

Chemical Composition and Antifungal Property of *Eucalyptus camaldulensis* Leaf Oils from Thailand

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Abstract: The present study was performed to evaluate antifungal activities of leaf essential oils from *Eucalyptus camaldulensis* Dehnh. originating from Thailand against 9 fungal strains. The leaf samples were collected from 3 different clones. The fungi examined in this study were (1) household molds: *Aspergillus niger*, *Cladosporium cladosporioides*, *Chaetomium globosum* and *Penicillium citrinum*, (2) wood rot fungi: *Fomitopsis palustris* and *Trametes versicolor*, (3) plant pathogenic fungi: *Fusarium oxysporum*, *Thanatephorus cucumeris* and *Rhizopus oryzae*. The results revealed that *E. camaldulensis* leaf oils provided 100% inhibition of the mycelial growth of *T. cucumeris* (5 mg/mL), and *C. globosum* (10 mg/mL). No inhibition effect was observed against *R. oryzae* even at the concentration of 10 mg/mL. A medium to low inhibitory activities against the mycelial growth of the six other fungi were found. The essential oils of *E. camaldulensis* leaf have potency as an antimicrobial agent especially against seedling blight pathogens and it could also act as moderate agents against household molds and wood rot fungi. Therefore, even if they need relatively higher concentration for the controlling agents, they deserve as the alternatives to hazardous synthetic fungicides from the ecological viewpoints.

Keywords: Antifungal property; *eucalyptus camaldulensis*; household molds; leaf essential oil; plant pathogenic fungi; wood rot fungi.

1. Plant Source

The genus *Eucalyptus*, which is indigenous to Australia, consists of over 800 species and spreads worldwide due to its easy adaptability and fast growth [1].

In Thailand, *Eucalyptus* plantations are distributed on several parts of the country and different species are planted depending on the climate, area, soil and usage. *Eucalyptus camaldulensis* Dehnh. is the most grown species in Thailand. It is mainly planted for use as pulpwood and utilized even at ages of 3-5 years due to high growth rate. During the process of papermaking, large amounts of waste such as leaves are generated and disposed of. The utilization of these waste materials is crucial from an ecological viewpoint.

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Three clones S1, S2, and S3 of *E. camaldulensis* were selected through several generations based on their pulp yields and adaptability to circumstance etc. by Siam Forestry, Kanchanaburi Province, Thailand. The leaves of each clone were collected in mid-April (summer season) 2007.

2. Previous Studies

Several studies reported that *E. camaldulensis* leaf essential oils contained bioactive compounds that displayed antibacterial [2], analgesic and anti-inflammatory effects [3], antitermitic activity [4], antioxidative and antiradical activities [5], larvicidal and mosquito repellent activities [6,7].

3. Present Study

About 1 kg of *E. camaldulensis* fresh leaves was extracted by water and steam distillation for 5 h (until no more essential oil was obtained). The essential oils were collected, dried over anhydrous sodium sulfate, and stored in sealed vials at low temperature before analysis.

The chemical compositions of the essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS) on a system consisting of a GC-17A gas chromatograph (GC) coupled to a QP5050A mass spectrometer (Shimadzu, Kyoto, Japan), and a fused-silica capillary column TC-1 (0.25 mm i.d. x 15 m, 0.25 μ m film thickness; GL Sciences, Tokyo, Japan) was used according to the literature method [5]. Identification of the compounds was based on comparison of GC-MS data with the NIST database library, and most compounds such as *p*-cymene, γ -terpinene, α -pinene, 1,8-cineole, terpinen-4-ol, and α -terpineol were directly compared with authentic compounds obtained as reagents. The chemical compositions of the oils were calculated based on the peak areas of GC chromatogram using 1-decanol as an internal standard.

Yield and compound identification: The essential oil yields of *E. camaldulensis* leaves ranged from 1.07% to 2.23% based on dry leaves. The results of GC-MS analyses of these essential oils are shown in Table 1. Six compounds were identified; three were major components (γ -terpinene, *p*-cymene, 1,8-cineole) and three others were minor components. Among the major components, γ -terpinene showed the highest content in the components of each oil sample, followed by 1,8-cineole and *p*-cymene. However, 1,8-cineole was not detected in the S3 oil sample. Terpinen-4-ol, one of the minor components, was also only detected in the S2 and S3 oil samples. The other minor components, α -pinene and α -terpineol were contained in only the S1 oil sample. The number of the identified compounds obtained from the present study is much less than those of 22-year old *E. camaldulensis* previously reported [8]. The variations in the *Eucalyptus* oil yields and chemical constituents have been well investigated. They were greatly influenced by several parameters, such as plant varieties, sampling season, location, climate, soil type, tree ages, fertility regime, drying method of the plant material, and oil extraction procedures [1, 9-12].

