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Research article

Physicochemical and antifungal properties of pyroligneous acid derived from macadamia (*Macadamia integrifolia*) nutshell and its application

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Abstract

Importance of the work: Macadamia nutshell (MNS) can be used as a bio-based fungicide, offering a natural alternative to synthetic chemicals and reducing waste.

Objectives: to determine the physicochemical properties and antifungal activities of pyroligneous acid (PA) extracted from MNS, to compare refinement methods and to assess its mango preservation efficacy.

Materials and Methods: MNS was pyrolyzed at 600-700° C to extract PA, which was then distilled, purified and analyzed for yield, specific gravity, tar, solids, color, volatile compounds and antioxidant activity. The antifungal activity of the PA was assessed *in vitro* and on mangoes to evaluate its effectiveness in disease control and fruit quality.

Results: Distillation-refined PA from MNS had superior properties compared to naturally refined PA, with an 87.55% yield, lower tar content (0.16%), higher antioxidant activity, a lower pH (2.33), higher phenolic content (138.7 g gallic acid equivalent/L) and reduced total soluble solids (3.70% Brix). *In-vitro* analysis showed effective antifungal activity against the tested fungi with complete inhibition of *Colletotrichum gloeosporioides* and *Colletotrichum musae* at 1.5%. Combining 1.5% PA with 1% chitosan significantly enhanced the postharvest quality of Nam Dok Mai Sithong mangoes, reducing decay and weight loss while maintaining firmness, indicating a synergistic effect in preserving fruit quality.

Main finding: Distillation-refined MNS PA had strong antioxidant and antimicrobial properties. PA at 1.5% concentration effectively inhibited *Colletotrichum* spp. and improved the postharvest quality of Nam Dok Mai Sithong mangoes, highlighting the potential use of PA as an eco-friendly biocide.

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Introduction

The global production of macadamia nuts has witnessed a dramatic increase in recent years (Tan et al., 2020). Approximately 80% of the nut is made up of unwanted parts, such as the hard shell and outer husk, which pose major waste management challenges and can harm the environment and human health (Poinerna et al., 2011). Some studies have explored various alternative methods for converting macadamia byproducts into valuable products such as fuels, organic fertilizers, biochar and activated carbon (Cox et al., 2004; Fan et al., 2018; Nekhavhambe et al., 2022). Pyrolysis, a process involving the thermal degradation of biomass in the absence of oxygen, has shown promise in managing these wastes by producing tar, solid char, gas and wood pyrolysis liquid (Aguirre et al., 2020; Heo et al., 2010; Tiilikkala et al., 2010). *Colletotrichum*, *Lasiodiplodia* and *Aspergillus* are common pathogens affecting tropical and subtropical fruits. Nam Dok Mai Si Thong, a prized Thai variety, faces challenges from mango anthracnose caused by *Colletotrichum gloeosporioides* that has severely constrained commercializing high-quality mango fruit as this disease often remains latent until fruit ripening (Siddiqui & Ali, 2014). Synthetic fungicides commonly used to address this postharvest disease are increasingly losing their effectiveness due to the development of resistance, along with rising consumer concerns about their environmental and health impacts (Arauz, 2000; Bluma et al., 2008; Radojević et al., 2011). This has led to a growing interest in the use of plant-based alternatives such as pyroligneous acid (PA) from wood and fruit shells, with this chemical containing 200 natural compounds, with a substantial concentration of phenolic compounds known for their antimicrobial properties (Ninomiya et al., 2004; Lee et al., 2010). While PA extraction from other nut shells has been studied, research on MNS is limited. Additionally, there has been no published research on comparing naturally refined and distillation-refined PA or on using PA combined with chitosan for fruit preservation. Therefore, the aims of the current study were: to assess the physicochemical properties of PA from MNS; to compare PA refining methods; to evaluate the *in vitro* antimicrobial activity of PA against postharvest pathogens such *as Colletotrichum gloeosporioides*, *Colletotrichum musae*, *Lasiodiplodia theobromae* and *Aspergillus niger*; and to test PA combined with chitosan for extending the shelf life of Nam Dok Mai Si Thong mango.

