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Antioxidant and Antimicrobial Activities of *Pisang Berangan (Musa paradisiaca)* Pulp and Peel Extracts

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ABSTRACT

Banana is a tropical fruit that has a diverse range of species belongings to the genus *Musa* and *Musaceae* family. *Pisang Berangan* or known as *Musa paradisiaca* is frequently sliced and fried and sold as popular street food in Malaysia. This produced banana waste from the banana peel that was not fully utilized, however, the peel and pulp of bananas are reported to have dominant antioxidant properties and organic materials. The study was carried out to determine the extraction yield, total phenolic content (TPC), and antioxidant activity of banana *Pisang Berangan (Musa paradisiaca*) pulp and peel extracted using different extraction solvents; ethanol and methanol at 70 % and 90 % concentrations. The antimicrobial properties were determined using the disc diffusion method (DDM), Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays. The highest extraction yield was from banana pulp (28.3 %) extracted using 90 % ethanol, followed by methanol 90 % (27.8 %). The total phenolic content (TPC) of alcoholic-extracted pulp and peel was in the range of 15.1 to 17.7 mg GAE/g. Among both pulp and peel extracts, the ethanol (90 % of solvent) extraction exhibited significantly higher antioxidant activity that has been analysed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay to determine the IC_{50}. The largest inhibition zone was observed for the ethanol extract of banana peel against *Salmonella typhi* at a concentration of 500 mg/mL. *Bacillus cereus* and *Staphylococcus aureus* exhibited the highest value of MIC and MBC while *Salmonella typhi* was the lowest of MIC and MBC value against banana pulp and peel alcoholic extracts. In general, the greater properties of pulp and peel extracts were obtained by using 90 % ethanol. The present study revealed that both ethanol and methanol extracts of the *Musa spp.* contain constituents with significant phenolic, antioxidant, and antibacterial properties for food processing explorations and sustainability.

Keywords: *Musa spp*, total phenolic content, antioxidant activity, alcohol extraction, antimicrobial activity

INTRODUCTION

Banana is a tropical climacteric fruit which is cultivated globally throughout tropical and subtropical areas and become the second most produced fruit, after citrus fruits (Acevedo et al., 2021). Global banana production has expanded at a compound annual rate of 3.2 % with an increment from approximately 67 to 114 million metric tonnes within eighteen years (2000 to 2017), as per the recent Food and Agriculture Organization (FAO) statistics. Banana is grown in more than 122 countries around the world such as Australia, Indonesia and Malaysia (Ehiowemwenguan et al., 2014).

Besides the sensorial, textural properties and distinguished flavour, bananas also provide high calories, with a small amount of fat, and are rich in dietary fibre, vitamin C, vitamin B6 and bioactive phytochemicals (Chala & Yetenayet, 2018; Singh et al., 2016). Banana fruit is divided into 2 parts which are pulp and peel. The banana pulp being a consumable portion of the fruit has been used in many food productions due to its high nutrients and healthy benefits. However, the peel contributes about 40 % of the overall mass of a banana a usually discarded as waste, as it serves no purpose. However, banana peels are potentially utilised in medicine, animal nutrition, skin tanning, soap production, and rubber falling (Baskar et al., 2011). Banana peel also is a good source of antioxidants for foods and functional foods against cancer and heart disease because it is an underappreciated source of phenolic chemicals. Gallocatechin and dopamine are the antioxidant chemicals found in the peel of the banana (Baskar et al., 2011).

Extraction techniques play a crucial role in isolating and purifying many bioactive compounds from food materials as these compounds are obtained inside different complex food matrices in small quantities (Sridhar et al., 2021). Solvent extraction is a preferable method because it is often the most efficient way to the separation of valuable bioactive compounds from complex food products. Methanol, ethanol and acetone are commonly used for extracting antioxidants either without the combination of an aqueous solution or in combination with an aqueous solution (Shian et al., 2012). This solvent selection is based on their efficiency in extracting polyphenols. Antioxidant activity of common cultivar of bananas are commonly determined by 2,2 – diphenyl – 1 – picrylhydrazyl (DPPH) stimulant, ferric reducing antioxidant power (FRAP) assays and a complete evaluation of total phenolic content (TPC). The evaluation of total phenolic content (TPC) in the extracted banana peel and pulp samples helps identify the solvents that yield the greatest amount of extractable phenols. Do et al., (2014) reported that acetone is better at extracting greater molecular weight flavanols, whilst methanol is better at extracting low molecular weight polyphenols. Another solvent which is ethanol is also an excellent solvent for polyphenol extraction and is also safer for human use.

