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ANTIFUNGAL ACTIVITY OF *Persicaria odorata* EXTRACT AGAINST ANTHRACNOSE CAUSED BY *Colletotrichum capsici* AND *Colletotrichum gloeosporioides*

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ABSTRACT

Anthracnose is the most serious problem in the degradation of fruit quality. Natural plant products are currently the alternative source of fungicides. *Persicaria odorata*, which is commonly found in Southeast Asia, exhibits antioxidant and antibacterial activity as well as being a source of phenolic compounds. The activity of *P. odorata* extract against anthracnose caused by *Colletotrichum capsici* and *Colletotrichum. gloeosporioides* was investigated in this study. The chemical compounds were tested by employing the TLC technique. Bioautography and microdilution bioassay were also employed for spore germination and mycelium growth, respectively. The results from the TLC technique showed that the chemical constituents of *P. odorata* were terpenoids, steroids, and other unidentified organic compounds but not alkaloids. The antifungal test of lipophilic extract showed clear zones on the TLC plate of *C. gloeosporioides* whereas there were no clear zones with *C. capsici.* With the result of microdilution bioassay, the lipophilic extract concentration inhibited the germination of *C. capsici* at a minimum inhibitory concentration (MIC) of 625 µg/ml, and 20,000 µg/ml at 24 and 72 hours, respectively. Whereas the minimum concentrations that inhibited the germination of *C. gloeosporioides* were 2,500 µg/ml, 10,000 µg/ml, 20,000 µg/ml and 20,000 µg/ml at 24, 72, 120, and 168 hours, respectively.

Key words: Antifungal activity, *Persicaria odorata*, *Colletotrichum capsici*, *Colletotrichum gloeosporioides*

INTRODUCTION

Persicaria odorata (Lour.) Sojak (Syn. *Polygonum odoratum* Lour.), family Polygonaceae, is a native plant in Southeast Asia known as "Vietnamese coriander" and is scattered throughout Asia (Rafi and Vastano, 2006). In Thailand it is known as "Phak phai". *P. odorata* is a perennial herb, 30-35 cm in height. The leaves are globous, 6-15 cm, with dark purple marking in the centre. It has filiform inflorescence and strong odour (Kantachot and Chantaranothai, 2010; Starkenmann *et al.*, 2006). *P. odorata* grows well in wet environments with a rich and moist soil as well as in semi-shade (Shavandil and Haddadian, n.d.). It is cultivated and consumed as a vegetable (Kantachot and Chantaranothai, 2010).

P. odorata is a potential source of natural aliphatic aldehydes and exhibits a range of beneficial properties such as antimicrobial, anti-inflammatory, antitumor-promoting, and antifeedant activities as well as an antioxidative property (Shavandil *et al.*, n.d.). The aerial part of this plant is highly aromatic and contains many organic compounds such as (*Z*)-3-hexenal, (*Z*)-3-hexenol and aldehydes like decanal, undecanal and dodecanal. This plant's extract has high phenolic content with the flavonoids like rutin, catechin, quercetin, kaempferol and isorhamnetin, which are found in this plant, also exhibit strong antioxidant and antibacterial activities against *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus* (Nanasombat and Teckchuen, 2009).

Anthracnose is a plant disease caused by fungi known as *Colletotrichum*, a common group of plant pathogens which attacks all plant parts at every growth stage. The symptoms are most visible on leaves and ripe fruits. At first, anthracnose generally appears on leaves as small and irregular yellow, brown, dark-brown, or black spots. Spores are released and spread by wind, rain, and insect. In addition, cool wet weather promotes spore development (Freeman *et al.*, 1998). This disease is a major pre- and post-harvest problem that degrades

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the quality of products. To control the disease and maintain the quality of agricultural product, many farmers and orchardists resort to synthetic fungicides to control the disease. However, these fungicides are toxic to crops and harmful to the environment. Therefore, natural plant products were investigated for potential alternative less-toxic sources of fungicide which are safer to the environment (Yoon *et al*., 2001). Chen & Dai (2012) reported that *Cinnamomum camphora* had strong inhibitory activity against *Colletotrichum lagenarium* in cucumbers anthracnose. Moreover, compounds obtained from hexane, ethyl acetate, and methanol extracts from pericarp of *Areca catechu* L. were also used against *C. gloeosporioides* (Yenjit *et al*., 2010).

Chili is an important vegetable in Asia; 25.9 million tons of fresh chili was produced in 2006 (Montri *et al*., 2009), while 41,163 tons of fresh or frozen mangoes worth 1.026 billion baht was exported from Thailand in 2011 (Centre for Agricultural Information, 2012). Anthracnose in chilies and mangoes is caused by *C. capsici* and *C. gloeosporioides* (Hindorf, 1999; Phoulivong *et al.*, 2010). It is the most serious problem in the reduction of their quality.

