

# DETERMINATION OF BIOACTIVE COMPOUNDS, ANTIMICROBIAL ACTIVITY, AND THE PHYTOCHEMISTRY OF THE ORGANIC BANANA PEEL IN THAILAND

Thanyarat NAKSING<sup>1</sup> , Jantima TEEKA<sup>2</sup> , Wutti RATTANAVICHAI<sup>3</sup> ,  
Prapaporn PONGTHAI<sup>2</sup> , Dolnapa KAEWPA<sup>2</sup> , Atsadawut AREESIRISUK<sup>2</sup> 

<sup>1</sup> Postgraduate program in Applied Biology, Division of Biology, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Khlong Luang, Pathum Thani, Thailand.

<sup>2</sup> Division of Biology, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Khlong Luang, Pathum Thani, Thailand.

<sup>3</sup> Department of Fisheries Technology, Faculty of Agricultural Technology, Kalasin University, Mueang Kalasin, Kalasin, Thailand.

## Corresponding author:

Atsadawut Areesirisuk

Email: atsadawut\_a@rmutt.ac.th

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## Abstract

Banana fruit is enriched with phytonutrients, minerals, and its peel, which is mostly discarded as waste. This research aimed to study its bioactive compound properties, antimicrobial activity, and identify and characterize the constituents of organic banana peel extract (BPE), composed of six species (i.e., Kluai Homthong, Kluai Namwa, Kluai Kai, Kluai Hukmook, Kluai Lebmuernang, and Kluai Homtaiwan). Total phenolic content (TPC), antioxidant content, and ferric-reducing antioxidant power (FRAP) were important in BPE of Kluai Kai. BPE of Kluai Hukmook could inhibit *Aeromonas hydrophila* and *Staphylococcus aureus*. The Fourier-transform infrared spectroscopy (FTIR) spectrum exposed diverse compounds of primary and secondary phytochemicals. Four main constituents, including acetic acid, formic acid, 1,2-benzenediol, 3-methyl-, and 4-hydroxy-2-methylacetophone derived from gas chromatography-mass spectroscopy (GC-MS), demonstrated their antioxidant properties and antimicrobial activity. This result suggests that organic banana peel can both be applied as an antioxidant and antimicrobial substance. BPE increases the value of banana peels (BPs) and reduces the burden of its waste disposal in the environment.

**Keywords:** Antioxidant. Antimicrobial Activity. Banana Peel Extract. Gas Chromatography-Mass Spectrometry (GC-MS). Phytochemical Components.

## 1. Introduction

Presently, the natural compound from herbs and plants are widely used as growth-stimulating compounds, antimicrobial substances, and nutrients (Sulaiman et al. 2011). They are enriched in bioactive compounds that include fiber and phenolic compounds, which are related to antioxidant capacity. They are better than synthetic antibiotics in treating infectious disease, as they limit many of the adverse effects. Moreover, the plant extract is used as an additive in animal feed, which may provide an inexpensive agent in aquaculture, promoting survival rate, stimulating immunity, and offering greater reliability than chemotherapeutic agents. However, the side-effects and consumed doses of plant extract should be considered before utilization.

Banana, belonging to the family Musaceae, is a very prevalent fruit around the world, and is eaten in many regions as a staple food (Singh et al. 2016). It is grown extensively and comprises the fifth largest agricultural food crop in terms of world trade, ranking alongside rice, wheat, and maize. It is a rich source of

significant phytonutrients, including vitamins and phenolic compounds. It is also notably enriched with minerals such as sodium phosphorus, magnesium, potassium, calcium, iron, copper, zinc, and manganese. It contains many bioactive components, such as phenolic, carotenoids, biogenic amines, and phytosterols, which are highly beneficial in the diet, as they have positive effects on human health and well-being (Singh et al. 2016). In the past, banana was effectively used in the treatment of various diseases, such as reducing the risk of chronic degenerative disorders. Phenolic compounds, secondary metabolites in banana, are known for their antioxidant properties in protecting the body against various oxidative stress (Amri and Hossain 2018). Total phenolic aspects of a banana are reported to be more abundant in the peel, consistent with its antioxidant activity. The banana peel (BP) is the major by-product obtained during banana processing. BP is a good source of polyphenol, carotenoids, and other bioactive compounds which process its beneficial effects on human health. Different components have antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. BP contains high levels of phytochemical compounds, mainly antioxidants. Phenolic compounds in BP (*Musa acuminata* Colla AAA) range from 0.90 to 3.0 g/100 g dry weight (Singh et al. 2016). The antioxidant capacity of banana is increased during flesh maturity, with a higher capacity than some berries, herbs, and vegetables (Ummarat et al. 2011). In the banana processing industry, significant quantities of BP, equivalent to 40% of the total weight of a fresh banana, are generated as waste up to 0.3 million tons (MT) per year, posing a disposal problem (Housagul et al. 2014).

