**Short Communication:**

# Control of anthracnose in "Kluai Hom Tong" banana (*Musa* (AAA group)) using mangosteen pericarp crude extract

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## ABSTRACT

Anthracnose is a postharvest disease caused by the fungus *Colletotrichum musae* that results in major economic losses during transportation and storage of bananas. Mangosteen fruit has been widely used as traditional medicine and also as an antimicrobial agent against plant pathogenic fungi. The effectiveness of mangosteen pericarp crude extract on controlling anthracnose in "Kluai Hom Tong" banana (*Musa* (AAA group)) was evaluated. The fruits were dipped in various concentrations (0, 1,000, 1,200 and 1,400 mg $L^{-1}$) of mangosteen pericarp methanol crude extract solution for 2 min and then sprayed with 1 mL of $10^6$ spores $mL^{-1}$ of *Colletotrichum musae.* Incubated fruits at 29 ± 2°C for 2 days and stored at 13 ± 2°C for 14 days. Banana fruits were further directly sprayed with 1 mL of 500 mg $L^{-1}$ of ethephon solution and incubated for one day at room temperature. Anthracnose disease percentage and quality of banana fruits as total soluble solids (TSS), titratable acid (%TA), fresh weight and color were recorded. Color measurements were recorded 2 days interval. The results showed that mangosteen pericarp crude extract treatment demonstrated a lower disease percentage than non-treated fruits and the lowest disease percentage was found in the 1,200 mg $L^{-1}$ treatment at 45% with 8 days of shelf life.

**Keywords:** Mangosteen, *Musa* (AAA group), postharvest disease, *Colletotrichum musae*

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## INTRODUCTION

Bananas are an important agricultural commodity in Thailand and are grown throughout the country (National Committee on Agricultural Commodity and Food Standards, 2008). The main variety of bananas is the "Hom" banana, especially the "Hom Thong" variety (Anuchai *et al*., 2018). In 2012, Thailand produced "Kluai Hom Tong" banana (*Musa* (AAA group)) at 231,031 Mt for domestic use and 2,169 Mt for export (OAE, 2012). Worldwide, postharvest losses of fruits and vegetables could average about 45% (Kitinoja and Kader, 2015). One limiting factor that influences the economic value of banana is its relatively short shelf-life caused by postharvest pathogen attack (Kuyu and Tola, 2018). The anthracnose is a postharvest disease of banana that can result in 30–40% losses of marketable fruit during transportation and storage (Ranasinghe *et al*., 2003). Anthracnose is caused by the fungus *Colletotrichum musae* (Jeffries *et al*., 1990). Antimicrobial activities in plant extracts occur in both *in vitro* and *in vivo* fungal development. Cruz *et al*. (2013) discovered that extracts from *Allium sativum*,

*Copaifera langsdorffii*, *Cinnamomum zeylanicum* and *Eugenia caryophyllata* gave effective control of postharvest anthracnose of banana fruit while Montri *et al*. (2010) found that *Stemona curtisii* Hook. f. crude extract inhibited the fungus *C. musae* and reduced anthracnose disease in "Kluai Hom Tong" banana.

Mangosteen is a tropical fruit from the family Guttiferae. Thailand has high annual mangosteen production at approximately three million Mt. Mangosteen pericarp is the largest part of the fruit, comprising 66.67% of the total fruit (Siripanich and Luckanatinvong, 1997). The pericarp is usually discarded as waste due to its unpleasant bitter taste (Pedraza–Chaverri *et al*., 2008). However, mangosteen pericarp has long been used as a traditional indigenous medicine across Southeast Asia, especially for anti–inflammatory (Chomnawang *et al*., 2005; Akao *et al*., 2008), anti-oxidant (Jung *et al*., 2006), anti–proliferative (Pedraza-Chaverri *et al*., 2008) and immunostimulatory biological activities (Matsumoto *et al*., 2003). It has also been used as an antimicrobial (Al–Massarani *et al*., 2013; Palakawong *et al*., 2013) against plant pathogenic fungi. Sangsuwan *et al*. (2011) reported that mangosteen pericarp powder inhibited anthracnose fungi in "Kluai Hom Tong" banana. The use of waste for the production of various crucial bioactive components is an important step toward sustainable development (Sagar *et al*., 2018). Here, the effectiveness of mangosteen pericarp crude extract was evaluated for the control of anthracnose to extend shelf life in free toxin "Kluai Hom Tong".

