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EXTRACTION OF FRUIT PEELS OF *POMETIA PINNATA* AND ITS ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

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This study investigated the extraction of matoa (*Pometia pinnata*) fruit peels, a fruit in the family of *Sapindaceae*. The extraction was performed through maceration method using three kinds of solvent: acetone, ethanol and water. The extracts were tested for their antioxidant and antimicrobial activities. DPPH radical scavenging assay was used in evaluating the antioxidant activities. The antimicrobial activity was evaluated using disc-diffusion and broth dilution method. The highest antioxidant activity was showed by acetone crude extract with IC_{50} value at 15.323 ppm, followed by ethanol and water crude extract at 143.23 ppm and 451.306 ppm, respectively. The antioxidant activities of the crude extracts were compared to L-ascorbic acid and it was found that acetone crude extract exhibited half the strength of the antioxidant activity of L-ascorbic acid. Antimicrobial activity of matoa fruit peel extracts were tested against *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus*. The results showed that all crude extracts showed antimicrobial activity with bacteriostatic characteristic. Therefore, Non-Inhibitory Concentration (NIC) was determined for all crude extracts. Acetone and ethanol crude extract possessed the strongest antimicrobial activity at NIC <0.5 ppm against all tested microorganisms, while water crude extract showed the weakest antimicrobial activity with NIC at 5 ppm. The total phenolic content of the crude extracts were measured using Folin-Ciocalteau method. Positive correlation between the antioxidant activities and the total phenolic content of the crude extracts was detected. Saponin and tannin test, as well as spectrometric analyses supported the presence of saponin, tannin and alkaloid in matoa fruit peel.

Keywords: Matoa, Pometiapinnata, Extraction, Antimicrobial, Antioxidant

ABSTRAK. Fransisca C. Faustina dan Filiana Santoso, 2014. Ekstraksi dan Pengamatan Aktivitas Antioksidan dan Antimikroba Dari Kulit Buah *Pometia Pinnata*. Studi ini meneliti ekstraksi kulit buah matoa (*Pometia pinnata*), buah dari famili *Sapindaceae*. Ekstraksi dilakukan dengan teknik maserasi menggunakan 3 pelarut: aseton, etanol dan akuades. Ekstrak tersebut kemudian dievaluasi aktivitas antioksidan dan antimikrobanya. Tes antimikroba menggunakan tes difusi lempeng dan difusi cairan. Aktivitas antioksidan tertinggi dihasilkan oleh ekstrak aseton dengan nilai IC_{50} 15.323 ppm, kemudian ekstrak etanol 143.23 ppm dan 451.306 ppm. Aktivitas antioksidan dari ketiga ekstrak tersebut dibandingkan dengan aktivitas antioksidan dari asam askorbat dan ekstrak aseton menunjukkan kekuatan antioksidan setara dengan 50% dari kekuatan antioksidan dari asam askorbat. Aktivitas anti mikroba dievaluasi terhadap *Escherichia coli, Bacillus cereus* dan *Staphylococcus aureus*. Hasil evaluasi menunjukkan bahwa semua ekstrak memiliki aktivitas antimikroba dengan karakter bakteriostatis. Karena itu nilai NIC (*Non-inhibitory Concentration*) ditetapkan untuk semua ekstrak. Aktivitas tertinggi ditunjukkan oleh ekstrak aseton dan etanol dengan NIC <0.5 ppm terhadapsemua bakteri tes, sementara aktivitas terlemah ditunjukkan oleh ekstrak akuades dengan NIC 5 ppm. Pengukuran kadar fenolik dari semua ekstrak dilakukan dengan tes Folin-Ciocalteau, dan ditemukan adanya korelasi positif antara kadar fenolik dan aktivitas antioksidan dari semua ekstrak. Analisa komponen dengan tes tannin, saponin dan spektrometri juga dilakukan dan hasil menunjukkan adanya kandungan tannin, saponin dan alkaloid di dalam kulit buah matoa.

Kata kunci: Matoa, *Pometia pinnata*, Ekstraksi, Antimikroba, Antioksidan

INTRODUCTION

Pometia pinnata is a typical plant from West Papua. It is a species of Sapindaceae, the same family to which the famous lychee and longan also belong. In Indonesia, the fruit is known as matoa. However, the use of *Pometia pinnata* in the food industry is not yet as much as litchi and longan.