In the present study, BP varieties were extracted with 50% methanol. Bioactive compounds of BPE were analyzed by the DPPH and FRAP methods. *Aeromonas hydrophila*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* were used as indicators for antimicrobial activity determination. Phytochemicals contained in BPE were examined by Fourier-transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS).

## 2. Material and Methods

### Location of organic banana farm and harvesting

The organic banana was cultured with a process as well as farming at Funkajorn Garden Organic Farm, Nong Suea, Pathum Thani Province, Thailand, at GPS: 14°10'12.6"N 100°48'00.4"E.

### Preparation of banana peel

The six most common banana strains found in Thailand include: Kluai Kai, Kluai Homthong, Kluai Homtaiwan, Kluai Lebmuernang, Kluai Hukmook, and Kluai Namwa, were collected in the raw stage. The raw banana was matured in a plastic box until the peel color index (PCI) reached a level of 7, yellow with brown speckles (Soltani et al. 2010). Matured banana fruits were cleaned with tap water, rinsed twice to remove dust, and other extraneous materials adhering to them, and soaked in soda water for 5 min (Rattanavichai and Cheng 2014). The washed BP was separated, cut into small pieces, and dried in hot-air oven at 50°C until moisture content decreased to approximately 10%. The dried BP was ground into a powder with an electronic grinder and stored at 4°C for further experiments.

### Extraction of banana peel

Ten grams of dried BP powder was added to 100 mL of 50%v/v methanol. The mixture was extracted in a water bath at 55°C for 120 min and continuously stirred. The mixture was left and precipitated at room temperature. The sample was centrifuged to collect the supernatant at 5,000 rpm for 20 min. The methanol portion in obtained supernatant was removed with a vacuum rotary evaporator until completely evaporated. The sticky crude extracted was collected from the extraction bottle by redissolved with H<sub>2</sub>O and kept at -20°C for the next experiments.

### Bioactive compound properties assay

The amount of total phenolic content in crude BPE was measured by modifying the method of Singleton and Rossi (Singleton and Rossi 1965). The antioxidant activity was determined by the DPPH

method according to Blis (1958). The ferric-reducing antioxidant power (FRAP) was evaluated following the method of Benzie and Strain (Benzie and Strain 1996).

### Antimicrobial activity

Antimicrobial activity of crude BPE was analyzed by the disc diffusion method (Rattanavichai and Cheng 2014) in the following pathogens: *A. hydrophila*, *S. aureus* and *V. parahaemolyticus*. The pathogen was spread on nutrient agar (NA). The sterilized paper disc was soaked in BPE and placed on the NA plate, smeared with a pathogen. The water and methanol were utilized as controls and incubated at 37°C for 24 h. The antimicrobial activity of BPE will be shown by clear zone and reported as positive (+) or negative (-) results.

### Phytochemical analysis: FTIR

The FTIR (iD7ATR Thermo Scientific, Waltham, MA, USA) was used to specify the functional groups of phytochemicals of different crude BPE. The translucent sample discs were prepared by mixing 10 mg KBr salt and 1 mg freeze-dried powder of different extracts of plant material. The mixtures were packed in a FTIR spectroscopy (Shimadzu Corp., Kyoto, Japan) (4 cm<sup>-1</sup> resolution) with a frequency range of 4,000 to 400 cm<sup>-1</sup>.

### Phytochemical analysis: GC-MS

The phytochemical components were detected by gas chromatography-mass spectroscopy (GC-MS) (GC-MSQP2010 SE, Shimadzu Corp., Kyoto, Japan). The chromatography column was DB-5MS (30 m x 0.25 mm i.d., 0.25 µm film thickness). The injector temperature was operated at 220°C. The oven temperature was programmed at 45°C, held for 5 min, increased to 60°C at 2°C/min, increased to 220°C at 3°C/min, and held for 10 min. The carrier gas was helium and analyzed by mass spectrometry (MS).

