meanwhile, the antioxidant capacity of the peels have a rangefrom 3601.11 to 2523.71 mg GAEAC /kg extract.The antioxidant capacity and antibacterial activity were positively correlated with the total flavonoid and phenolic contents. Based on the study, it can be concluded that the banana peels can be used for infectious medicinesand have antioxidant properties. These findings suggest that flavonoid and phenolic content could be used as antibacterial and antioxidant agent.

Keywords: Banana peels, Antibacterial activity, antioxidant capacity, Staphylococcus aureus, Escherichia coli.

1. INTRODUCTION

Bananas (*Musa* sp.) are a plant that is widely cultivated in Bali, both as a yard plant and as a field crop. This is because they are used as a mandatory fruit of religious ceremonies, beside to direct consumption and for food materials. However, the peels are just thrown away, which causes problems for the environment. Whereas these banana peels contain many bioactive compounds that have various activities such as antibacterial and antioxidant. In Indonesia, Bananas are called pisang. There are many kinds of Bananas cultivated in Bali Indonesia, such as PG (Pisang Gunung), PR (Pisang Raja), PS (Pisang Susu), PKp (Pisang Kepok), PK (Pisang Keladi), PH (Pisang Hijau Lumut), PP (Pisang Pecah Seribu), and others.

Bananas were a useful source of potassium for muscles and can help lower blood pressure. Potassium can also reduce the risk of stroke. Bananas are also a source of vitamins A, B6, C, and D which can keep the body healthy. Banana flowers can be used for drugs for dysentery, boils,

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bronchitis, and diabetes. Banana sap can be used for the treatment of leprosy, fever, digestive problems, nosebleeds, and insect bites. Banana peels have a function as an antifungal and antibiotic (Maya et al., 2015). Pyar and Peh (2018) reported that banana peels (*Musa sapientum*) contain carbohydrate,crude protein, lipid, fiber, total ash, and water.

Ehiowemwenguan et al. (2014) reported that the ethanol extract of *Musa sapientum* peel inhibited6 bacteria species with MIC (Minimum inhibitory concentration) of 16-512.5mg / mL.Noor and Apriasari (2014) reported that stems of Mauli banana (*Musa acuminata*) methanol extract was active as antibacterial againts *Streptococcus mutans* with inhibitory zone of 15 mm at concentration of 80%. Antibacterial assay of *Musa acuminata* peelethanol extract against *E. coli*, *S. aureus*, *P. Aerginosa* resulted that the peels can inhibit the bacteriagrowth at a concentration of 20 mg / mL(El Zawawy, 2015). Ananta et al. (2018) revealed that the peels of milk, gold (lady finger), and wood banana have antibacterial activity againts*E. coli* and *S. aureus*, where lady finger was the most active. Wahyuni et al. (2019) reported that n-butanol extract of yellow kepok banana peels inhibited the growth of *S. aureus* and *E. coli* with MIC of 0.5 and 0.1% respectively. The total flavonoid and phenolic contents were 0.06 and 0.15%. According to Susanah et al. (2018), between Flavonoid or phenolic content and antibacterial activity, there was a positif correlation.

The ability to inhibit bacterial growth and accelerate wound healing was possible due to the presence of active compounds contained in banana peels. Waghmare and Kurhade (2014) analyzed the content of bioactive compounds from *Musa sapientum* peels with GC-MS (Chromatography Gas-Mass Spectroscopy), the compounds contained were estragol, ethyl hexadecanoate, epicatechin, gallocatechin, methyl p-coumaric, 1,2 benzenedicarboxylic acid mono (2-ethylhexylester), beta-tocopherol, and vitamin E. Phytochemical assay conducted by Ehiowemwenguan et al. (2014) showed that the ethanol extract of *Musa sapientum* peels contained flavonoids, tannins,alkaloids, volatile oils, saponins, andglycosides.

