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Research paper

Determination of Phenolic Content, Ascorbic Acid, Antioxidant Activity and Antimicrobial Activity of Selected Fruit Waste

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Abstract

The aim of this study was to determine the total phenolic content (TPC), antioxidant activity, ascorbic acid content and antimicrobial activity of extracts obtained from cocoa pod husk, banana peel and pineapple peel. Banana peel had significantly highest total phenolic content (154.50 mg GAE/g) followed by pineapple peel (140.37 mg GAE/g) and cocoa pod husk (114.08 mg GAE/g). Antioxidant activity of these samples measured using DPPH assays. Banana peel showed significantly higher DPPH scavenging activity (95.74%) compared to pineapple peel (84.96%) and cocoa pod husk (68.33%). Pineapple peel resulted in significantly higher (44.19 ppm) ascorbic acid as measured using High performance liquid chromatography (HPLC) method compared to banana peel (28.56 ppm). Cocoa pod husk, banana peel and pineapple peel were observed for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *penicillium*. Samples extract at different concentrations in *E. coli*, *S.aureus* and *penicillium*-seeded Mueller-Hinton agar medium, resulted zone of inhibition after 24 h incubation in 37°C for bacteria and 72 h incubation in 28ºC. Banana peel at 20 and 25mg/ml against *S.aureus* resulted in zone of inhibition 9.67, 11.67 mm and cocoa pod husk with 8.00, 9.67 mm respectively. Cocoa pod husk at 15, 20 and 25mg/ml against *E.coli* resulted in zone of inhibition 7.33, 9.33 and 10.33 mm and banana peel with 6.67, 7.33 and 7.67 mm respectively. Pineapple peel does not showed any inhibition zone against tested bacteria and fungi.

Keywords: *antioxidant; banana peel; cocoa pod husk; pineapple peel; phenolics; antimicrobial*

1. Introduction

Food industry uses fruits as one of the raw material for food production where the main wastes of the production are the peel and the seed of the fruit. Utilization of waste from fruits has been increased and also important as it could be utilized for further industrial purposes such as extraction of functional ingredients. Functional properties of some peel components such as pectin, antioxidants, flavonoids, carotenoids and other bioactive compounds can be valorised [1]. Waste material from fruits may be in form of leaves, seed, peels and pulp. About 25 to 30% of non-edible product are yield by fruits and vegetables. These waste material can be successfully used as source of phytochemical and antioxidant [2]. Peel and seed of some fruits have the potential to be utilized as a resource of bioactive compounds such as natural antioxidants due to high content of phenolic compounds [3]. Antimicrobial play an important role in fruits that provide protection of the fruits against pathogenic microorganism [2].

Cocoa (*Theobroma cocoa* L.) pod husk comes from cocoa industry. It is thrown away after the seed is removed. Every ton of dry cocoa bean produced, 10 tons of CPH are generated which can cause serious problems in waste management [4]. Phenolic content of cocoa gives good effect towards ageing, oxidative stress, atherosclerosis and blood pressure regulation [5]. Utilisation of by-products from banana plant can reduce environmental issue [6]. Banana peels is part of the banana fruit that has ability to serve as antibacterial agent against microorganism but it is not widely studied and contain bioactive compound such as flavonoids, tannins and terpenoids [7]. Pineapple (*Ananas comosus* L.) by-product consist of residual pulp, peel, stem and leaves that are generated from the processing of pineapple [8]. Pineapple peel represent about 10% (w/w) of the weight of the original fruit [9].

Phenolic compounds can exhibit wide range of physiological properties and occurs mainly in plant foods i.e. anti-allergenic, anti-inflammantory, anti-microbial as well as antioxidant [10]. Antioxidant has ability to inhibit or delay oxidation process [11]. Synthetic antioxidants have shown toxic effect that can cause liver damage and also mutagenesis [10]. Antimicrobial are agent that are able to inhibit growth of microorganism especially pathogenic microorganisms. Food poisoning is related to the bacteria contamination on foods [7]. For example, gram negative bacteria are the most common bacteria that cause food poisoning. Gram negative bacteria that contribute to food poisoning includes *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*. On the other hand, gram positive bacteria also can cause food poisoning which includes *Staphylococcus aureus* and *Bacillus* spp. Thus, the aim of this study was to determine the total phenolic content (TPC), antioxidant activity using DPPH assay, ascorbic acid content, chlorogenic acid content, and antimicrobial activity of extracts obtained from cocoa pod husk, banana peel and pineapple peel.

