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Preliminary study on antimicrobial activity of crude extracts of pomelo albedo against *Colletotrichum gloeosporioides*

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Abstract

Preliminary study was undertaken to investigate the effect of crude dichloromethane extracts of pomelo albedo on radial growth and spore germination of *Colletotrichum gloeosporioides*. Crude extracts at concentrations of 25%, 50%, 75% and 100% were used in all trials. The results showed that crude extracts from pomelo albedo cv. Tub Tim Siam' did not affect radial growth for up to 6 days of incubation at $25\pm 2^\circ\text{C}$; however, crude extracts at the concentrations of 25% and 100% tended to reduce spore germination in comparison to control. The percentage of spore germination was 8.3, 10.9 and 14.2 for the concentrations of 100%, 25% and the control, respectively. Since factors such as solvent and method of extraction may influence the properties of derived crude extract, different solvents and extraction methods may be of interest for further trial.

Keywords: pomelo; albedo tissue; antimicrobial activity; *Colletotrichum gloeosporioides*;

Introduction

Synthetic chemicals have been used as the primary means for control of plant diseases. However, there have been public concerns over food safety and the development of chemical resistance by pathogens; attention has recently been turned into alternative means of using a natural substance. Thus, the screening of plant extracts for antimicrobial activity has been encouraged (Mokbel and Suganuma, 2006).

Reports on the antimicrobial activity of the extracts of several plant species have been established. These include, for example, lemon peels against *Penicillium digitatum* (Ben-

Yehoshua *et al.*, 1992), lime leaves against *Gloeosporium limeticola* (Martin *et al.*, 1966) and pomelo peels against *Cladosporium cladosporioides* (Srisajjalertwaja, 1996), *Bacillus cereus* and *Staphylococcus aureus* (Chaisawadi *et al.*, 2007). The need for new alternatives has also contributed to the search for antimicrobial property of other natural sources including waste residues.

The albedo (mesocarp) tissue of the Citrus family (Rutaceae) is one of the residues which have been screened for antimicrobial activity. Pomelo albedo extracts have been shown to have an antimicrobial activity against several bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella enteritidis* and *Escherichia coli* (Mokbel and Suganuma, 2006). However, its potential for suppression of fungal pathogens is still unclear.

The fungus *Colletotrichum gloeosporioides* is one of the most important plant pathogens and widely distributed. It is a causal agent of anthracnose disease in a wide range of economic crops and ornamentals. The objective of this study was, therefore, to investigate the antifungal property of crude extracts from pomelo albedo against *C. gloeosporioides*.

Materials and Methods

Plant materials and extraction.

Pomelo (*Citrus grandis* L. Osbeck cv. 'Tub Tim Siam') was purchased from Nakornsrithamarat province, THAILAND. The albedo tissues were removed from the fruit, blended and extracted with dichloromethane. The blended albedo was soaked in the solvent for 24 h. The extracts were then dried under a vacuum on a rotary evaporator at 50°C for further analysis. Four different concentrations of extracts were studied: 25%, 50%, 75% and 100%.

C. gloeosporioides isolation and maintenance.

A pure culture of *C. gloeosporioides* was isolated from an infected mango fruit and was cultured on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI). Pathogenicity was verified by inoculating the fungus into fruit before use in the experiment. Stock cultures were maintained by transferring mycelial plugs from the edge of actively growing cultures onto fresh PDA every two weeks. Virulence was maintained every four weeks by reinoculating as modified from the method described by Sosa-Alvarez *et al.* (1995). Disks (5 mm in diameter) were cut from banana fruit and placed on PDA prior to plugs (5 mm in diameter) cut from the two-week-old fungus grown on PDA being placed on the fruit disk. The pathogen was reisolated after it had grown through the fruit disk.

Radial growth.

