Research article

Efficiency of Crude Extract from Pummelo Peel on Controlling the Growth of *Colletotrichum gloeosporioides* (Penz.)

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Received 15 December 2014 Accepted 1 April 2015 (*Corresponding Author)

Abstract The objective of this research was to investigate the antifungal efficiency of pummelo peel extracted with different solvents on *Colletotrichum gloeosporioides*. Crude extracts from pummelo peels were eluted with four solvents, such as ethanol, hexane, dichloromethane and ethyl acetate. Chemical compounds of the crude extracts were analyzed by GC-MS and HPLC and inhibition the growth of *C. gloeosporioides* was tested by PDA culture. The result showed that limonene was the major compound in all extracts (79.55-89.91%). α-Phellandrene and 2, 6-octadien-1-ol, 3, 7-dimethyl were found only in crude extracts using ethyl acetate as a solvent. In addition, the extract was eluted with ethyl acetate also had the amount of flavonoids: nobiletin and 3-hydroxy-7, 3', 4', 5'-tetramethoxyflavone, higher than those eluted with other solvents. Inhibition the growth of *C. gloeosporioides* of pummelo peel extracted with dichloromethane, ethanol, hexane and ethyl acetate were 19.16, 52.81, 65.58, and 95.61%, respectively. Therefore, pummelo peel extracted with ethyl acetate was the highest efficiency on controlling *C. gloeosporioides* ($p \leq 0.05$).

Keywords Crude extract, pummelo, *C.gloeosporioides*

INTRODUCTION

Mango is one of the most popular and best known tropical fruits (MacLeod and Troconis, 1982; MacLeod and Pieris, 1984). It is a highly priced fruit due to its attractive color, delicious taste, and high nutritional values (Mitra and Baldwin, 1997; Lalel et al., 2003). Traditionally, the major mango exporting countries are the Philippines, Thailand, Mexico, and India (Jacobi et al., 2001). Many different ripening conditions, postharvest treatments, and processing methods are used to prepare mangoes for marketing to consumers (Moore, 2003). Its short shelf life limits the commercialization at long distances. It is also highly susceptible to damages caused by fungi, bacteria and fruit fly larvae (Diaz-Sobac et al., 1996).

Anthracnose is a major pre- and post-harvest disease on mangoes, causing direct yield loss in the field and packing plant, and quality and marketing issues (Ploetz, n.d.).Mango anthracnose is

caused by the fungus *Colletotrichum gloeosporioides* (Pitkethley and Conde, 2007). Ripe fruits affected by anthracnose develop sunken, prominent, dark brown to black decay spots before or after picking. The fruit spots can usually do coalesce and can eventually penetrate deep into the fruit, resulting in extensive fruit rotting. Most green fruit infections remain latent and largely invisible until ripening (Nelson, 2008). Post-harvest treatments are available for control of anthracnose in mango fruits. Prochloraz is used as a cold nonrecirculating spray. Hot water dips, used to control fruit flies, will also control anthracnose and stem end rots. Hot benomyl dips will control anthracnose and are useful where stem end rots are a problem (Pitkethley and Conde, 2007). However, there are many studies using plant extracts such as essential oils and pure compounds against plant pathogenic fungi, which have been conducted. Antifungal compounds have been found in plants, derived from secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids (Arif et al., 2009).

Pummelo (*Citrus grandis* or *Citrus maxima*) is an original citrus fruit, with the look of a big grapefruit, native to South and Southeast Asia. Citrus fruits contain several bioactive compounds such as flavonoids, limonoids, and organic acid. Polymethoxyflavones are group of flavonoids that present mainly in the fruit peel. There are some reports that show polymethoxyflavones, which are important components of plant defensive mechanism against various diseases causing pathogens such as phytophthora and *Colletotrichum gloeosporioides* (Del Rio et al., 1998; Ortuño et al., 2006; Uckoo et al., 2011). Thus, this research aimed to investigate the major volatile compounds, flavonoids and polymethoxyflavones as well as the antifungal efficiency of crude extract from pummelo peel on *C. gloeosporioides*.

OBJECTIVES

The study was conducted with the following objectives:

1. To determine the major volatile compounds and flavonoids of crude extract from pummelo peel; and
2. To investigate the effect of crude extracts from pummelo peel on inhibition the growth of *Colletotrichum gloeosporioides*.