Materials and Methods

Plant materials and chemicals

MNS samples were collected from Rai Mae Khao Mao 2, Mae Suai district, Chiang Rai province, Thailand. Analytical reagents included Folin-Ciocalteu reagent, gallic acid, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride, sodium hydroxide, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), ferrous sulfate, Trolox, sodium acetate and hydrochloric acid. Food-grade chitosan (91.4% deacetylated, viscosity ≤ 300 mPa s) was sourced from Sinudin Agriculture Products Ltd., Thailand. Pathogens (*C. gloeosporioides*, *C. musae*, *L. theobromae* and *A. niger*) were cultured on potato dextrose agar (PDA) for 7-9 d. Mangoes (*Mangifera indica* L. cv. Nam Dok Mai Si Thong), harvested 110 d post-bloom, were selected, surface sterilized with 0.5% sodium hypochlorite and used for analysis.

Preparation of crude pyroligneous acid

Crude PA was extracted according to Theapparat et al. (2018). A sample (20 kg) of dried MNS was heated to 600–700°C in a 200 L kiln and the condensed smoke was collected and stored in a light-protected container at ambient temperature. The crude extract was purified based on distillation at Mae Fah Luang University, Thailand, heated to 95 ± 2°C and filtered to separate the oil from the PA. The purified PA was stored at 17°C in light-protected containers and compared with a naturally extracted PA sample stored for 6 mth.

Determination of yield and physicochemical properties of pyroligneous acid

The yield of the refined-PA was compared to the original volume of the crude PA prior to purification based on equations 1 and 2:

$$\text{Crude PA yield (\% wt)} = \frac{m_{crude\ PA}}{m_{feedstock}} \times 100 \quad (1)$$

$$\text{Purified PA yield (\% wt)} = \frac{\text{Purified wood vinegar volume}}{\text{Wood vinegar volume}} \times 100 \quad (2)$$

where $m_{crude\ PA}$ is the mass (measured in grams) of crude PA produced and, is the mass (in grams) of MNS (feedstocks) used.

Specific gravity

The AOAC (2000) official method 920.212 was used to compare the weight of an equal volume of the sample (PA) and water in a standardized pycnometer at 25°C, using equation 3:

$$\text{Specific gravity (SG)} = \frac{W1 - W2}{W3 - W2} \quad (3)$$

where W1 is the weight of the sample-filled pycnometer, W2 is the weight of the empty pycnometer and W3 is the weight of the water-filled pycnometer, with all weights measured in grams.

Total soluble tar, total soluble solids, titratable acidity and color

The total soluble tar (TST) content was measured according to the method of Theapparat et al. (2018). In brief, a 0.5 g sample was heated at 105°C overnight and the residue was analyzed for tar content. The total soluble solids (TSS) were measured using a digital refractometer (Brix range: 0-90%; Atago PAL-BX/Acid F5; Tokyo, Japan) according to Theapparat et al. (2014), without dilution. The titratable acidity (TA) was determined following the AOAC (2000) method 942.15. The PA color was assessed using a ColorQuest XE colorimeter (Hunter Associates Laboratory; Reston, VA, USA) according to Brochier et al. (2018), using the CIELAB system and hue angle (h°) for comparison.

Volatile compounds

The chemical components of the PA were analyzed using gas chromatography-mass spectrometry (GC-MS; Agilent 6890N and 5973A; Santa Clara, CA, USA) according to the method of Yang et al. (2016). The mass spectra were compared to the NIST database (MS 20.0; Gaithersburg, MD, USA) for identification. Results were valid if constituent matches were over 80%.