A mycelial plug (5 mm diameter) taken from a two-week-old culture was placed on half of PDA plate and sterilised filter paper disk (5 mm in diameter) was placed on another side of the plate. A 10 µL of crude extract was pipetted onto a paper disk in a plate. Sterile distilled water was used as control. The plate was sealed with Parafilm® and incubated at 25±2°C. Plates in 9 replicates were used for each concentration. The diameter of fungal colony was measured everyday and compared to that of untreated control. The experiment was a completely randomised design and was repeated twice. The results were similar for both trials, the best results are presented.

Spore germination.

A two-week-old sporulating culture of *C. gloeosporioides* was flooded with Tween-80 solution (sterile distilled water containing 0.1% (v/v) Tween-80) and gently agitated to dislodge the spores. Spore suspension was then filtered through two layers of sterile cheesecloth, washed

three times by centrifugation and resuspended in fresh Tween-80 solution. Spore suspension was adjusted with sterile distilled water to the concentration of 10^6 spore/mL. A 0.5 mL of spore suspension was added and spread onto a Petri dish containing 15 mL hard PDA. Sterilised filter paper disks (5 mm in diameter) were impregnated with albedo crude extract (10 μ L/disk), allowed to dry completely for 5-10 min in laminar flow and then evenly placed on the surface of previously inoculated agar. The pure solvent of dichloromethane was added as a blank to the paper disk as a control. Five replicates of one plate were used for each concentration of crude extracts. Germination of 100 spores per plate was examined microscopically after incubation at $25\pm 2^\circ\text{C}$ for 24 h. A spore was considered germinated when the length of the germ tube equalled or exceeded the length of the spore. The experiment was a completely randomised design and was repeated twice. The results were similar for both trials, the best results are presented.

Statistical analysis.

Data were subjected to analysis of variance using GenStat (11th Edition, Rothamsted Experimental Station, UK) and the differences between the means of the treatments were compared by the LSD test at a significance level of 0.05. Means and standard errors were calculated using Microsoft Excel.

Results and Discussion

The antimicrobial activity was initially proposed to determine the appearance of clear inhibition zone around the disks. In preliminary trial, there was no clear zone produced, thus, the evaluation was changed to measure radial growth in subsequent trials.

Effect of pomelo albedo extracts on radial growth.

Dichloromethane extracts from 'Tub Tim Siam' pomelo albedo did not delay radial growth of *C. gloeosporioides* (Figure 1). The colony diameters were not different among the concentrations of albedo extracts during storage at $25\pm 2^\circ\text{C}$ for up to 6 days. Similar result was obtained in crude extracts from cultivars Tong Dee, Kao Yai and Kao Tang Gwa using dichloromethane in which the crude extracts did not affect the radial growth of *C. gloeosporioides* (data not shown). Likewise, Srisajjalertwaja (1996) reported that pomelo albedo extracts from four cultivars: Tong Dee, Kao Pan, Kao Yai and Pattavia, extracted by either 95% ethanol or dichloromethane did not inhibit growth of *C. cladosporioides*. Apparently, cultivar does not influence the efficacy of albedo extract on suppression of fungal pathogens; however, methanol extracts of pomelo albedo exhibited significant antimicrobial activity as determined by the appearance of clear zone (Mokbel and Sukanuma, 2006), suggesting solvent and extraction method may play a role in interfering quality, quantity and stability of the crude extracts.

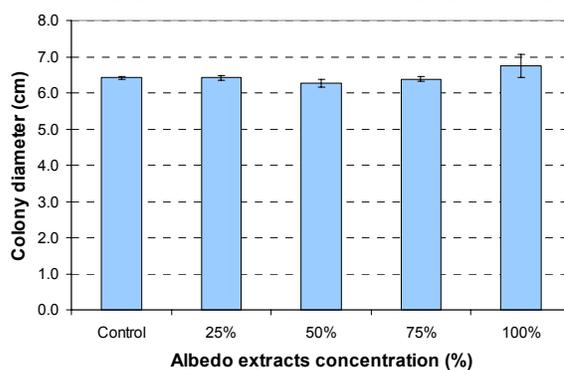


Figure 1 : Effect of pomelo albedo extracts on radial growth of *Colletotrichum gloeosporioides* after incubation at $25\pm 2^\circ\text{C}$ for 6 days. Data shown are means \pm SE from $n = 9$.

