

# Potential antioxidant and lipid peroxidation inhibition of *Phyllanthus acidus* leaf extract in minced pork

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**Objective:** This study investigated the effect of extraction solvents on antioxidant bio-active compounds as well as potential antioxidant and lipid peroxidation inhibition of *Phyllanthus acidus* (*P. acidus*) leaf extract in minced pork.

**Methods:** The effect of various solvent systems of water, 25%, 50%, 75% (v/v) ethanol in water and absolute ethanol on the extraction crude yield, total phenolic content, total flavonoid content and *in vitro* antioxidant activities of *P. acidus* leaves was determined. In addition, antioxidant activities of the addition of crude extract from *P. acidus* leaves at 2.5 and 5 g/kg in minced pork on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cation decolorization, reducing power and inhibition of lipid peroxidation (thiobarbituric acid reactive substances; TBARS) were determined. Moreover, sensory evaluation of the samples was undertaken by using a 7-point hedonic scale.

**Results:** The results showed that the highest crude yield (2.8 g/100 g dry weight) was obtained from water which also had the highest recovery yield for total phenolic content, total flavonoid content and the strongest antioxidant activity. The addition of crude water extract from *P. acidus* leaves was more effective in retarding lipid peroxidation and higher antioxidant activity than control and butylated hydroxytoluene in minced pork. In particular, the samples containing *P. acidus* extract had no significant effect on the sensory scores of overall appearance, color, odor, texture, flavor, and overall acceptability compared to the control.

**Conclusion:** Water solvent was an optimally appropriate solvent for *P. acidus* leaf extraction because of its ability to yield the highest amount of bio-active compounds and *in vitro* antioxidant property. Particularly, *P. acidus* crude water extract also strongly expressed the capacity to retard lipid oxidation, radical scavenging, radical cation decolorization and reducing power in minced pork. The results of this study indicated that *P. acidus* leaf extract could be used as natural antioxidant in the pork industry.

**Keywords:** Antioxidant; Lipid Peroxidation; Pork; *Phyllanthus acidus* Extract

## INTRODUCTION

Pork is a highly nutritious food, rich in proteins, essential amino acids, vitamins and minerals such as zinc and iron. However, minced pork contains high unsaturated fatty acid content, so it is easy to deteriorate caused by oxidation [1,2]. Lipid oxidation is the most common form of chemical deterioration in meat. The alterations of lipid oxidation in meat can negatively affect sensory attributes such as color, texture, odor, flavor, and structural damage on proteins [3]. In particular, the first impression consumers have of any meat product is its color and thus color is of utmost importance. The bright red color of fresh meat cuts is caused by the presence of oxymyoglobin - an oxygenated myoglobin, but myoglobin and oxymyoglobin have the ability to lose an electron (called oxidation) which transforms the pigment to brown color and yields metmyoglobin [4]. Moreover, these oxidative reactions can also diminish the nutritional quality of meat and form toxic compounds. Antioxidants are substances that can retard the oxidation of easily oxidizable

biomolecules such as lipids and proteins in meat, thus improving the shelf life of meats by protecting them from deterioration caused by oxidation [4]. Nowadays, two main solutions are employed to retard lipid oxidation and maintain the nutrients and sensory qualities of meat: synthetic and natural antioxidants [5]. In the meat industry, mainly synthetic antioxidants are used to slow the lipid oxidations such as butylated hydroxytoluene (BHT), butylated hydroxyanisole, tertiary butyl hydroquinone, and propyl gallate. However, the growing concern among consumers about synthetic antioxidants and their potential adverse effects coupled with the demand for natural antioxidants, especially of plant origin, has increased in recent years. In addition, consumers increasingly concern that foods contribute directly to their health because it was major the improvements in understanding the relationship between nutrition and health such as functional foods, which means a practical and new approach to get good health by raising the state of well-being and possibly reducing the risk of disease [6]. Food quality and food composition are the most important factors influencing consumer choice. In this regard, functional foods play a salient role.

Plants are a generous source of food with numerous valuable bioactive compounds. Bio-active plant compounds ranging from polar to non-polar have been found to possess potent antioxidant properties [7], and the solvent extraction plays an important role in extraction efficiency of bioactive compounds from the plant materials [8]. Different plant materials such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs are being evaluated due to their high content of polyphenols as natural antioxidants to preserve and improve the overall meat quality [8].