Pometia pinnata plants are used for many purposes. Matoa's taste is a mixture of longan, rambutan and durian taste. It is very sweet and watery, with strong durian flavor when eaten at a very ripe stage. The seed can be consumed after being roasted and baked. The wood is used for timber industry.

Some parts of the treehave been used by traditional communities such as in Papua, Papua New Guinea and Fiji for medicinal use. The citizens of Manokwari in Papua have been using the bark of *Pometia pinnata* to treat wounds, burns and lethargy [1].

Chemically, saponins, leucoanthocyanidins, and tannins are known to exist in the bark whereas the leaves are known to have antimicrobial activity. Researches on the stem bark and leaves of Pometia pinnata reveals the existense of saponin [2,3].

The heartwood, sapwood and bark of *Pometia pinnata* plant show antioxidant activities, antifungal activities and the existence of phenolic compound[4]. Ethanolic extract of *Pometia pinnata* leaves showed antifungal effect against some rice fungal pathogens: *Bipolaris setariae, Curvularia oryzae, Pyricularia oryzae*, and *Sclerotium rolfsii* [5].

Despite of all these uses, until now there is no research done on the peels of matoa fruit. The objective of this study is to investigate the extraction of matoa fruit peels, also to evaluate its antioxidant and microbial activities.

Safety Emphasis

All activities involving the use of volatile solvents were done in fume hood, including the usage of common safety equipments such as gloves and masker. Microbiological analyses were done in sterile enviroments, using bio-safety cabinet with proper act of sterilization before conducting experiments. Microorganisms were stored in incubator and throughout any experiment masker and gloves were equipped.

MATERIALS AND METHODS

Fruit source

Raw materials used were freshly harvested coconut matoa (*Pometia pinnata*), which were purchased from a private plantation in Sleman, East Java. The peels were collected, grinded, then dried in oven at 45°C for 24 hours.

Chemicals

Technical grade acetone, ethanol, methanol and hexane was purchased from BSD Kimia, Tangerang, Indonesia. DPPH used in antioxidant assay was purchased from Sigma-Aldrich, Germany. Müller-Hinton agar (Oxoid Ltd, England) and nutrient broth (Merck, Germany) were used in antimicrobial assays. Streptomycin antibiotic (Oxoid Ltd., England) was used as positive control in disc diffusion method. Total phenolic content analysis used Folin-Ciocalteau Phenol Reagent (Merck, Germany) and gallic acid (Sigma-Aldrich, Germany). Tannic acid (Sigma-Aldrich, Germany) and sodium carbonate (BDH, England) were used in tannin test. Silica gel 60 0.2-0.5 mm (Merck, Germany) as adsorbent in column chromatography, silica gel 60 F254 plates (Merck, Germany) for thin layer chromatography. Fourier-transform infrared spectroscopy (FTIR) used KBr powder (Sigma-Aldrich, Germany), deuterated acetone (Merck, Germany) was used as solvent for nuclear magnetic resonance (NMR) spectroscopy.

Microorganisms

Staphylococcus aureus and Bacilus cereus were purchased from Universitas Pertanian Bogor, Indonesia. Escherichia coli was purchased from Laboratoria Pengembangan Teknologi Industri Agro dan Biomedika (LAPTIAB) BPPT (Puspiptek, Tangerang, Indonesia).

Sample extraction

Dried peels was macerated with 3 kinds of solvents: acetone, ethanol and water with ratio 1:5. Maceration was done twice for each solvent, each time using fresh solvent. The extract was dried through vacuum distillation at -60 mmHg. The dried extract was stored in refrigerator at 4°C.

DPPH radical-scavenging assay

0.02 mM DPPH solution was prepared by diluting 10 mg DPPH with 126.8 ml 96% ethanol. 250µl samples with concentration of 100, 20, and 10 ppm were prepared and mixed with 350µl DPPH. The mixture was incubated in dark room at room temperature for

30 minutes. Absorbance at 515 nm was measured UV-Vis spectrophotometer (Thermo Electron Corporatino-Genesys 10uv, USA).