### Statistical analysis

The results were presented as the mean and standard deviation (SD) of triplicate experiments. Significant differences were determined by one-way analysis of variances (ANOVA) and by comparing the experiment by Duncan's Multiple Range Test (DMR) at  $\alpha < 0.05$  and performed by SPSS v. 15.0 (IBM, Armonk, NY, USA).

## 3. Results and Discussion

### Extraction of BP

The characteristics of fresh, hot air-dried, and powdered BP were illustrated in figures 1A-1C. The color of fresh BP changed from yellow to black and brown after hot air-drying, and powdering processes, respectively. Table 1 indicates the weight of BP before and after hot air-drying in the oven at 50°C for 48 h. The moisture content of dried BP ranged from 5.2 to 7.3% (wet basis), which depended on the thickness of each type. The moisture decreased from the surface of samples through capillary action. The unbound water was transported from the surface via material capillaries (Rahman et al. 2015). Next, the BP powder was extracted by 50% v/v of methanol. Results showed that the maximum extraction, for 3.50 g /10 g DM, yield was obtained from the BP of Kluai Homthong.



**Figure 1.** The characteristics of: A – fresh BP; B – hot air-dried BP; and C – powdered BP.

**Table 1.** The weight of BP, moisture content, and extraction yield.

Type of banana	Weight (g)		Moisture content (%)	Extraction yield (g/10 g DM)
	Fresh peel	Dry peel		
Kluai Kai	1,035.72	228.40	6.98	2.05 <sup>bc</sup>
Kluai Homthong	2,756.71	506.53	5.29	3.50 <sup>a</sup>
Kluai Homtaiwan	3,736.36	111.08	6.91	1.73 <sup>cd</sup>
Kluai Lebmuernang	1,027.68	120.21	4.99	2.09 <sup>b</sup>
Kluai Hukmook	5,902.21	615.22	7.30	1.49 <sup>de</sup>
Kluai Namwa	1,418.13	179.04	7.31	1.32 <sup>e</sup>

The letters are significantly different at  $\alpha \leq 0.05$ .

