Antifungal Activity of Extract of *Eugenia aromatica* (L.) Baill. (Myrtaceae) Against Some Plant Pathogenic Molds

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Abstract

Extract of *Eugenia aromatica* (L.) Baill. (Myrtaceae) was tested for antifungal activity. In this study, phytopathogenic molds, *Alternaria* sp. and *Fusarium* sp. causing leafspot and wilt disease in *Brassica* spp., and *Botrytis* sp. and *Septoria* sp., causing gray mold rot in roses and leafspot in chrysanthemums respectively, were used as test organisms. The extract of *Eugenia aromatica*, 0.01 - 1.00% concentration incorporated in PDA, provided inhibitory activity against all tested molds. *Botrytis* sp. and *Fusarium* sp. were completely inhibited at a concentration of 0.05%, while *Alternaria* sp. and *Septoria* sp. were completely inhibited at concentrations higher than 0.30%. Separation by preparative-TLC and confirmation by TLC-bioassay using *Cladosporium cladosporioides* as a diagnostic fungus distinguished two active compounds, eugenyl acetate and eugenol.

INTRODUCTION

Eugenia aromatica (L.) Baill. (Myrtaceae, common name: clove) is a tree reaching a height of 5-10 meters. It is native in Malacca and abundant in Malaysia, Sumatra, Africa, South America, and Thailand. In Thailand, flower buds of this species are used as a spice and traditional medicine, mainly as a carminative, stomachic, antidiarrheal, local anesthetic for toothache, and as a gargle for mouth-wash (Mahidol University, 1996). In spite of this widespread use, information concerning a marked antifungal property of the plant extract has been reported only quite recently (Lima et al., 1993; Tragoolpua, 1996; Wilson et al., 1997; Wonggiratthiti, 2000; Costa et al., 2000). In order to determine the antifungal activity, the dichloromethanic extract of *Eugenia aromatica* was tested against phytopathogenic molds and the active compounds were evaluated and identified.

MATERIALS AND METHODS

Plant Material

Dried flower buds of *Eugenia aromatica* were obtained from a grocery in Chiang Mai Province

Test Organisms

The fungi used for testing antifungal activity of the plant extract were: *Alternaria* sp. and *Fusarium* sp. causing leaf spot and wilt disease of cruciferous vegetables, *Septoria* sp. causing leaf spot of chrysanthemums and *Botrytis* sp. causing gray mold rot of roses. They were cultured on potato dextrose agar and maintained at 4-8°C.

Extraction Procedure

Dried flower buds of clove were crushed and extracted with dichloromethane using an ultrasonic bath (40°C) for 1 hour. The solvent was removed under reduced pressure in a rotary evaporator to give a dry crude extract, which was dissolved in a small amount of dichloromethane and mixed with sterile distilled water to obtain a 2% stock

solution.

Microbiological Test

1. Antifungal Activity The antifungal activity of *Eugenia aromatica* was conducted using a slide culture method (Liamthong et al., 1997). The crude extract from the stock solution was incorporated into potato dextrose agar at different concentrations (0.00, 0.01 – 1.00%). The plant-extract mixture was dropped on concave slides. A mycelial mass of 1 mm diameter was inhibited and incubated at 25°C. Growth of test molds was measured with a micrometer under a stereomicroscope after 2-3 days of incubation. According to Gamliel et al. (1989), the percent inhibition of fungal growth was calculated by

$$\text{Inhibition percentage} = 100 - [(r^2/R^2) \times 100]$$

when r and R represent the radii of the fungal colony in the treatment and control, respectively.

2. Bioassay for Detecting Active Compounds on TLC The crude extract was chromatographed separately on silica gel using toluene:ethyl acetate (93:7) as the mobile phase. *Cladosporium cladosporioides* spore suspension in potato dextrose broth (10^6 spores/ml) was used for bioassay by spraying it on the TLC plate, which was then incubated at 25°C for 3 days. R_f values of inhibition zones were measured in duplicate.