The aforementioned activities of *P. odorata* and the limited number of research papers on the subject (Starkenmann *et al.*, 2006; Nanasombat & Teckchuen, 2009) make the capabilities of *P. odorata* extract attractive. Therefore, the aim of this research was to investigate and extract the chemical compounds from *P. odorata* that show antifungal activities against *C. capsici* and *C. gloeosporioides*.

MATERIALS AND METHODS

Plant extraction and separation

Plant material is Phak phai (*Persicaria odorata*). The aerial parts of *P. odorata* were collected, dried, crushed, macerated in methanol for seven days, and then filtered through Whatman no. 1 filter paper to extract crude, which was partitioned into two parts: hydrophilic extract and lipophilic extract with distilled water and chloroform. The lipophilic extract was analysed by thin layer chromatography (TLC), and high performance liquid chromatography (HPLC), which was undertaken on Agilent 1100 Series at Scientific Instrument Center, Faculty of Science, Kasetsart University (Bangkhen Campus), Thailand. The extract was also used in biotest, bioautography, and microdilution methods to measure the effectiveness of the lipophilic extracts. Thin layer chromatography was used to investigate the chemical compounds by using a TLC plate (aluminum sheath coated with silica gel 60 f_{254} Merck) with solvent system chloroform: methanol (47.5:2.5).

Phytochemical Screening

Investigation of terpenoids, the essential oil, coumarins, sterols, steroids, and alkaloids presence was carried out using thin layer chromatography. TLC plate (aluminium sheath coated with silica gel 60 f_{254} Merck) with solvent system chloroform: methanol (47.5 : 2.5) was used.

The chemical characters of the lipophilic extract were investigated using UV cabinet under short (254 nm) and long (365 nm) wavelengths. Steroids and terpenoids were detected by spraying the TLC plate with anisaldehyde-sulfuric acid, followed by heating on a hot plate at 100-110°C for 5-10 minutes. As for essential oil, TLC plate was sprayed with vanillin and heated to 110°C. Coumarin was detected using 10% NaOH and inspected under 365 nm wavelengths. Organic compounds could be detected using an iodine vapour chamber. Sterols and steroids were detected using sulfuric acid and alkaloids by Dragendorff's reagent (Merck, 1980).

Fungal isolation

Fungal pathogens used in this study were *C. capsici* and *C. gloeosporioides*. *C. capsici* were obtained from the laboratory of the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University (Bangkhen campus), Thailand. *C. capsici* was isolated from chilli sample, whereas *C. gloeosporioides* was isolated from a mango sample. Both fungus were cultured on potato dextrose agar (PDA) and isolated using a tissue transplanting technique. Inhibitory effects of *Persicaria odorata* lipophilic extract against *C. capsici* and *C. gloeosporioides* were tested by using two biotest methods; bioautography and microdilution bioassay.

Bioautography was performed by developing the TLC plate with a solvent system (chloroform: methanol ratio : 47.5 : 2.5). After lipophilic extract of *P. odorata*, it was sprayed with spore suspension; *C. capsici* and *C. gloeosporioides,* and incubated at room temperature for 7 days. The inhibition areas on the plate were then compared with R_f value on the reference plate.

Microdilution bioassay started with serial dilution in 96-well microwell plates with 4 replications. The test substance was prepared with 80 mg stock solution and dissolved in acetone. The concentration of lipophilic extract in the first well was 20,000 µg/mL, with each successive well reduced by half. The spore concentration of *C. capsici* and *C. gloeosporioides* was 10^6 spore/ml for each well. The plates were kept at room temperature and in darkness. The results were checked by microscope and the lowest concentration showing spore germination was recorded as minimum inhibitory concentration (MIC) after 24, 72, 120 and 168 hours, respectively (Engelmeier, 2000).

RESULTS AND DISCUSSION

Phytochemical screening

For phytochemical screening, specific colour reagents were sprayed on the TLC plates to investigate the chemical groups. The results showed that *P. odorata* has terpenoids, sterols, steroids, phenols, coumarins, and other unidentified organic compounds, excluding alkaloids. As shown in Figure 1, all plates except for (f) showed positive result.

In related work, Nanasombat & Teckchuen (2009) found high phenolic content in *P. odorata*, while Starkenmann (2006) found aldehydes, along with Sasongko (2011) who found aldehydes and terpene. In this study, we used aerial parts in the same way as Starkenmann (2006) who reported the presence of aldehydes. The properties of essential oils, which is a source of aldehydes as stated by Sasongko (2011), implies that this plant exhibits antifungal activity, leading to the inhibition of the fungus species used in this work. This inhibition would be confirmed in the other experiments below.

