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Antifungal Activity and Phytochemical Analysis of Myrtaceae Leaf Extracts including the Induction of Defense Enzymes in Lettuce against *Alternaria* Leaf Spot

Suriyasit Somnuek*, Kamronwit Thipmanee, and Tanimnun Jaenaksorn

Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

*Corresponding author: suriyasitsom@gmail.com

Abstract

To avoid the risk of harmful effects from synthetic fungicides on human and natural environments, our study aimed to evaluate the potential use of the indigenous Thai Myrtaceae plants as a harmless means for controlling the plant diseases. Four Myrtaceae extracts showed no phytotoxicity towards lettuce cultivated in hydroponic conditions, with notable plant-growth-promoting potential observed in lettuce treated with *Callistemon viminalis* (EECV). With regard to a direct effect, *Melaleuca cajuputi* (95% EEMC) showed the strongest inhibition of mycelial growth of *A. brassicicola* (85–100%), followed by 95% EECV (40–63%), while both extracts significantly inhibited spore germination (80–100%). A GC/MS and LC/MS analysis revealed terpenoids as the predominant compounds at approximately 55% and 37% in EEMC and EECV, respectively, with high concentrations of phytol (14%) and 1,8-cineole (14%) being particularly notable. As an inducer of an indirect effect of the plant extracts, the results from the experiment with 95% EEMC (15,000 and 50,000 ppm) as well as 50 and 95% EECV (15,000 and 50,000 ppm) applications was in good agreement, demonstrating significant reductions in disease severity in hydroponically grown lettuce ranging from 50 to 70% together with the induction of defense enzymes (specifically β-1,3-glucanase, chitinase, and peroxidase) compared to those in the inoculated control and fungicide treatment groups. Furthermore, a correlation analysis revealed a negative correlation between disease severity and the three defense enzymes. Our findings underscore the considerable efficacy of the three botanical fungicides tested here (i.e., 95% EEMC as well as 95% and 50% EECV) for the management of *Alternaria* leaf spot in lettuce.

Additional key words: biofungicide, *Callistemon viminalis*, GC-MS/MS, LC-MS/MS, *Melaleuca cajuputi*, plant extract

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Introduction

For leafy vegetable production, synthetic fungicides have been widely employed to combat foliar diseases, including *Alternaria* leaf spot. However, the use of chemical fungicides is increasingly restricted due to concerns about food safety, particularly within organic farming practices (Horsfield

et al. 2010; Wang et al. 2016; Yang et al. 2019; Wu et al. 2023). Hence, a transfer from synthetic to natural safe fungicide is needed. Plant extracts are of great significance in this regard. Scientific studies have been carried out worldwide on the antifungal activities of plant extracts against the fungal pathogens of plant diseases, which have resulted in the development of promising sources of natural fungicides and botanical fungicides offering a potential solution to agricultural challenges (Jantasorn et al. 2016; Dethoup et al. 2018; Dethoup et al. 2019; Sukdee 2023). In general, plant extracts have secondary metabolites (e.g., phenolics, phenolic acids, terpenoids, essential oils, alkaloids, tannins, flavonoids, coumarins, lectins and polypeptide) which represent biologically active substances (Gurjar et al. 2012; Pinto et al. 2018). Several researchers have uncovered the potential of medicinal plants to control plant pathogens, both by direct fungal toxic actions and due to the ability to stimulate the accumulation of molecules with elicitor features capable of inducing defense responses (Bonaldo et al. 2004; Celoto et al. 2008; Bulhões et al. 2012; Pinto et al. 2018). These resistance mechanisms may include the accumulation of phenolic compounds, phytoalexins and pathogenesis-related proteins such as β-1,3-glucanase, chitinase, peroxidase, phenylalanine ammonia lyase and polyphenol oxidase (Barros et al. 2010). Plant extract enhancers of the defense response in Ponkan mandarin seedlings against *Alternaria alternata* f. spp. citri infection were reported by Pinto et al. (2018). In 2012, Gurjar et al. reviewed the efficacy of plant extracts in plant disease management, finding that thousands of phytochemicals with inhibitory effects on all types of microorganisms in vitro should be subjected to in vivo testing to evaluate how well they control the incidence of disease in crops. The latest studies of natural alternatives for chemical synthetic pesticides, including the application of neem extract to manage postharvest losses due to postharvest diseases (e.g., *Aspergillus flavus*, *A. niger* and *Botrytis cinerea*) of fresh produce in tropical and subtropical fruits, were reviewed by Tzortzakis and Proestos (2024). In Thailand, the popularity of botanical fungicides is also increasing. Quite a number of phytochemical extracts have been evaluated during the search for plant-based antifungal agents for plant disease management, some recently reported studies include those on indigenous plants, such as *Hydnocarpus anthelminthicus*, *Crateva magna*, *Caesalpinia sappan* (Jantasorn et al. 2016), *Acorus calamus* (Dethoup et al. 2019) and black pepper (Kunasakdakul and Suwitchayanon 2012).

