

Antifungal Properties of Essential Oils Derived from Three Plants of Zingiberaceae Family against *Phytophthora parasitica* Dastur †

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Abstract: The purpose of the study was to investigate the antifungal activity of essential oils derived from the Zingiberaceae family, such as *Zingiber officinale* Roscoe, *Alpinia officinarum*. Hance, and *Curcuma longa* Linn., against *Phytophthora parasitica* Dastur, (the pathogen that causes root and stem rot diseases). In vitro antifungal activity was measured using poisoned food technologies at a final concentration of 1000 mg/L in a completely randomized design with triplications. The essential oil of *A. officinarum* demonstrated the highest antifungal activity against *P. parasitica*, according to findings ($p < 0.05$). Gas chromatography-mass spectrometry was also used to analyze the oil. Twenty constituents representing 99.1% of the total content were identified. Eucalyptol was the most abundant component in *A. officinarum* rhizome oil (52%). The oil's half-inhibitory concentration (IC₅₀) of 432.89 mg/L had higher antifungal activity than eucalyptol (>1000 mg/L). The results suggested that *A. officinarum* oil should be expanded further for a new generation of fungicides as an environmentally acceptable agent and to reduce the use of chemicals in crop protection.

Keywords: antifungal activity; Zingiberaceae; essential oil; *Phytophthora parasitica*; IC₅₀ values

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1. Introduction

Phytophthora parasitica is a phytopathogenic fungus and a widespread oomycete that infects a wide range of crop plants. The fungus can attack and damage the root tissues [1]. The fungus has been successfully controlled by chemical fungicides. Synthetic agrochemicals have direct effects on non-target organisms and the surrounding environment [2].

Essential oils are complex aromatic chemical agents extracted from traditional medicinal plants distributed in many tropical countries. However, the oils are also secondary metabolites with a few understandings about their modes of action [3]. Many researchers are still searching for the new generation of fungicides derived from plants. The Zingiberaceae family consists of about 47 genera and 500 plant species distributed throughout the tropical and subtropical regions. Many members of the family have been studied for antifungal activity against dermatophytes, filamentous fungi, and yeast-like fungi [4]. The main groups of compositions, including terpenes and terpenoids, coumarins, and phenylpropanoids, have been reported [5].

In this work, the aim was to investigate the *in vitro* antifungal activity of essential oils derived from the Zingiberaceae family, including *Zingiber officinale*, *Alpinia officinarum*, and *Curcuma longa*, against *P. parasitica* mycelia growth. The effective oil was also to determine the chemical compositions of effective oils.

2. Methods

2.1. Plant Materials

Fresh rhizomes of *Z. officinale*, *A. officinarum*, and *C. longa* were purchased from the Or Tor Kor Market, Bangkok, Thailand. The plants tested were identified and confirmed by the herbarium of the Kasin Suyathabandhu Herbarium (HCU-Herbarium) at the Department of Botany, Faculty of Science, Chulalongkorn University.

2.2. Hydro-Distillation

Five kilograms of the fresh rhizomes of these plants were washed, cut into small pieces (5×5 cm²), and dried. Each plant was then subjected to hydrodistillation in a Clevenger-type apparatus for 4 h. The oils were separated and dried over anhydrous sodium sulfate, and obtained. Each oil was placed in a paper-wrapped container to protect it from oxidation (kept at 4 °C) [6].

2.3. Compound

Eucalyptol (95%) was purchased from Sigma-Aldrich.

2.4. Fungal Strain

P. parasitica was supplied by the Center of Excellence in Chemistry of Natural Products, Faculty of Science, Chulalongkorn University. The fungus was maintained on carrot agar (CA) at 30 °C in the dark.

2.5. Antifungal Activity Assay

The oils were filtered after being dissolved in 20 percent DMSO at 10⁵ mg/L. The melted CA was combined with 0.1 mL of different oils with a final concentration of 1000 mg/L and placed into petri plates. The control treatment consisted of a CA mix containing only 20% DMSO. This research employed the poisoned food approach [7].

By testing multiple concentrations of the effective oil and the main component, the half-maximal inhibitory concentration (IC₅₀) was calculated. The percentage mycelia growth inhibition of each concentration was plotted against the concentration using a linear equation method, yielding a 50% suppression of mycelia growth.

2.6. Gas Chromatography-Mass Spectroscopy Detection Analysis

An agilent 6890 NGC and an Agilent 5973 MS were used for the GC-MS study. An HP-5MS fused-silica capillary column with dimensions of 30 m × 0.250 mm id and a film thickness of 0.25 μm was utilized to examine the samples. With a constant flow of 1 mL/min, an injector temperature of 270 °C, and a splitless ratio of 1:100, helium was employed as the carrier gas. The column oven's temperature was supposed to rise from 60 to 280 °C in 1 min and then remain isothermal for 25 min. The ion source was 250 °C, the transfer line was 280 °C, and the ionization energy was 70 electron volts. Electron-impact mass spectra were collected from 20 to 550 amu.

The chemical components of the oil were evaluated by comparing their retention times and mass spectra to the Wiley 7N electronic libraries, as performed by the Central Instrument Facility, Faculty of Science, Mahidol University, Bangkok, Thailand.