Identification of Antifungal Compounds

The silica gel at the inhibition zones was scraped off and the compounds present dissolved using a small amount of distilled dichloromethane and then subjected to Gas Chromatography-Mass Spectrometry (GC-MS). Antifungal compounds were detected by GC-MS; HP6890 plus version A 03.07 Hewlett Packard 5973 Mass Selective Detector instrument employing the following condition : Column : HP-5MS, 30x0.25 mm ID, 0.25 μm film thickness; carrier gas: Helium (1.3 ml/min) ; injector and ion source temperatures were 250°C and 230°C respectively. The oven temperature was programmed from 100°C (isothermal for 1 min), with an increase of 25°C/min to 240°C ending with 10 min isothermal at 240°C. The active compounds were identified by comparison of mass spectra with the library database.

RESULTS AND DISCUSSION

The results of antifungal activity of *Eugenia aromatica* extracts against tested molds are summarized in Table 1. All the four test molds were sensitive to the plant extract. *Fusarium* sp. and *Botrytis* sp. were more sensitive and completely inhibited by 0.05% crude extract. *Alternaria* sp. and *Septoria* sp. were somewhat resistant but almost completely inhibited at higher concentrations (>0.3-0.5%). Meena and Sethi (1994), Wendorff and Wee (1997), Hitokoto et al. (1980), Hasan and Mahmoud (1993), Patkar et al. (1993), Sinha et al. (1993) have also reported the efficacy of clove extract against some food-borne microorganisms, especially pathogenic molds.

After extraction and evaporation of the dichloromethanic extract using a rotary evaporator, 12.2 g of crude extract was obtained from 100 g of *Eugenia aromatica* buds. Separation of the extracts by TLC gave bands with R_f values of 0.62-0.8, 0.53-0.62 and 0.47-0.53 (referred to as samples A, B and C, respectively in Fig. 2-4), which showed antifungal activity against *Cladosporium cladosporioides*. Two active compounds identified by GC-MS were eugenyl acetate and eugenol. The results indicated that these compounds could be responsible for the antifungal activity against some phytopathogenic molds.

Our results are similar to those from some other investigations which stated that eugenol was the main substance in clove which gave the distinct antifungal effect against toxin-producing *Aspergillus* spp. (Bullerman et al., 1977; Hitokoto et al., 1980; Martini et al., 1996)

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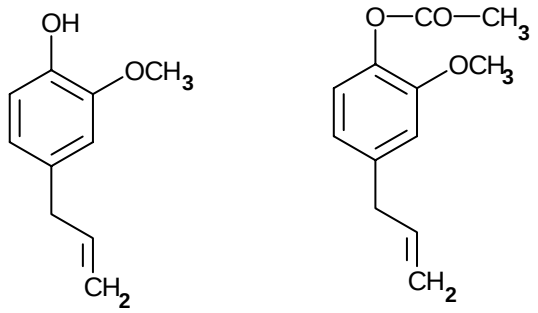
Tables

Table 1. Inhibition percentage of clove extract against tested phytopathogenic molds

Organisms	% Concentration	Inhibition percentage	Standard deviation
Alternaria sp.	0.00	0.00 (a)*	0.00
	0.01	14.25 (ab)	4.12
	0.03	16.59 (abcd)	8.18
	0.05	86.98 (ghi)	3.61
	0.10	88.59 (hi)	0.26
	0.15	90.19 (hi)	2.65
	0.30	95.10 (i)	8.49
	0.50	100.00 (i)	0.00
	0.80	100.00 (i)	0.00
	1.00	100.00 (i)	0.00
Fusarium sp.	0.00	0.00 (a)	0.00
	0.01	25.32 (bcd)	43.83
	0.03	56.07 (e)	23.26
	0.05	100.00 (i)	0.00
	0.10	100.00 (i)	0.00
	0.15	100.00 (i)	0.00
Septoria sp.	0.00	0.00 (a)	0.00
	0.01	32.22 (d)	17.29
	0.03	23.84 (bcd)	14.81
	0.05	64.83 (ef)	4.87
	0.10	69.66 (efg)	5.32
	0.15	76.08 (fgh)	4.95
	0.30	74.33 (fgh)	10.02
	0.50	100.00 (i)	0.00
	0.80	100.00 (i)	0.00
	1.00	100.00 (i)	0.00
Botrytis sp.	0.00	0.00 (a)	0.00
	0.01	25.01 (bcd)	13.95
	0.03	26.48 (bcd)	15.38
	0.05	100.00 (i)	0.00
	0.10	100.00 (i)	0.00
	0.15	100.00 (i)	0.00

* The same letter indicates no significant value at 95% confidence by LSD.