Myrtaceae plants, specifically *Callistemon viminalis*, *Melaleuca cajuputi*, *Syzygium jambos*, and *Syzygium malaccense*, are naturally found in Australia, southeast Asian countries, including Thailand, Malaysia and Indonesia (Bharat and Praveen 2016; Salem et al. 2017; Patel et al. 2019; Isah et al. 2023). Various plant species from the Myrtaceae family have long been widely used for medicinal purposes given their antimicrobial, anti-inflammatory and antioxidant activities (Imatomi et al. 2013; Al-Abd et al. 2015; Rita et al. 2017; Salem et al. 2017; Puig et al. 2018; Patel et al. 2019; Vasconcelos et al. 2022). The phytochemical extract of *C. viminalis* demonstrated strong to moderate antibacterial effects against certain plant bacterial pathogens (El-Hefny et al. 2017) as well as in vitro antifungal effects (Somnuek et al. 2020; Somnuek et al. 2021). Essential oil extracted from *M. cajuputi* has been widely used worldwide for many purposes. Most of the compounds found in this plant possess aromatic, antibacterial and insecticide properties (Sharif et al. 2019). Keereedach et al. (2020) reported that Thai cajuput oil can be used to create new potential combination therapies to combat the antifungal resistance of *Candida albicans*. In addition, several studies have found the excellent antimicrobial effects of *M. cajuputi* extracts against bacteria, viruses, protozoa and fungal species due to the presence of specific phytoconstituents (Isah et al. 2023). *S. jambos* extract showed antimicrobial activity on the growth of both gram-positive and gram-negative bacteria (Mohanty and Cock 2010), while only gram-negative bacteria were susceptible to the extract of *S. malaccense* (Bouzada et al. 2009). Although multiple studies have analyzed chemical compounds and the antibacterial

activities of extracts from different Myrtaceae family plants (Al-Abd et al. 2015; Salem et al. 2017; Isah et al. 2023), few studies have evaluated the potential of these extracts in in vivo testing to control the incidence of fungal plant diseases.

Therefore, the objective of our research was to investigate the possibility of employing ethanolic extracts from Myrtaceae plants as an alternative approach for managing *Alternaria* leaf spot in lettuce. For this purpose, an experimental series was carried out to determine the following: (1) the phytotoxicity of Myrtaceae plant extracts on the cultivated crop, (2) the direct effects of Myrtaceae extracts on mycelial growth and spore germination of a fungal pathogen of lettuce leaf spot, (3) the phytochemical profile of Myrtaceae plant extracts according to liquid chromatography (LC) / mass spectrometry (MS) and gas chromatography (GC) / MS, (4) the potential of Myrtaceae plant extracts as an inducer for defense-related enzymes for controlling *Alternaria* leaf spot in lettuce grown in hydroponics and (5) the correlation between disease severity and defense enzymes.

Materials and Methods

Myrtaceae extracts preparation

Four Myrtaceae plants were studied here. *Callistemon viminalis* (CV), *Syzygium jambos* (SJ), and *Syzygium malaccense* (SM) were collected from an area in Bangkok province, while *Melaleuca cajuputi* (MC) was collected from Chumphon province in Thailand.

Crude extracts using 95% ethanol

Fresh leaves of the Myrtaceae plants were cleaned with tap water and dried in open air. The leaf samples were then completely dried using a hot air oven (Memmert) at 50°C. The dried leaves were ground and extracted using the Soxhlet extraction technique with 95% ethanol, after which the ethanol solvent was evaporated with a rotary evaporator (Buchi, Rotavapor R300) at 50°C. The sticky extracts were kept at 4°C for further study.

Crude extracts using 50% ethanol

The method used to extract the *Callistemon viminalis* leaves was identical to that used with the 95% ethanolic crude extracts but with 50% ethanol instead as a solvent. This extract as well was kept at 4°C for further study.

Identification of *Alternaria* species by sequence analysis

The *Alternaria* isolate in this experiment obtained from our previous research (Somnuek et al. 2020) was morphologically identified and already proven with regard to its pathogenicity. Here, the identification of *Alternaria* species was confirmed by molecular identification. The genomic DNA of the tested fungus was extracted and the extracted DNA was used as the template for amplification with the ITS1 primer (5’ TCCGTAGGTGAACCTGCGG 3’) and ITS4 primer (5’ TCCTCCGCTTATTGATATGC ’3) of the internal transcribed spacer (ITS) of rDNA regions via polymerase chain reaction (PCR), with the process conducted according to the work of Mohammadi and Bahramikia (2019). The purified PCR products were sequenced by Bionics Co., Ltd. of Korea. Sequence similarity analyses were conducted using the Basic Local Alignment Search Tool (BLAST) in the GenBank NCBI database.

Preliminary phytochemical screening of leaf extracts from four Myrtaceae plants

Phytochemical compounds such as phenols, flavonoids, tannins, alkaloids, and terpenoids showed antifungal properties on plant pathogenic fungi, and they acted in a synergistic manner (Tiwari et al. 2011; Gurjar et al. 2012). Therefore, this part of the experiment was undertaken to screen the targeted phytochemical compounds from the ethanolic crude extracts of *Callistemon viminalis* (95% EECV), *Melaleuca cajuputi* (95% EEMC), *Syzygium jambos* (95% EESJ), and *Syzygium malaccense* (95% EESM). Phytocompounds were detected according to a slightly modified version of the methods of Harborne (1998), Iqbal et al. (2015) and Dubale et al. (2023). The qualitative results were assessed by means of colorimetric reactions and were presented here as positive (+) for the presence of and negative (−) for the absence of phytochemicals.