Kinetic reactions between radicals and antioxidants in plant materials are influenced by solvent types (Tavasi et al., 2009). A higher yield of phenolic compounds and greater solubility properties occurred in organic solvents (Hameed et al., 2020). Moreover, the polarity of solvents (Veta, et al., 2013) and solid-solvent ratio (Mustapa et al., 2015) lead to variants in amounts of polyphenolics. While the different plant parts also contribute to variations in phenolic contents (Galvan D'Alessandro et al., 2012).

This research aims to study the extraction of *Pisang Berangan (Musa paradisiaca)* pulp and peel using methanol and ethanol at 70 % and 90 % concentrations and the extraction yields, total phenolic content (TPC), antioxidant activity and antimicrobial activities were determined.

MATERIALS AND METHODS

Sample collection and preparation

The *Pisang Berangan* (*Musa spp.*) was bought from local farmers in Besut, Terengganu, Malaysia. The bananas were chosen based on their maturity stage, which were observed by visual and instrumental colour measurement using a colorimeter of banana peels. The bananas were then immediately processed. The banana pulps and peels were cut into 1 cm slices before being dried in a cabinet dryer at 40 °C for 48 hours. The dried pulps and peels were grounded and sieved throughout a mesh of 0.5 mm. The ground powder was stored in amber glass bottles at room temperature until further steps.

Banana Extraction

Thirty-five grams (35 g) of banana pulp and peel powders were dissolved in 350 mL of different extract solutions. The extract solutions used were ethanol-water and methanol-water at concentrations of 70 % and 90 % (*v*/*v*). The powder sample to solvent was set at a 1:10 ratio (Ramaiya et al., 2013). The mixture was stirred vigorously for 60 minutes until the mixture was homogenised and allowed to stand for 48 hours under dark conditions at room temperature. Next, the mixture was re-stirred and filtered using Whatman No.1 filter paper to separate filtrates and sediments. The filtrate was concentrated at 40ºC using a rotary evaporator. The crude extracts then were transferred into clean amber bottles and stored in a freezer at setting -20 ºC until further use (Ehiowemwenguan et al., 2014).

Determination of Total Extraction Yield

The banana extracts were weighed before drying and after filtrate evaporation to determine the weight of crude extract and total extraction yield percentage. Total extraction yield is a measurement of extract mass compared with the original mass of raw material (Samudin et. al., 2022). The percentage of extraction yield was calculated using the following formula:

$$\text{Extraction yields (\%)} = \frac{W1-W2}{W2}\ x\ 100\% \qquad \textbf{Eqn.1}$$

Where; W_1 = Weight of crude extract after solvent evaporation (g)
W_2 = Weight of raw material (g)

Determination of Total Phenolic Content

The total phenolic content (TPC) of the banana peel and pulp extracts was determined using the Folin-Ciocalteu assay (Singleton et. al., 1999). The external calibration was carried out using 7 different concentrations of the gallic acid standard (0.08 to 500 µg/ml) and the linearity for the standard curve was determined (R2 = 0.996). Briefly, 25 µg of banana pulp and peel extracts were mixed with 725 µL distilled water and continued mixed with 125 µL of Folin-Ciocalteaus reagent and 500 µL of 20 % sodium carbonate. The mixtures were allowed to stand for 1.5 hours in a dark place. Then, 100 µL mixture was pipetted to the 96 well-plates and the absorbances were read at 765 nm using a microplate reader. The TPC was calculated as gallic acid equivalent (GAE) in milligrams per gram of dry weight (mg GAE/g DW) by using a standard gallic acid calibration curve prepared before the analysis.