Total phenolic content of pyroligneous acid

The total phenolic content (TPC) was measured using a modified Folin-Ciocalteu method (Senter et al., 1989; Oramahi et al., 2018). PA (1 mL) was diluted 100-fold and mixed with Na_2CO_3 and Folin-Ciocalteu reagent. Absorbance was read at 750 nm, with gallic acid as the standard. The TPC, expressed as grams of gallic acid equivalent (g GAE) per liter, was calculated from a calibration curve obtained using equation 4:

$$\text{TPC} = \frac{\text{(Concentration from standard curve} \times \text{Extract volume} \times \text{Dilution factor)}}{\text{(Weight of sample} \times 1{,}000)} \quad (4)$$

Antioxidant capacity of pyroligneous acid

The DPPH radical scavenging ability of the PA was assessed following the methods of Brand-Williams et al. (1995) and Xiao et al. (2020), with slight modifications. Various concentrations of PA (20–120 µL/mL) were added to separate 60 mM DPPH solutions and incubated for 30 min in darkness. Absorbance was measured at 515 nm using a microplate spectrophotometer (Thermo Fisher Scientific; Multiskan GO; Waltham, MA, USA), with ascorbic acid (AA) as the standard. The inhibition percentage was calculated using equation 5:

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_S) / A_0 \times 100 \quad (5)$$

where A_O is the absorbance of the control (no sample, DPPH solution only) and A_S is the absorbance in the presence of the sample. The radical scavenging activity of the PA was expressed in milligrams per liter, as the concentration needed to scavenge 50% of DPPH radicals (IC_{50}) at 515 nm.

The ferric reducing power ability (FRAP) assay (Benzie and Strain, 1999) involved preparing a reagent from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ (25:2.5:2.5, v/v/v) and then warming to 37°C. PA (10 µL, diluted 10-fold) was mixed with 1.990 mL of the FRAP reagent and incubated at 37°C for 30 min. Absorbance at 593 nm was measured, using ferrous sulfate as the standard. The results, expressed as grams of ferrous sulfate equivalent per liter of sample (g $FeSO_4$/L sample), were obtained in triplicate.

In vitro antifungal activity of pyroligneous acid

The antifungal activity of PA was evaluated against *C. gloeosporioides*, *C. musae*, *L. theobromae* and *A. niger* using the poison food technique (Saberi et al., 2015; Rahmat et al., 2020). PA concentrations of 0.5% volume per volume (v/v), 1.0% (v/v), 1.5% (v/v) and 2.0% (v/v) were mixed with molten PDA. Chitosan (1%) was used in combination with PA (1.5%). Fungal inoculum (5 mm) was placed in Petri dishes with treatments and incubated at 25 ± 2°C for 10 d. The negative controls were untreated PDA and the positive controls were 0.1% carbendazim. Fungistatic activity (FA) was calculated using equation 6:

$$\text{FA} = 1 - \frac{CT}{CC} \times 100 \quad (6)$$

where CT is the colony diameter in the treatment and CC is the colony diameter in control (both measured in millimeters).

Antifungal activity on postharvest Nam Dok Mai Sithong mango

The treatments consisted of: 1) negative control; 2) 1.5% PA; 3) 1% chitosan; 4) 1.5% PA + 1% chitosan; and 5) positive control (0.1% carbendazim). The 1.5% PA concentration was selected based on effective *in vitro* results. Chitosan (1%) was prepared by dissolving 1 g chitosan in 100 mL of 1.0% glacial acetic acid, stirring overnight and adjusting the pH to 5.6 with 1 M NaOH. Treatments were applied according to the procedure described by Shi et al. (2018): (1) control, fruits dipped in sterile distilled water for 10 min; (2) 1.5% PA, fruits dipped in 1.5 mL/100 mL (v/v); (3) chitosan (1%), fruits dipped in 10 g/L (w/v) chitosan for 10 min; (4) PA (1.5%) + chitosan (1%), fruits dipped in 1.5% PA for 5 min, air-dried for 1 h and then dipped in 10 g/L chitosan for 5 min; (5) 0.1% carbendazim, Fruits dipped in 100 µL/100 mL (v/v). All fruit samples were air-dried and stored at 13 ± 2°C and 85–95% relative humidity for 20 d with sampling every 5 d. The trial was repeated twice. Disease incidence was assessed every 5 d during storage by calculating the number of infected fruits showing symptoms among the total sampled fruits (Ogbo and Oyibo, 2008) using Equation 7:

$$\text{Disease incidence (\%)} = \frac{\textit{Number of infected fruits}}{\textit{Total number of fruit assessed}} \times 100 \quad (7)$$

Disease severity in the naturally infected mangoes was evaluated by measuring decayed areas using a scale of 1–4 (Klangmuang and Sothornvit, 2018): 1 for no symptoms; 2 for black spot area < 40 mm²; 3 for black spot area 40-60 mm²; and 4 for black spot area > 60 mm². Observations were made every 5 d on 25 fruits per treatment.