Phytotoxicity of four Myrtaceae extracts on lettuce grown in hydroponics

Plant extracts, an important source of bioactive compounds, have been suggested as a viable, environmentally friendly option for plant disease control. However, plant extracts may also have negative impacts on cultivated crops. Therefore, a preliminary test was conducted here to find the extract with the least negative effect on lettuce grown in a hydroponic system. The experiment utilized a 4 × 4 × 2 factorial in a completely randomized design (CRD) with 3 replications (3 plants per replicate). Factor A was the type of plant extract (EECV, EEMC, EESM, and EEMJ), Factor B was the concentration of the extract used (0, 5,000, 25,000 and 50,000 ppm), and Factor C was the number of applications (one time and two times). The tested lettuce plants were grown in a deep water culture (DWC) system, which was constructed from a plastic box (25 × 34 × 15 cm) and filled with a nutrient solution (EC = 1.6 − 1.8 mS/cm, pH = 5.8 − 6.2) (Benoit and Ceustermans 1995). Subsequently, 2 ml of each prepared extract was sprayed on 15-day-old lettuce seedlings, and this step was repeatedly sprayed on 30-day-old lettuce seedling group. Phytotoxicity was evaluated according to the incidence of necrosis and discoloration at 1 to 3 days after the foliar spray. Moreover, the lettuce growth parameter of leaf greenness of the lettuce under test was monitored weekly using a chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) after foliar spraying, whereas the fresh weights of the plants were recorded at the end of the cultivation period.

In vitro antifungal activity of Myrtaceae extracts on the growth of *Alternaria* sp.

Effect of four Myrtaceae extracts on mycelial growth

The effect of the ethanolic extracts from the four Myrtaceae plants on the mycelium growth of *Alternaria brassicicola* was investigated using 4 × 4 factorials in a completely randomized design (CRD). Factor A indicated the 4 Myrtaceae plants, and Factor B represented the 4 concentrations of the extracts used. The crude extracts were tested with regard to the mycelial growth of *A. brassicicola* by a poisoned food technique. A fungus was cultured on potato dextrose agar (PDA; Sisco Research Laboratories Pvt. Ltd., India) at room temperature (~25°C). Mycelial disks, 0.5 cm in diameter, were cut from the margins of the colonies and inoculated onto PDA mixed with each extract. The sizes of *A. brassicicola* colonies in the control plate and the plate containing the extract were determined at 3, 5, and 7 days after inoculation. Mycelial growth inhibition was calculated using the following formula: Mycelial growth inhibition (%) = (DC − DT)/DC × 100 where DC = diameter of the *Alternaria* colony on the control plate

DT = diameter of the *Alternaria* colony on the plate containing the extract

Effect of potentially selected Myrtaceae extracts on spore germination

Two plant extracts (95% EEMC and 95% EECV) showing high-potential antifungal activity in the mycelium growth test described above were selected and tested further for their effect on *A. brassicicola* spore germination. The experiment was carried out by CRD. One mL of spore suspension (approximately 10^5 spores/mL) was inoculated into 1 mL of PDB in test tubes with 5 extract concentrations (1,000, 5,000, 10,000, 15,000 and 50,000 ppm). Sterilized water and mancozeb (500 ppm) were used as inoculated and chemical controls, respectively. Then, spore germination of the tested fungus was observed under a light microscope at 24, 48, and 72 h.

GC/MS and LC/MC-TOF analyses of the Myrtaceae extracts

GC/MS and LC/MS have often been used to analyze phytochemical compounds in plant extracts. The two methods are more alike than different. The only key difference between the systems is that GC/MS uses a gas mobile phase while LC/MS uses a liquid mobile phase. Therefore, GC/MS appears to be more applicable to more volatile and gas samples.

GC/MS analyses of the 95% EEMC and 95% EECV were carried out according to a slightly modified method based on Al-Abd et al. (2015) and Hassan et al. (2022). A gas chromatograph-mass spectrometer (7890 B GC–5977A MSD, Agilent Technologies, Inc., USA) was employed for these analyses. A HP-5 Ultra Inert column (30 m × 250 μm × 0.25 μm, Agilent Technologies) with helium (He, flow rate = 40 cm/s) as the carrier gas was used. The oven was initially programmed to run at a temperature of 60°C (hold time 2 min), a rate of 7°C/min to 150°C (hold time 1 min), a rate of 2°C/min to 230°C (hold time 1 min), and a rate of 15°C/min to 300°C (hold time 15 min). The injection volume of 1 μL operated in the split mode with a ratio of 10:1. The MS transfer line and ion source temperatures were maintained at 230°C and 150°C, respectively, and the mass spectra detector voltage was set at 70 eV. The scan range was from 35 to 600 *m/z.* Peaks and chemical constituents were identified by a MassHunter Workstation Software Quantitative Analysis Version B.09.00 Unknown Analysis.