Sundaram et al. (2011) reported that raw, mature, and very mature banana (*Musa paradisica*) peels have the potential as antioxidants. The antioxidant activity of the peel was evaluated by red cell hemolysis test, free radical scavenging (1,1-diphenyl-2-picrillhidrazi), and superoxide dismutase activity. The results show that raw banana peels are the most active as compared to other banana peels. Determination of total flavonoids shows a correlation between total flavonoid content and its activity as an antioxidant. Alamsyah *et al*. (2016) reported that banana peels (*Musa paradisica*) have potential as antioxidants with IC_{50} of 64.03 ppm. Baskar et al.(2011) investigated the antioxidant potential of 9 local banana peel varieties in Coimbatore, India. The results showed that the banana peel extract showed significant antioxidant activity. This study shows that the extract of this banana variety can be useful for treating free radical mediated diseases.Aboul-Enein et al. (2016) reported that banana peel (*Musa paradaisica* L.) acetone extract showed the highest antimicrobial and antioxidant activity at 600 ppm. The main phenolic compounds from the extract was catchin,qurectein and chyrsin. The high phenolic and flavonoid content increased percentage of free radical scavenging activities (Azim et al., 2018).This study aims to investigate the antibacterial efficacy and antioxidant capacity of selected local banana peels cultivated in Bali. In addition, determination of total flavonoid and phenolic contents was conducted to investigate the correlation to their activity.

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2. MATERIALS AND METHODS

2.1 Plant Material

Selected banana peelswere collected around Bali. Fresh peels were cut and thenair dried for 3week. After that, the peels were powdered and and stored for extraction.

2.2 Bacterial Agents

Pure cultures of *Staphylococcus aureus* and *Escherichia coli*were purchased from the Clinical Microbiology Laboratory of Region Public Hospital Sanglah Denpasar. The isolates were then purified and maintained at 4 °C until use.

2.3Extraction

Each selected banana peel powder (PKp, PS, PH, PP, PG, PK, dan PR) was weighed as much as 200 g, then extracted with methanol for 2 x 24 h atroom temperature (25 °C). The extract was filtered throughfilter paper (Whatman No.4), thenevaporated under vacuum. The extracts were stored at 4 °C untilanalysis.

2.4 Antibacterial Activity Assay

Antibacterial activity was evaluated by agar diffusion method (Rita et al., 2015 with modification) using media of nutient agar (NA). 10 mL of NA solution were poured into petri dish, then bacterial suspension of 200 µL was inoculated, homogenized, and then allowed until solidified. Four wells were made to each petri dish. Antibacterial activity assay of the peels methanol extract was performed at concentration of 20 and 50 % (w/v). 20 µL of the extract was put into each well, then incubated at 37°C for 24 hours. The inhibitory zone was evaluated in diameter. Each assay was carried out3 times.

2.5 Antioxidant Capacity

Antioxidant capacity was determined by UV-vis spectrophotometer using gallic acid standards. Gallic acid standards were made at concentrations of 0, 2.5, 5.0, 7.5, and 10 ppm. 0.5 mL of each standard was added 3.5 mL of DPPH. The solution was allowed to stand for 30 minutes and the absorbance was measured at a wavelength of 517 nm. Inhibition was calculated based on the following equation:

$$I = \frac{Ac \cdot}{A} \quad \text{.....(1)}$$

Where I = Inhibition, Ac= absorbance of control, and As= Absorbance of sampel.

Then a regression curve was made to get the line equation y = ax + b. 25 mg of banana peel extract was dissolved with 10 mL of methanol, centrifuged at 3000 rpm for 15 minutes, and then filtered. 0.5 mL of filtrat was added 3.5 mL of DPPH. The solution was allowed to stand for 30 minutes and the absorbance was measured at a wavelength of 517 nm. Antioxidant capacity were expressed as mg/kg GAEAC (Gallic Acids Equivalent Antioxidant Capacity). Antioxidant concentrations were determined based on linear regression equations obtained from standard solutions. Antioxidant capacity was measured based on the following equation:

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$$AC = \frac{C}{} \quad \text{..... (2)}$$

Where AC = Antioxidant Capacity (mg GAEAC /kg); C = Antioxidant concentration (ppm); V = Total Volume (mL); F = the dilutionfactor and W = weight of sample (g).

