Journal of Functional Food and Nutraceutical

STABILIZATION OF RED MELINJO PEEL (*GNETUM GNEMON* L.) ETHYL ACETATE EXTRACT AS ANTIBACTERIAL AGENT

Adolf J.N. Parhusip[1]
Nuri A. Anugrahati[1]
Wenny S.L Sinaga[1]
Johan Honga[1]
Veliana Angel[1]

[1] Food Technology Study Program of Pelita Harapan University

ABSTRACT

Melinjo (*Gnetum gnemon* L.) is a typical Indonesian plant that has many benefits such as antimicrobial agent. The aim of this study was to determine the antimicrobial activity of red melinjo peel extract. In this study, extraction was conducted by maceration using ethyl acetate as solvent for 24 hours at room temperature. 4-16% red melinjo peel extract (w/v) could inhibit the growth of *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 7644 and *Salmonella* Typhi ATCC 14028. However, 4-16% red melinjo peel extract could not inhibit the growth of *Candida albicans* ATCC 10231. In stability test, the selected extract had a stable inhibition at pH 4-7, heat treatment 65 - 95 °C for 30 minutes, salt 1%-5%, and sugar 10%-50%. The selected extract produced the biggest inhibition diameter at low pH (pH 4) and produced the smallest inhibition diameter at neutral pH (pH 7). Heat treatment 65 °C for 30 minutes produced the biggest inhibition diameter among tested bacteria and decreased with increasing heating temperature. Addition of 1-5% NaCl and 10-50% sucrose worked synergistically with the selected extract in inhibit the growth of the tested bacteria.Abstract in English.

Keywords: *Melinjo, extraction, antibacterial, stabilization, cell damage.*

ABSTRAK

Melinjo (*Gnetum gnemon* L.) merupakan tanaman khas Indonesia yang mempunyai banyak manfaat, salah satunya adalah sebagai senyawa antimikroba. Tujuan penelitian ini adalah mengetahui aktivitas antimikroba dari ekstrak kulit melinjo merah. Ekstraksi dilakukan dengan metode maserasi menggunakan pelarut etil asetat yang berlangsung selama 24 jam pada suhu ruang. Ekstrak kulit melinjo merah konsentrasi 4% (w/v) hingga 16% (w/v) mampu menghambat pertumbuhan *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 7644, dan *Salmonella* Typhi ATCC 14028. Ekstrak kulit melinjo merah 4-16% (w/v) tidak mampu menghambat pertumbuhan *Candida albicans* ATCC 10231. Ekstrak terpilih memiliki kemampuan penghambatan yang stabil pada pH 4-7, suhu 65 - 95 °C 30 menit, konsentrasi garam 1%-5%, dan konsentrasi gula 10%-50%. Ekstrak terpilih menghasilkan diameter penghambatan terbesar pada pH rendah (pH 4), sedangkan pH netral (pH 7) menghasilkan diameter penghambatan terkecil. Pemanasan pada suhu 65 °C selama 30 menit menghasilkan diameter penghambatan terbesar pada bakteri uji dan diameter penghambatan semakin menurun seiring dengan meningkatnya suhu pemanasan. Penambahan NaCl 1-5% dan sukrosa 10-50% pada ekstrak bekerja sinergis dengan kemampuan ekstrak dalam menghambat pertumbuhan bakteri uji.

Kata kunci: *Melinjo, ekstraksi, antibakteri, stabilitas, kerusakan sel.*

Article Information

Article Type: Research Article
Journal Type: Open Access
Volume: 1 Issue 1

Manuscript ID
v1n11928-2

Received Date
23 March 2019

Accepted Date
13 June 2019

Published Date
21 August 2019

DOI:
10.33555/jffn.v1i1.7

Corresponding author:
Johan Honga
Tangerang, Indonesia, 15811
Email: johanhonga@gmail.com

Citation:
Parhusip, A.J.N., Anugrahati, N.A., Sinaga, W.S.L., Honga, J., Angel, V. 2019.Stability of Red Melinjo Peel (*Gnetum gnemon* L.) Ethyl Acetate Extract as Antibacterial Agent. J. Functional Food & Nutraceutical, 1(1), pp.1-12.

INTRODUCTION

Melinjo (*Gnetum gnemon* L.) is a typical Indonesian plant which is rich in flora diversity. Melinjo belongs to *Gnetaceae* family that is originated from Indo-Malaya and Melanesia and widely cultivated from Southeast Asia to Fiji (Manner and Elevitch, 2006). In Indonesia, melinjo is commonly processed into *emping* (chips from melinjo seeds) and vegetables in various soup (Kato *et al*., 2009).

According to BPS or Indonesian Central Bureau of Statistics (2016), melinjo production increased about 7.78% from 197,648 tons in 2014 becoming 213,025 tons in 2015. This increasing of melinjo production consequently will increase the melinjo peel waste as well. Unfortunately, the utilization is still limited. In fact, melinjo peel has many benefits, one of them is as an antimicrobial agent. Parhusip and Sitanggang (2011) explains that melinjo peel had antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*.