#### Antioxidant assay of BPE: Total phenolic content (TPC)

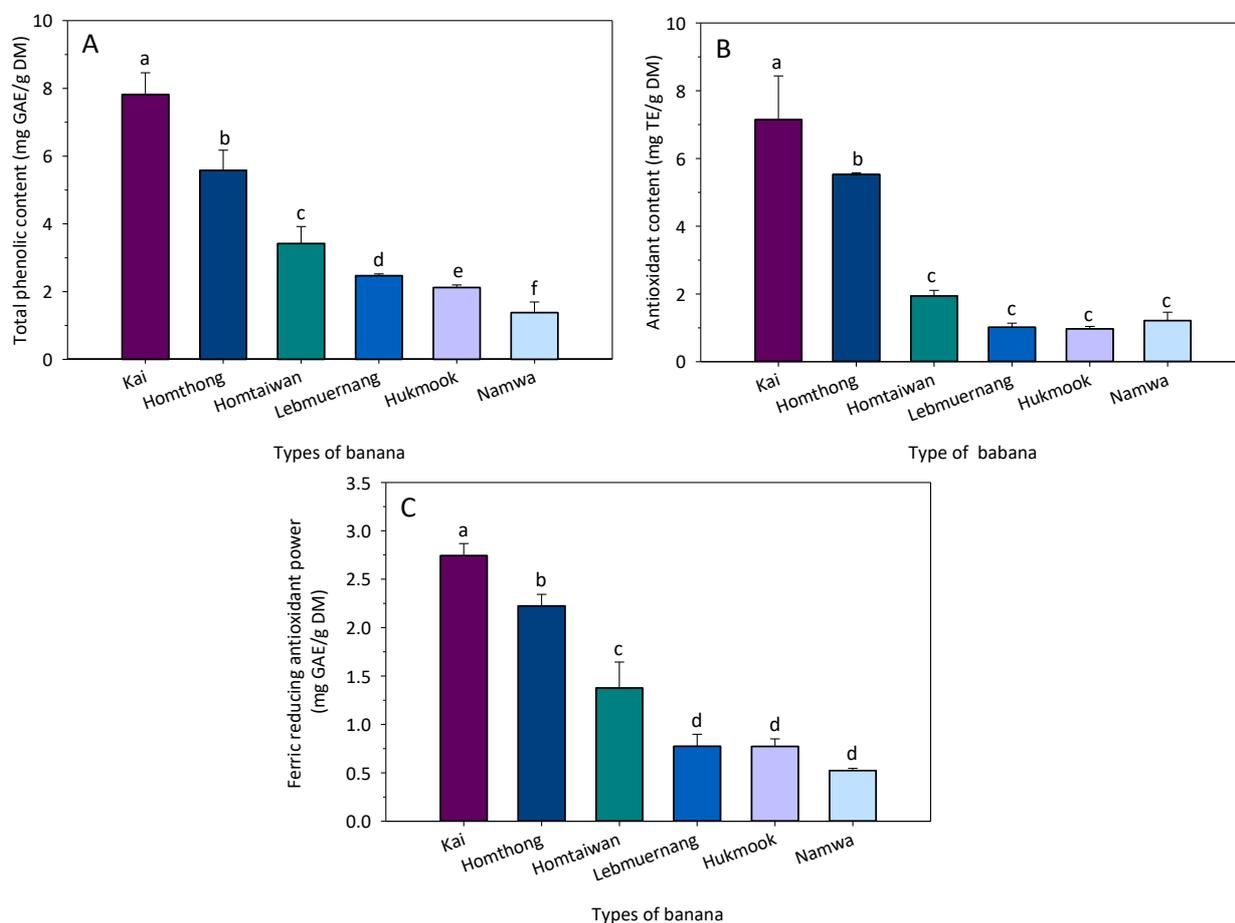
The TPC of obtained crude BPE is presented in figure 2A. The results showed that Kluai Kai provided the highest TPC of  $7.82 \pm 0.64$  mg GAE/g DM, followed by Kluai Homthong, Kluai Homtaiwan, Kluai Lebmuernang, and Kluai Hukmook ( $5.58 \pm 0.60$ ,  $3.42 \pm 0.50$ ,  $2.47 \pm 0.05$  and  $2.12 \pm 0.07$  mg GAE/g DM), respectively, while Kluai Namwa gave the lowest TPC of  $1.38 \pm 0.31$  mg GAE/g DM. These results demonstrated that the TPC depended on the type of banana. González-Montelongo et al. (2010) measured the total phenolic content of BPE, extracted by 50%v/v of methanol at 55°C for 120 min. Two different varieties, Gruesa and Grande Naine (*M. acuminata* Colla AAA) provided the TPC of 16.0 and 17.0 mg GAE/g DM, respectively. This result suggests that the quantity and quality of phenolic compounds from plants are influenced by geographical origin, plant genetics, cultivar, soil composition, growing conditions, state of maturity, post-harvest processing, drying, and extraction methods (Jeffery et al. 2003; Mallavadhani et al. 2006).

#### Antioxidant assay of BPE: Antioxidant content

The amount of antioxidant content was measured by the DPPH radical method, according to Blios (1958). The maximum amount of antioxidant content of BPE was detected in Kluai Kai ( $7.15 \pm 1.30$  mg TE/g DM), followed by Kluai Homthong, Kluai Homtaiwan, Kluai Namwa, and Kluai Lebmuernang ( $5.52 \pm 0.05$ ,  $1.94 \pm 0.17$ ,  $1.22 \pm 0.24$ , and  $1.02 \pm 0.12$  mg TE/g DM), respectively (Figure 2B), while the minimum antioxidant content was seen in Kluai Hukmook ( $0.97 \pm 0.07$  mg TE/g DM). The antioxidant content of BPE in this study was lower than that of Gruesa and Grande Naine (9.4 and 8.8 mg TE/g DM, respectively) due to the difference in species and extraction method (González-Montelongo et al. 2010). The antioxidant compounds were widely known to prevent or inhibit the oxidative reduction in the human body. Moreover, antioxidants can retard aging, prevent coronary heart disease, cancer, and neurodegenerative disorders related to oxidative stress, caused by reactive oxygen species (ROS) (Singh et al. 2016). Polyphenols are antioxidant compounds in a plant's innate defense system; the antioxidant content is synthesized under stress conditions, such as temperature alterations, UV exposure, and pathogenic attacks (Dixon and Paiva 1995).