*Phyllanthus acidus* (*P. acidus*) is commonly known as star gooseberry. It is a tree with small, yellow, edible berries in the Phyllanthaceae family and is quite commonly found in the tropics [5]. *P. acidus* leaves are 2 to 7.5 cm long and thin; they are green and smooth on the upper side and blue-green on the underside. *P. acidus* has long been used as an analgesic, antipyretic, and anti-rheumatic; it has also been used to treat jaundice, small pox, urticaria, gum infection, liver disease, and as a blood purifier [9]. A large number of previous studies demonstrated that *P. acidus* leaves contain some important chemical constituents including kaempferol, hypogallic acid, gallic acid, quercetin, alkaloid, tannin, flavonoid, phenolic, and terpene compounds [5]. The antioxidant activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging aqueous and ethanol extract from *P. acidus* have been investigated [9].

However, to our knowledge, there is still no information available on the application of *P. acidus* leaf extract in minced pork as natural antioxidant. Therefore, the objective of this study was the investigation of the best solvent for the extraction of bio-active compounds that contributes directly to antioxidant properties and the application of *P. acidus* leaf extract in minced pork as a natural antioxidant on DPPH radical scavenging assay, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

(ABTS) radical cation decolorization, reducing power and inhibition of lipid peroxidation (thiobarbituric acid reactive substances; TBARS) as well as develop a new functional food product.

## MATERIALS AND METHODS

### Plant materials and solvent extraction of *Phyllanthus acidus* leaves

Fresh *P. acidus* leaves were collected from the local area in Ladkrabang, Bangkok, Thailand. After washing under running tap water, they were dried in a hot-air oven at 45°C for 3 days, and then ground to small pieces by using an electric blender (Tefal Model Moulinette DPA1, Saint-Martin, France), and then sieved by using 40 mesh (420 µm). The dried leaves were extracted with different solvent systems (water, 25%, 50%, 75% [v/v] ethanol in water and absolute ethanol) using 1 part of powder to 9 parts of each solvent (w/v) for 72 h at 4°C. Extraction was repeated three times and the extracts were then combined after being filtered through three layers of cheesecloth to remove large debris and re-filtered through Whatman No.1 filter paper. The filtrates were evaporated in a rotary evaporator (BUCHI, Lausanne, Switzerland) at 45°C, to leave a sticky residue and stored at 4°C until use. The sticky crude extracts (CE) were dissolved in their extraction solvent before use.

### Total phenolic content

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method [10]. The calibration curve was established using gallic acid (0 to 200 mg/L). One ml of each extract solution (1,000 mg/L) or gallic acid was individually combined with 0.5 mL of 2 N Folin-Ciocalteu reagent and 4 mL of 7.5% (w/v) sodium carbonate solution. The mixtures were allowed to stand for an hour at room temperature in the dark, and then measured at 765 nm using a spectrophotometer (Shimadzu model UV-1601, Shizuoka, Japan). TPC was calculated on the basis of the calibration curve of gallic acid standard. The results were expressed as mg gallic acid equivalent per 1 gram of CE (mg GAE/g CE).

### Total flavonoid content

Total flavonoid content (TFC) was measured with the aluminum chloride colorimetric assay [11]. The calibration curve was established using quercetin (0 to 200 mg/L). In brief, 0.5 mL of each stock extract solution (1,000 mg/L) or quercetin was individually mixed with 0.1 mL of 10% (w/v) aluminum nitrate, 0.1 mL of 1 M potassium acetate; the total volume was then added to more ethanol to reach a total of 10 mL. The combination was thoroughly mixed and allowed to stand for 40 min at room temperature. The maximum absorbance of the mixture was measured at 415 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed as mg quercetin equivalent per 1 gram CE (mg QE/g CE).

### Determination of *in vitro* antioxidant activity of crude extracts from *Phyllanthus acidus* leaves

There are abundant antioxidant methods for determination of antioxidant activity. Among all, inhibition of lipid peroxidation, reducing power, DPPH radical scavenging activity and metal chelating were used to determine the antioxidant activity of *P. acidus* CE. The CE was dissolved in their extraction solvent to contain the stock extract solution at concentrations of 100, 250, 500, 750, and 1,000 mg/L (w/v) for the following antioxidant determination.

**Inhibition of lipid peroxidation:** Lipid peroxidation inhibition of CE in egg yolk extract was performed according to the previous method with minor modifications [12]. In brief, one ml of each concentration (100, 250, 500, 750, and 1,000 mg/L [w/v]) of each CE was individually added to 50 mL of egg yolk prepared in phosphate buffered saline (PBS) at aE ratio of 1:4 (w/v), then 0.5 mL of 24 mM ferrous sulfate and 0.5 mL of PBS were added. The mixture was shaken vigorously and incubated at 37°C for 15 min. Next, 0.5 mL of 20% (w/v) trichloroacetic acid and 1 mL of 0.8% (w/v) thiobarbituric acid was added to the mixture. After boiling at 95°C and cooling for 30 min, the mixture was centrifuged at 2,200×g for 20 min at 25°C. The absorbance was measured at 532 nm by spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed as the sample concentration providing 50% inhibition (IC<sub>50</sub>) values, which required 50% inhibition of lipid peroxidation.