High Performance Liquid Chromatography (HPLC)

The chromatogram of *P. odorata* lipophilic extract from the analysis with HPLC shows a specific pattern as shown in Figure 2.

Bioautography

The inhibitory activities of lipophilic extract from *P. odorata* against *C. capsici* and *C. gloeosporioides* were investigated using the bioautography method (Thin layer chromatography technique with a solvent system, chloroform: methanol ratio; 47.5 : 2.5). The occurrence of a clear zone on the TLC plate at $R_f = 0.76$ could be detected for *C. gloeosporioides*, but no clear zone for *C. capsici* was observed (Figure 3 (a) and (b)).

The microdilution bioassay of lipophilic extract from *P. odorata* against *C. gloeosporioides* and *C. capsici* spores yielded results as displayed in Table 1.

The lipophilic extract from *P. odorata* could inhibit the spore germination of *C. gloeosporioides* from mango at the minimum inhibitory concentration (MIC) = 2,500 g/ml, 10,000 g/ml, 20,000 g/ml, and 20,000 g/ml after 24, 72, 120, and 168 hours, respectively.

The lipophilic extract from *P. odorata* could inhibit the spore germination of *C. capsici* from chili at the minimum inhibitory concentration (MIC) = 625 g/ml, 20,000 g/ml, >20,000 g/ml, and >20,000 g/ml after 24, 72, 120, and 168 hours, respectively. From the results of the bioautography and microdilution bioassay, the lipophilic extract of *P. odorata* showed more potential to inhibit spores of *C. gloeosporioides* compared to *C. capsici*. This is demonstrated by clear zones indicating the inhibition of the fungus that could be detected for *C. gloeosporioides* but not for *C. capsici*. Additionally, the MIC value of the extract against *C. gloeosporioides* was lower than *C. capsici*.

Results from this study suggest that further step can be extended to extract pure compounds which could assist identification of the compounds or groups that inhibit the fungus spores. Increasing the concentration of the lipophilic extract could also increase the inhibitory effects.

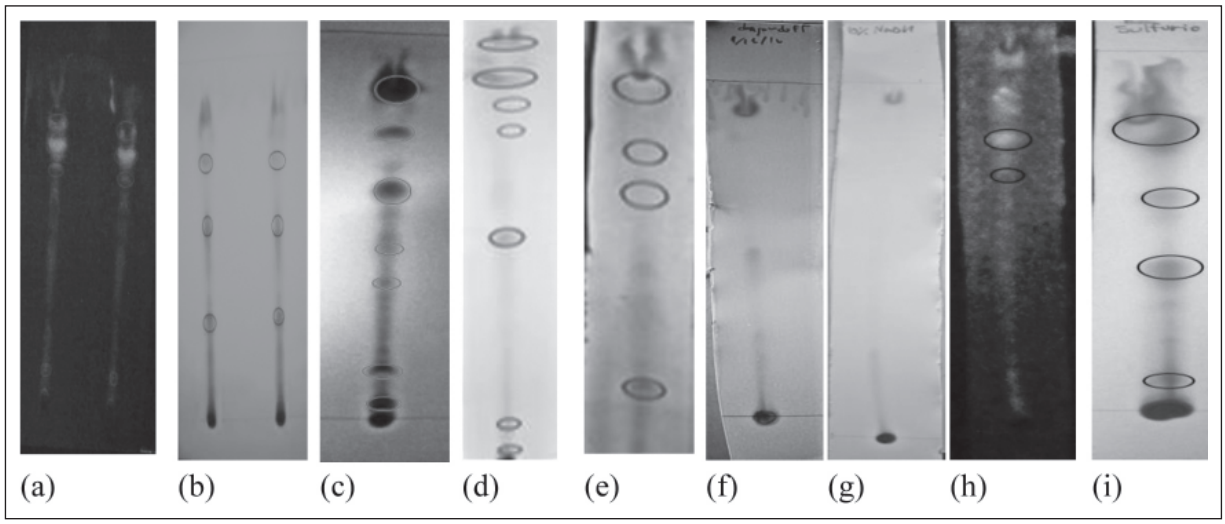

Fig. 1. (a) and (b), TLC plate under UV 365 nm and 254 nm; (c) Steroids and terpenoids screening using anisaldehyde-sulfuric acid; (d) Steroid and phenols screening with vanillin-sulfuric acid; (e) Organic compound screening using iodine; (f) Alkaloids screening using Dragendorff's reagent; (g) and (h) Coumarin with 10% NaOH under natural light and 365 nm UV respectively; (i) steroids and sterols with sulfuric acid.