METHODOLOGY

Pummelo was purchased from a commercial market in Phathumtani province, Thailand. The flavedo of the fruit was cut into small pieces and then immersed with a solvent for 24 hours and the ratio of peel and solvent was 1:3. The four solvents were ethanol, dichloromethane, hexane and ethyl acetate which were used in this experiment. The extracts were pooled and evaporated at 40 ^{o}C using a rotary evaporator. Then the extracts were stored at freezer until used. Volatile compounds and polymethoxyflavones in the extracts were analyzed by GC-MS and HPLC. Each sample was analyzed in triplicate.

C. gloeosporioides was isolated from infected mango fruits with typical symptoms and maintained on potato dextrose agar (PDA) at 25 ^{o}C for seven days. The pathogen was inoculated into mango and re-isolated on PDA before the experiment.

The effect of extracts from pummelo peel on mycelial growth of *C. Gloeosporioides* was assayed by modifying the method of Liu et al., (2007). Mycelial disks (5 mm in diameter) from one-week-old culture of *C. gloeosporioides* were placed in the center of Petri dishes containing 20 ml of PDA with the 10,000 ppm extracts eluted with different solvents, and then incubated at 25^{o}C. The plate without the extract served as the control. Mycelial growth was determined by measuring colony diameter (mm) each day after inoculation. Each treatment was five times replicated, and the experiment was repeated thrice. The inhibition rate on mycelial growth was calculated according to the following formula: Inhibitory rate (%) = 100 x (colony diameter of the control − colony diameter of the treatment) / (colony diameter of the control).

RESULTS AND DISCUSSION

Pummelo peel in this study had the color L* a* and b*, which the values were between 62.8-67.6, 3.1-8.7 and 47.3-48.7, respectively. Chemical compounds of the crude extracts were analyzed by GC-MS (Table 1) and HPLC (Table 2). The result showed that the extract from pummelo peel was eluted with ethyl acetate that could identify volatile components and flavonoids better than those extracted with dichloromethane, hexane and ethanol (Table 1). Terpenes were the major components in extracts of pummelo peel. Volatile compounds were always found in citrus such as pinene, sabinene, myrcene, phellandrene, limonene, ociemene, linalool, terineol, carvone, caryophyllene, and geraniol. Ester such as geranyl acetate was also found in the extracts. Limonene was the major compound in all extracts (79.55 - 89.91%) and the result was consistent with Hosni et al., (2010). However, this research could identify the compounds of the extracts less than another research. This might be due to the difference of solvents and extraction methods. Hosni et al., (2010) used both of pummelo flavedos and leaves in their research as well. Linalool was also found in all extracts; however, it was presented in very small amounts (0.20 - 2.22% peak area). Jabalpurwala et al., (2009) found that linalool was the main compound in pummelo blossom. α-Phellandrene and 2, 6-octadien-1-ol, 3, 7-dimethyl were found only in crude extracts using ethyl acetate as a solvent.

Table1 Volatile compounds (% peak area) of extracts from pummelo peel eluted with four solvents

Volatile Compounds	Solvent			
	Ethanol	Hexane	Dichloromethane	Ethyl acetate
α-Pinene	nd	0.42	nd	0.72
Sabinene	nd	0.98	nd	nd
β-Pinene	nd	nd	1.52	2.26
β-Myrcene	0.35	1.49	nd	2.54
α-Phellandrene	nd	nd	nd	0.20
Limonene	79.55	81.52	84.62	89.91
β-Ociemene	nd	0.19	0.167	0.15
Linalool	2.22	0.20	0.63	0.28
α-Terpineol	nd	nd	0.76	nd
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethene)-cis	nd	nd	0.21	nd
D-Carvone	nd	nd	0.28	nd
2,6-Octadien-1-ol, 3,7-dimethyl	nd	nd	nd	0.58
Geraniol	nd	nd	0.44	0.30
Geranyl acetate	0.47	nd	nd	0.12
Caryophyllene	0.55	0.25	nd	0.10
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)	0.73	1.17	nd	0.71