## MATERIALS AND METHODS

### Raw Material

Toxic-free "Kluai Hom Tong" banana (*Musa* (AAA group)) fruits at the 70% mature stage were purchased from Thung Kha Wat farmers, Lamae District, Chumphon Province to avoid bruising and injury from the harvesting process. The fruit bunches were de-handed and selected for fruit weight (110 ± 10 g) with uniformity of shape and size. The crown portions were wrapped up to the mid-portion of the hand with paper to prevent abrasion and latex staining of other fruits. Fruits were cleaned following the process to export free toxin "Kluai Hom Tong" bananas to Japan by removal of hull and dirt of sap, removal of insect, sap washing after cutting, cleaning the fruits with tap-water and air-drying at room temperature.

### Isolation of the Pathogen and Inoculum Preparation

Banana fruits showing anthracnose disease symptoms were collected from the "Kluai Hom Tong" banana orchard in Chumphon and used to isolate *Colletotrichum musae*. The pure culture was maintained on potato dextrose agar (PDA) medium. The inoculum was prepared by flooding the surface of 8 days old culture with sterile distilled water, scraping the surface gently with a glass rod. Spores were counted using a hemacytometer (Hausser Scientific, Horsham, PA, USA) and the spore concentration was adjusted to $10^6$ spores $mL^{-1}$.

### Mangosteen Pericarp Methanolic Crude Extract

Mangosteen fruits at maturity stage 4–5 were bought from a farmer in Lang Suan District, Chumphon Province, Thailand. Fruits were stored at the ambient temperature till the color turns to stage 6 or when the skin color turns to blackish purple. The crude extract was prepared by cutting the fruit pericarp into small pieces. Pieces of fruit pericarp were dried in a hot air oven at 60°C for 24 h, then ground by using a blender. The dried powder was macerated in 95% methanol for 72 h (3 times) and the extracted was concentrated by using a rotary evaporator. A stock solution of crude extract was prepared at the concentration of 10,000 mg $L^{-1}$ and kept in the refrigerator.

### Treatments and Applications of Mangosteen Pericarp Extract

The experiment was conducted in a completely randomized design (CRD) with 4 treatments and 10 replications. A cluster of three fingers was considered as a replicate. After

cleaning and drying, the fruits were dipped into various concentrations of mangosteen pericarp crude methanolic extract solutions at the concentrations of 0, 1,000, 1,200 and 1,400 mg $L^{-1}$ for 2 min then sprayed with 1 mL of $10^6$ spores $mL^{-1}$ of *Colletotrichum musae* spore suspension, incubated for 2 days at room temperature (29 ± 2°C) for fungal growth, and following the transportation process to Japan, the fruit clusters were stored in the control room at 13 ± 2°C for 14 days. Banana fruits were further directly sprayed with 1 mL of 500 mg $L^{-1}$ of ethephon solution and incubated for 1 day at room temperature. Anthracnose disease in each fruit was measured in 2 days interval for incidence and lesion. Quality of banana fruits as total soluble solids (TSS), titratable acid (%TA), fresh weight and color were recorded. The percentage of weight loss and disease incidence were calculated.

**Weight Loss (%)**

Banana fruits were weighed before and after storage at an interval of 2 days. The percentage of weight loss was calculated using the expression given below.

Weight loss (%) = Initial weight (g) – Weight (g) at sampling date / Initial weight (g) × 100

**Color Measurement**

Peel and pulp color of banana fruits were measured using a HunterLab MiniScan Photometer (MiniScan@ XE Plus; Hunter Associates Laboratory Inc.; Reston, VA, USA). The CIELAB color scale which is lightness (L*), greenness (–a*), redness (+a*) and yellowness (b*) values were recorded (Boun and Huxsoll, 1991). The color determination was performed at the top, middle and bottom regions from three different faces of an individual finger.

**Firmness Measurement**

Three fingers from each hand were used for firmness measurement. The peeled banana was placed in a Texture Analyzer (TA Plus; Lloyd Limited; Fareham, UK) with a 1 mm cylindrical probe at the middle part of the fruit. The firmness value was recorded in Newtons and reported as mean of three readings.

**Total Soluble Solids (%) and Total Acidity Measurements**

Ten grams of banana pulp tissue were homogenized with 30 mL of distilled water and filtered. Total soluble solids of banana fruit pulp were measured with a refractometer (Model no. ATC–1090, Atago Co., Ltd., Tokyo, Japan) with readings expressed as a percentage. Readings were recorded by pouring two drops of filtrate on the glass prism of the refractometer. Then, a 5 mL aliquot was titrated with 0.1 N NaOH using 1% (w/v) phenolphthalein as an indicator, the results were expressed as a percentage of malic acid in flesh pulp (AOAC, 1990).

**Statistical Analysis**

All data analyses were carried out in ten replications and results were submitted for analysis of variance (ANOVA) with a significance level of 95% ($P < 0.05$).