**Reducing power ability:** The reducing power of CE was measured by the method described by Kim [13]. In brief, one mL of each concentration (100, 250, 500, 750, and 1,000 mg/L [w/v]) of each CE was individually mixed with 2.5 mL of 0.2 M phosphate buffer pH 6.6 and 2.5 mL of 1% (w/v) potassium ferricyanide, then incubated at 50°C for 20 min. Next, 2.5 mL of 10% (w/v) trichloroacetic acid was added and centrifuged at 2,200×g for 10 min at 25°C. Finally, 2.5 mL of upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride. The absorbance was measured at 700 nm by a spectrophotometer against a blank. The results were expressed as IC<sub>50</sub> values, which required 50% inhibition of reducing power activity.

**DPPH radical scavenging:** DPPH radical scavenging activity was determined using the method described by Ebrahimzadeh et al [7]. In brief, 2 mL of each concentration (100, 250, 500, 750, and 1,000 mg/L [w/v]) of each CE was individually mixed with 2 mL of 100 μM DPPH prepared in ethanol. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed as IC<sub>50</sub> values, which required 50% scavenging inhibition of DPPH radical.

**Metal chelating:** The chelating ability of CE for ferrous ions (Fe<sup>2+</sup>) was quantified according to the modified method of Jamma et al [14]. Briefly, one ml of each concentration (100, 250, 500, 750, and 1,000 mg/L [w/v]) of each CE was individually

mixed with 50 μL of 2 mM ferrous sulfate heptahydrate, and then added 100 μL of 5 mM ferrous solution. The mixture was shaken vigorously and incubated at room temperature for 10 min. The absorbance was then measured at 562 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed as IC<sub>50</sub> values, which required 50% inhibition of chelate ferrous ions.

### Preparation of meat samples

Raw pork and fat were obtained from Makro supermarket, Thailand. After being cut to small pieces, the pork was combined to contain 30% fat (w/w), and then the mixture was minced by a houlinette homogenator (Tefal Model Moulinette DPA1, France). The experiment consisted of four treatments: none added (control); 0.2 g BHT/kg meat (positive lipid peroxidation); minced pork plus 2.5 and 5.0 g CE/kg meat; and mixed vigorously. Cooked meat samples were prepared by boiling at 95°C for 20 min. Both raw and cooked meat samples were assayed for antioxidant activity as described below.

Meat extractions were prepared by the method described by Jung et al [15]. Briefly, exactly 3 g of meat samples were homogenized in 12 mL of food-grade ethanol for 10 s at top speed by using a homogenizer (T-18 Ultra tarrax, Staufen, Germany). Lipids and the supernatant were separated by centrifuged machine (Avanti JA-20, Beckman Coulter, Brea, CA, USA) at 4°C, 5,160×g for 5 min. The meat extract was filtered through a Whatman No.1 filter paper before use.

### Determination of antioxidant activity of minced pork extract

**DPPH radical scavenging activity:** The radical scavenging activities of the meat samples were measured according to the method of Qwele et al [16]. Briefly, two ml of the meat extracts was individually added to 2 mL of 0.2 mM DPPH prepared in ethanol. The mixture was vortexed and left to stand in the dark at room temperature for 30 min. The mixture of ethanol (2 mL) and meat extract (2 mL) serve as a blank. The control solution was prepared by mixing ethanol (2 mL) and DPPH radical solution (2 mL). The absorbances were measured at 517 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed as inhibiting percent and calculated as in (1):

$$\text{DPPH activity (\%)} = \left[ 1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \right] \times 100 \quad (1)$$

**Reducing power ability:** Reducing power ability was measured by the method described by Gallego et al [17]. Briefly, one mL of the meat extracts was individually mixed with 2.5 mL of 0.2 M phosphate buffer pH 6.6, 2.5 mL of 1% potassium ferricyanide and then incubated at 50°C for 30 min. Afterwards, the mixture was added to 2.5 mL of 10% trichloroacetic acid and centrifuged at 2,200×g for 10 min at 25°C. Finally, 2.5 mL of upper layer solu-

tion was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the sample solutions were measured at 700 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed as the absorbance values.