The use of red melinjo peel in this study was based on the study conducted by Cornelia *et al*. (2009) that it had the highest phenolic compounds (0.386 mg GAE/g samples) compared to yellow and green melinjo peels. The phenolic compounds can inhibit the growth of Gram positive bacteria (Septiadi *et al*., 2013). In this study, the melinjo peel extract will be used to test its antimicrobial activity toward *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* Typhi, and *Candida albicans* which represented Gram positive bacteria, Gram negative bacteria, and yeast. The four types of microbes are pathogenic microbes that often contaminate food products and cause disease if consumed (Parhusip and Sitanggang, 2011)

The melinjo peel extract was obtained through extraction and maceration using ethyl acetate as a solvent. Parhusip and Sitanggang (2011) reported that ethyl acetate extract of red melinjo peel produced a better inhibition diameter compared to ethanol extract. In addition, ethyl acetate has low toxicity, and is able to attract polar and nonpolar compounds, easily evaporated, inexpensive, and easily obtained (Putri, 2013).

In this study, ethyl acetate extract of red melinjo peel will be tested to inhibit *S. aureus*, *Listeria monocytogenes*, *Salmonella* Typhi, and *Candida albicans*, then the optimum concentration of ethyl acetate will be determined to inhibit the growth of the tested micorbes. Stability test will be tested using the selected extract which is carried out at pH of 4-5, temperature of 65-95 °C for 30 min, and addition of 1-5% NaCl solution, and 10-50% sucrose. Stability test is required to see its inhibition in certain pH, heat temperature, salt, and sugar concentration as an initial step to be applied on food products. Based on the previous study, the 1-5% salt concentration was chosen in this study because in general the addition of 1-2% salt gives a salty taste in food products. Salt with a concentration of 5-15% used to preserve food or food products (Yusmita, 2018). Meanwhile, the sugar concentration was chosen from 10% to 50%, because in general the addition of sugar concentration up to 40% gives a sweet taste to food products. If the concentration is more than 40%, the sugar will act as a preservative in food products (Utomo *et al*., 2015).

METHODOLOGY

Materials and Tools

The materials used in this study were red melinjo peel (*Gnetum gnemon* L.) that was obtained from Bogor-West Java (evenly perfect red/merah sempurna dan merata), ethyl acetate, distilled water, Gram positive bacteria cultures (*S. aureus* ATCC 6538 and *L. monocytogenes* ATCC 7644), Gram negative bacteria culture (*S.* Typhi ATCC 14028), and yeast culture (*C. albicans* ATCC 10231), Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), tartaric acid solution, K2PO4, HCl, NaOH, NaCl, Tween 80, sucrose, and aluminum foil.

Material Preparation and Red Melinjo Peel Extraction (Parhusip and Sitanggang, 2011)

Melinjo seed was sorted, washed, and drained, then, the peel was separated from its seed. Melinjo peel was dried in a cabinet dryer until its moisture content was around 10%. The dried peel was grinded using a blender and sifted to obtain red melinjo peel powder.

The dry powder of melinjo peel was mixed with ethyl acetate as an extraction solvent with a ratio of (1:4 w/v). The mixture was stirred using a shaker at 150 rpm for 24 h at room temperature, then filtered using a Whatman filter paper No. 1 and vacuum pump. The filtrate was concentrated using a rotary evaporator at temperature of 55 °C and blown with Nitrogen to obtain a crude extract. The crude extract was diluted into 4-16% concentration by weighting 0.4, 0.8, 1.2, and 1.6 mg in 10 mL ethyl acetate.

Antimicrobial Activity Test of Melinjo Extract with Well Diffusion (Parhusip and Sitanggang, 2011)

A 40 µL culture of each test microbe was distributed into the media on a petri dish and contacted with the extract, so the concentration of each test microbe is about 10^4-10^5CFU/40 Ml. Let the media solidify for 30 minutes and make 5 holes with 6 mm diameter each. A 0.5 mL of each concentration of red melinjo peel extract was aseptically inserted into wells 1-4 (4%, 8%, 12%, and 16% (w/v) respectively). Well 5 was a control which ethyl acetate solution was used as a control. Then, the dish was incubated for 24 h at 37 °C and observed their inhibition diameter on the next day by measured to the nearest mm. The most effectiveness extract concentration obtained in this test will be further used for stability test, it is done by statistical analysis.

Stability Test of the Selected Extract at pH, Temperature, Salt and Sugar Concentration (Romson *et al.*, 2011, Winarti *et al.*, 2008, and Adriansyah *et al.*, 2003)

The selected extract was put into a test tube containing a different pH solution on each tube. The four pH levels were pH 4, 5, 6 and 7. The temperatures for stability were 65, 75, 85, and 95 °C for 30 minutes.

The sugar stability test was done by inserting the selected extract into a tube containing 10%, 20%, 30%, 40% and 50% sucrose, respectively. The stability test for salt was done by inserting the selected extract into 6 tubes containing NaCl solution with different concentrations (1%, 2%,

3%, 4% and 5%). The inhibition diameter was observed using well diffusion method with the selected extract (no sucrose or NaCl added) as a control.

RESULTS AND DISCUSSION

Antimicrobial Activity of Red Melinjo Peel

The inhibition diameter observed on the NA media indicated the antimicrobe activity of the melinjo peel extract. Ethyl acetate extract of red melinjo peel showed the ability to inhibit the growth of test bacteria from concentrations of 4% to 16%. However, the extract was not able to inhibit the growth of *C. albican*s at such Concentrations (Table 1 and Fig. 1).