2.6 Total Flavonoid and Phenolic Content Determination

Total flavonoid and phenolic contents were determined by Spectrofotometer UV-vis using aluminum chloride and Folin-Ciocalteu reagents for the total flavonoid and the total phenol respectively according to Rita et al. (2015). Flavonoid contents were determined at a wavelength of 415 nm. The total flavonoid content were expressed as mg quercetin / 100 mg extract. Meanwhile, the phenolic contents were measured at a wavelength of 760 nm. The total phenolic contents obtained was equivalent to mg gallic acid equivalents /100 g of extract.

3. RESULTS AND DISCUSSION

The result of antibacterial assay of the banana peel methanol extract can be seen at Table 1. The selected banana peels inhibited the growth of *E. coli* with the inhibitory zone ranged from 9.2 to 13.37 mm at concentration of 20%, from 10.03 to 13.97 mm at concentration of 50%, while those of *S. aureus*at concentration of 20 and 50%, the inhibitory zone ranged from 11 to 14.77 mm and from 12.23 to 15.17 mm respectively.PKp (pisang kepok) was the most active to inhibit the growth of the bacteria,while PR (pisang raja) had the lowest zone. However, there was no significant difference between PH and PP.

Table1. Antibacterial assay results of selected banana peel methanol extracts against *E.coli* and *S. aureus*at concentration of 20 and 50%

No.	Kind of Bananas	Average inhibitory zone (mm)			
		E. coli		*S.aureus*	
		20%	50%	20%	50%
1	PKp	$13.37 \pm 0.06^{a*}$	13.93 ± 0.55^{a}	14.77 ± 0.06^{a}	15.17 ± 0.42^{a}
2	PS	12.30 ± 0.10^{b}	13.37 ± 0.12^{b}	13.73 ± 0.12^{b}	14.03 ± 0.12^{b}
3	PH	11.67 ± 0.12^{c}	12.63 ± 0.06^{c}	12.23 ± 0.38^{c}	13.50 ± 0.20^{bc}
4	PP	11.87 ± 0.12^{c}	12.73 ± 0.23^{c}	12.27 ± 0.06^{c}	13.60 ± 0.10^{cd}
5	PG	10.93 ± 0.12^{d}	11.27 ± 0.25^{d}	11.44 ± 0.12^{d}	13.00 ± 0.50^{de}
6	PK	10.33 ± 0.29^{e}	11.00 ± 0.20^{d}	11.23 ± 0.21^{d}	12.50 ± 0.20^{e}
7	PR	9.20 ± 0.17^{f}	10.03 ± 0.25^{e}	11.00 ± 0.20^{d}	11.23 ± 0.21^{f}

* The same letters in the same column showsno significantly different according to the Duncan's Multiple Range Test at P<5%.

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Overall, the inhibitory zone of the extracts against *E. coli*(gram negative bacteria) was lower than *S. aureus* (gram positive bacteria).This indicates that gram-negativebacteria were more resistant compared to those ofgram-positive. These findings were in line with the findings of Okigbo and Mmeka (2008) who reported that *S. aureus* was moresusceptible to the plantextracts than *E. coli*. This is due to differences in the complexity of the cell walls of the both bacteria (Pelczar, 2010). *E coli* contains peptidoglycan (2-7 nm) between the inner and outer membranes, and the outer membrane (7-8 nm thick) was derived from lipids, proteins, and lipopolysaccharides. Polysaccharides played a role inprevent the entry of hydrophobic compounds intoin cell membranes, whereas lipidsplayed a role in preventing entry hydrophilic compound (Prescott *et al*., 2002; Pelczar et al., 2002).In contrast Susanahet al. (2018) revealed that *E. coli* was more suscetible to the *Acorus calamus*ethanol extract than *S. aureus*, but the reason why *E. coli* was more sensitive is not yet known.

Regression equation obtained from the reduction of DPPH by gallic acid standard solution wasy = -0,038x + 0,554 (R^2 = 0,991) (Figure 1). The antioxidant capacity of 7 types of banana peels successively from high to low werePKp, PS, PH, PP, PG, PK, and PR, with a range from 3601.11 to 2523.71 mg GAEAC /kg extract (Table 2).Regression equation obtained from quercetin standard solution to determine the total flavonoid content was y = 0,0004x - 0,0001 (R^2 = 0,9992) (Figure 2), while that of gallic acid standard solution to determine total phenolic content was y = 0,0332x - 0,0244 (R^2 = 0,9978) (Figure 3).