Broth-dilution method

Culture broth was made by inoculating one colony of microorganism into sterile nutrient broth, then incubated at 37°C for 3 days. After incubation the culture was diluted to match 0.5 MCFarland standard. 1 mL of sample with concentration 500, 50,5, and 0.5 ppm were added into 1 ml of diluted culture broth, each sample was done as duplo. Positive control was made by adding 1 ml of sterile nutrient broth into 1 ml of diluted culture broth, and as negative control, 2 ml of sterile nutrient broth was used. Incubation lasted 24 hours at 37°C, then the absorbance of each sample was measured at 625 nm using UV-Vis spectrophotometer (Thermo Electron Corporatino-Genesys 10uv, USA).

Disc-diffusion method

Culture broth was centrifuged at 8000 rpm, 4-8°C for 10 minutes. Supernatant was replaced with saline solution. This mixture was centrifuged twice, then supernatant was removed. Precipitated microorganisms were dissolved in 5 ml saline solution and vortexed. 0.1 ml of suspended microorganism was pipetted onto hardened Müller-Hinton agar (Oxoid Ltd, Germany) and spread using L-rod. Paper discs were dipped into sample with concentration 500, 50, 5 and 0.5 ppm, then placed on agar surface. As positive control, disc was dipped into streptomycin (Oxoid Ltd., England), whilst as negative control, sterilized disc was put on the agar surface. The petri dishes were incubated for 3 days at 37°C then the inhibition zone was measured.

Total phenolic content

1.5 ml Folin-ciocalteau solution (1:10) was mixed with 0.3 ml sample and 1.2 ml of 7.5% sodium carbonate, consecutively. The mixture was incubated for one hour at room temperature and the absorbance was measured using UV-Vis spectrophotometer (Thermo Electron Corporation-Genesys 10uv, USA) at 765 nm.

Tannin test

Stock solution of tannic acid was prepared by dilutiong 100mg tannic acid (Sigma-Aldrich, Germany) in 1 l H_2O to make 0.1 mg/ml solution and was diluted to make concentration of 2.5, 5, 7.5, 10, 20, 30 and 40 µg/ml. 1 ml tannic acid solution was added with 0.5 ml Folin-Denis reagent and 1 ml Na_2CO_3 solution then diluted to 10 mL in volumetric flask. The absorbance at 760 nm was measured using spectrophotometer (Thermo Electron Corporation-Genesys 10uv, USA).

Saponin test

Dried matoa fruit peels was mixed with water at ratio 1:10, w/v. The mixture was shaken vigorously and left for 30 minutes. Drops of HCL 2N was added and remaining stable foam was observed.

Analysis of components

Chromatographic column 15x300 mm (Iwaki, Indonesia) was filled with silica gel 60 0.2-0.5 mm (Merck, Germany) using slurry technique. Mobile phase used was n-Hexane: ethyl acetate: ethanol (6:3:1; v/v). Isolate obtained was further analysed with FTIR using IR Prestige-21 (Shimadzu, Japan) and NMR spectroscopy using NMR spectrometer JNM-ECA (JEOL, Japan) at 500 MHz.

RESULTS AND DISCUSSION

The peels of matoa fruits were collected and dried in oven at 45°C for 24 hours. The dried peels were blended to produce matoa fruit peel powder with bright brown color. Maceration was done using three kinds of solvent: acetone, ethanol and water. The pooled extracts showed distinct color difference. Ethanol extract was bright yellow and transparent, water extract was brown and turbid, while acetone extract had bright green color with a slight turbidity. All extracts were dried through vacuum distillation and the result is shown in table 1 below.

Antioxidant Activity

Antioxidant activities of the crude extracts were measured using DPPH radical-scavenging assay. Recorded absorbance of the samples were converted into % inhibition and compared to each other (Figure 1), and it was shown that acetone extract showed the highest inhibition towards active radicals compared to other crude extract with the same concentration. The weakest antioxidant activity was shown by water extract. This analysis was also supported by two-way ANOVA which indicated significant difference between the % inhibitions of the crude extracts.