Effect of pomelo albedo extracts on spore germination. Since we have encountered the problem regarding the separation of crude extracts and PDA, the spore germination was determined using a paper disk method as described above and 100 spores around the paper disk impregnated with crude extracts were randomly examined for germination. The percentage of spore germination were not significantly different between the extract concentrations and the untreated control; however, albedo extracts at the concentration of 25% and 100% tended to inhibit germination of spores in comparison to the control (Figure 2). The percentage of spore germination was 8.3, 10.9 and 14.2 for the concentrations of 100%, 25% and the control, respectively. The preliminary results in our study suggested that although albedo extracts did not suppress radial growth, they showed an apparent potential in inhibiting spore germination. To our knowledge, there is no report on the effect of pomelo albedo extracts on spore germination. Thus, further study focusing on the potential of crude extracts from pomelo albedo tissues for inhibiting spore germination should be conducted. The utilisation of albedo tissues leads to minimise agricultural and industrial wastes, subsequently solves environmental problem.

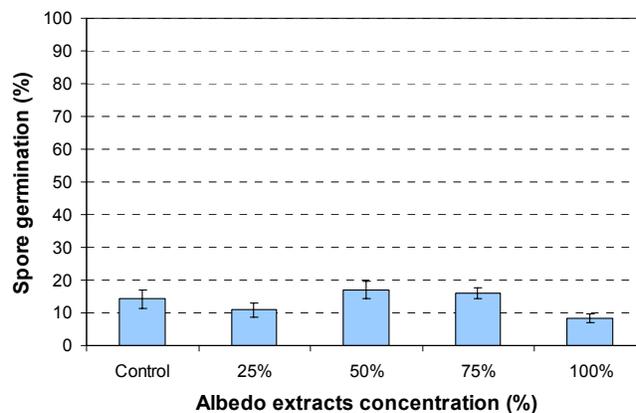


Figure 2 : Effect of pomelo albedo extracts on spore germination of *Colletotrichum gloeosporioides* after incubation at $25\pm 2^{\circ}\text{C}$ for 24 h. Data shown are means \pm SE from $n = 5$.

Conclusion

Crude extract of pomelo albedo cv. ‘Tub Tim Siam’ using dichloromethane did not exhibit antifungal activity against radial growth of *C. gloeosporioides*; however, it tended to inhibit spore germination.

We are now investigating the efficacy of methanol extracts from pomelo albedo tissues against *Colletotrichum musae* and *Lasiodiplodia theobromae*.

Acknowledgements

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References

Ben-Yehoshua, S., Rodov, V., Kim, J.J. and Carmeli, S. (1992). Preformed and induced antifungal materials of citrus fruits in relation to the enhancement of decay resistance by heat and ultraviolet treatments. *Journal of Agricultural and Food Chemistry* 40, 1217-1221.

Chaisawadi, S., Methawiriyasilp, W. and Bualouy, P. (2007). Preliminary study of antimicrobial activities on citrus fruit peel. Proceeding of the 33rd Congress on Science and Technology of Thailand 2007.

Martin, J.T., Baker, E.A. and Byrde, R.A.W. (1966). The fungitoxicities of cuticular and cellular components of citrus lime leaves. *Annals of Applied Biology* 57, 491-550.

Mokbel, M.S. and Suganuma, T. (2006). Antioxidant and antimicrobial activities of the methanol extracts from pummelo (*Citrus grandis* Osbeck) fruit albedo tissues. *European Food Research and Technology* 224, 39-47.

Sosa-Alvarez, M., Madden, L.V. and Ellis, M.A. (1995). Effects of temperature and wetness duration on sporulation of *Botrytis cinerea* on strawberry leaf residues. *Plant Disease* 79, 609-615.

Srisajjalertwaja, S. (1996). Antifungal compounds from pomelo (*Citrus grandis*, L., Osbeck) peels. Master thesis, Discipline of Chemistry, Chiang Mai University, Chiang Mai, Thailand.