## Antioxidant assay of BPE: Ferric reducing antioxidant power (FRAP)

Figure 2C presents the amount of FRAP of BPE, analyzed with the method used by Benzie and Strain (1996). The maximum FRAP was found in BPE of Klwai Kai for  $2.74 \pm 0.13$  mg GAE/g DM, followed by Klwai Homthong and Klwai Homtaiwan. However, the FRAP of Klwai Lebmuernang, Klwai Hukmook, and Klwai Namwa was not significantly different. Moreover, the FRAP of BPE allows reductants to be electron donors, which is able to convert them into more stable constituents and terminate the free radical reaction (Aboul et al. 2016).



**Figure 2.** A – total phenolic content; B – antioxidant content; C – ferric reducing antioxidant power of BPE. Mean values with different superscripts (a, b, c, d, and e) made a significant difference ( $\alpha < 0.05$ ) with the error bar  $\pm$  SD.

## Antimicrobial activity

Three pathogens of aquatic animals were chosen to indicate the antimicrobial activity in this study. The inhibition zone of a pathogen by BPE is presented in table 2. The results demonstrated that *A. hydrophila* was inhibited by all BPE except that of Klwai Kai. In addition, the BPE of Klwai Hukmook could also inhibit *S. aureus*, while *V. parahaemolyticus* could not be inhibited by any BPE. It has been suggested that the antimicrobial activity of BPE is related to the type of banana. The previous study reported on the antibacterial activity of BP (Musa, AAA cv. Cavendish), which shows that the BP used ethyl acetate to extract the inhibited *Bacillus subtilis*, *Bacillus cereus*, *Salmonella enteritidis*, *Escherichia coli*, and *S. aureus* (Mokbel and Hashinaga 2005). Normally, plant extracts have antioxidant and antibacterial properties. The hydroxyl group of the phenolic compound can have an inhibitory effect on target bacteria. Gram-negative bacteria are more sensitive than gram-positive bacteria, with differences in their cell wall structures. The gram-positive bacteria have a thick multilayer peptidoglycan cell wall, which is an obstacle to environmental materials, including natural matter and antibiotics. In contrast, the cells of gram-negative bacteria have single peptidoglycan in the outer layer. As such, gram-negative bacteria have a penetrability barrier lower than gram-positive bacteria (Saleem and Saeed 2020).

Furthermore, gram-negative bacteria have a low resistance to physical disruption due to a weak cell wall structure. In a previous study, the extract of pomegranate peels, yellow lemon peels, orange peels, and banana peel containing phenolic compounds was responsible for excellent antimicrobial activities (Al-zoreky 2009; Mehrotra et al. 2017; Saleem and Saeed 2020). However, the modification of outer membrane permeability and porin mutation increases the resistance ability in gram-negative bacteria. On the other hand, this important layer is absent in gram-positive bacteria. Thereby, gram-negative bacteria can be more resistant to antibiotics and cause health problems than gram-positive ones (Breijyeh et al. 2020).

**Table 2.** The antimicrobial inhibition of BPE.

Type of banana	Pathogen inhibition		
	<i>A. hydrophila</i>	<i>S. aureus</i>	<i>V. parahaemolyticus</i>
Kluai Kai	Negative	Negative	Negative
Kluai Homthong	Positive	Negative	Negative
Kluai Homtaiwan	Positive	Negative	Negative
Kluai Lebmuernang	Positive	Negative	Negative
Kluai Hukmook	Positive	Positive	Negative
Kluai Namwa	Positive	Negative	Negative