Effect of pyroligneous acid on physicochemical qualities of Nam Dok Mai Sithong mango

The mango peel color was measured using a reflectance spectrophotometer (CM-600d; Konica Minolta; Tokyo, Japan), following the method of Peralta-Ruiz (2021), with the hue angle (h^0) used for color analysis. Fruit firmness was assessed using a texture analyzer (TA-XT plus; Stable Micro Systems; Godalming, UK) according to the method of Klangmuang and Sothornvit (2018). Five fruits per treatment were randomly selected for firmness measurement at each sampling, with average values recorded. Weight loss was determined by weighing the same marked mangoes (five per treatment) from the start to the end of storage using a digital balance (PB3002-S / FACT; Mettler Toledo Inc.; Columbus, OH,, USA), using Equation 8:

$$WL = \frac{IW - FW}{IW} \times 100 \quad (8)$$

where WL is the weight loss as a percentage, IW is the initial weight in grams of the mango and FW is the final weight in grams of the mango on the sampling date.

The TSS and TA were analyzed following the method of Srisawat et al. (2022). The TSS was measured in undiluted juice using a portable Brix-Acidity Meter (Model PAL-BX/ACID1; ATAGO; Tokyo, Japan), calibrated with distilled water. For determination of the TA, juice was diluted 1:50 with distilled water and the results were expressed as a percentage.

Statistical analysis

A completely randomized design with three replications was used. PA properties were analyzed using t-tests. The SPSS software (Version 20; IBM Corp.; Armonk, NY, USA) was used for the analysis of variance and data were expressed as mean ± SD values, with Tukey's honest significant difference test ($p < 0.05$) for treatment differences.

Results and Discussion

Yield and physicochemical properties of refined pyroligneous acid

Table 1 summarizes the physicochemical properties of the refined PAs. The yield of PA from distillation (87.55%) was similar to that of the naturally extracted PA (88.16%),

Table 1 Physicochemical properties of refined pyroligneous acid (PA) obtained using distillation and naturally

PA sample	Yield (ns)	Color (h°)	pH	TAC (%/weight) (ns)	SG	TST (%/weight)	TSS (°Brix)
Distillation-refined PA	87.55 ± 0.40	108.96 ± 0.96^b	2.33 ± 0.01^b	5.44 ±1.05	1.01 ±0.00^b	0.27 ± 0.21^b	3.70 ± 0.22^b
Naturally refined PA	88.16 ± 0.14	305.64 ± 2.07^a	3.52 ± 0.01^a	5.08 ± 0.09	1.02 ± 0.00^a	2.13 ± 0.05^a	6.70 ± 0.00^a

TAC = titratable acid content; SG = specific gravity; TST = total soluble tar; TSS = total soluble solid; ns = not significantly different.
Data analyzed using t test and shown as mean ± SD values from triplicate analysis. Means with different lowercase letters in same column indicate significant ($p < 0.05$) differences among parameters.

with no significant difference. The ColorQuest XE colorimeter analysis revealed significant differences, with the distillation-refined PA having a hue angle of 108.96°, indicating a yellow-green hue due to the distillation process, while the naturally refined PA had a hue angle of 305.64°, suggesting varied chemical compositions. The lower pH of the distillation-refined PA (2.33) was attributed to heat-induced water loss and the concentration of phenolic components (Ratanapisit et al., 2009). The distillation-refined PA had a slightly higher TAC (5.44) than the naturally refined PA (5.15). This inverse relationship between the pH and organic acid content reflected the complex interactions in the pyrolyzates (Mun et al., 2007). The SG values were 1.01 for the distilled PA and 1.02 for the naturally refined PA, indicating differences in the tar and organic acid content (Wada, 1997; Mun et al., 2007). The distillation-refined PA had a lower total soluble tar content (0.16) than the naturally refined PA (2.13). The TSS were 3.70% for the distillation-refined PA and 6.70% for the naturally refined PA, indicating that the distillation had effectively removed unwanted tar and inorganic solids.