The inability melinjo peel extract to inhibit *C. albicans* caused by the presence of chlamydospore in yeast, an asexual spore at the end of hyphae which forms a thick wall thus cannot penetrate by the antimicrobial compounds (Jawetz *et al.*, 2005). Besides, yeast is a eukaryotic organism which has more stable membrane than prokaryotic organisms due to sterol component in its cytoplasm. This caused the red melinjo extract hardly interfere the yeast cell permeability (Madigan et al., 2009). The 16% extract concentration showed the largest diameter inhibition compared to other concentrations seen in three microbes

Table 1. Results of various concentration level of red melinjo extract affected the inhibition diameter produced on the test microbes

Microbes	Extract concentration (%)	Inhibition diameter (mm)
S. aureus	0 (Control)	0 ± 0
	4	10.08 ± 0.60^{cd}
	8	11.59 ± 0.47
	12	14.23 ± 0.93^{fg}
	16	16.65 ± 0.31
L. monocytogenes	0 (Control)	0 ± 0
	4	9.25 ± 0.82
	8	10.54 ± 0.60
	12	13.94 ± 0.90^{fg}
	16	14.88 ± 1.07
S. Typhi	0 (Control)	0 ± 0
	4	7.74 ± 0.93
	8	9.74 ± 0.89
	12	11.06 ± 0.98^{de}
	16	13.45 ± 1.18
C. albicans	0 (Control)	0 ± 0
	4	0 ± 0
	8	0 ± 0
	12	0 ± 0
	16	0 ± 0

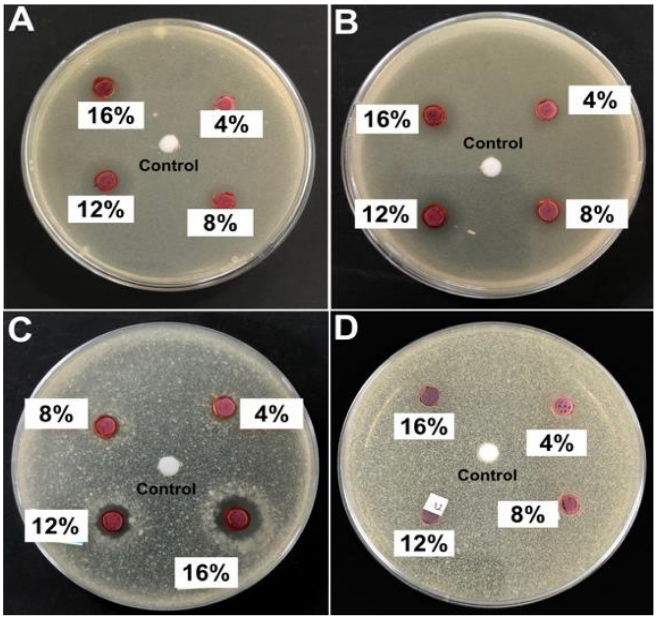

Figure 1. Results of inhibition diameter produced on *S. aureus* (A), *S.* Typhi (B), *L. monocytogenes* (D), and *C. albicans* (D) at 4-16% red melinjo peel extract

The inability melinjo peel extract to inhibit *C. albicans* caused by the presence of chlamydospore in yeast which is difficult to penetrate with antimicrobial compounds. Chlamydospore is an asexual spore at the end of hyphae which forms a thick wall so that it is difficult to penetrate antimicrobial compounds (Jawetz *et al*., 2005). Besides that, yeast is eukaryotic organism which has more stable membrane than prokaryotic organisms due it has a sterol component in its cytoplasm. This caused the red melinjo ethyl acetate extract at concentrations of 4%, 8%, 12%, and 16% couldn't interfere yeast cell permeability (Madigan et al., 2009).

The 16% extract produced the largest diameter inhibition compared to other concentrations. *S. aureus* and *L. monocytogenes* had a larger inhibitory diameter than *S.* Typhi. This result was match with theory that Gram negative bacteria have more complex cell walls composed of lipopolysaccharide, proteins and have peptidoglycan (Madigan *et al*., 2009). From the results of visual observations and statistical analysis showed that each concentration could be used as the chosen concentration. The selection of selected extract will be based on extract efficiency and suitability with the increase in inhibition diameter produced. The selection of extracts concentrations referred to Widyasanti *et al*. (2016) theory, that the inhibition diameter of 20 mm or more classified as very strong, the inhibition diameter is 10-20 mm classified as strong category, the inhibition diameter is 5-10 mm classified as medium category, and the inhibition diameter is 5 mm or less classified as weak category. Based on the theory, red melinjo peel ethyl acetate extract was classified as strong category. Table 1 showed that at 12% concentration, all test bacteria produced inhibition diameters greater than 10 mm. Therefore, the extract selected in this study was 12% concentration extract.

Extract Stability Test on pH

Testing the extract at the pH aims to see the stability of the selected extract in various acidic to neutral conditions (pH 4-7). The selected extract itself has a pH of 4.93 before it was tested into various pH solutions. Most foods are produced at that pH range, thus was chosen in this study (FDA, 2008).