Table 1. Yield of Extraction

Extract	Yield (g)	% Yield
Acetone	5,301	5,30
Ethanol	5,211	5,21
Water	6,990	6,99

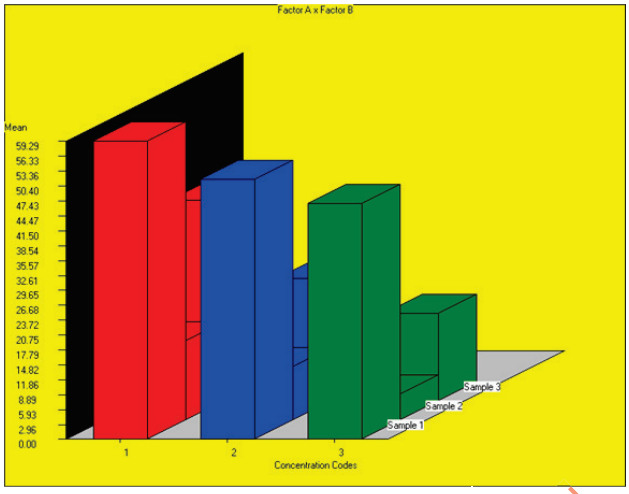

Figure 1. Antioxidant Activity of Matoa Fruit Peel Crude Extracts

To measure the strength of antioxidant agents in the crude extracts, comparison to L-ascorbic acid was done. Identical procedure was executed towards L-ascorbic acid and instead of determining %inhibition, IC_{50} values were calculated (Table 2).

At concentration of 6.22 ppm, L-ascorbic acid is able to eliminate 50% active radicals in the sample. Acetone crude extract is able to eliminate the same amount of active radicals in the sample at concentration 15.323 ppm, or only twice as the amount of L-ascorbic acid. This indicated that the antioxidant agents in acetone crude extract possess half the strength of the antioxidant activity of L-ascorbic acid.

Antimicrobial Activity

The antimicrobial activity of matoa fruit peel extract was tested towards gram positive (*Staphyloccocus aureus* and *Bacillus cereus*) and gram negative bacteria (*Escherichia coli*).Two methods were used: disc diffusion and broth dilution method. From disc diffusion method the zone of inhibition was measured. The formation of inhibition zone indicated that the extract was able to inhibit the growth of microorganism. The larger the inhibition zone, the higher the sensitivity of the microorganism against the extract.

The broth dilution method observed the amount of microorganism present after the culture had been added with various concentration of extract. The number of microorganism was known through its absorbance. The culture used in broth dilution method was standardized according to the turbidity of 0.5 McFarland standard. Culture having turbidity similar to 0.5 McFarland standard should have absorbance between 0.08-0.13 at 625 wavelength. A culture in this range should have concentration about 10^8 CFU/ml.

By doing both of these methods, the characteristic of the antimicrobial agents in the crude extracts were able to be observed. In broth dilution method, number of microorganisms exist in the tube added with extract increased, however, it was still lower than the control tube, which indicated the normal growth of microorganism after overnight incubation. This means that the antimicrobial agents in the crude extracts only inhibited the growth (bacteriostatic), instead of killing them (bactericidal).

This behavior was observed both against gram positive and gram negative microorganisms at extract concentration ranging from 0.5 to 500 ppm. Considering this characteristics, value of NIC (non-inhibitory concentration) was determined instead of MIC (minimum inhibitory concentration). NIC is the minimum concentration below which the growth of microorganism would no longer be slowed down by antimicrobial agent.

Table 3 summed up the NIC values for all crude extracts and both grams. It is shown that the strongest antimicrobial activities were exhibited by acetone and ethanol crude extracts at NIC <0.5 ppm for both gram positive and gram negative microorganism. On the other hand, water crude extract was the weakest among the others with NIC at 5 ppm.

Table 2. IC50 Values

Sample	IC50 (ppm)
L-ascorbic acid	6.220
Acetone extract	15.323
Ethanol extract	143.13
Distilled water extract	451.306

Table 3. Antimicrobial Activities of Matoa Fruit Peel Crude Extracts

Sample	Non-Inhibitory Concentration (ppm)		
	Gram Positive		Gram Negative
	S.aureus	*B. cereus*	*E. Coli*
Acetone Crude Extract	<0.5	<0.5	<0.5
Ethanol Crude Extract	<0.5	<0.5	<0.5
Water Crude Extract	5	5	5

Total Phenolic Content

Measurement of total phenolic content in matoa fruit peel crude extracts used the Folin-Ciocalteau method. Absorbance produced by the samples were extrapolated to gallic acid standard curve in order to determine the concentration of phenolic content.