Volatile compounds

There was a subtle difference between the compositions of the distillation-refined and the naturally refined PA. Table 2 shows the GC-MS chromatogram peak areas as a percentage of the total area, reflecting relative quantities (w/w). For the distillation-refined PA, 91.79% of the peaks were identified, with 20 components, predominantly phenolic compounds (64.26%). In contrast, for the naturally refined PA 82.25% of the peaks were identified, with 18 components, including 49.0% phenolics. The higher phenolic content in the distillation-refined PA was likely due to the extraction techniques used. The MNS, with a lignin content of about 47.6% (Wechsler et al., 2013), contributed to the higher phenolic yields in the refined PA, as confirmed by the GC-MS analysis. Both PAs contained G-phenols, with 2-methoxyphenol (guaiacol) as a major component. The absence of anhydro sugars in the distillation-refined PA may have resulted from sugar degradation during distillation (Mora et al., 2022), while the catechol in the naturally refined PA likely came from the demethylation of guaiacol (van Bergen et al., 2000). Few organic acids were detected in the refined PA, differing from other sources where acetic acid is more common, likely due to the low hemicellulose content (11.7%) in the MNS (Toles et al., 1998) and the high pyrolysis temperatures (500-600°C). The distillation-refined PA contained organic acids, such as methylenecyclopropane carboxylic acid and 2-pyridinecarboxylic acid (6-methyl), indicating high acidity. Small amounts of furans from holocellulose decomposition and minimal esters due to cross-linking during pyrolysis (Worasuwannarak et al., 2007) were also observed.

Table 2 Comparative analysis of chemical compositions in distillation-refined and naturally refined pyroligneous acid (PA) identified based on gas chromatography-electron microscopy

Grouping	Chemical compound	Relative quantity (% weight per weight)	
		Distillation-refined PA	Naturally refined PA
Ketones			
	1-Penten-3-one	0.30	ND
	2-Cyclopenten-1-one, 2-methyl	1.24	ND
	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	ND	6.09
	∑Ketones (%)	1.54	6.09
Esters			
	2-Furanmethanediol, dipropionate	0.08	ND
Acids			
	1-Methylcyclopropanecarboxylic acid	2.39	ND
	2-Pyridinecarboxylic acid, 6-methyl-	0.09	ND
	Pentanoic acid	0.13	ND
	∑Acids (%)	2.61	ND
Furan and pyran derivatives			
	3-Furaldehyde;	22.13	ND
	Furfural	ND	8.99
	2-Furanmethanol	ND	2.14
	2-Furancarboxaldehyde, 5-methyl-	ND	3.76
	1-(2-Furanyl)-ethanone	0.98	ND
	Maltol	ND	2.00
	3-Methyl-3-butene-1,2-diol;	0.47	ND
	∑Furan and pyran derivatives (%)	23.58	16.91

Table 2 Comtinued

Grouping	Chemical compound	Relative quantity (% weight per weight)	
		Distillation-refined PA	Naturally refined PA
Heterocyclic compounds			
	Pyridine	ND	0.99
	Pyridine, 2-methyl-	ND	0.47
	∑Heterocyclic compound (%)	ND	1.45
Hydrazide			
	2-Furancarboxylic acid, hydrazide;	0.09	ND
Phenols and derivatives			
	Phenol	4.53	4.45
	Creosol	19.12	12.46
	Catechol/ 1,2-Benzenediol	ND	3.22
	Phenol, 2-methyl-; (o-Cresol)	2.12	1.35
	(p-Cresol;	3.95	2.97
	(Phenol, 2-methoxy-;	28.15	15.52
	Benzaldehyde, 3-hydroxy-4-methoxy-/ Isovanillin	ND	0.97
	Phenol, 3,4-dimethyl-(para cresols)	0.38	ND
	Acetovanillone/ 1-(4-Hydroxy-3-methoxyphenyl)-ethanone	ND	1.67
	Phenol, 4-methoxy-3-methyl-;	0.49	ND
	(Phenol, 4-ethyl-2-methoxy-;	5.07	4.12
	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	ND	2.27
	∑Phenol and derivatives (%)	63.79	49.00
Sugar			
	β-D-Glucopyranose, 1,6-anhydro-	ND	8.80
Other compounds			
	1,3-Dimethyl-3-propylazetidine-2,4-dione;	0.05	ND
	(2S,6R,7S,8E) -(+)-2,7-Epoxy-4,8-megastigmadiene;	0.06	ND
	∑Other compounds (%)	0.11	ND