The LC/MS analyses of the 95% EEMC and 95% EECV were conducted according to a slightly modified method based on Al-Abd et al. (2015). The system for analyzing the samples consisted of a high-performance liquid chromatograph-mass spectrometer-quadrupole time-of-flight [ExonLCTM AD Series (LC) and X500R QTOF system (QTOF),-SCIEX] with dual electrospray ionization (ESI). The LC separation steps were performed using a 2.1 mm (i.d.) narrow-bore SB-C18 (length 150 mm, particle size 3.5 mM) analytical column. The LC parameters used were: 25°C for the autosampler temperature, 1 μL for the injection volume, 25°C for the column temperature, and 0.4 mL/min for the flow rate. A gradient system consisting of 0.1% formic acid in water as solvent A and 0.1% formic acid in acetonitrile as solvent B was used. The mass spectra data were acquired using an ESI capillary voltage of (+) 4000 V in the positive ion mode with the fragmentor set to 125 V. Other conditions were set to 45 psi for the liquid nebulizer, a flow rate of 10 L/min with the drying gas, and vaporizer temperatures maintained at 300°C for the nitrogen drying gas. Moreover, the ionization interface was operated in positive mode. The data were collected and analyzed using SCIEX OS 2.1.0.

Evaluation of EEMC and EECV as an inducer on development of *Alternaria* leaf spot and the induction of defense enzymes in lettuce grown in a hydroponic system

In addition to a direct effect evaluation, we also focused on an indirect effect of the extracts as inducers on *Alternaria*

leaf spot disease severity as well as on the activity of defense enzymes. Two experiments were separately conducted, one to assess the effect of 95% EEMC (15,000 and 50,000 ppm) and the other to test 95% EECV (15,000 and 50,000 ppm). Moreover, 50% EECV, given its potential to stimulate lettuce growth as determined in our previous research (Somnuek et al. 2020), was also included in the 95% EECV experiment for further evaluation as an inducer on defense enzyme activity.

The EEMC effect

Preparation and experimental design

In order to make certain of the indirect effect of EEMC as an inducer, a hydroponic experiment with lettuce was carried out. First, lettuce seeds were germinated in a plastic tray on a moist sponge with nutrient solution (EC = 1, pH = 5.8 − 6.2) for 14 days. The seedlings were then transferred to grow in DWC with nutrient solution (EC = 1.6 − 1.8 mS/cm, pH= 5.8 − 6.2). The 95% EEMC at 15,000 and 50,000 ppm were prepared and foliar sprayed on the above lettuce seedlings (1 mL/plant). The treated seedlings were inoculated with a 100 μL of tested spore suspension (approximately 10^6 spores/mL) a day after spraying with the plant extract. CRD with 3 replications (3 plants per replicate) was used in this experiment, as follows:

T1 = Healthy control, T2 = Inoculated control, T3 = Chemical control (mancozeb 500 ppm)

T4 = 95% EEMC (15,000 ppm), T5 = 95% EEMC (50,000 ppm)

Determination of disease severity

The treated leaves were observed at 1, 3, 5 and 7 days after inoculation (DAI), and a disease index was scored using a scale of 0 to 4 for the lesion size: 0 = no infection, 1 = 1 to 5 mm, 2 = 5 to 10 mm, 3 = 10 to 15 mm, and 4 ≥ 15 mm. Disease severity (DS) of the tested lettuce leaves was analyzed using the following equations:

% DS = [$\sum$ (number of infected leaves × disease index) / number of total leaves × the highest disease index] × 100.

In addition, the plant growth parameters, such as the greenness value (by chlorophyll meter; SPAD-502, Konica Minolta Sensing, Inc., Japan) and fresh weight were checked at 1, 3, 5, 7 days after spraying and at harvest.

Induction of plant defense enzymes due to the effect of the inducer

During pathogenesis, plant defense enzymes were also determined. The aforementioned treated lettuce leaves were collected at 1, 3, 5, and 7 days after foliar spraying. Three treated leaves were sampled from each replication of the treatment and were ground using liquid nitrogen. Then, 1 g of the tested leaf sample in each case was homogenized with 2 mL of 0.1 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged for 20 min at 10,000 rpm in a cooling centrifuge at 4°C. Subsequently, peroxidase, chitinase, and β-1,3-glucanase were detected according to a modified version of the method introduced by Verburg and Huynh (1991), Boller and Mauch (1988), Gupta et al. (2013) and Selvaraj and Ambalavanan (2013).

β-1,3-glucanase activity

The homogenate of 62.5 μL was mixed with laminarin (4% w/v in 0.05 M sodium acetate buffer, pH 5.0) of 62.5 μL for 10 min at 40°C. The reaction was then stopped by adding 375 μL of DNS and heating this solution for 5 min in a boiling

water bath. The absorbance was detected by a spectrophotometer at 500 nm. The enzyme activity was expressed as μmol glucose/g of fresh leaves.

Chitinase activity

The homogenate of 0.4 ml was mixed with colloidal chitin (0.1% w/v in 0.05 M sodium acetate buffer, pH 5.0) at a ratio of 1:1 and incubated at 37°C for 2 hrs with the product of N-acetyl glucosamine (GlcNAc). Then, the chitinase activity was detected with a spectrophotometer at 585 nm. The enzyme activity was expressed as μmol GlcNAc/g of fresh leaves.