The results of determination of antioxidant capacity, total flavonoid and phenolic contents were presented at Table 2, while chart of the antioxidant capacity, the total flavonoid and phenolic contents can be seen at Figure 4. The figures show that the total flavonoid and phenolic contents shows the same trend with the antioxidant capacity, where PKp peel was the highest flavonoid and phenolic contents, followed by PS, PH, PP, PG, PK, dan PR respectively. Piluzza and Bullitta (2011) investigated the correlations between antioxidant properties and phenolic content in 24 plant species. The result shows that Trolox equivalent antioxidant capacity (TEAC) were positively correlated with total phenolic content.Esmaeili *et al*. (2015) reported that the antioxidant activity of extracts were significantly correlatedwith their content of phenolic and flavonoid compounds.

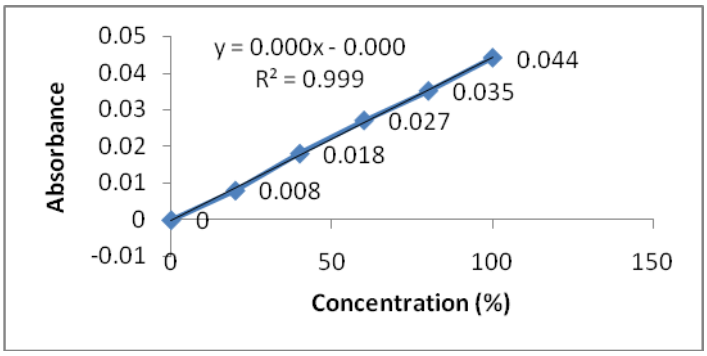

Figure 1. Regression equation of the reduction of DPPH by gallic acid standard solution

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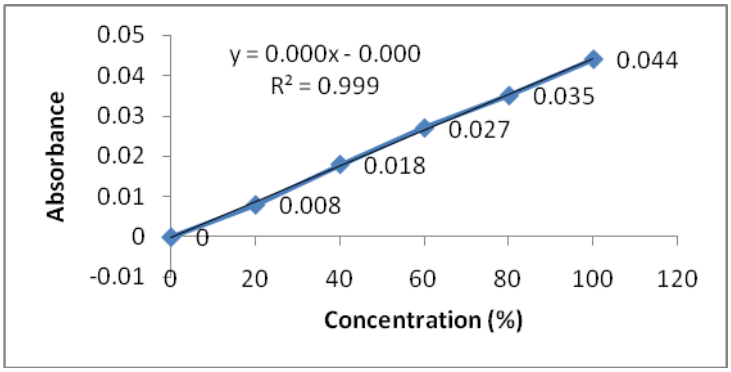

Figure 2. Regression equation ofquercetin standard solution to determine total flavonoid content

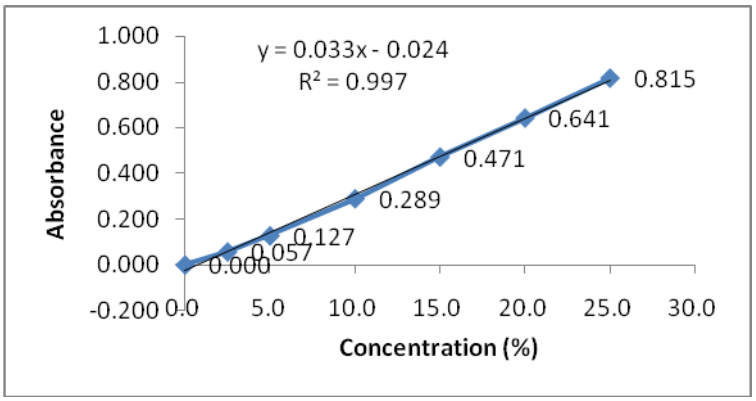

Figure 3. Regression equation ofgalic acid standard solution to determine total phenolic content

An antioxidant is usually associated with free radicals. Antioxidant capacity is a measure of the amount of free radicals captured by antioxidants. compounds containing phenol groups inhibit free radicals by releasing radical hydrogen atoms from their phenol groups to neutralize free radicals, then the chain reaction of free radicals can be terminated (Santos-Sánchez *et al*., 2019).