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2. Materials and methods

2.1. Preparations of samples

The cocoa waste sample (cocoa pod husk) was collected from Lembaga Koko Malaysia (Jengka, Pahang) and banana and pineapple peel was collected from local market Seksyen 6, Shah Alam. Fruit waste samples (cocoa pod husk, banana peel and pineapple peel) was dried by using cabinet dryer (60 ºC, 24 hours) then grind to fine powder. Ground samples was extracted with 70% aqueous ethanol for 2 hours at 50 ºC using an orbital shaker [5]. Mixture then was filtered through filter paper (Whatman No 1) using a funnel. The filtrate was stored in freezer (-20 ºC) for further used.

2.2. Total phenolic content

The total phenolic content of the fruit waste was determined by the Folin-Ciocalteu method [12]. 200µL of the extracts was mixed with 1.5mL Folin-Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 minutes. 1.5mL of sodium bicarbonate solution (0.566M) was added. After 90 minutes, the absorbance was measured by using spectrophotometer at 725nm. Results expressed as gallic acid equivalents.

2.3. Ascorbic acid and chlorogenic acid content

The sample extracts prepared in Section 2.1 was further subjected to rotavapouriser to remover remaining ethanol solvent and used for ascorbic acid and chlorogenic acid analysis. The analysis was carried-out using a combination of chromatographic separation Agilent HPLC 1200 series as described previously [13]. The chromatographic separation was performed using GL Sciences - Inertsustain Column C-18 (250 mm × 4.6 mm, i.d., 5 mm) and temperature set at 40ºC for both left and right side. The liquid chromatography (LC) parameters such as injection volume was set for 5ul at auto sampler (G1367D), binary pump was set till 45.01 min with post time 4.99 min, flow rate 0.6 ml/min, minimum pressure 1 bar and maximum pressure 400 bar, max flow gradient 100 ml/min. Two set of solvents used known as solvent A and B. Solvent A and solvent B consist of H_2O: MeOH (8:2) with 0.1% formic acid. Combination of both solvent in LC system was set at a ratio of solvent A: solvent B, 95:5 with gradient elution: from 5% solvent B at 0 min, 55% solvent B at 30 min to 100% solvent B at 40 min. The eluent was monitored with a diode array detector at 254 and 280 nm, respectively, for ascorbic acid and chlorogenic acid. Ascorbic acid and chlorogenic acid was used as standard.

2.4. Antioxidant activity using DPPH radical scavenging assay

Antioxidant activity was determined by using method described previously [14]. Different dilutions of the fruit waste extract was prepared (50, 100, 150, 200, 250 µg/mL). DPPH solution was also prepared by dissolving 6.0 mg of DPPH in 100 mL methanol. Then, 1 mL of extract from each dilution was added into the test tube containing 2 mL of DPPH solution. Control was prepared by adding 1 mL of ethanol to 2 mL of DPPH solution. Gallic acid were used as standards. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured using spectrophotometer at 517 nm.

2.5. Antimicrobial activity

Antimicrobial activity of the samples was tested by using disc diffusion method. After incubation, zone of inhibition was identified from circular transparent area that is free from bacteria colonies. The test was carried out using cocoa pod husk, banana peel and pineapple peel extracts at concentration of 15, 20 and 25 mg/mL.

2.5.1 Minimum inhibition concentration (MIC)

MIC is the lowest sample concentration capable of inhibiting any visible growth of the germ. It measures a bacteriostatic effect and does not provide information on the status of the bacterial population. MIC was measured by the method described [15] using 96-well microplates according to the following steps: 200 µL of cocoa waste, banana and pineapple peel extract was deposited in columns 3; a dilution series of factor 2 was carried out by taking 100 µL of column No. 3 and adding them in column No. 4 and so on to column No. 12. The last 100 µL of the wells of column No. 12 were discarded. Then 20 µL of the microbial suspension were deposited in the various wells to which 10 µL of resazurin (growth indicator which is initially blue and turns pink in case of cell growth) was added. In column 1 an antibiotic (ampicilin) was used (positive control). The test was repeated three times. The broth dilution from MIC test that showed clear turbidity was chosen for MBC test.

2.5.2 Minimum bactericidal concentration (MBC)

Minimum bactericidal concentration (MBC) is the minimum extract concentration that caused bacterial elimination. The clear broth dilution from MIC was sub-cultured onto Muller Hilton (MH) agar using streak plate method and incubated for 24 hours at 37 ˚C. After the incubation, the MH agar plate was observed for any growth of bacteria. The selected concentration of ethanolic extract of fruit waste used for MBC is said to be bactericidal if the MH agar plate shows no growth of organism after incubation.

2.6. Statistical analysis

All data was expressed as mean ± standard deviation. All statistical analysis was conducted by using SPSS 16.0 (Statistical Programme for Scientific Students) for windows. Tukey's multiple-range test was used to access the differences between means. A significant difference was considered at the level of $p < 0.05$.