Fig. 2 shows that the average inhibition diameter for each pH level was greater than 10 mm, thus can be classified as a strong inhibitory category (Widyasanti *et al*., 2016). This means that red melinjo ethyl acetate extract had a good stability and strong inhibition capacity, even though the pH varied from 4 (acid) to 7 (neutral). pH 4 gave the highest value, while other pH levels were not significant. According to Silhavy *et al*. (2010), bacteria would be more difficult to grow at acidic condition. This stability test reflects that red melinjo extract is potential to be applied for food products that have pH ranging from 4 to 7, such as meat, fish, milk, corn, spinach, asparagus, beets, and yellow walnut (FDA, 2008).

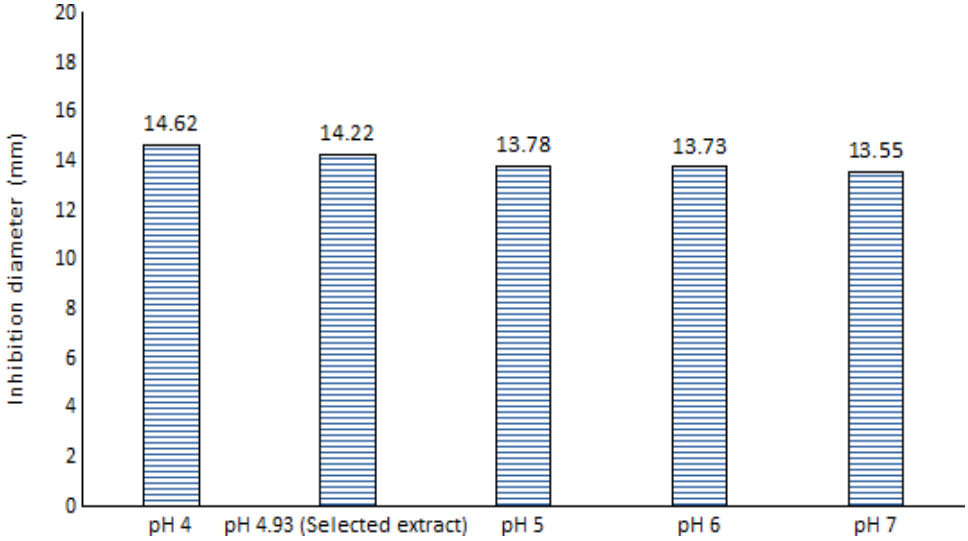

Figure 2. Test results of pH level affect the average of inhibition diameter on pH stability test

There was no interaction between the type of test bacteria and the pH level on the inhibitory diameter., However, significant effect was seen on the inhibitory response of the test bacteria (Fig. 3). *S. aureus* had the highest inhibition diameter (14.81 mm), followed by *L. monocytogenes* and S. Typhi (both were not significant) with inhibition diameter of 13.57 mm (Fig. 4). These results are in accordance with the study of of Silhavy *et al*. (2010) that the growth of Gram-positive bacteria was easier to be inhibited than Gram negative due to differences in the composition of membrane cell.

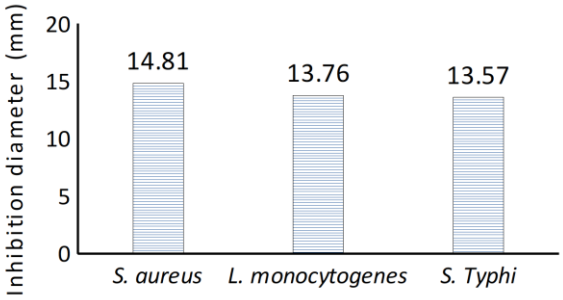

Figure 3. Test result of bacteria type affect the average of inhibition diameter on pH stability test

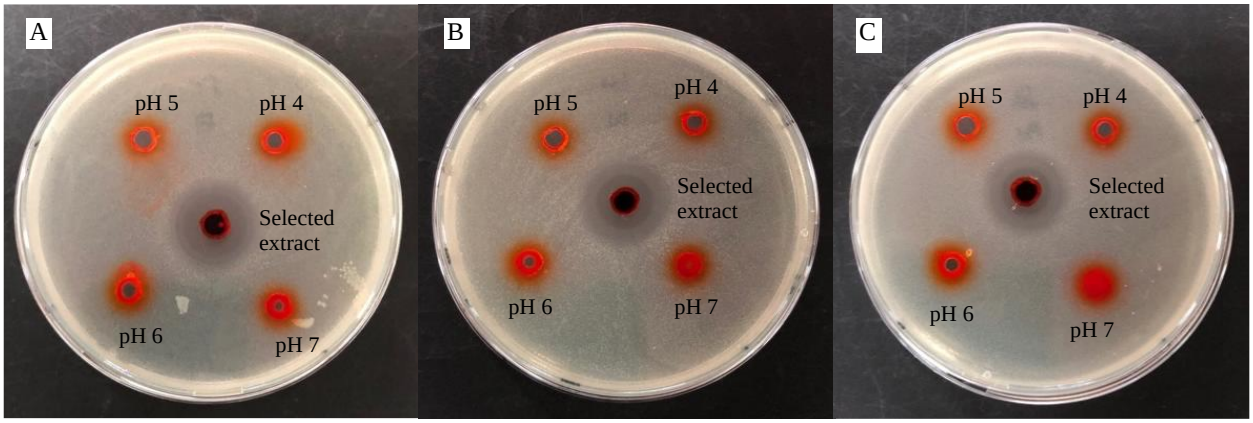

Figure 4. Inhibition diameter of *S*. Typhi (A), *L. monocytogenes* (B), and *S. aureus* (C) at 4-7 pH

Extract Stability Test on Temperature

Heating temperature of the selected extract was chosen between 65 and 95 °C to see extract ability for heat treatment, especially for pasteurization and blanching. Table 2 and Fig. 5 showed that heating temperature at 65 °C for 30 minutes produced the largest inhibition diameter compared to other temperature.