### Phytochemicals in BPE: FTIR analysis

FTIR measurements were taken to identify the major functional groups of the BPE. The infrared spectra of Kluai Kai, Kluai Homthong, Kluai Homtaiwan, Kluai Lebmuernang, Kluai Hukmook, and Kluai Namwa were similar to each other, conveying that they contained similar functional groups (Figure 3 and Table 3). The strong peaks, at frequencies between 3,328 and 3,344  $\text{cm}^{-1}$ , were assigned to O–H stretching, which represents the free hydroxyl group of the polymer: lignins and BP pectins (Deshmukh et al. 2017), phenols, (González-Cabrera et al. 2018) and polysaccharides (Nogales et al. 2017). At these frequencies, it was attributed to the presence of O–H stretching of a carboxylic group from the two main constituents of GC-MS: acetic acid and formic acid. The distinctive peak at around 1,636  $\text{cm}^{-1}$  was assigned to the stretching C=C ring and the COO<sup>-</sup> antisymmetric stretching of aromatic and carboxylate ions, (González-Cabrera et al. 2018) and amides' C=C stretching present in phytoconstituents of BPE (Thomas and Johny 2017). Gnanasambandam and Proctor (2000) found the stronger bands between 1,640 and 1,620  $\text{cm}^{-1}$  to be an important region that identified and quantified pectin samples. The prominent peak of ester linkage of the carboxylic group from lignin or hemicellulose appears at around 1,730  $\text{cm}^{-1}$ , normally found in BPE; this disappeared, which might be due to the chemical treatment of BPs (Alemdar and Sain 2008). The weak absorption band between 1,395 to 1,412  $\text{cm}^{-1}$  was characteristic of C=C in aromatic rings and COO<sup>-</sup> symmetric stretching of carboxylate ions (Kamsonlian et al. 2011; González-Cabrera et al. 2018; Gnanasambandam and Proctor 2000). The IR spectrum of BPE of Kluai Kai, Kluai Homthong, and Kluai Lebmuernang exhibited C-O stretching and C-C stretching between 1,058 to 1,061  $\text{cm}^{-1}$ , indicating the existence of cellulose and phenol (González-Cabrera et al. 2018; Patial et al. 2019). Lu et al. (2011) specified the wavenumber between 950 and 1,200  $\text{cm}^{-1}$  as the main functional group of carbohydrates, whereas Khamsucharit et al. (2018) found that the pectin of BP was the “fingerprint” region between 800 to 1,300  $\text{cm}^{-1}$ . The FTIR absorption peak of six BPE indicates the existence of functional groups, like hydroxyl and carboxyl, in agreement with BPE of other studies (Memon et al. 2009; Alejandra et al. 2015; El-nafaty et al. 2013; Thomas and Johny 2017).