**ABTS<sup>+</sup> decolorization radical cation:** The total antioxidant activity of the meat samples was measured by the method described by Re et al [18]. The stock (2,2'-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid]) ABTS<sup>+</sup> solution was produced by mixing 7 mM ABTS<sup>+</sup> aqueous solution with 2.4 mM potassium persulfate in the dark for 12 to 16 h at room temperature. The mixture was incubated in the dark at room temperature 12 to 16 h before use. Prior to assay, this solution was diluted in 80% ethanol and equilibrated at 30°C to give an absorbance of 0.70±0.02 at 734 nm. Then, 3 mL of diluted ABTS<sup>+</sup> solution was added to 300 µL of the meat extract. After 6 min of incubation at room temperature, the absorbance were recorded at 734 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). against a blank (3 mL of ethanol plus 300 µL of sample extract), and a control (3 mL of ABTS<sup>+</sup> solution plus 300 µL of ethanol). The scavenging activity of meat samples against ABTS radical cation decolorization was expressed as inhibiting percent and calculated as in (2):

$$\text{ABTS activity (\%)} = \left[ 1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \right] \times 100 \quad (2)$$

### Inhibition of lipid peroxidation

The TBARS were measured by the method described by Ortuno et al [19]. Briefly, 2 g of meat samples was individually homogenized with 10 mL of thiobarbituric acid solution reagent (a mixture of 0.375% [w/v] 2-thiobarbituric acid, 0.25 N hydrogen chloride, and 15% [w/v] trichloroacetic acid was prepared with a silent crusher for 1 h at high speed), and heated at 95°C for 15 min in a boiling water bath. Subsequently, the mixtures were cooled in ice-cold water and centrifuged at 1,500×g for 15 min at 4°C. The absorbances were measured at 532 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were reported as mg malondialdehyde per kg meat sample (mg MDA/kg meat).

### Sensory evaluation

Sensory evaluation was carried out to evaluate the overall appearance, color, odor, texture, flavor, and overall acceptability of each minced pork sample, following a descriptive hedonic scale method of Beinner et al [20]. The sensory evaluation consisted of 30 consumer panels who were invited from the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Thailand. They were trained to follow the procedure of sensory evaluation before starting to evaluate the pork samples. The minced pork samples were boiled in a water bath at 95°C for 20 min, including time for defrosting and evaluating as soon as the

samples were completed. Water was provided for cleaning the palate between samples. Panelists were asked to evaluate the sensory properties of each sample on overall appearance, color, odor, texture, flavor, and overall acceptability using a 7-point descriptive hedonic scale ranging from extremely like to extremely dislike: 1 = extremely dislike; 2 = very dislike; 3 = dislike; 4 = neither like nor dislike; 5 = like; 6 = very like; 7 = extremely like was used to score the samples.

### Statistical analysis

The experimental design was carried out as a completely randomized design with three replications and was repeated three times using one-way analysis of variance. Analysis of variance was performed using raw data with the mean values and standard deviation was calculated. Differences among the means were analyzed using the Tukey's multiple range tests with a significance defined at p<0.05 level.

## RESULTS AND DISCUSSION

### Effect of solvent system on extraction yield and bioactive compounds from *Phyllanthus acidus* leaves

**Extraction yield:** The yield of CE significantly depended on the solvent system of mixture of ethanol in water. Selection of solvent is an important step for obtaining extracts with acceptable yields and strong antioxidant activity [21]. In this study, the solvent extracts had significantly (p<0.05) affected the amount of the extraction yields (Table 1). The extraction yields of various solvents decreased in the following order: water >25% ethanol >50% ethanol >75% ethanol >absolute ethanol. It could be seen that the extraction yield of water was the highest (2.80 g/100 g dry weight) and the extraction yield of absolute ethanol was the lowest (0.53 g/100 g dry weight). This finding was in agreement with the extraction yields of Zielinski and Kozłowska [22], who reported that water extracted more crude yield than organic solvent. These studies investigated the increasing water concentration in the solvent increase extraction yield, which can be explained by the increased solubility of other components such as proteins and

**Table 1.** Effect of crude extracts from *Phyllanthus acidus* leaves by various solvent systems on crude yield, total phenolic and flavonoid contents<sup>1)</sup>

Solvent systems (% ethanol)	Crude yield (g/100 g DW)	TPC (mg GAE/g CE)	TFC (mg QE/g CE)
Water	2.80 ± 0.03 <sup>a</sup>	49.87 ± 0.23 <sup>a</sup>	0.70 ± 0.04 <sup>a</sup>
25	1.93 ± 0.04 <sup>b</sup>	49.82 ± 0.10 <sup>a</sup>	0.66 ± 0.03 <sup>ab</sup>
50	1.77 ± 0.05 <sup>bc</sup>	49.46 ± 0.17 <sup>ab</sup>	0.52 ± 0.11 <sup>b</sup>
75	1.59 ± 0.05 <sup>c</sup>	48.54 ± 0.13 <sup>bc</sup>	0.50 ± 0.01 <sup>b</sup>
100	0.53 ± 0.07 <sup>d</sup>	48.04 ± 0.92 <sup>c</sup>	0.51 ± 0.05 <sup>b</sup>

DW, dry weight; TPC, total phenolic content; GAE, gallic acid equivalent; CE, crude extracts; TFC, total flavonoid content; QE, quercetin equivalent.