Peroxidase (PO) activity

The enzyme supernatant of 100 μl was taken along with 0.05 M pyrogallol. To initiate the enzyme reaction, 1% H_2O_2 in an amount of 0.5 ml was added. The change in the absorbance was recorded by a spectrophotometer at 420 nm at 30 sec intervals for 3 min from zero seconds of incubation at room temperature. The result was expressed as the change in unit/g of fresh leaves.

The EECV effect

In this experiment, the preparation and experimental design, including the determination of disease severity and the plant defense enzyme activity, were carried out in the same manner as in the 95% EEMC experiment. Treatments in the experiment were as follows:

T1 = Healthy control, T2 = Inoculated control, T3 = Chemical control (mancozeb 500 ppm)
T4 = 50% EECV (15,000 ppm), T5 = 50% EECV (50,000 ppm), T6 = 95% EECV (15,000 ppm),
T7 = 95% EECV (50,000 ppm)

Correlation of disease severity and plant defense enzymes

In order to understand the relationship between the severity of leaf spot disease and the induced plant defense enzymes, as well as to confirm that these defense enzymes have a role in leaf spot management in lettuce, the correlation coefficients (r) between disease severity and the defense enzymes of β-1,3-glucanase, chitinase and peroxidase were estimated by standard statistical calculations. Simple regression equations (Y= a + bx) were also developed for all variables.

Results and Discussion

Identification of *Alternaria* species by sequence analysis

Alternaria species (Somnuek et al. 2020) was confirmed by ITS identification. The nucleotide sequences in the tested isolate were approximately 568 bp in size. The nucleotide sequence of the tested *Alternaria* isolate Alt-LL1 (OR226735) allowed an identification of *Alternaria brassicicola*. Our findings were in agreement with those of many researchers who reported that *A. brassicicola* was the causal agent of leaf spot disease in a number of vegetable crops, including lettuce (Pattanamahakul and Strange 1999; Dethoup et al. 2018; O'Neill 2019; Blagojević et al. 2020).

Phytochemical screening of four Myrtaceae leaf extracts

Our study confirmed the presence of qualitative phytochemical compounds, specifically phenol, flavonoids, and tannins, in all tested extracts (Table 1). High levels of phenols and flavonoids were especially detected in the 95% EEMC. Terpenoids were found only in the 95% EEMC and 95% EECV while alkaloids were not found in any of the extracts. Our findings were in accordance with those of many researchers who reported that the targeted phytochemical compounds, i.e., phenol, flavonoids, tannins, alkaloids and terpenoids, were found in several Myrtaceae plants, such as the fruit extract of *M. cajuputi* (Isnaini et al. 2023), *C. viminalis* extract (Salem et al. 2017), an extract from the leaves and bark of *S. jambos* (Wamba et al. 2018), the leaf extract of *S. malaccense* (Patel et al. 2019) and an extract from *Psidium cattleianum* leaves (Faleiro et al. 2016). These targeted phytocompounds were detected in other plant extracts as well (Tiwari et al. 2011; Gurjar et al. 2012).

Phytotoxicity of four Myrtaceae extracts on lettuce grown in hydroponics

The phytotoxic effect of the four Myrtaceae extracts on lettuce was assessed by the foliar spray technique. Of the 4 Myrtaceae extracts evaluated here, none of the treatments showed phytotoxicity (such as browning and discoloration) on lettuce at 15 and 30 days (Table 2). Regarding the plant growth parameters, the SPAD values did not differ significantly ($p < 0.05$) from control and the other treatments, while the fresh weights were dependent on the plant extracts and corresponding concentrations but not on the application times. The 95% EECV at all concentrations significantly ($p < 0.05$) stimulated the fresh weight of lettuce (65.5 − 70.2 g/plant) compared to those treated with other plant extracts (39 − 43.5 g/plant). In addition, an increase in the extract concentration appeared to stimulate lettuce growth more strongly (Table 2 and Fig. 1). This result also agreed with our previous research (Somnuek et al. 2020), which reported that 3 ethanolic extracts from *C. viminalis* (50%, 70% and 95% EECV) at 5,000 − 50,000 ppm showed no phytotoxic effect and still increased seed germination and the growth of lettuce. More notably, the water extract of this plant showing no phytotoxicity could still promote the growth of rice (Bali et al. 2017). Apart from the 95% EECV, the research on the phytotoxic effect of the other 3 tested plant extracts was rather limited, most likely due to the fact that these plants were Thai native plants. Furthermore, no phytotoxicity of extracts from other plants within Myrtaceae, such as *Myrcia tomentosa* (Imatomi et al. 2013), *Myrciaria dubia* (Kunth) McVaugh (Rita et al. 2017), *Eucalyptus globulus* (Puig et al. 2018), clove, tea tree, jaboticaba and guava (Carmello and Cardoso 2018; Teixeira et al. 2018), was also noted together with the stimulating effects on growth parameters (e.g., germination percentage, germination speed index, leaf number,

Table 1. Phytochemical compounds of 95% ethanolic crude extracts from the leaves of 4 Myrtaceae plants

Phytochemical compound	Plant extract			
	EEMC	EECV	EESJ	EESM
Phenols	++[z]	+	+	+
Flavonoids	++	+	+	+
Tannins	+	+	+	+
Alkaloids	−	−	−	−
Terpenoids	+	+	−	−

[z]++ = high presence, + = moderate presence, − = absence.