nd: Not detected

Citrus peel was rich in flavanones and polymethoxlated flavones (Ortuño et al., 2006). Nobiletin in the extracts were eluted with ethanol, dichloromethane, hexane, and ethyl acetate were 4.87, 0.20, 0.24 and 0.68%, respectively. Tangeretin of pummelo peel extracted with ethanol, dichloromethane, hexane and ethyl acetate were 7.39, 4.36, 0.10 and 7.80%, respectively. Polymethoxyflavones were groups of flavonoids that presented mainly in the fruit peel. These compounds had two or more methoxyl's on their basic flavonoid structure (Uckoo et al., 2011). Pummelo peel extracted with ethanol had very small amount of 3, 6, 3', 4'-tetramethoxyflavone (0.66%), when compared to other solvent extractions. And 3-hydroxy-7,3',4',5'-tetramethoxyflavonein the extract were eluted with hexane, and dichloromethane were found only 0.34 and 0.47%, respectively. Whereas extraction pummelo peel with ethanol and ethyl acetate had 3-hydroxy-7, 3', 4', 5'-tetramethoxyflavone higher than 7.00% (Table 2).

Table2 Flavonoids and polymethoxyflavones (% peak area) of extracts from pummelo peel eluted with four solvents

Flavonoids and Polymethoxyflavones	Solvent			
	Ethanol	Hexane	Dichloromethane	Ethyl acetate
Nobiletin	4.87	0.20	0.24	0.68
3, 6, 3', 4'-Tetramethoxyflavone	0.66	77.44	70.59	75.72
3-Hydroxy-7, 3', 4', 5'- tetramethoxyflavone	8.35	0.34	0.47	7.67
Tangeretin	7.39	4.36	0.10	7.80

Figure 1 showed that mycelial growth of *C. gloeosporioides* on PDA plate without the extract served as the control. Antifungal efficiency of crude extract from pummelo peel on *C. gloeosporioides* was showed in table 3 and figure 2. Inhibition the growth of *C. gloeosporioides* of pummelo peel extracted with dichloromethane, ethanol, hexane and ethyl acetate were 19.16, 52.81, 65.58 and 95.61%, respectively. Thus, pummelo peel extracted with ethyl acetate was the highest efficiency on controlling *C. gloeosporioides* ($p \leq 0.05$). Polymethoxyflavones are important component of plant defensive mechanism against various disease causing pathogens such as *Phytophthora* and *C. gloeosporioides* (Del Rio et al., 1998). The present study found two compounds of polymethoxyflavones; 3, 6, 3', 4'-tetramethoxyflavone and 3-hydroxy-7, 3', 4', 5'-tetramethoxyflavone. Extraction of pummelo peel with hexane and dichloromethane had the high peak area of 3, 6, 3', 4'-tetramethoxyflavone (more than 70%), while 3-hydroxy-7, 3', 4', 5'-tetramethoxyflavone were only 0.34 and 0.47%, respectively. On the contrary, the extract eluted with ethanol had a higher 3-hydroxy-7, 3', 4', 5'- tetramethoxyflavone (8.35%), whereas 3, 6, 3', 4'-tetramethoxyflavone was very low. Extraction of pummelo peel with ethyl acetate had the high peak area of both 3, 6, 3', 4'-tetramethoxyflavone and 3-hydroxy-7, 3', 4', 5'-tetramethoxyflavone were 75.72 and 7.67%, respectively, which was different from the extracts eluted with other solvents. Therefore, this might be the cause of effectiveness in inhibiting the growth of *C. gloeosporioides*.

Table 3 Effect of crude extracts from pummelo peel eluted with four solvents on the growth of *C. gloeosporioides*

Solvent	Inhibitory rate (%)
Ethanol	52.81^{b}
Hexane	65.58^{b}
Dichloromethane	19.16^{c}
Ethyl acetate	95.61^{a}

Mean of the same column with different superscripts indicating significantly differences ($p \leq 0.05$)

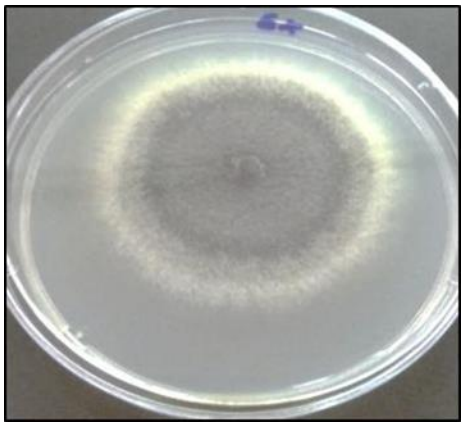

Fig. 1 Mycelial growth of *C. gloeosporioides* (on plate without crude extract from pummelo; control) after incubated at 25°C for 7 days