ND = not detected.

Total phenolic content of pyroligneous acid

The TPC of the refined PA derived from MNS (Table 3) was notably higher than in other reported plant sources (Yang et al., 2016; Mathew et al., 2015). The distillation-refined PA had a phenolic content of 138.70 g GAE/L PA, compared to 103.0 g GAE/L PA in the naturally refined PA. This increase may have been due to differences in the extraction techniques, as discussed in Section 3.3. Phenolic compounds are primarily produced through lignin degradation at temperatures between 280 and 500°C (Kartal et al., 2004; Chen et al., 2012). Reheating the crude PA at 95 ± 2°C during distillation likely broke down the weak lignin bonds from the initial pyrolysis, a finding supported by Ratanapisit et al. (2009), who highlighted the significant influence of temperature and heating rate on phenolic production during crude PA distillation.

Table 3 Total phenolic content and radical scavenging activity of refined pyroligneous acid (PA)

Sample	TPC g GAE/L	DPPH (IC_{50}) mg/L	FRAP (gM $FeSO_4$/ L sample)
Distillation-refined PA	138.7 ± 4.41	7.3 ± 1.91	1320.9 ± 4.84
Naturally refined PA	103.0 ± 2.76	15.4 ± 0.95	1015.0 ± 4.28
Ascorbic acid standard	-	48.3 ± 9.79	-

TPC = total phenolic content; DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = ferric reducing power ability; IC_{50} = concentration needed to scavenge 50% of DPPH radicals.
Data are presented as mean ± SD values from triplicate analysis.

Determination of antioxidant activity of pyroligneous acid

The distillation-refined PA had superior antioxidant activity with a significantly lower IC_{50} value of 7.3 mg/L than the 15.4 mg/L for the naturally refined PA. (Table 3) In addition, the distillation-refined PA had greater reducing power, with 1,320.9 gM $FeSO_4$/L, while naturally-refined PA had a reducing power of 1,015 gM $FeSO_4$/L. The higher antioxidant capacity of the distillation-refined PA was likely due to its greater content of phenolic compounds (Loo et al., 2007).

In vitro antifungal activity of pyroligneous acid

Significant fungal growth inhibition was observed at various PA concentrations (0.5%, 1.0%, 1.5%, 2%), chitosan (1%), a combination of PA (1.5%) + chitosan (1%), as well as for carbendazim (0.1%) compared to the negative control (Table 4). PA at 1.5% completely inhibited *C. gloeosporioides* and *C. musae*, while higher concentrations produced increased inhibition of *A. niger*. *L. theobromae* was only fully inhibited at 2%. PA's effectiveness was attributed to its phenolic components and aldehydes (Liu et al., 2008; Ma et al., 2011). Additionally, its low pH might have played a role as an antimicrobial factor. Chitosan has been reported to affect fungal growth by disrupting cell wall morphogenesis (Kalagatur et al., 2018; (Lo et al., 2020). The combination of PA (1.5%) + chitosan (1%) resulted in complete mycelial inhibition. Carbendazim (0.1%) partially inhibited *C. gloeosporioides* by 61.25% but was more effective against the other fungi, indicating possible resistant strains (Ma and Michailides, 2005).