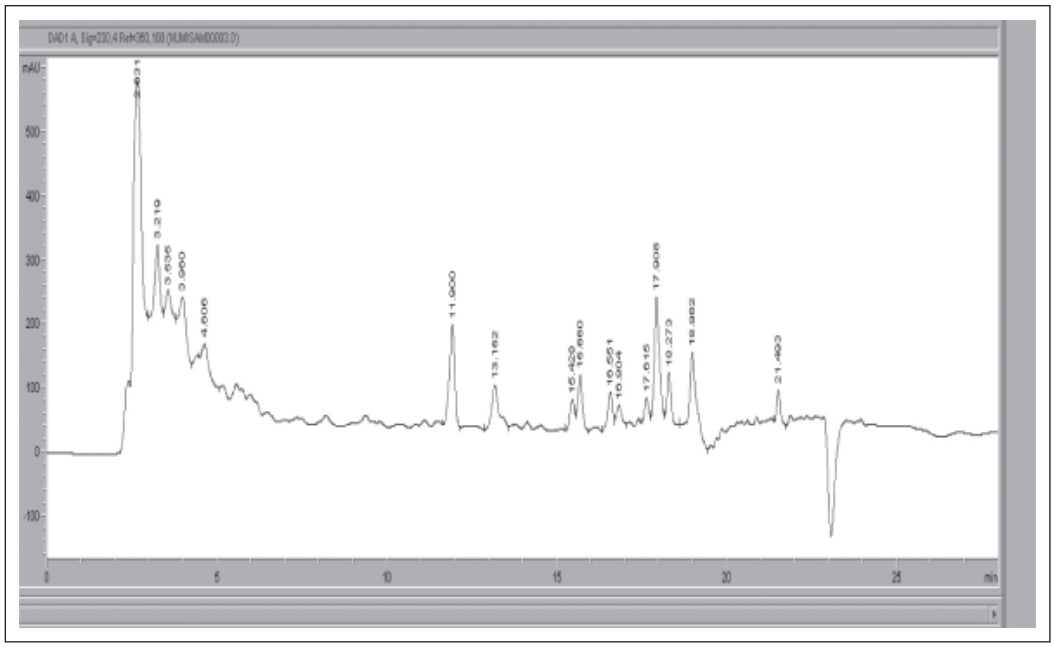

Fig. 2. HPLC profile of *Persicaria odorata* lipophilic extract

Table 1. Minimum inhibitory concentration (MIC) of lipophilic extract against *C. gloeosporioides* and *C. capsici* obtained by microdilution bioassay

	24 hours	72 hours	120 hours	168 hours
C. gloeosporioides	2,500 µg/ml	10,000 µg/ml	20,000 µg/ml	20,000 µg/ml
C. capsici	625 µg/ml	20,000 µg/ml	>20,000 µg/ml	>20,000 µg/ml

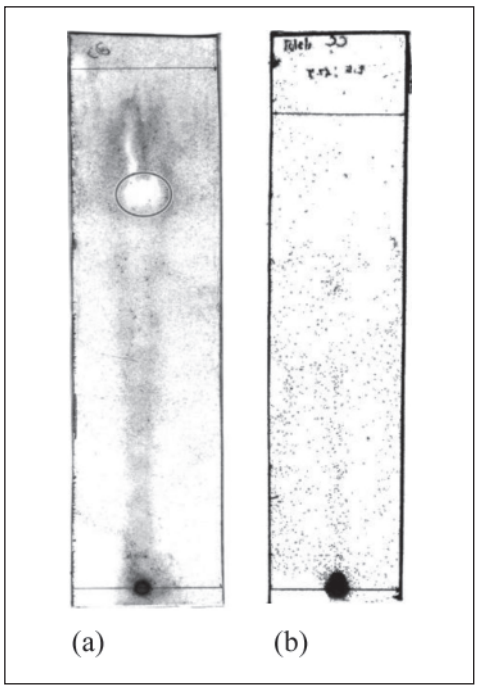

Fig. 3. Inhibitory activity test of lipophilic extract from *P. odorata* against (a) *C. gloeosporioides* and (b) *C. capsici*

CONCLUSIONS

The results of the screening test using thin layer chromatography indicated the chemical constituents of *P. odorata* included terpenoids, steroids, and other unidentified organic compounds. However, there was no indication of alkaloids.By using bioautography and microdilution, lipophilic extract of *P. odorata* showed inhibitory activity against the germination of *C. capsici* and *C. gloeosporioides.* In addition, lipophilic extract of *P. odorata* could inhibit spore germination of *C. gloeosporioides* more effectively than *C. capsici* from bioautography (indicated by the appearance of a clear zone). Furthermore, microdilution bioassay showed that lipophilic extract of *P. odorata* could inhibit spore germination of *C. gloeosporioides* more effectively compared to *C. capsici*.

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