**Table 3.** Phytochemistry of BPE as analyzed by FTIR and GC-MS.

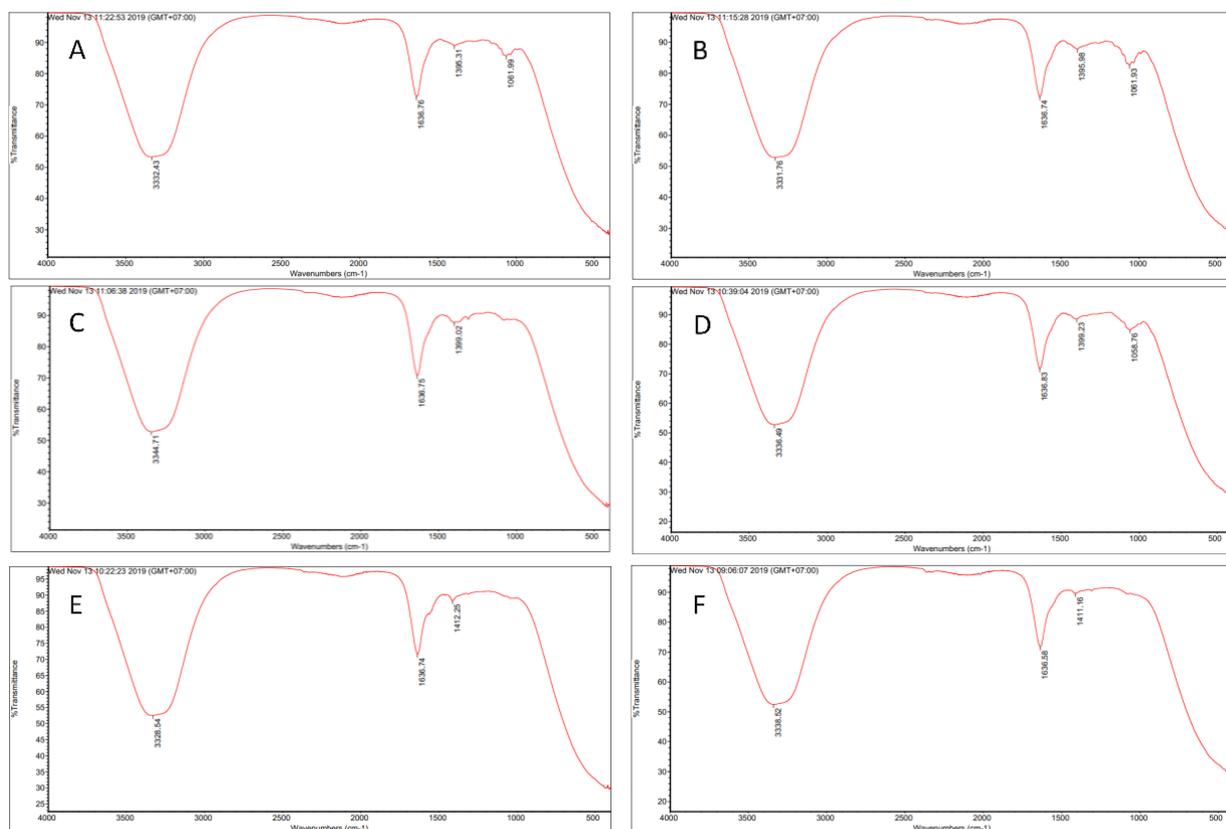
Type of banana	Functional group <sup>a</sup>	Phytochemical <sup>b,c</sup>		Area (%)	Biological activities
		Common name	IUPAC		
Kluai Kai	OH stretching	Ethanoic acid	Acetic acid	43.61	Antimicrobial and preservative in food
	(C=C) stretching and (N-H) bending	Methanoic acid	Formic acid	3.57	Antibacterial agent in livestock feed and retain the nutritive value longer
	COO-symmetric stretching and CHC bending				
	Alcohol C-O stretching, C-C stretching and C-N stretching				
Kluai Homthong	OH stretching	Ethanoic acid	Acetic acid	49.94	Antimicrobial and preservative in food
	(C=C) stretching and (N-H) bending	Methanoic acid	Formic acid	7.73	Antibacterial agent in livestock feed and retain the nutritive value longer
	COO-symmetric stretching and CHC bending	3-methylcatechol	1,2- benzenediol,3-methyl-	1.28	Degrade nitroaromatic compound and antioxidant
	Alcohol C-O stretching, C-C stretching and C-N stretching				
Kluai Homtaiwan	OH stretching	Ethanoic acid	Acetic acid	38.78	Antimicrobial and preservative in food
	(C=C) stretching and (N-H) bending				
	COO-symmetric stretching and CHC bending				
Kluai Lebmuernang	OH stretching	Ethanoic acid	Acetic acid	48.25	Antimicrobial and preservative in food
	(C=C) stretching and (N-H) bending	3-methylcatechol	1,2- benzenediol,3-methyl-	1.00	Degrade nitroaromatic compound and antioxidant
	COO-symmetric stretching and CHC bending				
	Alcohol C-O stretching, C-C stretching and C-N stretching				
Kluai Hukmook	OH stretching	Ethanoic acid	Acetic acid	5.66	Antimicrobial and preservative in food

Type of banana	Functional group <sup>a</sup>	Phytochemical <sup>b,c</sup>		Area (%)	Biological activities
		Common name	IUPAC		
	(C=C) stretching and (N-H) bending	2-methyl-5-(1-methylethyl) phenol	4-hydroxy-2-methylacetophone	3.79	Inhibit the growth of several bacterial strains, antiseptic, antibacterial, and antifungal agent
	C-C aromatic, Asymmetric bend methyl (C-H) and CH <sub>2</sub> scissoring				
Kluai Namwa	OH stretching	Ethanoic acid	Acetic acid	25.64	Antimicrobial and preservative in food
	(C=C) stretching and (N-H) bending				
	C-C aromatic, Asymmetric bend methyl (C-H) and CH <sub>2</sub> scissoring				