Determination of Antioxidant Activity

The antioxidant activity of banana pulp and peel extracts was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method was used in determining the antioxidant activity. 0.1mM DPPH stock solution was prepared by dissolving 39.4 mg of DPPH in 1 L methanol. Ascorbic acid was used as a standard and diluted in a methanolic solution of DPPH to prepare 7 different concentrations (0 to 300 µg/mL). About 3 mL of sample extracts or standard solution mixed with 1 mL of DPPH solution. An equal amount of methanol in the DPPH solution was used as a blank (Control). The mixtures were incubated in the dark for 30 minutes at room temperature before being transferred for UV/VIS spectrometry reading at a wavelength of 517 nm. DPPH scavenging activity was determined using the equation:

$$\text{DPPH scavenging activity \%} = \frac{A1-A2}{A1}\ x\ 100\% \qquad \textbf{Eqn.2}$$

Where; A_1 = Absorbance of the blank sample
A_2 = Absorbance of the test sample

The results were reported as IC_{50} value, where the IC_{50} is the concentration of the sample extract required to scavenge 50 % of DPPH free radical. The IC_{50} values were expressed as mg of dry matter per 1 mL of DPPH solution (mg/mL).

Antimicrobial Activities Determination

Disc Diffusion Method (DDM)

The determination of antibacterial activity was done using the dis diffusion method (DDM). Four strains were used in the assay; *Bacillus cereus, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*; were inoculated into the sterile nutrient broth and incubated for 24 hours at 37 °C. Wells with 5 mm diameter were made in sterile nutrient agar plate using a sterile cork borer by flame sterilized and inoculum containing 10^7 CFU/ml of test bacteria were spread on solid plates with the aid of sterile swab moisture. Then, about 50µl of banana pulp and peel alcohol extracts (90 % of concentration of solvent) were placed in the wells made in inoculated plates. Then, an antibiotic disc (Ampicillin) was dispensed in the inoculated agar plates. The plates were incubated at 37 °C for 24 hrs. The zones of inhibition around the discs were recorded in millimetres (mm) on the next day (Girish and Satish, 2008).

Minimum Inhibitory Concentration (MIC) Determination

Determination of the Minimum Inhibitory Concentration (MIC) of the extracts was carried out using the tube-dilution technique. A double-fold serial dilution was made using Muller Hinton broth (MHB). The following concentrations were obtained which were 500 mg/ml, 125 mg/ml, 62.5 mg/ml, 32 mg/ml, 16 mg/ml, 8 mg/ml, and 4 mg/ml. About three to five(3-5) single colonies from nutrient agar culture were suspended in MHB. Then an equal volume of extract and MHB (2 ml) was dispensed into sterilized test tubes. A tube containing broth, extract solvent and colonies was labelled as positive growth control meanwhile a tube containing broth and extracts without inoculum was known as negative growth control. All the tubes were incubated overnight at 37° C. The MIC values explain the lowest concentration of the extracts at which microbial growth is completely inhibited (Kowalska-Krochmal & Dudek-Wicher, 2021).

Minimum Bactericidal Concentration (MBC) Determination

One loop of the suspension samples from the previous MIC test that showed no visible growth was transferred to sterile Muller Hinton agar plates. The agar plates were then incubated at 37 °C for 24 hours. The lowest concentration of the extract yielding no growth was recorded as the minimum bactericidal concentration (MBC) (Ehiowemwenguan et al., 2014).

Statistical Analysis

All tests were carried out in triplicate. The statistical analysis for the data was carried out using SPSS version 26 (SPSS Inc, Chicago, Illinois, USA) and was expressed as mean and standard deviation. T-test was used for percentage yield and analysis of variance (ANOVA) were used for TPC and antioxidant analysis to compare any significant difference between samples at $p < 0.05$.