<sup>1)</sup> All values were expressed as mean ± standard deviation.

<sup>a-d</sup> Means sharing different letters in the same column are significantly different (p < 0.05).

carbohydrates [23].

**Total phenolic content and total flavonoid content**

The recovery amount of TPC and TFC is expressed in Table 1. The results found that the TPC and TFC were increased with increasing water concentration in solvent. TPC of CE ranged from 48.04 mg GAE/g crude (absolute ethanol extract) to 49.87 mg GAE/g crude (water extract). The TFC values of CE range from 0.51 mg QE/g crude for 100% ethanol extract for to 0.70 mg QE/g crude for water extract. The increase of TPC and TFC was in the following order: water >25% ethanol>50% ethanol>75% ethanol>absolute ethanol. These results were in agreement with the study of Jagajothi et al [5] and Munuswamy et al [24], who found flavonoids and phenols in *P. acidus* leaf extract. Moreover, the previous studies also demonstrated that extraction solvents significantly influenced the recovery amount of bioactive compounds from plant materials [21,25]. These results could be explained that polyphenols are often extracted in higher amounts in more polar solvents (polarity index of water: 9>polarity index of ethanol: 5.2) [26]. Therefore, water was the best solvent for bioactive compounds in this study. However, in the cases of Do et al [23], who found that the highest TPC and TFC was obtained in the absolute ethanol extract compared to the water solvent, and Sun et al [27], who suggested that 75% ethanol/water solvent can be the best extraction solvent for phenolic compounds in propolis, could be explained by the differences about the presence and position of hydroxyl groups, and the molecular size and the length of constituent hydrocarbon chains [23].

**In vitro antioxidant activity of Phyllanthus acidus leaf extracts**

The *in vitro* antioxidant activity of the extracts from *P. acidus* leaves is presented in Table 2. The results are expressed as IC<sub>50</sub> values, which are defined as the concentration of the sample necessary to cause 50% inhibition and are obtained by interpolation from linear regression analysis. A lower IC<sub>50</sub> value is associated with higher activity. In this study, the antioxidant activity of the extracts from *P. acidus* leaves was increased with increasing water concentration in solvent. The highest antioxidant activity was found in water extract and the highest IC<sub>50</sub> values were 716.32, 932.25, 88.68, and 1,311.17 mg/L for lipid peroxidation, reducing

power, DPPH scavenging, and metal chelating, respectively. Whereas, the lowest IC<sub>50</sub> values were 1,006.15, 1,462.27, 95.32, and 6,442.60 mg/L for lipid peroxidation, reducing power, DPPH scavenging, and metal chelating, respectively.

These results are supported by studies of Kim [13], who reported that the scavenging activity of water extract was significantly higher than absolute ethanol extract from dried boxthorn (*Lycium chinensis*) fruit. Jia et al [28], who demonstrated that absolute ethanol had lower reducing power, DPPH and ABTS scavenging water than among extraction system of ethanol such as water, 20%, 40%, 60%, and 80% ethanol. The antioxidant activities of *P. acidus* leaf extracts could be estimated by the phenolic compounds present in the extracts played an important role in antioxidant activity directly through the reduction of oxidized intermediates in the chain reaction [29].

Notably, the antioxidant activity of the extracts from *P. acidus* leaves was enhanced with increasing water concentration in solvent, which may be directly correlated with the phenolic and flavonoid compounds of the extracts. It could be inferred that the increasing ratio of phenolic and flavonoid compounds in turn improves the lipid peroxidation, reducing power, DPPH scavenging and metal chelating abilities of the extracts [24]. This may be caused by *P. acidus* leaf water extract may either contain more bioactive compounds or possess phenolic compounds that contain a larger number of active groups than the other solvents. Thus, the water extract was selected as an optimally appropriate solvent for the following study showing the strongest antioxidant activity.

**Antioxidant activity of minced pork extract**

**DPPH Radical scavenging activity:** Generally, the antioxidant activity of bio-active compounds is due to their radical scavenging effects. The DPPH assay is one of the most widely employed and preferred methods for measuring the radical scavenging activity of samples. DPPH is a stable nitrogen-centered free radical that produces a violet color in methanol solution. When DPPH radicals react with suitable reducing agent as antioxidants, the solution loses its color depending on the number of electrons absorbed. The DPPH radical scavenging activities of the meat samples are shown in Table 3.