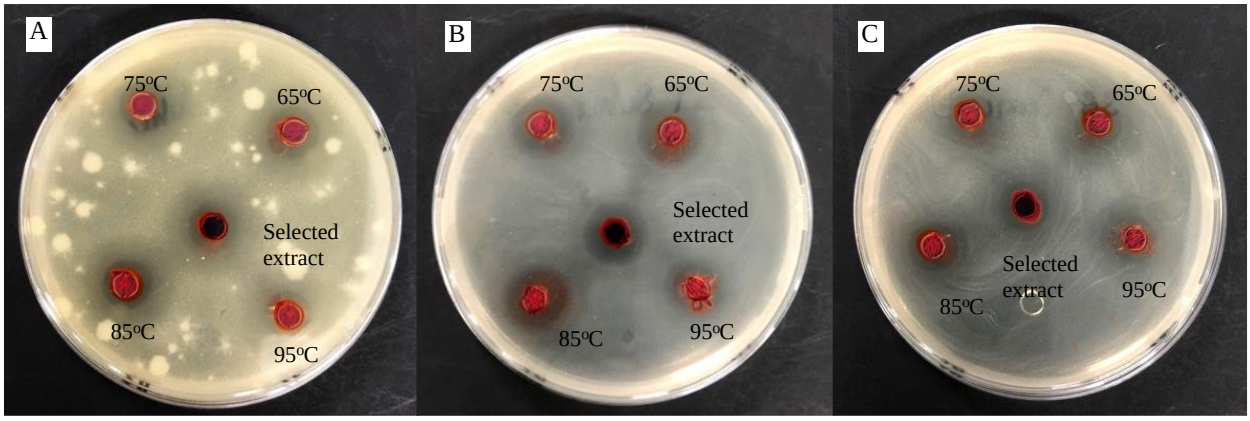

Figure 5. Inhibition diameter of *L. monocytogenes* (A), *S.* Typhi (B), and *S. aureus* (C) at 65-95ºC heating temperature

Table 2 Impact of heating temperature to the average of inhibition diameter on heating temperature stability test

Bacteria	Heating temperature (°C)	Diameter inhibition (mm)
S. aureus	Selected extract (without heating)	14.23 ± 0.93^{efg}
	65	15.60 ± 1.01^{g}
	75	14.34 ± 1.05^{efg}
	85	13.89 ± 0.93^{def}
	95	13.51 ± 0.60^{de}
L. monocytogenes	Selected extract (without heating)	13.94 ± 0.90^{ef}
	65	14.99 ± 0.58^{fg}
	75	13.20 ± 1.0^{de}
	85	12.53 ± 1.07^{cd}
	95	11.75 ± 0.46^{bc}
S. Typhi	Selected extract (without heating)	11.06 ± 0.98^{b}
	65	15.10 ± 0.59^{fg}
	75	13.31 ± 0.98^{de}
	85	10.44 ± 1.01^{ab}
	95	9.69 ± 0.61^{a}

Increasing the diameter caused by the breakdown of bioactive components to be antibacterial agent during heating. The antibacterial activity from heated bioactive component is usually higher than the initial bioactive component in inhibiting bacteria (Kyung *et al*., 1997). From the statistical

analysis showed that there is an interaction between the type of bacteria and the diameter of inhibition produced. *S.* Typhi produced the smallest diameter average (resistance) to the heating temperature and *S. aureus* produced the largest (vulnerable) average diameter of the heating temperature. This results are in accordance with the study of Silhavy *et al*. (2010) that Gram positive bacteria are easier to inhibit growth than Gram negative

Elevated heating above 65°C showed a decrease in the inhibition diameter of each test bacteria. The heating treatment at 95 °C produced the smallest inhibition diameter compared to the control or other heating temperatures. This was caused by bioactive component damage when exposed to high temperatures (Ewald *et al.*, 1999). From the stability test of extracts to heating temperature, red melinjo ethyl acetate extract had to be applied for pasteurized food products, such as milk and fruit juice drinks, as well as leaf products, such as fruit and vegetables on blanching process (Singh and Lovedeep, 2009).