Table 2. Phytotoxicity test of 95% ethanolic crude extracts from 4 Myrtaceae plants on lettuce grown in a hydroponic system

Factor A (Plant extract)	Factor B (Concentration)	Factor C (Application time)[z]	Phytotoxicity[y] (3 DAT)	SPAD value	Fresh weight (g/plant)
EECV	0 ppm	One time	−	31.6 a[x]	40.9 c
		Two times	−	30.2 a	42.5 c
	5,000 ppm	One time	−	31.4 a	65.5 b
		Two times	−	31.5 a	67.3 ab
	25,000 ppm	One time	−	30.8 a	68.9 ab
		Two times	−	32.4 a	67.4 ab
	50,000 ppm	One time	−	32.5 a	68.3 ab
		Two times	−	30.7 a	70.2 a
EEMC	0 ppm	One time	−	33.4 a	40.9 c
		Two times	−	32.2 a	42.5 c
	5,000 ppm	One time	−	33.2 a	39.0 c
		Two times	−	31.6 a	43.5 c
	25,000 ppm	One time	−	30.6 a	40.1 c
		Two times	−	32.2 a	41.1 c
	50,000 ppm	One time	−	31.3 a	40.6 c
		Two times	−	31.9 a	40.1 c
EESJ	0 ppm	One time	−	32.0 a	40.9 c
		Two times	−	30.6 a	42.5 c
	5,000 ppm	One time	−	33.8 a	40.4 c
		Two times	−	33.2 a	39.6 c
	25,000 ppm	One time	−	32.0 a	41.1 c
		Two times	−	32.0 a	39.4 c
	50,000 ppm	One time	−	32.0 a	40.2 c
		Two times	−	30.8 a	41.2 c
EESM	0 ppm	One time	−	32.0 a	40.9 c
		Two times	−	32.8 a	42.5 c
	5,000 ppm	One time	−	32.0 a	39.0 c
		Two times	−	30.5 a	39.2 c
	25,000 ppm	One time	−	32.0 a	40.2 c
		Two times	−	30.2 a	40.0 c
	50,000 ppm	One time	−	32.3 a	40.6 c
		Two times	−	32.5 a	40.4 c
C.V. (%)				4.56	7.59
A				ns	ÚÚ
B				ns	ÚÚ
C				ns	ns
A × B				ns	ÚÚ
A × C				ns	ns
B × C				ns	ns
A × B × C				ns	ns

[z] Application time: For one time group, the spray was made at 15 days. For two times group, sprays were made at 15 and 30 days.

[y] − showing non-phytotoxicity, + showing phytotoxicity.

[x] Values are the means of 3 replicates. Values in each column followed by the same letter are not significantly different according to LSD ($p > 0.05$).

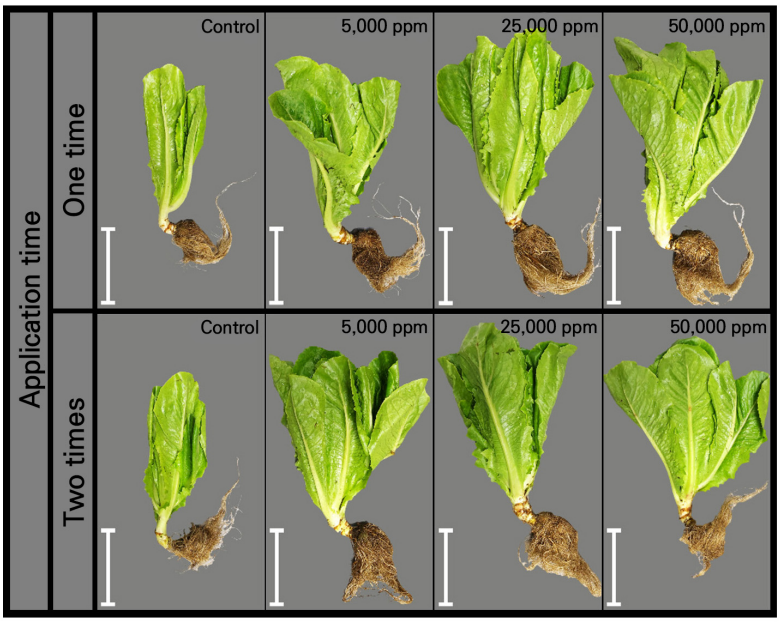

Fig. 1. A photo (at harvest) showing the non-phytotoxicity and plant growth stimulating effect of the 95% ethanolic extract of *C. viminalis* (95% EECV) spraying onto lettuce in a hydroponic system. Bar = 20 cm.

root length, and yield) in lettuce. In contrast, phytotoxic effects of *Myrcia vittoriana* (Myrtaceae) in a plant model (lettuce and onion) have recently been reported by Vasconcelos et al. (2022). Because the 4 Myrtaceae plant extracts have been proven so far from this experiment to have no negative effects on lettuce, their antifungal activities against *Alternaria* sp. in vitro and against leaf spot of lettuce grown in a hydroponic system should be investigated further.