**Table 2.** *In vitro* antioxidant activities of crude extracts from Phyllanthus acidus leaves by various solvent systems<sup>1)</sup>

Solvents (% ethanol)	IC <sub>50</sub> (mg/L)			
	DPPH activity	Reducing power	Metal chelating	Lipid peroxidation
Water	88.68 ± 0.04 <sup>c</sup>	932.25 ± 0.16 <sup>e</sup>	1,311.17 ± 0.70 <sup>e</sup>	716.32 ± 0.95 <sup>d</sup>
25	89.03 ± 0.87 <sup>c</sup>	988.21 ± 0.06 <sup>d</sup>	2,512.29 ± 0.31 <sup>d</sup>	885.56 ± 0.68 <sup>c</sup>
50	92.47 ± 0.59 <sup>b</sup>	1,084.95 ± 0.87 <sup>c</sup>	3,677.13 ± 0.02 <sup>c</sup>	983.20 ± 0.91 <sup>b</sup>
75	94.66 ± 0.61 <sup>a</sup>	1,288.50 ± 0.16 <sup>b</sup>	4,599.27 ± 0.17 <sup>b</sup>	1,004.87 ± 0.25 <sup>a</sup>
100	95.32 ± 1.22 <sup>a</sup>	1,462.27 ± 0.03 <sup>a</sup>	6,442.60 ± 0.53 <sup>a</sup>	1,006.15 ± 0.22 <sup>a</sup>

IC<sub>50</sub>, concentration providing 50% inhibition.

<sup>1)</sup> All values were expressed as mean ± standard deviation.

<sup>a-e</sup> Means sharing different letters in the same column are significantly different (p < 0.05).

The meat samples containing water extract from *P. acidus* leaves at the concentration of 2.5 g CE/kg meat (raw sample: 57.56%, cooked sample: 61.15%) and 5 g CE/kg meat (raw sample: 71.66%, cooked sample: 78.20%) were significantly ( $p < 0.05$ ) higher DPPH scavenging activity than the control (raw sample: 16.33%, cooked sample: 22.16%) and 0.2 g BHT/kg meat (raw sample: 27.20%, cooked sample: 38.72%). This result could be explained by the increasing phenolic and flavonoid compounds in the meat samples. Do et al [23], who demonstrated that flavonoids are highly scavengers of most oxidizing molecules, including singlet oxygen and various other free radicals implicated in several diseases.

Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses [30]. Similarly, phenolics confer oxidative stress tolerance on plants. Moreover, following cooking of meat, the overall DPPH scavenging activity of the samples increased significantly ( $p < 0.05$ ). This finding was similar to that found by Fasseas et al [8], who reported that the DPPH scavenging activity of meat substitute was enhanced with increasing heating temperature and time. This could be due to reactions between reducing sugars and free amino acids or free amino groups in proteins - the Maillard reaction. As in previous studies, the Maillard reaction products have antioxidant activity by scavenging oxygen radicals or chelating metals [30].

**ABTS<sup>+</sup> radical cation decolorization assay:** The ABTS radical cation decolorization of the meat samples are shown in Table 3. The meat samples containing water extract from *P. acidus* leaves at the concentration of 2.5 g CE/kg meat (raw sample: 68.24%, cooked sample: 73.52%) and 5 g CE/kg meat (raw sample: 85.39%, cooked sample: 88.41%) were significantly ( $p < 0.05$ ) higher ABTS radical cation decolorization than the control (raw sample: 36.75%, cooked sample: 38.58%) and 0.2 g BHT/kg meat (raw sample: 59.44%, cooked sample: 65.13%). This finding also could be explained as the DPPH scavenging activity phenomenon cause the difference of phenolic and flavonoid compounds in the meat samples [3]. Radical scavenging was also affected over time in all untreated meat samples, perhaps because oxidation of the meat leads to the creation of compounds that scavenge free radicals.

Moreover, following cooking of the meat, the overall ABTS radical cation decolorization activity of the samples increased significantly ( $p < 0.05$ ). This finding could be due to reactions between reducing sugars and free amino acids or free amino groups in proteins - the Maillard reaction.

**Reducing power:** The reducing power ability was expressed as the absorbance at 700 nm (Table 3). The results showed that the meat samples treated with water extract from *P. acidus* leaves at concentration of 2.5 g CE/kg meat (raw sample: 0.57, cooked sample: 0.72) and 5 g CE/kg meat (raw sample: 0.77, cooked sample: 0.84) significantly ( $p < 0.05$ ) increased reducing power (higher absorbance) compared to the control (raw sample: 0.32, cooked sample: 0.42) in both raw and cooked samples. This finding could be explained by the presence of reductants in meat causes the reduction of Fe<sup>3+</sup>/ferric cyanide complex to the ferrous form. Therefore, the Fe<sup>2+</sup> complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [13]. *P. acidus* extract treatment gave a significantly higher absorbance than the control and BHT treatments due to the high polyphenolic content of the *P. acidus* extract.