Result shown in table 4 stated that the highest total phenolic content was found in the acetone crude extract, came second was the ethanol crude extract, and the lowest was water crude extract.The result showed positive correlation with the total phenolic content of the extract. As shown in Table 2, the order of IC_{50} values from the extract, starting from the strongest, was acetone, ethanol and water extract.

This result also confirms with other research which stated that the amount of phenolic content reflected its antioxidant activity.Research on the antioxidant activity of basil (*Ocinum basilicum* L.) showed linear positive relationship between the antioxidant activity and the total phenolic content[6]. A study on the antioxidant activity of turmeric leaf, pandan leaf and torch ginger flower also showed positive correlation between total phenolic content and antioxidant activity [7].

Tannin Test

Tannins were known to exist in the tree bark of *Pometia pinnata* and kaempferol was found in the leaves[3]. Other fruits sharing the same family such as rambutan and lychee were also confirmed to contain tannins. Two types of ellagitannin, geraniin and corilagin were found in the peels of rambutan [8]. Peels of lychee contains epicathecin, procyanidin and high quantity of condensed tannin[9]. Referring to the previous studies mentioned abovve, it was assumed that matoa fruit peels also contains tannins.

The tannin content of matoa fruit peels was determined using Folin-Denis method. Standard curve for detemination of tannin content was made using tannic acid of known concentration. Various concentration of tannic acid was made and their absorbances were measured at 760 nm. A standard curve was made, giving the linear equation y=0.0187x + 0.0402 where x is the concentration of tannic acid and y is the absorbance. In the tannin content analysis, the dried matoa fruit peel was used as sample. Absorbances of the sample was measured and the concentration of tannin was calculated using the equation. From the analysis, the tannin content measured from matoa fruit peels was 7.864±0.2372%.

It is possible that tannin was the biggest contributor in the antioxidant activity of acetone extract. A research on polyphenols and tannins from burs of Castanea mollissima Blume showed that the antioxidant activity and the total tannin content in extract had positive linear correlation [10].

Saponin Test

Two saponins were previously discovered from *Pometia pinnata*, one triterpenoid saponin from the stem bark and one monodesmosidic triterpenoid saponin from the leaves [2,3]. The presence of saponin in the matoa fruit peels was confirmed qualitatively by performing saponin test. Dried matoa peels and hot water were mixed with ration 1:10 and shaked vigorously. The presence of saponin was known by the formation of stable foam [11].

Analysis of Components

Isolate obtained from column chromatography was analysed by some spectral methods, i.e. FTIR and Nuclear Magnetic Resonance (NMR). The IR spectra of is shown by Figure 2 below. Broad peak at 3381cm^{-1} indicated the presence of -OH functional group. Signals at 2964 cm^{-1} and 2922 cm^{-1} represented –CH stretching of alkanes. The CH stretching of alkenes was assumed to be the slight bending at the region between 3203 cm^{-1} and 2954 cm^{-1}.

The appearance of carbonyl peak was assumed in the IR spectra of crude extract (Figure 20), and the stretching appeared in the IR spectra of Isolate 1 with one sharp peak at 1753 cm^{-1}. Signal from a sp^2-C-O appeared

Table 4. Total Phenolic Content of Crude Extract

Solvent for Extraction	Total Phenolic Content (mg/l) ± sd
Acetone	714.851 ± 5,6
Ethanol	246.040 ± 4,2
Water	207.921 ± 1,4