RESULTS AND DISCUSSION

Yield of Extraction

The extraction of banana was conducted with the different ratio of water and extraction solvent (methanol and ethanol) because water have a greater polarity than solvents and these solvents are miscible in water. Table 4.1 show the extraction yield of banana pulp and peel by using 70 % and 90 % of methanol and ethanol. The result showed that the extraction yield for banana pulp was significantly higher with a value ranging from 19.47 % to

28.26 %. Meanwhile, banana peel samples showed lower yields in both extraction solvents compared to banana pulp. This could be attributed to the presence of compounds that are not extractable by the solvent. Banana peel contains about 60 % of carbohydrates, fibre (30 %), water and trace amounts of protein and fat (Franklin-Cheung, 2019). Due to its thickness and high fibre content, the toughness of banana peel results in a lower extraction yield compared to banana pulp. The variations in extraction yields can be attributed to the different polarities of both ethanol (0.65) and methanol (0.76). Ethanol is the most effective extraction solvent because ethanol was able to dissolve the polar and nonpolar compounds at the same time. Thus, the yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time, the composition of the sample, and ratio sample to solvent (Abubakar & Haque, 2020; Do et. al., 2014).

Table 1. The percentage yield of banana *Musa spp.* pulp and peel extracts

Extraction solvent	Concentration of solvent (%)	Percent Yield (%)	
		Pulp	Peel
Methanol	70	19.47±0.02[a]	11.44±0.06[a]
	90	27.83±0.06[c]	11.66±0.01[b]
Ethanol	70	21.42±0.01[b]	16.60±0.07[c]
	90	28.26±0.01[d]	18.21±0.01[d]

Values were given as mean ± SD from triplicate determination (n=3).
Values of different letters in the same column are significantly different at $p<0.05$.

Total Phenolic Content

Table 4.2 shows the TPC value of banana pulp and peel alcoholic extracts. The pulp contained higher TPC than the peel extract in both ethanol and methanol extract of *Musa spp.* In the pulp and peel sample, there was a significant increase of TPC between 70 % with 90 % of methanol and ethanol. As a result, the TPC in the pulp and peel samples extracted from 90% concentration of methanol and ethanol showed the highest ranging from 16.71 to 17.68 mg GAE/g. Meanwhile, 75 % methanol for both pulp and peel samples indicated the lowest TPC with a value ranging from 15.16 to 15.39 mg GAE/g. Hence, 90 % of ethanol is the most effective solvent for the extraction of phenolic compounds from the banana pulp and peel samples.

Table 2. Total phenolic content of pulp and peel extracts of banana *Musa spp.*

Extraction solvent	Concentration of solvent (%)	Total phenolic content (mg GAE/g)	
		Pulp	Peel
Methanol	70	15.16±0.32[a]	15.39±0.48[a]
	90	17.23±0.14[b]	16.71±0.30[b]
Ethanol	70	15.47±0.36[a]	16.35±0.36[b]
	90	16.83±0.39[b]	17.68±0.19[c]

Values were given as mean ± SD from triplicate determination (n=3).
Values of different letters in the same column are significantly different at $p<0.05$.

A previous study reported that polyphenols are often most soluble in organic polar solvents and ethanol is one of the most effective solvents for extracting phenols from plant materials (Azwanida et al., 2015). Hence, the statement above supported the result of this research as this research also concluded that ethanol solvent was the highest in extracting phenolic compounds from banana pulp and peel samples. The total phenolic concentration of fresh and dried samples of banana cultivars of pulps and peels was also influenced by the extraction solvents. It may be established that within each type of extraction, the phenolic compounds of pulps and peels are differed according to their polarity (Sulaiman et al., 2011).

Antioxidant Activity

Table 3 shows the antioxidant activity expressed as the IC_{50} value of banana pulp and peel samples. In the pulp sample, there was a significant increase of antioxidant activity between 75 % with 90 % of methanol and ethanol solvent. In addition, the pulp extracts had significantly higher antioxidant activities than the peel extracts of bananas. For the peel sample, antioxidant activity between 75 % with 90 % of methanol and ethanol solvent was increased significantly. As a result, the antioxidant in the pulp and peel samples extracted from 75 % concentration of methanol showed the highest antioxidant activity as its IC50 value was the lowest with a value of 242.74 mg/L and 245.26 mg/L respectively. Meanwhile, 90 % of ethanol in pulp and peel samples extract resulted in the lowest antioxidant activity with a value of 393.318 mg/L and 371.79 mg/L respectively due to its IC50 being the highest. Hence, the most effective solvent for the extraction of bioactive compounds in antioxidant activity from the banana pulp and peel samples is methanol solvent.