Extract Stability Test on Salt

The addition of 1-5% salt concentration was chosen in this study because in general the use of 1-2% salt gives an acceptable salty taste in food products. Meanwhile, salt with a concentration of 5-15% is used to preserve food or food products (Yusmita, 2018). Table 3 and Fig. 6 showed the greater salt concentration added to the selected extract, the greater inhibition diameter produced in each test bacteria. There is an interaction between salt concentration and the response of each bacteria. Statistical analysis showed that the inhibition diameters produced by the control differed significantly from the inhibition diameter at 1-3% salt concentration. Meanwhile there was no significant difference in inhibition diameter produced at concentrations of 1-3%, but differed significantly from the concentration of 4%. The diameter of inhibition produced at a concentration of 4% was significantly different from the concentration of 5% ($p \leq 0.05$). These results reaffirm that the ethyl acetate extract of red melinjo peel had a good stability and works synergistically with salt concentration.

Table 3. Test results of salt concentration affect the average of inhibition diameter on salt stability test

Bacteria	Salt concentration (%)	Inhibition diameter (mm)
S. aureus	Selected extract	12.93 ± 0.66^{c}
	1	15.89 ± 0.19^{e}
	2	16.19 ± 0.23^{e}
	3	16.19 ± 0.21^{e}
	4	18.25 ± 0.53^{fg}
	5	18.78 ± 0.26^{gh}
L. monocytogenes	Selected extract	10.93 ± 1.21^{b}
	1	16.16 ± 0.75^{e}
	2	16.49 ± 0.19^{e}
	3	16.64 ± 0.79^{e}
	4	18.14 ± 0.87^{fg}
	5	19.44 ± 0.53^{gh}
S. Typhi	Selected extract	9.73 ± 0.44^{a}
	1	14.41 ± 0.65^{d}
	2	14.53 ± 0.61^{d}
	3	14.58 ± 0.29^{d}
	4	17.66 ± 0.29^{f}
	5	17.96 ± 0.53^{fg}

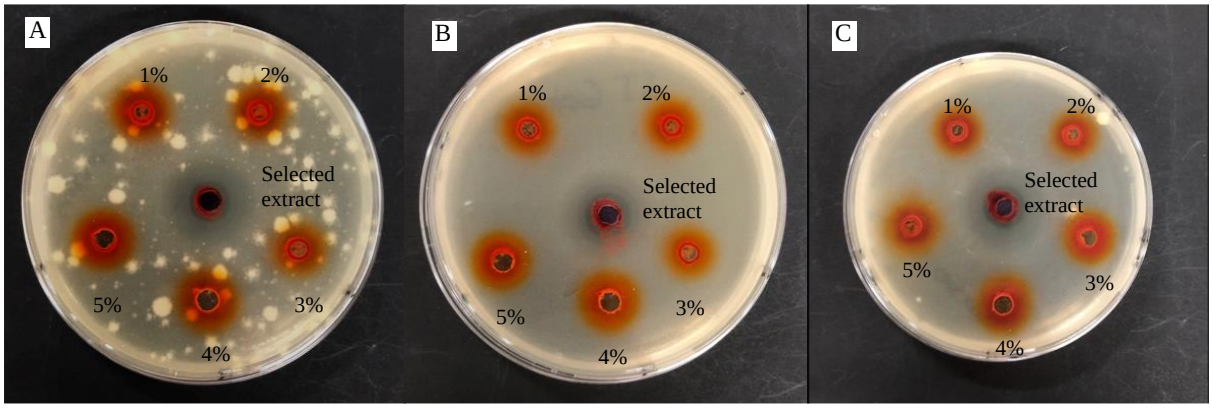

Figure 6. Inhibition diameter of *L. monocytogenes* (A), *S.* Typhi (B), and *S. aureus* (C) at 1-5% NaCl solution

Salt works synergistically with the extract in inhibiting the growth of test bacteria as salt has a high osmotic pressure that causes plasmolysis in bacterial cells (Ahillah *et al*., 2017). In addition, NaCl is hydroscopic thus it can bind water molecules, resulting in lower Aw (Madigan *et al*., 2009).

Based on this stability test, red melinjo extract had a potential to be applied for food products that contain 1-5% salt, such as biscuits, extruded foods, cakes, noodles, porridge, chocolate drinks, canned tuna, and selected seafoods (NHRI, 2018).

Extract Stability Test on Sugar Addition

The sugar concentration was chosen from 10% to 50% in this study, because the addition of sugar concentration up to 40% to food products generally gives an acceptable sweet taste. If the concentration is more than 40%, the sugar will act as a preservative in food products (Utomo et al., 2015).

Based on the stability test (Table 4 and Fig. 7), the greater the concentration of sugar added to the extract, the greater diameter of inhibition produced in each test bacteria. The presence of sugar works synergistically with the extract in inhibiting the growth of test bacteria as it can reduce the water content of bacterial cells, thus limits the microbial living activities. This result in disruption of cell metabolism which leads to cell death. When sugar with high concentrations added to food, it can block the microbial growth and decrease the water activity (a_w) (Buckle et al., 2009).

There is an interaction between the sucrose concentration and inhibition diameter of test bacteria (Fig. 10). *S. aureus* produced the largest (sensitive) average inhibition diameter when added with sugar concentration, while *S.* Typhi produced the smallest inhibition diameter (resistant). Statistical analysis showed that both controls and 10-50% concentrations did not differ significantly.