In vitro antifungal activity of Myrtaceae extracts against growth of *Alternaria* sp.

Effect of four Myrtaceae extracts on mycelial growth

The antifungal activities of crude ethanolic extracts from the four Myrtaceae plants, i.e., *Callistemon viminalis* (95% EECV), *Melaleuca cajuputi* (95% EEMC), *Syzygium jambos* (95% EESJ) and *Syzygium malaccense* (95% EESM) at different concentrations were significantly ($p < 0.05$) noted against *A. brassicicola* mycelial growth. Moreover, their effect was dependent on the concentration of the extract and on the Myrtaceae plant species (Fig. 2). The 95% EEMC at all tested concentrations (5,000, 15,000 and 50,000 ppm) statistically ($p < 0.05$) showed the highest inhibitory effect (85 − 100%), while 95% EECV was the next most effective extract, with activity in the range of 40 − 63%. The 95% EESM and 95% EESJ presented inhibitory effects in the corresponding ranges of 15 − 80% and 30 − 40% (Fig. 2). The antifungal activity found in this experiment is attributable to the presence of phytochemical compounds such as phenolics, flavonoids, tannins and terpenoids. These phytocompounds were reported to have good antifungal effects on plant pathogens (Al-Abd et al. 2015; Bharat and Praveen 2016; Salem et al. 2017; Patel et al. 2019). The mode of action of the phytochemicals includes membrane disruption, enzyme inactivation, protein binding and toxicity (Tiwari et al. 2011; Gurjar et al. 2012). Moreover, the significant in vitro antifungal activities of 4 extracts were in good agreement with the aforementioned phytochemical screening test, which showed the highest phytochemical substance levels in the 95% EEMC and 95% EECV. This study was in line with those of other researchers who found that *M. cajuputi* (essential oil)

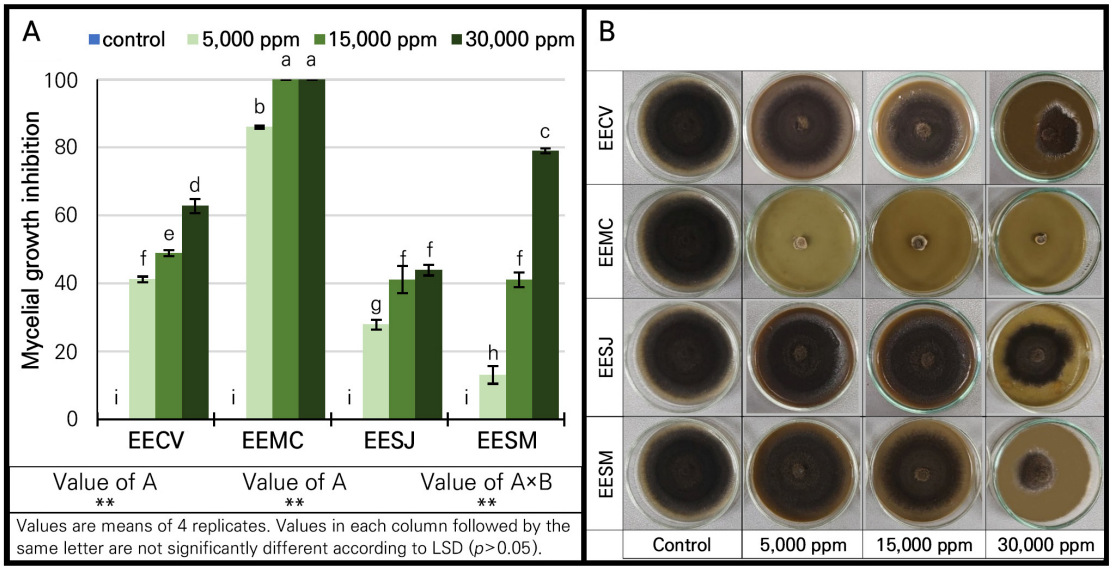

Fig. 2. Mycelial growth inhibition of *A. brassicicola* by different concentrations of 95% ethanolic extract of 4 Myrtaceae plants (EECV = *Callistemon viminalis*; EEMC = *Melaleuca cajuputi*, EESJ = *Syzygium jambos*; EESM = *Syzygium malaccense*) (A) and corresponding growth outcomes on Petri dishes (B).

had strong inhibitory effects on *Alternaria* spp. (Pawar and Thaker 2007; Siddique et al. 2018), *Aspergillus* spp. (Thanaboripat et al. 2007; Thanaboripat 2011; Bharat and Praveen 2016; Thanaboripat et al. 2016; Siddique et al. 2018), *Fusarium* spp. (Pawar and Thaker 2007; Siddique et al. 2018) and *Penicillium digitatum* (Siddique et al. 2018). Apart from having antifungal activity, the methanolic extract of *M. cajuputi* also exhibited antimicrobial activity against certain bacteria, such as *Bacillus* spp., *Enterococcus faecalis* (Ukit et al. 2019), *S. aureus*, *Vibrio cholerae*, *Shigella dysenteriae*, and *Staphylococcus epidermidis* (Al-Abd et al. 2015; Ukit et al. 2019). Of the 4 extracts tested here, the 95% EECV result confirmed the inhibitory effect, as noted in our previous research (Somnuek et al. 2020), against *Alternaria* sp. For 95% EESJ, our result agreed with other studies testing seed and leaf extracts. The seed extract was effective at controlling certain fungi, e.g., *Candida albicans*, *Microsporum canis*, and *M. gypseum* (Sakander et al. 2015), while the leaf extract inhibited the bacteria, namely *Salmonella typhi* (Murugan et al. 2011), *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Bacillus cereus* and *Staphylococcus aureus* (Mohanty and Cock 2010). In the 95% EESM case, *S. malaccense* leaf extracts showed inhibition activity against *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Shigella sonnei* (Bouzada et al. 2009).