## RESULTS AND DISCUSSION

Results showed that mangosteen pericarp crude methanolic extracts had no effect on TSS, TA, pulp firmness and weight loss percentage, however represented potential inhibitory activity against *Colletotrichum musae* (Table 1). Lower disease incidence percentage and fewer disease lesions were achieved in treated fruits compared with non–treated fruits as control. The lowest disease incidence of 45% was found at the concentration of 1,200 mg $L^{-1}$ treatment. The disease incidence percentage was high when using a higher concentration of crude extract at the concentration of 1,400 mg $L^{-1}$ (Figure 1). The quality of ripening banana fruits was controlled by ethylene treatment in commercial practices. During banana ripening, peel color changes from green to yellow, the flavor develops and the pulp softens. However, the results showed that

non-treated fruits with shelf life of 4 days improved to 6 days for mangosteen pericarp methanolic crude extract treated fruits at the concentrations of 1,000–1,200 ppm (Figure 2). The quality of banana fruit also improved in peel firmness (Table 1) and peel color (Table 2) at the concentration of 1,000 mg $L^{-1}$ treatment, with 8 days of shelf life.

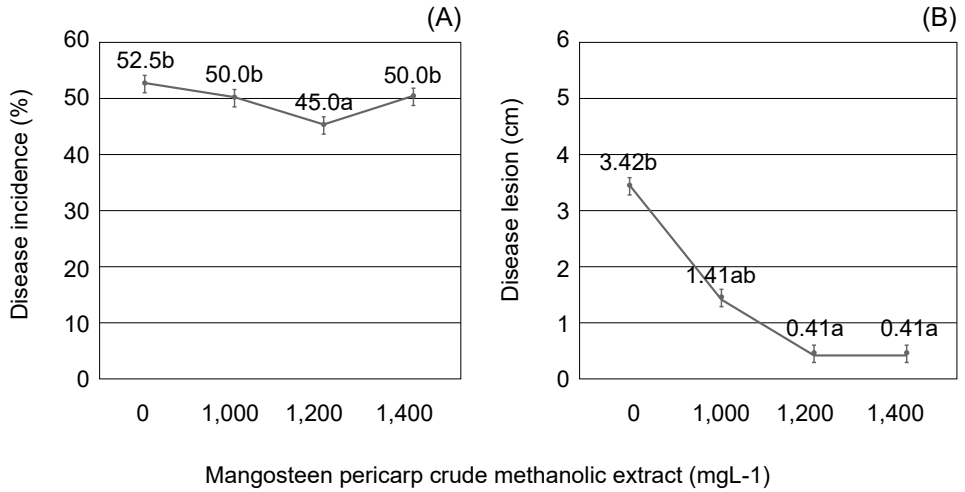


**Figure 1** Disease incidence percentage (A) and disease lesions (B) of treated banana fruits with various concentrations of mangosteen pericarp crude methanolic extract before spraying with spore suspension of *Colletotrichum musae* at 8 days of shelf life

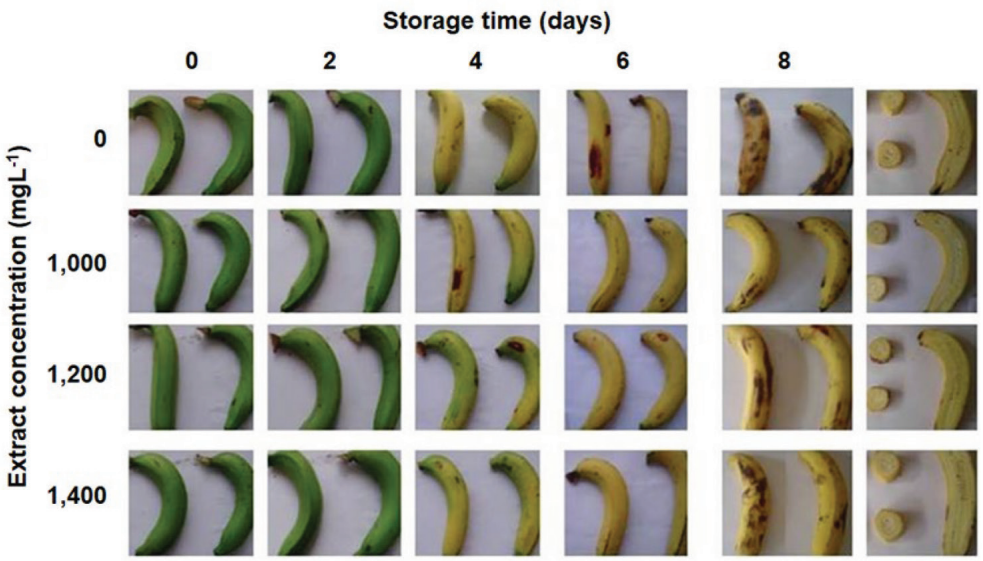


**Figure 2** External and internal appearances of banana fruits after soaking with different concentrations of mangosteen pericarp crude methanolic extract before spraying with spore suspension of *Colletotrichum musae*