The flavonoid and phenolic contents also provided a positive correlation with antimicrobial activity. Mahboubi et al. (2015) and Susanah et al. (2018) reported that there was a positive correlation betweenantimicrobial activity and their flavonoid and phenolic contents. This is confirmed by research conducted by. Rita *et al*. (2019) who revealed that total flavonoid and phenolic content of *Acorus calamus*rhizome extract increased with increasing antimicrobial activity. Baba and Malik (2015) stated that the total phenolic and flavonoid contentswere positively associated with the antimicrobial and antioxidant activities. Pelima et al. (2020) also reinforced the results of this study, it found that the antioxidant of sweet potatoes was correlated with the

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Table 2. The results of antioxidant capacity, total flavonoid and phenolic contents of selected banana peels

Kinds of Banana	Antioxidant Capacity mg GAEAC /kg	Total Flavonoid mg QE /100g	Total Phenolic mg GAE /100g
PKp	3601.11 ± 0.46[a*]	2258.77± 0.10[a]	275.97± 0.02[a]
PS	3565.53 ± 0.64[b]	2056.55 ± 0.09[b]	274.92± 0.51[b]
PH	3315,49 ± 0.27[c]	2040.96± 0.12[c]	252.65± 0.21[c]
PP	3313.89 ± 0.16[d]	2033.53± 0.12[d]	250.25± 0.05[d]
PG	3217.88 ± 0.80[e]	1808.95 ± 0.25[e]	242.39± 0.10[e]
PK	2698,67 ± 0.21[f]	1763.46± 0.11[f]	214.70± 0.08[f]
PR	2523.71 ± 0.58[g]	1756.35 ± 0.09[g]	176.94± 0.18[g]

* The same letters in the same column showsno significantly different according to the Duncan's Multiple Range Test at P<5%.

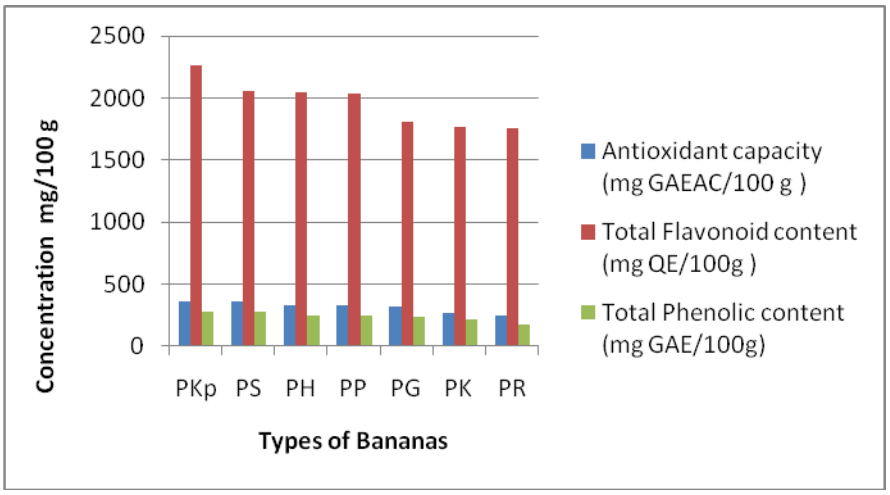

Figure 6. Antioxidant capacity, total flavonoid and phenolic contents of the selected banana peels

The ability of phenolic compounds to inhibit bacterial growth is due to the hydrogen bonding between phenol groups and proteins, which causes the bacterial protein structure to be damaged. These bonds affect the permeability of cell walls, resulting in an imbalance of macromolecules and ions in the cell. As a result, bacterial cells become lysis (Pelczar et al. 2002).

4. CONCLUSION

The banana peels can be used for infectious medicinesand have antioxidant properties. A positive correlation was found between the activity and the content of flavonoid and phenolic compounds.These findings suggest that flavonoid and phenolic content could be used as antibacterial and antioxidant agent.

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