Table 3. Antioxidant activity of pulp and peel extracts of banana *Musa spp.*

Extraction solvent	%	Antioxidant activity IC_{50} DPPH (mg/L)	
		Pulp	Peel
Methanol	70	242.74±3.19[a]	245.26±2.67[a]
	90	254.38±3.30[b]	243.79±5.22[a]
Ethanol	70	385.46±2.84[c]	340.23±2.88[b]
	90	393.18±3.04[c]	371.79±1.69[c]

Antioxidant activity is expressed as an IC_{50} value
Values were given as mean ± SD from triplicate determination (n=3).
Values of different letters in the same column are significantly different at $p<0.05$.

Phenolic compounds are although considered as most important antioxidants of plant materials, no significant relationship could be established between TPC and antioxidant activity in this study. For example, eethanol of pulp and peel showed the highest phenolic content however low antioxidant activity IC_{50}. This suggests that phenolics are not the only contributor to the antioxidant activities found in the various extracts. In the DPPH radical scavenging capacity assay, this radical is conducted to assess the ability of antioxidants to quench the DPPH radical. Due to the delocalization of a spare electron over the molecule, the DPPH radical remains stable, inhibiting dimer formation. When DPPH is reduced to its nonradical form, it loses its dark purple colour. It is a stable organic nitrogen-centred free radical with a dark purple colour that becomes colourless when reduced to its nonradical form by antioxidants. The colour of the reaction mixture changes from purple to yellow as the DPPH radical is scavenged with decreasing absorbance at wavelength 517 nm (Gangwar et al., 2014). The type and polarity of the solvent used to extract antioxidants from banana samples can have an impact on single-electron transfer and hydrogen atom transfer, both of which are important aspects of antioxidant capacity assays (González-Montelongo et al., 2010). The polarity of the solvent has an important effect in enhancing the phenolic content and increasing the antioxidant activity of the samples. The higher polarity of solvents usually can extract more phenolic compounds (Che Sulaiman et al., 2017). In the DPPH

free radical scavenging method, the IC_{50} (Half maximal Inhibitory Concentration) value is the concentration of the sample that can scavenge 50% of the DPPH free radical. As IC_{50} is inversely proportional to the antioxidant activity of the sample the higher IC_{50} value will indicate the lower antioxidant activity and vice versa (Noipa et al., 2011). Moreover, many studies found that banana pulps exhibit many antioxidant properties, including phenolic compounds and vitamins (e.g. catechin, epicatechin, lignin and tannin, anthocyanin, ferulic, sinapic, salicylic, gallic, p-hydroxybenzoic, vanillic, syringic, gentisic, and *p*-coumaric) (Borges et al., 2014; Russell et al., 2009).

Antimicrobial Activities

Due to the highest TPC of banana pulp and peel samples with ethanol solvent (90% concentration of solvent), thus the antimicrobial activities of ethanolic extracts was measured. Table 4 and Table 5 show the antibacterial activities in ethanolic extract of banana pulp and peel extracts, respectively. After 24-hour incubation, at a concentration of 500mg/ml, the highest zone of inhibition resulted against *Salmonella typhi* with a diameter of 40mm followed by *Escherichia coli* (30mm) and *Bacillus cereus* (12mm). Meanwhile, the zone of inhibition against *Staphylococcus aureus* shows the lowest (11mm) at this concentration. Thus, at the low concentration ranging from 4mg/mL to 8mg/mL of extraction, there was no microbial growth indicated by all the isolates. The same trend occurred in peel extracts as the highest zone of inhibition occurred at the highest concentration (500 mg/mL) and gradually decreased against four bacterial isolates.