Disease incidence and severity caused by natural infection

The PA and chitosan coatings significantly reduced mango postharvest decay compared to uncoated fruits. The initial signs of decay emerged at around 10 d for both the uncoated and the 0.1% carbendazim-dipped mangoes, while PA (1.5%) effectively prevented fungal infection. The PA (1.5%) application effectively prevented fungal infection on fruits until 10 d (Fig. 1a), owing to its phenolic, aldehyde and alcohol compounds, which have been recognized for their antioxidant and antimicrobial properties (Nomura, 2004). In contrast to the uncoated fruits, all the other coating treatments helped maintain quality, with disease severity scores below the acceptable limit of 3 (Fig. 1b). Minor blemishes on the PA-coated fruits after 15 d were likely the result of volatilization of chemical components. Reapplying PA after 10 d might have sustained its efficacy. Chitosan alone provided a 40% reduction in disease severity; combining it with PA enhanced efficacy to 45%, offering broader protection and multifunctional preservation. This synergy arose from PA's antifungal and antioxidant properties complementing chitosan's protective coating. The observed low antifungal activity of 0.1% carbendazim aligned with the *in vitro* results.

Table 4 In vitro antifungal activity of pyroligneous acid (PA) against phytopathogens

Treatment	% Growth inhibition			
	Colletotrichum gloeosporioides	*Colletotrichum musae*	*Aspergillus niger*	*Lasiodiplodia theobromae*
Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.300^{e}	0.00 ± 0.00^{c}
0.5% PA	28.75 ± 3.31^{d}	22.22 ± 0.00^{c}	44.07 ± 0.64^{d}	0.00 ± 0.00^{c}
1% PA	37.50 ± 0.00^{c}	56.30 ± 1.28^{b}	50.00 ± 1.11^{c}	0.00 ± 0.00^{c}
1.5% PA	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	55.56 ± 0.00^{b}	34.82 ± 1.29^{b}
2% PA	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	56.67 ± 1.11^{b}	100 ± 0.00^{a}
Chitosan (1%)	100.00 ± 0.00^{a}	NT	NT	NT
PA (1.5%) + chitosan (1%)	100.00 ± 0.00^{a}	NT	NT	NT
0.1% Carbendazim (Positive control)	61.25 ± 1.25^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100 ± 0.00^{a}

NT = not tested.

Data presented as mean ± SD (n = 5). Values in same column with different lowercase superscripts indicate significant ($p < 0.05$) differences among parameters based on Tukey's honest significant difference test.

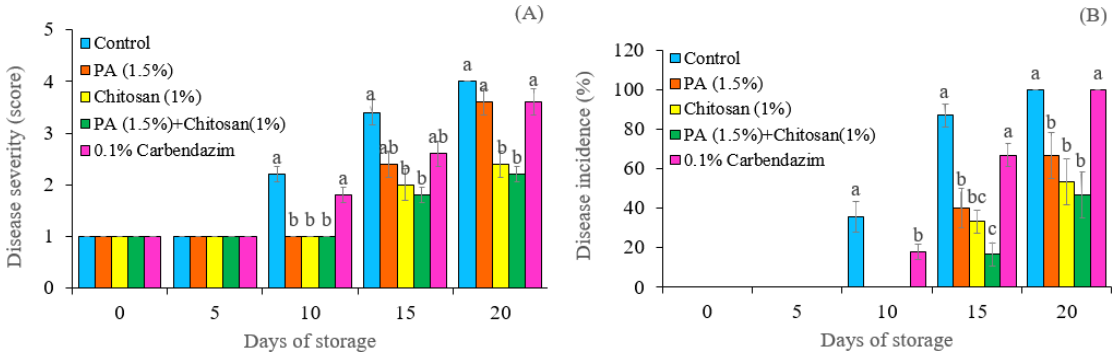

Fig. 1 Effect of pyroligneous acid (PA) on: (A) disease incidence; (B) disease severity of Nam Dok Mai Sithong mango fruit during storage at 13°C for 20 d, where each data point represents mean ± SD (n = 5) and different lowercase letters represent significant ($p < 0.05$) differences

Effect of pyroligneous acid on postharvest qualities of Nam Dok Mai Sithong mango