3. Results and discussion

3.1. Total phenolic content, antioxidant, ascorbic acid and chlorogenic acid in selected fruit waste

The results shown that banana peel has the highest total phenolic content with 154.50 ± 1.04 mg GAE/g dried weight as indicated in Table 1. Comparing the results obtained for ethanolic extract of banana peel [16], total phenolic content obtained was lower with 15.21 ± 0.09 mg GAE/g dry weight. Total phenolic content of pineapple peel obtained was 140.37 ± 1.10 mg GAE/g. TPC recorded for pineapple peel was slightly lower than banana peel. Study conducted by [17] found that the TPC of pineapple peel was 7.89 mg GAE/g or dry weight and 148.91 mg GAE/100 g of fresh weight. For cocoa pod husk, the total phenolic content obtained was 114.08 ± 2.33 mg GAE/g. The TPC of cocoa pod husk was very lower than banana and pineapple peel. Highest TPC of cocoa pod husk obtained was 49.54 ± 3.69 mg GAE/g [18].

Table 1: Total phenolic content, ascorbic acid and chlorogenic acid of selected fruit waste

Sample	Total phenolic content (mg GAE/g)	Ascorbic acid (ppm)	Chlorogenic acid (ppm)
Banana peel	154.50 ± 1.04^{a}	28.56 ± 8.59^{b}	0
Pineapple peel	140.37 ± 1.10^{b}	44.19 ± 4.93^{a}	2.72^{a}
Cocoa pod	114.08 ± 2.33^{c}	29.25 ± 4.18^{ab}	3.73^{a}

husk

As for antioxidant activity measured using DPPH assay, scavenging activity increased as the concentration of extracts increased. At concentration 250 ppm, banana peel extract showed highest scavenging activity with 95.74%. [16] found that the scavenging activity of banana peel extract at 50 µg/mL was 40.45%. For pineapple peel, at highest concentration 250 ppm, it showed significantly lower scavenging activity than banana peel with 84.96%. Cocoa pod husk showed lowest scavenging activity even at highest concentration with 68.33%. Highest scavenging activity of cocoa pod husk was 79.6% [18]. One of the reasons for differences of antioxidant activity maybe because of variation of phenolic content in the waste extracts.

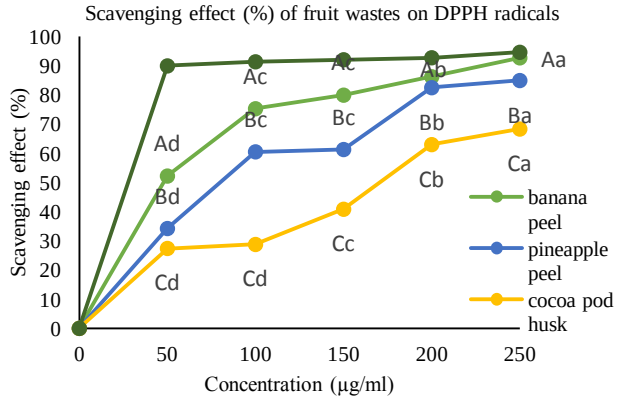

Fig 1: Scavenging effect of Cocoa pod husk, banana peel and pineapple peel.

Meanwhile, ascorbic acid and chlorogenic acid were determined by HPLC. Peak obtained were identified was by comparing its retention time. To conduct quantification of each compound obtained, calibration curve was constructed. Pineapple peel was found to have highest concentration of ascorbic acid (44.19 ppm) as shown in Table 1. Lower amount of ascorbic acids were detected in the banana peel (28.56 ppm) and cocoa pod husk (29.25 ppm). Previous research found that ascorbic acid in pineapple stem (0.73 mg/100mL) were higher compared to its core (0.33 mg/100 mL) after subjected to 380 W microwave drying [20].
Chlorogenic acid (CGA) is an important biologically active dietary polyphenol that is produced by certain plant species. Small amount of chlorogenic acid was identified in the cocoa pod husk and pineapple peel. However, the amount of CGA detected was not significant.
Effective concentration (EC_{50}) was determined for banana peel, pineapple peel and also for cocoa pod husk extracts. Table 2 shows the summary of the effective concentration of the samples. The results showed that banana peel has the lowest EC_{50} compared to pineapple peel and cocoa pod husk. Banana peel extract have powerful antioxidant activity compared to pineapple peel and cocoa pod husk. The strong antioxidant properties of banana peels could be an attributed to the presence of different antioxidant components. It is good to mention that a lower EC_{50} value represents more potent free radical inhibitory activity.

Table 2: Effective concentration

Sample	EC_{50} (µg/ml)
Banana peel	49
Pineapple peel	80
Cocoa pod husk	170

3.2. Antimicrobial activity

Insignificant differences were detected for antimicrobial properties of banana peel and cocoa pod husk against *Staphylococcus aureus* at concentration 25 mg/mL and 20 mg/mL as indicated in Table 3. Cocoa pod husk also showed good antimicrobial properties against *S. aureus* with 9.67 mm at 25mg/ml and 8.00 mm at lower concentration 20mg/ml.