Figures



(a) (b)

Fig. 1. The structures of eugenol (a) and eugenyl acetate (b)

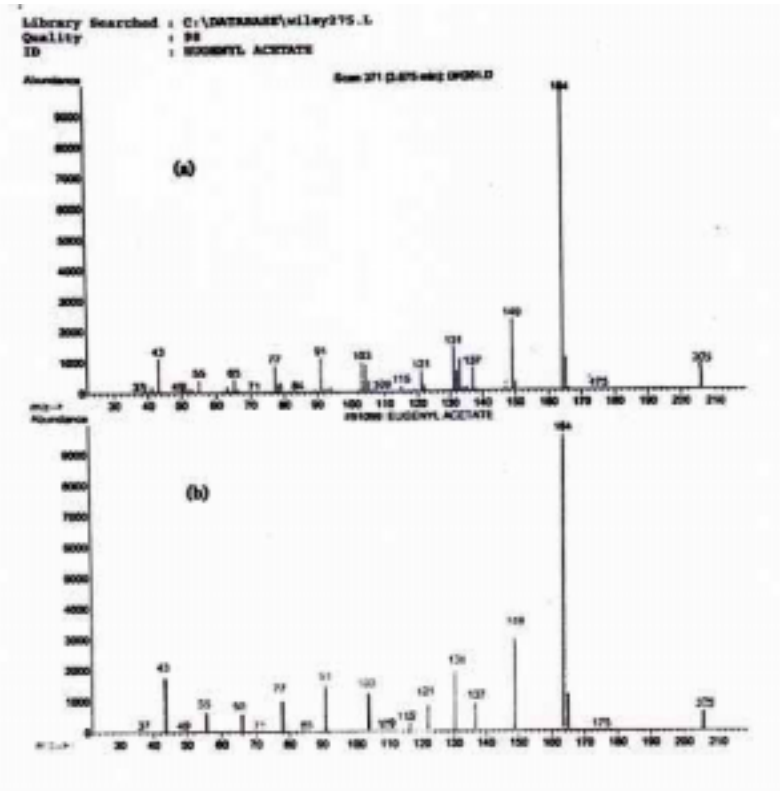


Fig. 2. Mass spectrum of Sample A (a) and eugenyl acetate from the library (b)