### Inhibition of lipid peroxidation

The TBARS method has been widely used to determine the degree of lipid oxidation through MDA compound, which results from lipid peroxidation of polyunsaturated fatty acids. Table 4 represented the influence of adding *P. acidus* water CE on the lipid oxidation of raw and cooked minced pork. The results revealed

**Table 4.** Effect of water crude extract from *Phyllanthus acidus* leaves on total MDA in minced pork<sup>1)</sup>

Treatments	Total MDA (mg/kg meat)	
	Before cooking	After cooking
Control	2.89 ± 0.17 <sup>a</sup>	13.03 ± 0.39 <sup>a</sup>
0.2 g BHT/ kg meat	2.71 ± 0.01 <sup>a</sup>	6.69 ± 0.13 <sup>b</sup>
2.5 g CE/kg meat	2.68 ± 0.24 <sup>a</sup>	4.61 ± 0.14 <sup>c</sup>
5 g CE/kg meat	2.76 ± 0.44 <sup>a</sup>	2.95 ± 0.14 <sup>d</sup>

MDA, malondialdehyde; BHT, butylated hydroxytoluene; CE, quercetin equivalent.

<sup>1)</sup> All values were expressed as mean ± standard deviation.

<sup>a-d</sup> Means sharing different letters in the same column are significantly different ( $p < 0.05$ ).

**Table 3.** Effect of water crude extract from *Phyllanthus acidus* leaves on antioxidant activities in minced pork<sup>1)</sup>

Treatments	DPPH activity (% activity)		ABTS activity (% activity)		Reducing power (OD at 700 nm)	
	Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking
Control	16.33 ± 0.15 <sup>d</sup>	22.16 ± 0.06 <sup>d</sup>	36.75 ± 0.07 <sup>d</sup>	38.58 ± 0.12 <sup>d</sup>	0.32 ± 0.17 <sup>c</sup>	0.42 ± 0.09 <sup>b</sup>
0.2 g BHT/kg meat	27.20 ± 0.19 <sup>c</sup>	38.72 ± 0.09 <sup>c</sup>	59.44 ± 0.02 <sup>c</sup>	65.13 ± 0.01 <sup>c</sup>	0.47 ± 0.12 <sup>bc</sup>	0.60 ± 0.31 <sup>a</sup>
2.5 g CE/kg meat	57.56 ± 0.08 <sup>b</sup>	61.15 ± 0.03 <sup>b</sup>	68.24 ± 0.01 <sup>b</sup>	73.52 ± 0.18 <sup>b</sup>	0.57 ± 0.04 <sup>b</sup>	0.72 ± 0.17 <sup>a</sup>
5 g CE/kg meat	71.66 ± 0.01 <sup>a</sup>	78.20 ± 0.08 <sup>a</sup>	85.39 ± 0.36 <sup>a</sup>	88.41 ± 0.01 <sup>a</sup>	0.77 ± 0.36 <sup>a</sup>	0.84 ± 0.32 <sup>a</sup>

DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; OD, optical density; BHT, butylated hydroxytoluene; CE, quercetin equivalent.

<sup>1)</sup> All values were expressed as mean ± standard deviation.

<sup>a-d</sup> Means sharing different letters in the same column are significantly different ( $p < 0.05$ ).