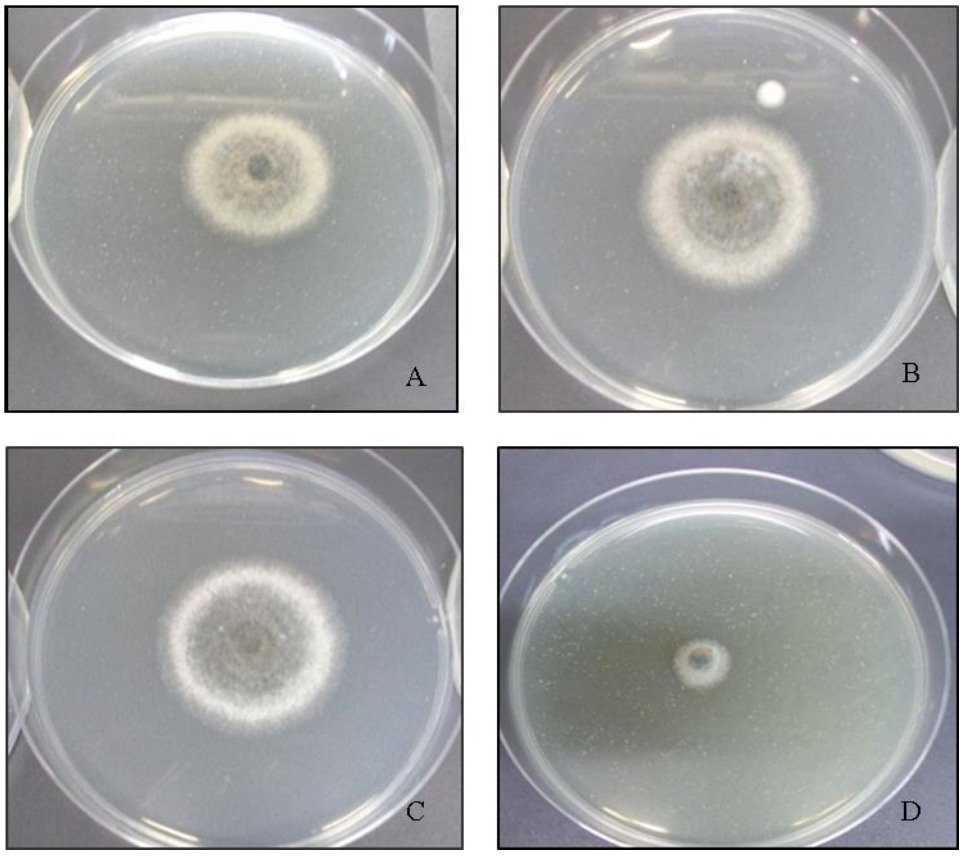

Fig. 2 Effect of extracts from pummelo peel eluted with ethanol (A), hexane (B), dichloromethane (C) and ethyl acetate (D) on mycelial growth of *C. gloeosporioides* after incubated at 25°C for 7 days

CONCLUSION

The major volatile compound of crude extract from pummelo peel was limonene. The extract eluted with ethyl acetate had the most volatile components, followed by the extraction with dichloromethane, hexane, and ethanol, respectively. Flavonoids (nobiletin and tangeretin) and polymethoxyflavones (3, 6, 3', 4'-tetramethoxyflavone and 3-hydroxy-7, 3', 4', 5'-tetramethoxyflavone) were found in all solvent extractions. However, the extract eluted with ethyl acetate had the amount of nobiletin and 3-hydroxy-7, 3', 4', 5'- tetramethoxyflavone, which were higher than those eluted with other solvents.

Inhibition the growth of *C. gloeosporioides* of pummelo peel extracted with dichloromethane, ethanol, hexane and ethyl acetate were 19.16, 52.81, 65.58, and 95.61%, respectively. The crude extract of pummelo peel eluted with ethyl acetate was the highest efficiency on controlling mycelial growth of *C. gloeosporioides*.

ACKNOWLEDGEMENTS

This work was supported by National Research Council of Thailand (NRCT) and Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi (RMUTT).

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