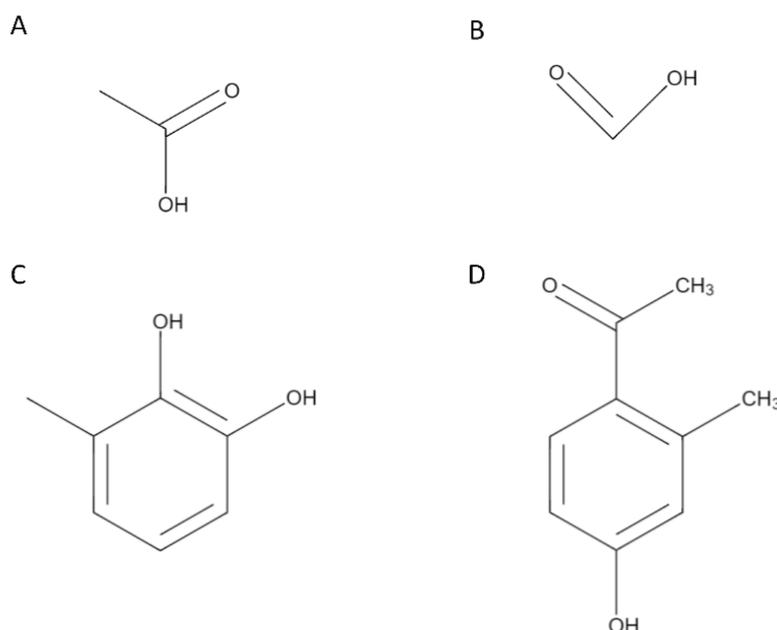
<sup>a</sup>FTIR, <sup>b</sup>GC-MS, and <sup>c</sup>only phytochemical constituents present biological activities were reported.

### Phytochemicals in BPE: GC-MS analysis

GC-MS was used to investigate the phytochemical composition in crude BPE of six banana types. The results of numerous BPE by GC-MS contained 1 to 3 main chemical constituents. The different components of BPE would be affected by the species of banana and cultivation conditions, maturity, and extraction method (Vu et al. 2018). The main constituents were identified from the data of various BPE and shown in table 3 and figure 4. The major compounds were acetic acid, formic acid, 1,2-benzenediol,3-methyl-, and 4-hydroxy-2-methylacetophone. These major compounds have various biological activities, such as antibacterial, antifungal, and antioxidant properties. The acetic acid, formic acid, and 4-hydroxy-2-methylacetophone were the antibacterial and antifungal properties (Fraise et al. 2013; Cheremisinoff and Rosenfeld 2010; Mordi et al. 2016). The 4-hydroxy-2-methylacetophone in *Musa acuminata* Colla demonstrated the inhibition on several bacterial strains: *Bacillus* spp., *Staphylococcus aureus*, *Pseudomonas* spp., *E. coli*, *Streptococcus* spp., *Klebsiella* spp., and *Proteus* spp. (Mordi et al. 2016). Acetic acid was shown to have good antibacterial activity against microorganisms, while formic acid functioned as an important intermediate in chemical synthesis, and an antibacterial and preservative agent in livestock feed. The 1,2-benzenediol,3-methyl- is one of the phenolic compounds in the flavan-3-ol group, which also has antioxidant activity (Fraise et al. 2013; Cheremisinoff and Rosenfeld 2010).



**Figure 3.** FTIR-Spectrum of different crude BPE of: A – Kluai Kai; B – Kluai Homthong; C – Kluai Homtaiwan; D – Kluai Lebmuernang; E – Kluai Hukmook; F – Kluai Namwa.



**Figure 4.** Structure of compounds identified from BP extracts of GC-MS. A – acetic acid; B – formic acid; C – 1,2-benzenediol,3-methyl-; D – 4-hydroxy-2-methylacetophenone.

#### 4. Conclusions

In summary, the present study exposed bioactive compound properties and identified the phytochemical aspect of the most popular banana from a local organic farm in Thailand. BPE showed a difference in TPC, antioxidant activity, and FRAP. Moreover, antimicrobial activity was affected by the type of banana. The acetic acid, formic acid, 1,2-benzenediol,3-methyl-, and 4-hydroxy-2-methylacetophenone were phytochemicals found in BPE and represent antioxidant activity and antimicrobial activity. There is the possibility of applying BPE to supplement animal and aquatic feed to improve growth and immunity.

**Authors' Contributions:** NAKSING, T.: conception and design, acquisition of data, interpretation of data, drafting the manuscript; TEEKA, J.: conception and design, interpretation of data; RATTANAVICHAI, W.: conception and design, interpretation of data; PONGTHAI, P.: interpretation of data; KAEWPA, D.: interpretation of data; AREESISIRUK, A.: conception and design, acquisition of data, analysis and interpretation of data, drafting the manuscript. All authors have read and approved the final version of the manuscript.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**Ethics Approval:** Not applicable.

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