that total MDA content was similar for raw samples (control: 2.89, 0.2 g BHT/kg, 2.71, 2.5 g/kg meat: 2.68 and 5 g/kg meat: 2.76 mg MDA/kg meat) ( $p < 0.05$ ). Whereas, in cooked meat samples, total MDA content in the control (13.03 mg MDA/kg meat) and 0.2 g BHT/kg meat (6.69 mg MDA/kg meat) were higher than samples containing 2.5 g CE/kg meat (4.61 mg MDA/kg meat) and 5 g CE/kg meat (2.95 mg MDA/kg meat) ( $p < 0.05$ ). This is generally in agreement with other research studies that have investigated the effects of plant extract on lipid peroxidation in meat [7,30]. The results of the present study show that adding phenolic-rich extracts protects minced pork samples against lipid peroxidation. Other previous studies have reported on the relationship between phenolic content and antioxidant activity. The phenolic compounds are associated with the hydroxyl group linked to the aromatic ring, which is capable of donating hydrogen atoms with electrons and neutralizing free radicals. This mechanism blocks further degradation to more active oxidizing forms, such as MDA. Particularly, Lee et al [31] have observed an increase in the hue-angle (arc tan  $b^*/a^*$ ) of stored meat products, suggesting a degree of change from redness ( $0^\circ$ ) to yellowness ( $90^\circ$ ), an indication of increased oxidation during storage time. In our study, the hue-angle value of minced pork with *P. acidus* extract supplementations (73.61 to 77.11) was significantly lower ( $p < 0.05$ ) than the control (78.52 to 81.47) at 6 to 8 days after storage (data not shown). Regarding to discoloration among samples ( $\Delta E$ ), the addition of *P. acidus* extract in mined pork (2.63 to 3.64) could prevent discoloration of the samples rather than the control (4.77 to 6.30) at 6 to 8 days ( $p < 0.05$ ) (data not shown). This result was in agreement with the study of Bowser et al [32], who reported that hue-angle showed a trend to decrease in samples, which became darker with extending time but low mean values (more reddish samples) were observed for meat product with added rosemary extract. This result may due to the difference metmyoglobin concentration which related to brown pigment among the samples and indicated that *P. acidus* extract supplementation has the better effect on the color stability than the control during the storage up to 8 days [31]. Therefore, the strong *in vitro* antioxidant activity shown by *P. acidus* extracts also had a protective role in real meat products.

Moreover, heat treatment of meat revealed significant differ-

ences between raw and corresponding cooked samples with much higher MDA values were noted for the cooked meat samples than for the corresponding raw ones. These findings could be explained by the production of pre-oxidized myoglobin that is susceptible to further oxidation [33] or by cooking's decreased ferric ion reducing capacity, but increases non-heme iron of meat, resulting in comparatively lower ferric ion reducing capacity in cooked than raw meat. Therefore, heat stable ferric ion capacity may be primarily responsible for the regeneration of ferrous ion to increase total MDA in cooked meat.

### Sensory evaluation

Result of sensory evaluation of overall appearance, color, odor, texture, flavor, and overall acceptability of the minced pork samples was shown in Table 5. In overall, addition of *P. acidus* extract in the minced pork samples had no significant effect on the sensory scores of overall appearance, color, odor, texture, flavor, and overall acceptability compared to the control. This result suggested that *P. acidus* extract could be applied as a natural product in minced pork.

### CONCLUSION

Water was an optimally appropriate solvent for *P. acidus* leaf extraction because of its ability to yield the highest amount of bio-active compounds and *in vitro* antioxidant property. Particularly, *P. acidus* crude water extract also strongly expressed the capacity to reduce lipid oxidation, radical scavenging activity, radical cation decolorization and reducing power ability in minced pork. Moreover, addition of *P. acidus* extract in the minced pork samples had no significant effect on sensory evaluation of overall appearance, color, odor, texture, flavor, and overall acceptability. The results of this study indicate that *P. acidus* leaf extract could be used as a natural antioxidant in the pork industry.

### CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

**Table 5.** Effect of water crude extract from *Phyllanthus acidus* leaves on sensory evaluation in minced pork<sup>1)</sup>

Items	Control	0.2 g BHT/ kg meat	2.5 g CE/kg meat	5 g CE/kg meat
Overall appearance	6.20 ± 0.70 <sup>a</sup>	5.05 ± 1.19 <sup>b</sup>	6.00 ± 0.92 <sup>a</sup>	5.90 ± 1.11 <sup>a</sup>
Color	6.05 ± 0.94 <sup>a</sup>	5.60 ± 0.82 <sup>a</sup>	5.95 ± 0.82 <sup>a</sup>	6.00 ± 0.79 <sup>a</sup>
Odor	6.25 ± 0.91 <sup>a</sup>	6.25 ± 1.02 <sup>a</sup>	6.30 ± 0.80 <sup>a</sup>	6.25 ± 0.72 <sup>a</sup>
Texture	6.00 ± 0.73 <sup>a</sup>	5.85 ± 0.99 <sup>a</sup>	5.95 ± 0.76 <sup>a</sup>	5.90 ± 0.85 <sup>a</sup>
Flavor	5.90 ± 1.17 <sup>a</sup>	4.40 ± 0.99 <sup>b</sup>	5.35 ± 1.39 <sup>ab</sup>	5.55 ± 1.32 <sup>a</sup>
Overall acceptability	6.15 ± 0.74 <sup>a</sup>	4.65 ± 1.31 <sup>b</sup>	5.65 ± 1.04 <sup>a</sup>	5.75 ± 0.91 <sup>a</sup>

BHT, butylated hydroxytoluene; CE, quercetin equivalent.

<sup>a,b</sup> Means sharing different letters in the same row are significantly different ( $p < 0.05$ ).

<sup>1)</sup> All values were expressed as mean ± standard deviation.

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