The effect of PA on the postharvest qualities of Nam Dok Mai Sithong mangoes was assessed using the 20 d data (Table 5). All treatments showed a steady decrease in h^0, likely due to increased carotenoid levels (Klangmuang & Sothornvit, 2022; Srisawat et al., 2022). The combination of 1.5% PA with 1% chitosan, or the 1% chitosan alone, significantly slowed this decline. Treated fruits were significantly firmer (0.39 to 0.70 N) than the untreated control (0.18 N). The PA-coated mangoes had about 10% lower weight loss ($p < 0.05$), attributed to the PA's antioxidant properties (Liu et al., 2020). The PA (1.5%) + chitosan (1%) combination produced the greatest reduction in weight loss. The TSS content increased variably, with the lowest TSS in fruits treated with PA (1.5%) + chitosan (1%) (18.3 ± 0.05), followed by chitosan (1%) (19.1 ± 0.21), PA (1.5%) (19.3 ± 0.30%), fungicide (0.1% carbendazim) (20.2 ± 1.14) and the control (21.1 ± 0.82). The TA decreased in all treatments due to acid reduction or conversion to sugars (Khosroshahi et al., 2007); however, the PA (1.5%) + chitosan (1%) delayed this decrease. At the end of storage, the TA values were 0.68 ± 0.01 for PA (1.5%), 0.73 ± 0.04 for chitosan (1%), 0.78 ± 0.01 for the combination and 0.68 ± 0.02 for the fungicide, compared to 0.61 ± 0.01 in the control. The distillation-refined PA from MNS had antioxidant and antimicrobial properties. Combining PA with chitosan further improved postharvest mango quality, highlighting its potential use as an eco-friendly biocide.

Conclusion

Based on the study results, the distillation-refined PA derived from macadamia nutshells had significant antioxidant and antimicrobial properties. Specifically, PA had strong inhibition of *Colletotrichum* spp. and partial inhibition of *Aspergillus niger* and *Lasiodiplodia theobromae in vitro*. Tests on stored mangoes indicated that PA delayed metabolic processes. When combined with chitosan, the efficacy of the PA improved, providing broader protection and sustained quality preservation. These findings underscored the potential of PA as an eco-friendly and cost-effective solution for postharvest mango preservation. Integrating it with chitosan could be a promising approach to extending the shelf life of mangoes, contributing to food security, reducing agricultural waste and offering an alternative to conventional chemical treatments. The current study should provide a robust foundation for future studies exploring this synergistic effect across different pathogens and fruit varieties.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Table 5 Effect of pyroligneous acid (PA) on postharvest quality of Nam Dok Mai Sithong mango after 20 d of storage at 13°C

Treatment	Parameter				
	Color (h°)	Firmness (N)	Weight loss (%)	TSS (°Brix)	TA (%)
Control	69.2 ± 1.49^{c}	0.18 ± .07^{b}	18.5 ± 1.95^{a}	21.1 ± 0.82^{a}	0.61 ± 0.00^{c}
PA (1.5%)	74.2 ± 2.10^{abc}	0.59 ± .13^{a}	9.1 ± 1.15^{bc}	19.3 ± 0.30^{ab}	0.68 ± 0.01^{b}
Chitosan (1%)	76.5 ± 0.96^{ab}	0.69 ± .19^{a}	7.6 ± 0.76^{c}	19.1 ± 0.21^{b}	0.73 ± 0.04^{ab}
PA (1.5%) + chitosan (1%)	77.6 ±1.24^{a}	0.70 ± 0.17^{a}	6.7 ± 0.53^{c}	18.3 ± 0.05^{b}	0.78 ± 0.01^{a}
Carbendazim (0.1%)	70.1 ±1.43^{bc}	0.39 ± .04^{ab}	11.8 ± 2.04^{b}	20.2 ± 1.14^{ab}	0.68 ± 0.02^{b}

TSS = total soluble solid, TA = titratable acidity

Data are presented as mean ± SD (n = 5). Means with different letters in same column indicate significant differences among parameters at $p < 0.05$.

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