2.7. Statistical Analysis

The SPSS software for Windows version 20.0 was used to analyze the data. The Duncan's Multiple Range Test (DMRT) was used to compare the results, and significance was determined at the $p \leq 0.05$ level. The experiment used a general linear model with duplication within a completely randomized design.

3. Results and Discussion

3.1. Percentage Yields

The percentage yields of the hydro-distilled oils are shown in Table 1. The rhizome oil from *C. longa* obtained the highest yield.

Table 1. Percentage yields of the oils.

Plant	%Yield (v/w)	Color Characteristics
<i>Z. officinale</i>	1.04	Pale-yellow clear liquid
<i>A. officinarum</i>	1.30	Pale-yellow clear liquid
<i>C. longa</i>	1.60	Pale-yellow clear liquid

3.2. Antifungal Susceptibility

The antifungal activity of the rhizome oils against *P. parasitica* are shown in Table 2. The oil from *A. officinarum* displayed completely antifungal activity on the tested fungus. Thus, the oil was chosen for checking the chemical compositions.

Table 2. Antifungal activity of essential oils against *P. parasitica* at 1000 mg/L.

Essential Oil	% Mycelia growth Inhibition * (% Mean \pm SD), $n = 3$
<i>Z. officinale</i>	20.55 \pm 1.47 ^b
<i>A. officinarum</i>	100.00 \pm 0.00 ^a
<i>C. longa</i>	21.67 \pm 1.46 ^b

* Mean values within a column followed by a different letter are significantly different ($p < 0.05$; DMRT) ($R^2 = 0.99$).

3.3. Chemical Compositions of the *A. officinarum* Oil

The GC/MS analysis revealed 21 principal compositions, accounting for 99.1% of the oil. Among 21 compounds, eucalyptol (52%) was the major component, followed by α -fenchyl acetate (9%), β -pinene (6%), α -terpineol (6%), β -caryophyllene (4%) and Terpinen-4-ol (3%).

3.4. The Half Maximal Inhibitory Concentration (IC_{50})

The oil from *A. officinarum* oil was used for the IC_{50} study, comparing the main compound with eucalyptol in different concentrations. The results are displayed in Figure 1.

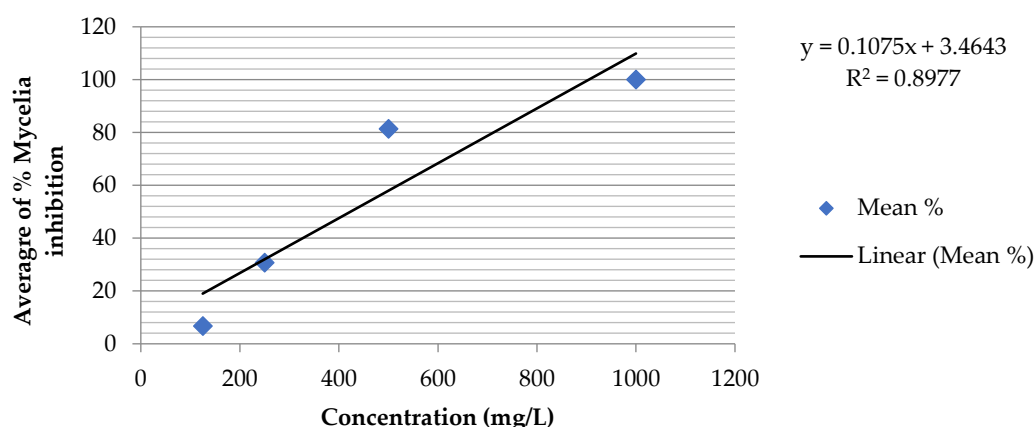


Figure 1. IC₅₀ value of the *A. officinarum* oil against *P. parasitica*

The oil's IC₅₀ was calculated as 432.89 mg/L using the linear equation formula: $y = 0.1075x + 3.4643$. Thus, 898.01 mg/L could be used to achieve complete inhibition of *P. parasitica* mycelia. The IC₅₀ value for eucalyptus could not be calculated because the only compound in various concentrations supported the mycelia growth of the fungus tested. The findings suggested that the oil's synergistic compounds could boost antifungal activity against the fungus.

4. Conclusions

The essential oils of rhizomes of *Z. officinale*, *A. officinarum*, and *C. longa* showed antifungal activity against *P. parasitica*. The *A. officinarum* oil displayed the most antifungal activity. The major composition of the effective oil was eucalyptol. The synergistic agent as the oil presented more mycelia growth inhibition than the eucalyptol. The *A. officinarum* oil could be feasible to use as a natural agrochemical for prevention of the growth of *P. parasitica*.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, P.C.; methodology, S.T. and P.C.; software, S.T. and P.C.; validation, P.C.; formal analysis, S.T. and P.C.; investigation, S.T. and P.C. resources, S.T. and P.C.; data curation, S.T. and P.C.; writing—original draft preparation, S.T. and P.C.; writing—review and editing, P.C.; visualization, S.T. and P.C.; supervision, P.C.; project administration, P.C.; funding acquisition, S.T. and P.C. All authors have read and agreed to the published version of the manuscript.

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