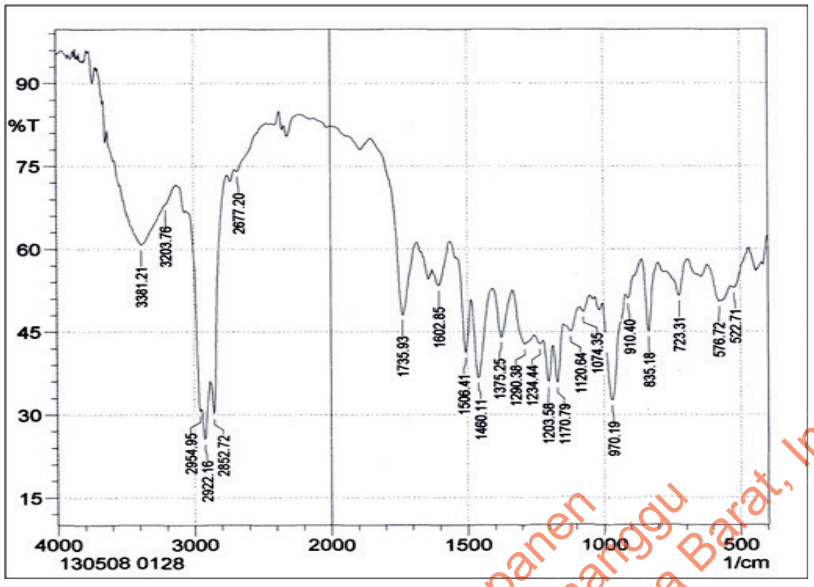

Figure 2. IR Spectra of Isolate

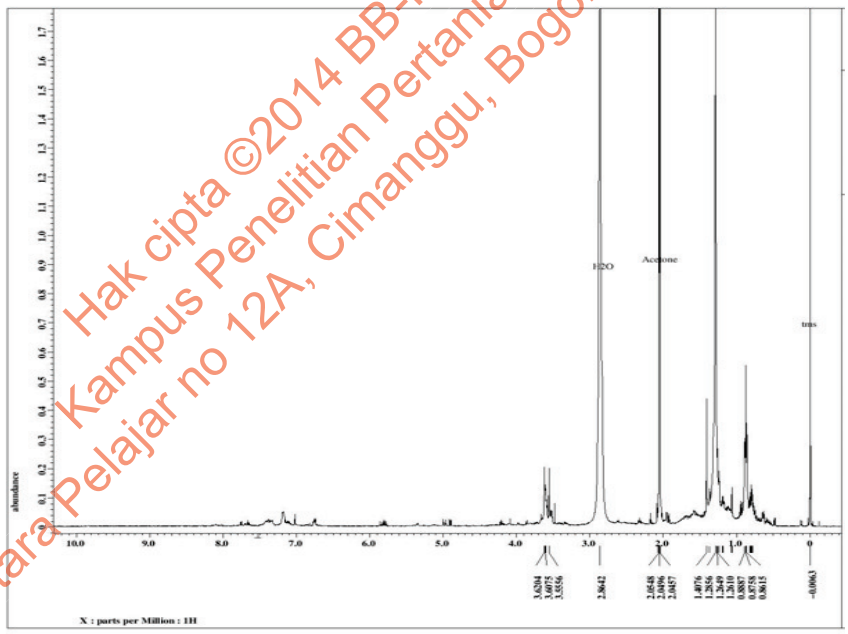

Figure 3. NMR Spectra of Isolate 1

at 1203 cm^{-1} and from =C-O at 1074 cm^{-1}. Combination of these three peaks confirmed the presence of ester. A signal for ether functional group appeared at 1170 cm^{-1}.

NMR analysis was also performed on Isolate 1 at 500 MHz using deuterated acetone as solvent. The result is shown in Figure 22 below.Peak at δ 2.0 ppm was the peak of the solvent used for NMR (deuterated acetone). Water still existed in Isolate 1 as a signal peak from water appeared at δ 2.8 ppm. Strong signals appeared at region δ 0.8-1.4 ppm, which is the region for the protons of alkane. Moderate intensity of signals at region δ 3.5 ppm and 3.6 ppm suggested the presence of alcohol ($R-CH_2-OH$) and/or ether ($R-CH_2-OR$). Signals in this region can also belong to amine groups. Signal from carbon protons bound to ester group was observed at δ 4.1-4.7 ppm ($R-COO-CH_2-R$). Evidence of ester group in the isolate was also supported by the fact that no signals appeared at δ > 9.5 ppm, area of which is typical for the presence of aldehydes and/or carboxylic acid. Alkene protons ($R_2C=CH_2$ and $R_2C=CHR$) were detected by the appearance of signals at 4.6-5.0 ppm. Small signals at δ 7.0-8.0 ppm came from the protons of aromatic rings.