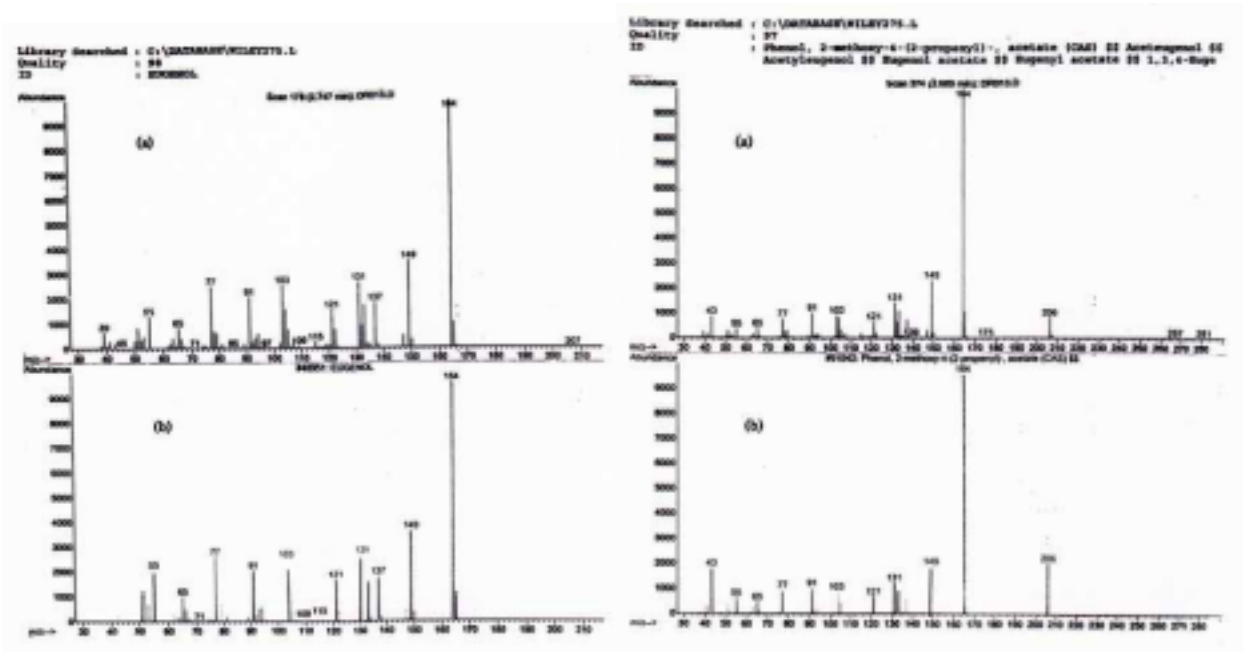


Fig. 3. Mass spectrum of sample B at Rt = 2.747 (a) and eugenol from library (b) (left) and spectrum at Rt = 3.689 (a) and eugenyl acetate from library (b) (right)

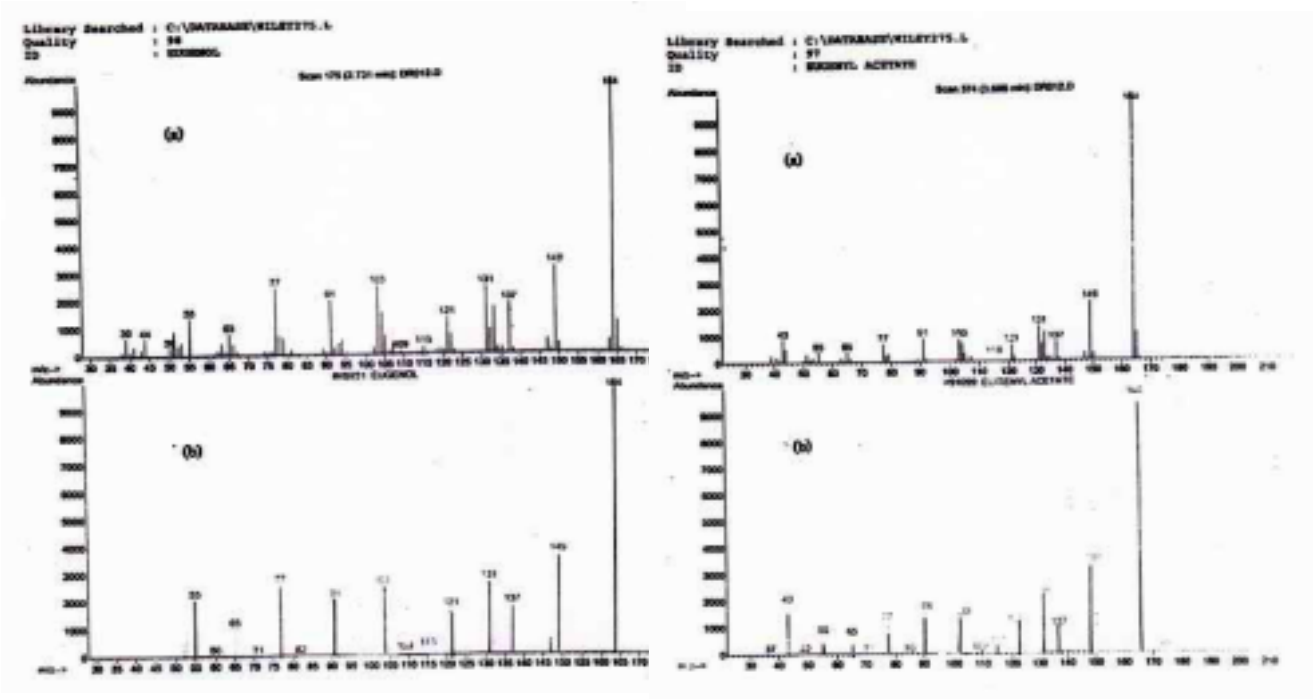


Fig. 4. Mass spectrum of sample C at Rt = 2.731 (a) and eugenol from library (b) (left) and spectrum at Rt = 3.688 (a) and eugenyl acetate from library (b) (right)