Antifungal assay: Nine fungi were obtained from the department of biotechnology, national institute of technology and evaluation, Chiba, Japan, namely *Aspergillus niger* NBRC 6342, *Cladosporium cladosporioides* NBRC 6348, *Chaetomium globosum* NBRC 6347, *Fomitopsis palustris* NBRC 30339, *Fusarium oxysporum* NBRC 31213, *Penicillium citrinum* NBRC 6352, *Rhizopus oryzae* NBRC 31005, *Thanatephorus cucumeris* NBRC 30937, and *Trametes versicolor* NBRC 4937 were used in the experiments.

Antifungal activity in the present study was evaluated according to the literature method [13] with slight modification. Potato dextrose agar (PDA, Difco Co.) plates were prepared on Petri dishes with 9 cm diameter. The known weights of oil samples were dissolved in methanol. These solutions were serially diluted and then added to 20 mL of PDA to final concentrations of 5 and 10 mg/mL, respectively. For antifungal tests, mycelium discs (5 mm diameter) were taken from the periphery of the colony cultivated for 4-14 days and placed on the center of each PDA plate in Petri dish.

Table 1. Tree ages, oil yields, and their chemical compositions of three clones of *Eucalyptus camaldulensis*

Clone Sample	Tree age (years)	Oil yield (%) ^a	Chemical composition of oil						Total (%) ^b
			<i>p</i> -Cymene (%) ^b	γ Terpinene (%) ^b	1,8-Cineole (%) ^b	Terpinen-4-ol (%) ^b	α -Pinene (%) ^b	α -Terpineol (%) ^b	
S1	1.0	1.07	19.16	33.03	27.22	-	5.25	2.91	87.57
S2	1.5	2.23	17.50	42.49	33.60	3.92	-	-	97.51
S3	1.0	1.95	18.79	75.50	-	3.90	-	-	98.19

^a Based on dry leaves^b Based on chromatogram peak areas. Dashes indicate no detection

Three replicate plates were set up for each concentration and the plates were incubated in the dark at 27°C. The area (cm²) of the mycelial colony was measured everyday for seven days, the antifungal index was calculated as follows:

$$\text{Antifungal index (\%)} = (1 - \text{Sa/Sb}) \times 100$$

Sa: Area of mycelial colony grown with oil sample (cm²)Sb: Area of mycelial colony grown in control (cm²)

PDA plates containing methanol without essential oil solutions were used as a control. Each experiment was performed three times, and the data were averaged.

The Scheffe method was used to evaluate the differences in antifungal indices in the antifungal tests. Results with $P < 0.05$ were considered statistically significant.

Antifungal activities: Nine fungi tested here represented three fungal groups namely, (1) household molds or allergy inducing molds: *A. niger*, *C. cladosporioides*, *C. globosum* and *P. citrinum*, (2) wood rot fungi: *F. palustris* and *T. versicolor*, (3) plant pathogenic fungi: *F. oxysporum*, *T. cucumeris* and *R. oryzae*. The results of antifungal activities of *E. camaldulensis* leaf oils against nine fungal strains are shown in Table 2. The essential oils from *E. camaldulensis* leaves are able to inhibit the mycelial growth of eight fungi tested, but the inhibitory effects vary relatively according to the chemical ingredients of the oils and the fungal species.

The essential oils of *E. camaldulensis* leaves used here are the most effective for retarding plant pathogenic fungi except fast growing species (*R. oryzae*). They show good inhibitory effects against two damping-off pathogens [*T. cucumeris* (100% at 5 mg/mL) and *F. oxysporum* (more than 84% at 5 mg/mL)] and one household mold [*C. globosum* (100% at 10 mg/mL)]. These results are in agreement with the previous findings that the essential oils from *E. camaldulensis* significantly inhibited the mycelial growth of wood rot fungi [14]. It was also reported that the oils were able to inhibit the seed-borne fungi such as *Colletotrichum graminicola*, *Phoma sorghina*, and *Fusarium moniliforme* [15].