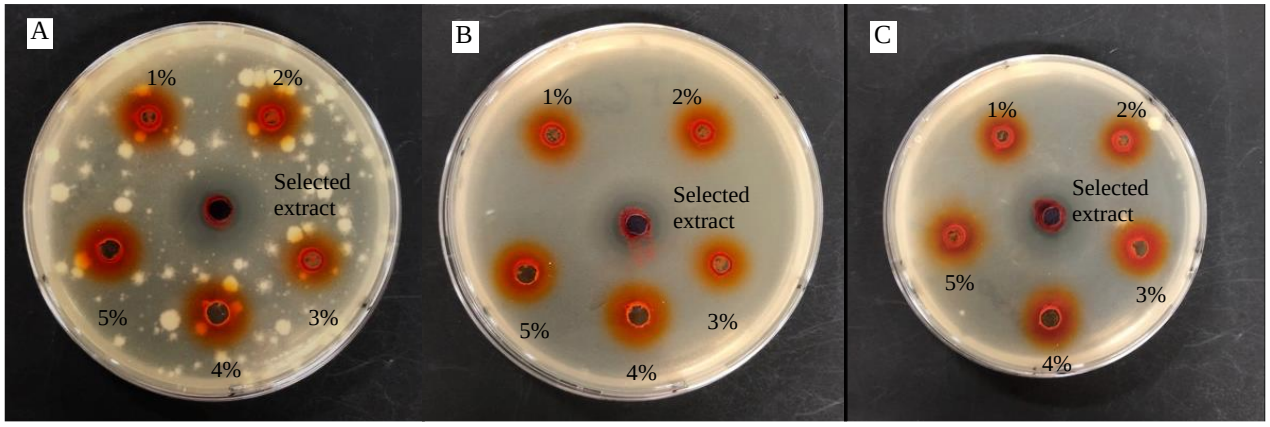

Figure 7. Inhibition diameter of *S.* Typhi (A), *L. monocytogenes* (B), and *S. aureus* (C) at 1-5% sucrose solution

Table 4. Test results of sugar concentration affect the average of inhibition diameter on sugar stability test

Bacteria	Sugar concentration (%)	Inhibition diameter (mm)
S. aureus	Selected extract	13.64 ± 0.38^{abcd}
	10	13.29 ± 0.88^{abc}
	20	13.79 ± 0.40^{bcde}
	30	15.09 ± 0.96^{ghi}
	40	15.33 ± 0.61^{hi}
	50	15.91 ± 0.63^{i}
L. monocytogenes	Selected extract	13.84 ± 0.98^{bcde}
	10	14.57 ± 0.32^{defgh}
	20	14.61 ± 0.30^{defgh}
	30	14.20 ± 0.53^{cdefg}
	40	14.74 ± 0.60^{efgh}
	50	14.93 ± 0.24^{fgh}
S. Typhi	Selected extract	12.76 ± 0.71^{a}
	10	13.06 ± 0.65^{ab}
	20	13.11 ± 0.29^{ab}
	30	13.99 ± 0.97^{bcdef}
	40	14.59 ± 0.42^{defgh}
	50	15.33 ± 0.38^{hi}

This confirmed that the red melinjo peel ethyl acetate extract had a good stable at 10-50% sugar concentration and synergistic with extract. Silhavy *et al*. (2010) revealed that Gram positive bacteria are more sensitive to the antimicrobial agent than Gram negative because the differences in their cell membrane structures. From this stability test, red melinjo ethyl acetate extract had a potential to be applied as an antimicrobial agent to food products that have 10- 50% sugar content, such as carbonated drinks, fruit juices (Walker, 2014), formula milk, yogurt, wafers, brownies, crackers, donuts, and some baby foods (Walker and Goran, 2015).

CONCLUSION

A 12% red melinjo peel ethyl acetate extract was able to inhibit the growth of *S. aureus*, *L. monocytogenes*, and *S.* Typhi with categorized strong inhibitory strength (average inhibition diameter was 13.08 mm). The selected extract had a good stability at 4-7 pH, 65-95 ^{o}C for 30 minutes heating temperature, 1-5% NaCl concentration, and 10-50% sucrose concentration. So, red melinjo peel ethyl acetate extract had a potential to be applied to food products widely. Further research is needed regarding quantitative phytochemical testing to see the percentage of bioactive components contained in the extracts. In addition, an *in vivo* toxicity test is needed to be carried out as an initial step for the extract application as an antibacterial agent in food products.

ACKNOWLEDGEMENTS

The authors thank Universitas Pelita Harapan (UPH) for funding this research and Steven Wielard, Caroline Wangsawan and Yosafat Rudju for their assistance and support.

REFERENCES

Ahillah, Nurul, Aoda Rusdanillah, Windi Afiana, Reni Sulistiani, and Rita Puspa Lestari Mail. 2017. Pengaruh Konsentrasi Garam pada Fermentasi Ikan Wader (*Rasbora lateristriata*). J. Bioedukasi, Vol. 10, No. 2: Hal. 12-17.

Ardiansyah, Lilis Nuraida, and Nuri Andarwulan. 2003. Aktivitas Antimikroba Ekstrak Daun Beluntas (*Plucea indica* L.) dan Stabilitas Aktivitasnya pada berbagai Konsentrasi Garam dan Tingkat pH. J. Teknologi dan Industri Pangan, Vol. 14, No. 2: hal. 90-97.