CONCLUSIONS

Matoa fruit peels extract were proven to exhibit antioxidant activity with positive correlation to its total phenolic content. Strongest antioxidant activity was performed by acetone crude extract at IC_{50} 15,323 ppm, which was half the strength of L-ascorbic acid's antioxidant activity at 6,220 ppm.

Antimicrobial activity with bacteriostatic character was observed from matoa fruit peels extract. Antimicrobial test was performed towards both gram positive and gram negative microorganisms, revealing equal strength of acetone and ethanol crude extract. Whilst water crude extract showed the weakest antimicrobial performance.

Analysis towards the components in matoa fruit peels showed the presence of tannins, saponins and alkaloids. Their exact types and structures are not yet to be discovered and should be investigated thoroughly.

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APPENDIX

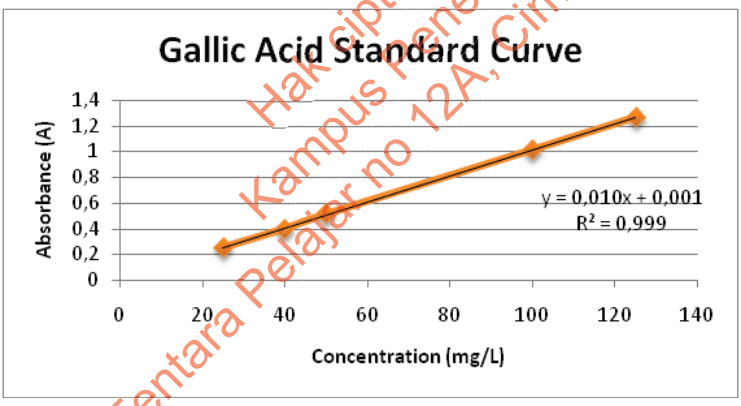
Data Result of Antioxidant Activities

Absorption of Control (0.02 mM DPPH) = 1.044

Sample	Concentration	Absorption	% Inhibition	Mean	Standard Deviation
Acetone Extract	100 ppm	0.408	60.920	59.291±2.303	2.303
		0.442	57.663		
	20 ppm	0.510	51.149	51.268±0.677	0.677
		0.500	52.107		
	10 ppm	0.563	46.073	46.839±1.084	1.084
		0.547	47.605		
Water Extract	100 ppm	0.881	15.613	15.661±0.068	0.068
		0.88	15.709		
	20 ppm	0.935	10.441	10.489±0.068	0.068
		0.934	10.536		
	10 ppm	0.992	4.981	5.125±0.203	0.203
		0.989	5.268		
Ethanol Extract	100 ppm	0.631	39.559	39.847±0.406	0.406
		0.625	40.134		
	20 ppm	0.789	24.425	24.186±0.339	0.339
		0.794	23.946		
	10 ppm	0.862	17.433	14.751±0.203	0.203
		0.865	17.146		

Gallic Acid Standard Curve and Total Phenolic Content Calculation

Gallic Acid Standard Calibration Curve



Total Phenolic Content Data of Matoa Fruit Peel Extract

Data Result of Total Phenolic Content of Matoa Fruit Peel Crude Extract

Solvent for Extraction	Absorbance (A)	Total Phenolic Content (mg/L)	Average	Standard Deviation
Acetone	0.719	710.891	714.851	5.601
	0.727	718.812		
Water	0.214	210.891	207.921	4.201
	0.208	204.950		
Ethanol	0.274	270.297	269.307	1.400
	0.272	268.317		

Total Phenolic Content (mg/L)= (Absorbance (A))/(m (A.L/mg))×dilution factor

y= 0.0101x + 0.001
Absorbance = 0.719
Total Phenolic Content = (0.719-0.001)/0.0101×10= 710.891
Dilution factor = 10

Tannic Acid Standard Calibration Curve
Data Result of Tannin Content from Matoa Fruit Peels

Sample Weight (g)	Dilution (times)	Absorbance (A)	Mean of Absorbance (A)	Tannin Content (g/mL)	% Tannin	Mean	
0.1044	10	0.372	0.384	1.614×10-5	7.730	8.031	7.864 ± 0.2372
		0.395					
	4	0.876	0.844	4.35×10-5	8.330		
		0.842					
0.1035	10	0.357	0.356	1.458×10-5	7.050	7.696	
		0.354					
	4	0.842	0.838	4.319×10-5	8.350		
		0.834					