Extracts from mangosteen peels contain various secondary metabolites such as polyphenols (Moosophin *et al*., 2010) and prenylated and oxygenated xanthones (Ji *et al*., 2007). Disease incidence and quality were improved by several active compounds contained in mangosteen peel extract with 95% ethanol as xanthones, flavonoids, tannins, terpenoids and saponins (Saepudin *et al*., 2019). Several studies showed that xanthones from mangosteen have antibacterial, antifungal and antiviral biological activities (Vishnu *et al*., 2010; Al-Massarani *et al*., 2013; Saepudin *et al*., 2019). Tannins and flavonoids are a class of complex molecules of polyphenols (Saepudin *et al*., 2019). They can slow down and inhibit the growth of many fungi, yeasts, bacteria and viruses (Kaka *et al*., 2019), while tannins inhibit the protein transport enzymes through cell membranes. Sangsuwan *et al*. (2011) reported that fruits coated with mangosteen pericarp powder inhibited anthracnose in "Kluai Hom Tong" banana (*Musa* (AAA group)). Moreover, antifungal activity of the extract from *Garcinia mangostana* against the three phytopathogenic fungi *Fusarium oxysporum*, *Alternaria tenuis* and *Drechslera oryzae* was reported by Gopalakrishnan *et al*. (1997). Our results indicated that mangosteen pericarp crude extract showing the effectiveness of disease control and fruit quality of "Kluai Hom Tong" banana (*Musa* (AAA group)). However, disease incidence percentage was higher when increased the concentrations of the crude extract due to the negative effect resulting from enhanced total polyphenols (Afifah and Niwat, 2015).

**Table 1** Total soluble solids (TSS), titratable acid (%TA), peel and pulp firmness and weight loss percentage of treated banana fruits with various concentrations of mangosteen pericarp crude methanolic extract before spraying with spore suspension of *Colletotrichum musae* at 8 days of shelf life

| Crude extract (mg $L^{-1}$) | TSS (%) | TA (%) | Peel firmness (N) | Pulp firmness (N) | Weight Loss (%) |
|---|---|---|---|---|---|
| 0 | 5.75 | 0.36 | 14.94[b] | 5.23 | 5.50 |
| 1,000 | 6.30 | 0.32 | 19.74[a] | 5.71 | 6.36 |
| 1,200 | 6.75 | 0.31 | 16.07[b] | 5.97 | 6.15 |
| 1,400 | 6.60 | 0.34 | 15.10[b] | 6.23 | 5.93 |
| F-test | ns | ns | * | ns | ns |
| CV (%) | 14.02 | 12.76 | 18.94 | 16.85 | 67.90 |

**Note:** CV = coefficient of variation, mean values in columns followed by the same small letter are not statistically different using Duncan's test at 5% probability, ns = non-significant, * $P < 0.05$

**Table 2** Peel and pulp color (L*, a* and b*) of treated banana fruits with various concentrations of mangosteen pericarp methanolic crude extract before spraying with spore suspension of *Colletotrichum musae* at 8 days of shelf life

| Crude extract (mg $L^{-1}$) | Peel color | | | Pulp color | | |
|---|---|---|---|---|---|---|
| | L* | a* | b* | L* | a* | b* |
| 0 | 63.68[a] | 43.97 | 10.73 | 75.73[a] | 26.65[b] | 2.21[b] |
| 1,000 | 52.00[b] | 35.24 | 11.69 | 75.38[a] | 26.81[b] | 2.47[ab] |
| 1,200 | 57.90[ab] | 38.69 | 10.97 | 73.05[b] | 30.60[a] | 2.84[a] |
| 1,400 | 53.03[b] | 35.84 | 11.27 | 76.07[a] | 23.64[c] | 1.96[b] |
| F-test | * | ns | ns | * | * | * |
| CV (%) | 14.75 | 20.91 | 11.46 | 1.35 | 8.86 | 20.01 |

**Note:** CV = coefficient of variation, mean values in columns followed by the same small letter are not statistically different using Duncan's test at 5% probability, ns = non-significant, * $P < 0.05$

## CONCLUSIONS

Our results suggested that crude methanolic extracts of mangosteen pericarp were effective for disease control of banana fruit. Treatments preserved acceptable and better peel color with a lower incidence of anthracnose disease.

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