BPS [Badan Pusat Statistik]. 2016. Statistik Tanaman Buah-buahan dan Sayuran Tahunan. Badan Pusat Statistik Republik Indonesia. Jakarta.

Buckle, K.A., R.A. Edwards, G.H. Fleet and M. Wootton., 2009. Ilmu Pangan. Terjemahan: Purnomo H. dan Adiono. Penerbit Universitas Indonesia (UI- Press) Jakarta.

Cornelia, M., Siregar, T. M., and Ermiziar. 2009. Studi Kandungan Karotenoid, Vitamin C dan Aktivitas Antioksidan Kulit Melinjo (*Gnetum gnemon* L.). Laporan Penelitian Universitas Pelita Harapan, Tangerang.

Ewald, C. *et al.* 1999. Effects of Processing on Major Flavonoids in Processed Onion, Green Beans, and Peas. Food Chem 64: 231-235.

FDA [Food and Drug Administration]. 2008. Approximate pH of Foods and Food Products. U.S. FDA/Center for Food Safety & Applied Nutrition. USA.

Jawetz, E., J. L. Melnick and E. Adelberg. 2005. Mikrobiologi Kesehatan. Penerbit Buku Kesehatan. Jakarta.

Kyung, K. H., D. C. Han and H. P . Fleming. 1997. Antibacterial Activity of Heated Cabbage Juice, S-methyl-L-cysteine sulfoxide and Methyl Methanethiosulphate. Journal of Food Science 62(2):406-409.

Madigan, M. T., Martinko, H. M., Dunlap, P. V., and Clark, D. P. 2009. Brock Biology of Microorganisms 12th ed. California:Pearson/Benjamin Cummings.

Manner HI, Elevitch CR. 2006. *Gnetum gnemon* (gnetum). In: Traditional Trees of Specific Islands: Their Culture, Environment And Use. Holualoa: CR Elevitch.

National Health Research Institutes. 2018. Elements 25: Sodium (online): http://apjcn.nhri.org.tw/server/info/books-phds/books/foodfacts/html/data/data5a.html. Accessed 4 Oktober 2018.

Parhusip A. J. N., and Sitanggang A. B. 2011. Antimicrobial Activity of Melinjo Seed and Peel Extract (*Gnetum gnemon*) Against Selected Pathogenic Bacteria. Microbiol. Indonesia Vol. 5 No. 3: 103-112.

Putri, W. S., Warditiani, N. K., Larasanty, L. P. F. 2013. Skrining Fitokimia Ekstrak Etil Asetat Kulit Buah Manggis (*Garcinia mangostana* L.) Jurnal Farmasi Udayana:56-60.

Romson, S., Sunisa S., and Worapong U. 2011. Stability of Antioxidant and Antibacterial Properties in Heated Tumeric-Chili Paste and Its Ingredients. International Food Research Journal 18: 397-404.

Septiadi, T., D. Pringgenies & O. K. Radjasa. 2013. Uji Fitokimia dan Aktivitas Ekstrak Teripang Keling (*Holoturia atra*) dari Pantai Bandengan Jepara terhadap Jamur Candida Albicans. *Journal of Marine Research*. 2(2):76-84.

Silhavy, T. J., D. Kahne and S. Walker. 2010. The Bacterial Cell Envelope. Cold Spring Harbour Perspectives in Biology 2(5): hal. 1-16.

Singh, J., and Lovedeep K. 2009. "Advances in Potato Chemistry and Technology." Elsevier Inc., India.

Utomo, T. P., Argo, B. D., and Nugroho, W. A. 2015. Pengaruh Penambahan Gula dan Asam Askorbat pada Pengolahan Minimal Terhadap Kualitas Fisik Buah Apel Manalagi (*Malus sylvestris* Mill). Jurnal Keteknikan Pertanian Tropis dan Biosistem Vol. 3 No. 2, Juni 2015, 192-198.

Walker, R. W. Dumke, K. A. Goran M. I. 2014. Fructose Content in Popular Beverages Made with and without High-fructose Corn Syrup. Nutrition 30 (2014): 928–935.

Walker, R. W. Goran M. I. 2015. Laboratory Determined Sugar Content and Composition of Commercial Infant Formulas, Baby Foods and Common Grocery Items Targeted to Children. Nutrients 7 (2015): 5850–5867.

Widyasanti, Asri, Andita Mega Prianti, and Dadan Rohdiana. 2016. Aktivitas Antibakteri *Bacillus cereus* dan *Shigella dysenteriae* Ekstrak Teh Putih dalam Variasi Jenis Pelarut. Jurnal Penelitian Teh dan Kina, Vol. 1 (19): hal. 41-56.

Winarti, S., Sarofi, U, and Anggrahini, D. 2008. Ekstraksi dan Stabilitas Warna Ubi Jalar Ungu (*Ipomoea batatas* L.) sebagai Pewarna Alami. J. Teknik Kimia. 3: 207- 214.

Yusmita, L. 2018. Identifikasi Konsentrasi Natrium Klorida (NaCl) pada Jahe dan Lengkuas Giling di Beberapa Pasar Tradisional di Kota Padang. Jurnal Teknologi Pertanian Andalas Vol. 21 No. 2 September 2017: 122-126