Table 4. The antibacterial activities of ethanolic extracts of banana *Musa spp.* pulp

Concentration (mg/ml)	Zone inhibition (mm) of bacterial isolates			
	B. cereus	***E. coli***	***S. aureus***	***S. typhi***
500	12	30	11	40
250	5	18	6	31
125	0	13	0	23
62.5	0	9	0	17
32	0	5	0	10
16	0	0	0	5
8	0	0	0	0
4	0	0	0	0

Table 5. The antibacterial activities of ethanolic extracts of banana *Musa spp.* peel

Concentration (mg/ml)	Zone inhibition (mm) of bacterial isolates			
	B. cereus	***E. coli***	***S. aureus***	***S. typhi***
500	8	24	10	45
250	2	18	0	37
125	0	12	0	26
62.5	0	8	0	18
32	0	0	0	10
16	0	0	0	0
8	0	0	0	0
4	0	0	0	0

Table 6 shows the results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Musa spp.* pulp and peel on the test bacteria. The ethanolic extract of the pulp had MIC values ranging from 16mg/ml to 500mg/ml while peel extract ranged from 32mg/ml to 250mg/ml. The lowest MIC in banana pulp was 16mg/ml against *Salmonella typhi* while *Bacillus cereus* and *Staphylococcus aureus* showed the highest MIC of 250mg/ml. The highest MIC in banana peel was obtained against *Staphylococcus aureus* with a value of 500mg/ml while the lowest MIC obtained was 32mg/ml by *Salmonella typhi*. The MBC values of the banana pulp ranged between 32mg/ml to >500mg/ml while the banana peel ranged between 64mg/ml to 500mg/ml. The highest MBC in banana pulp was >500mg/ml against *Bacillus cereus* and *Staphylococcus aureus* while Salmonella typhi showed the lowest MBC of 32mg/ml. The lowest MBC in banana peel was obtained against *Salmonella typhi* with a value of 64mg/ml while the highest MBC obtained was >500mg/ml by *Staphylococcus aureus* and *Bacillus cereus*.

Table 6. The minimum inhibitory and bactericidal concentrations of ethanolic extracts of banana *Musa spp.* peel on bacterial isolates

Isolates	Pulp		Peel	
	MIC*	***MBC****	***MIC****	***MBC****
B. cereus	250	>500	250	>500
E. coli	32	125	62.5	125
S. aureus	250	>500	500	>500
S. typhi	16	32	32	64

MIC = Minimum inhibitory concentration
MBC = Minimum bactericidal concentration
*Unit used for MIC and MBC is mg/mL

MIC and MBC tests were used to examine the antibacterial activities of banana pulp and peel. The MIC is the lowest concentration of antimicrobial agent that inhibits observable microorganism growth under

specified conditions. The antimicrobial activity against bacterial isolates shows low activity as indicated by the small inhibition zone diameter. Due to differences in cell wall composition, Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) demonstrated greater inhibition zones than Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*). Gram-positive bacteria have a thick peptidoglycan layer that consists of linear polysaccharide chains cross-linked by short peptides, resulting in a more rigid structure whereas Gram-negative bacteria have a thinner peptidoglycan layer (Ibrahim, 2015). MBC is the lowest concentration of antimicrobial agent required to prevent microbe growth after culturing onto MHB (Ibrahim, 2015). The susceptibility of microorganisms to antimicrobial agents differs widely. Low activity indicates a high MIC value and vice versa (Ehiowemwenguan et. al., 2014). Thus, extracts from the banana peel and pulp has been demonstrated in this study that they have antibacterial properties and possibly can be used to treat infections caused by *Salmonella typhi, Escherichia coli, Bacillus cereus*, and *Staphylococcus aureus*.

CONCLUSION

The study found that alcohol type and concentration play important roles in the quality of *Pisang berangan* (*Musa paradisiaca)* pulp and peel extractions. The extraction yield of pulp and peel was significantly higher in ethanol at a concentration of 90 % (*v*/*v*) compared to methanol extraction. The TPC concentration in both pulp and peel extracts are in the order of $MetOH_{70}$< $MetOH_{90}$< $EtOH_{70}$< $EtOH_{90}$. Hence, this study recommended ethanol at higher concentrations is suitable to be used as an extraction solvent for banana *Musa spp*. The results of microbial activities showed that *Bacillus cereus* and *Staphylococcus aureus* exhibited the highest value of MIC and MBC while *Salmonella typhi* was the lowest of MIC and MBC value against banana pulp and peel. The findings of this study also highlight the potential of *Musa spp*. as a potential ingredient with a higher source of polyphenols, natural antioxidants and antimicrobial properties.

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