Table 3: Antimicrobial activity against *Staphylococcus aureus*

Sample	Inhibition zone (mm)				
	15 mg/ml	20 mg/ml	25 mg/ml	Positive	Negative
Banana peel	NIZ	9.67 ± 0.58^{ab}	11.67 ± 1.53^{a}	35	NIZ
Pineapple peel	NIZ	NIZ	NIZ	35	NIZ
Cocoa pod husk	NIZ	8.00 ± 1.73^{b}	9.67 ± 0.58^{ab}	35	NIZ

Banana peel showed lower inhibition zone against *E. coli* at high concentration (25 mg/ml) with 7.67 mm compared to cocoa pod husk (10.33 mm) as shown in Table 4. Pineapple peel does not showed any inhibition zone against both tested bacteria.

Table 4: Antimicrobial activity against *Escherichia coli*

Sample	Inhibition zone (mm)				
	15mg/ml	20mg/ml	25mg/ml	Positive	Negative
Banana peel	6.67 ± 0.58^{c}	7.33 ± 0.58^{bc}	7.67 ± 0.58^{bc}	17.5	NIZ
Pineapple peel	NIZ	NIZ	NIZ	17.5	NIZ
Cocoa pod husk	7.33 ± 0.58^{bc}	9.33 ± 0.58^{ab}	10.33 ± 1.53^{a}	17.5	NIZ

Antimicrobial activity of the extracts also was tested against fungi (*Penicillium*). Positive control used was antibiotic known as nystatin. Nystatin showed great inhibition zone with 10.2 mm diameter. The result showed that cocoa pod husk, banana peel and pineapple peel does not show any inhibition (Table 5). However, methanolic extract of banana leaves showed stronger antifungal properties as compared to nystatin [19].

Table 5: Antimicrobial activity against *Penicillium*

Sample	Inhibition zone (mm)				
	15mg/ml	20mg/ml	25mg/ml	Positive	Negative
Banana peel	NIZ	NIZ	NIZ	10.2	NIZ
Pineapple peel	NIZ	NIZ	NIZ	10.2	NIZ
Cocoa pod husk	NIZ	NIZ	NIZ	10.2	NIZ

3.2.1 Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibition concentration (MIC) refer to the least concentration of antibacterial agent that affects bacteria resistance and supress colonies growth. MIC of the extracts was tested by choosing the lowest concentration of extracts that showed inhibition zone. Table 6 shows that banana peel and cocoa pod husk extracts at 7.5mg/ml shows clear broth indicates the lowest concentration that able to inhibit growth of *E. coli*. Banana peel at 5mg/ml able to inhibit the growth of *S. aureus* and cocoa pod husk at 10mg/ml able to inhibit growth of *S. aureus*.

Table 6: Minimum inhibition concentration of banana peel and cocoa pod husk extract

	Minimum Inhibition Concentration (MIC)	
	Escherichia coli	*Staphylococcus aureus*
Banana peel	7.5mg/ml	5mg/ml
Cocoa pod husk	7.5mg/ml	10mg/ml

Table 7 showed the Minimum bactericidal concentration (MBC). The lowest concentration of extract that yield no growth was rec-

orded as MBC. In other word, MBC is the lowest concentration that able to kill the growth of bacteria. Broth dilution from MIC was subculture onto MHA plate and plate that does not show any bacterial growth indicates as MBC. Banana peel and cocoa pod husk showed potentially bactericidal activity against the tested pathogenic bacteria *E.coli* with MBC 15mg/ml and while MBC against *S.aureus* was 10mg/ml.

Table 7: Minimum bactericidal concentration (MBC) of banana peel and cocoa pod husk extract

	Minimum Bactericidal Concentration (MBC)	
	Escherichia coli	***Staphylococcus aureus***
Banana peel	15mg/ml	10mg/ml
Cocoa pod husk	15mg/ml	10mg/ml

4. Conclusion

In conclusion, banana peel exhibited antioxidant activity with promising amount of total phenolic contents (154.50 mg GAE/g). Banana peels have higher antioxidant activity (92.74% at a concentration of 250µg/mL) in comparison with pineapple peel and cocoa pod husk. Banana peel can be considered as a good antioxidant which can be developed as new food product. Significantly higher ascorbic acid was found in pineapple peel (44.19 ppm) compared to banana peel (28.56 ppm). Banana peel and cocoa pod husk showed excellent antimicrobial activity by inhibiting growth of *Escherichia coli* and *Staphylococcus aureus* in agar diffusion method. Both of banana peel and cocoa pod husk further can be considered as a good antimicrobial material against both gram positive and negative bacteria that can be developed as antimicrobial agent in food preservation.

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