The essential oils from S1 and S2 showed the best inhibitory effect at the concentration of 10 mg/mL against the growths of *P. citrinum* and *C. cladosporioides*, respectively, and it was statistically significant. The main components of the oils obtained from three different clones are considerably different. S1 and S2 contain 1,8-cineole and γ -terpinene, but S3 only contains γ -terpinene. Therefore, higher activities of S1 and S2 oils were probably due to the existence of 1,8-cineole. The leaf oils of three different clones show wide range of inhibition activities against 5 fungi tested. The inhibition indices at the concentration of 10 mg/mL were as follows: *A. niger* (45.4 to 48.8%), *C. cladosporioides* (53.9 to 89.5%), *F. palustris* (80.8 to 90.9%), *P. citrinum* (29.1 to 82.0%) and *T. versicolor* (35.3 to 43.6%).

Table 2. Antifungal indices of *E. camaldulensis* leaf oils at 5 and 10 mg/mL against 9 fungi

Sample	Concentration (mg/mL)	Antifungal index ^{a, b} (%)								
		A. <i>niger</i>	C. <i>globosum</i>	C. <i>cladosporioides</i>	F. <i>palustris</i>	F. <i>oxysporum</i>	P. <i>citrinum</i>	R. <i>oryzae</i>	T. <i>cucumeris</i>	T. <i>versicolor</i>
S1	5	19.0 ± 1.0b	60.9 ± 0.7b	58.0 ± 1.2b	60.3 ± 5.0c	91.3 ± 2.8ab	66.7 ± 4.0b	nd ^c	100.0 ± 0.0a	0.0 ± 0d
S1	10	45.4 ± 4.1a	100.0 ± 0.0a	62.5 ± 0.7b	80.8 ± 0.0ab	95.7 ± 2.3a	82.0 ± 7.0a	nd ^c	100.0 ± 0.0a	39.5 ± 5.3a
S2	5	22.5 ± 4.3b	43.2 ± 7.6c	34.9 ± 7.9c	76.6 ± 5.7b	84.0 ± 4.5b	24.1 ± 3.5c	nd ^c	100.0 ± 0.0a	15.6 ± 0c
S2	10	48.8 ± 6.4a	100.0 ± 0.0a	89.5 ± 1.4a	83.2 ± 2.2ab	97.7 ± 1.4a	53.9 ± 5.9b	nd ^c	100.0 ± 0.0a	43.6 ± 3.2a
S3	5	27.9 ± 6.5b	37.8 ± 2.2c	48.7 ± 0.9bc	63.2 ± 0.0c	90.5 ± 1.0ab	22.6 ± 3.9c	nd ^c	100.0 ± 0.0a	28.6 ± 1.3b
S3	10	47.8 ± 5.5a	100.0 ± 0.0a	53.9 ± 9.9b	90.9 ± 4.7a	97.7 ± 0.1a	29.1 ± 2.7c	nd ^c	100.0 ± 0.0a	35.3 ± 3.3ab

^aData collected after 7 days of incubation.

^bEach experiment was performed three times, and the data were averaged ($n = 3$). Values are means of three replication ± SD.

Numbers followed by different alphabetical among the oil samples tested are significantly different at $P < 0.05$ according to the Scheffe test.

^cnd: No detection of antifungal activity.

In conclusion, the essential oils obtained from *E. camaldulensis* leaves have a potential as the antifungal agents especially against the seedling blight pathogens. Furthermore, they are able to act as a moderate antifungal agent against household molds and wood rot fungi. Gentle and versatile natural substances such as essential oils are recently favored due to low toxicity, eco-friendliness, and comfort etc. Even if they need relatively higher concentration for the antifungal agents, they deserve as the alternatives to hazardous synthetic fungicides from the ecological viewpoints.

Eucalyptus camaldulensis is being extensively planted in Thailand in order to fulfill the increasing demand of pulpwood. It means that huge amount of leaves will be remained as the industrial wastes, therefore, effective treatment of these wastes is urgent matter. The antifungal agent can be one of the promising and value-added materials derived from the wastes.

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