Effect of potentially selected Myrtaceae extracts on spore germination

Potentially selected Myrtaceae extracts tested here, i.e., 95% EEMC and 95% EECV, at different concentrations (1,000, 5,000, 10,000, 15,000 and 50,000 ppm) were further evaluated for their antifungal activities on spore germination of *A. brassicicola*. Both 95% EEMC and 95% EECV at all tested concentrations indicated a significant ($p < 0.05$) inhibitory effect on spore germination (Fig. 3). Complete inhibition was noted upon the application of 95% EEMC at 5,000 − 50,000 ppm (Fig. 3), while abnormal spores were detected at all tested concentrations. At 72 hrs, abnormalities of *A. brassicicola* spores were detected, taking the forms of collapsed spores, degraded cell walls, and ungerminated spores (Fig. 3). For

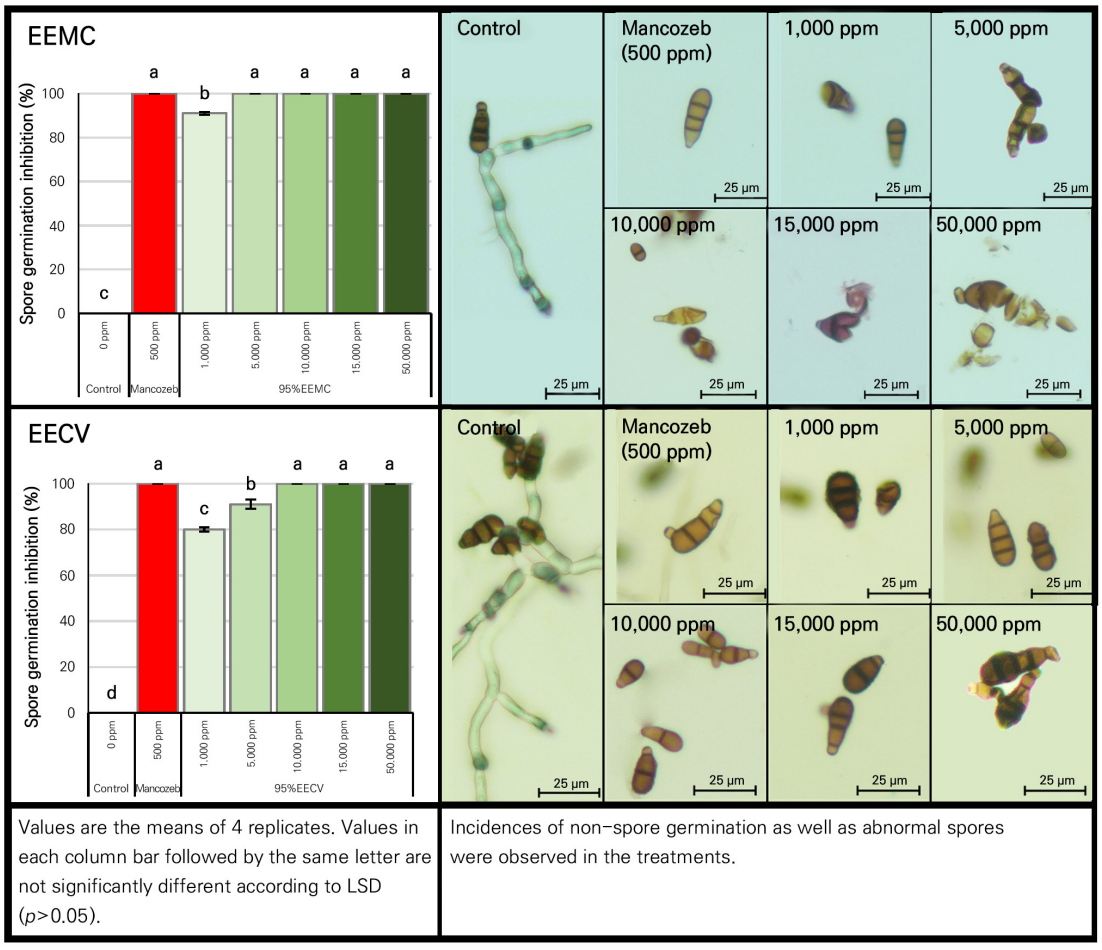

Fig. 3. Bar graph showing the inhibition effects of different concentrations of 95% ethanolic crude extracts from *M. cajuputi* (95% EEMC) and *C. viminalis* (95% EECV) on spore germination of *A. brassicicola*.

95% EECV, complete inhibition of spore germination was initially noted at 10,000 ppm and up. However, abnormal spores were only found at the highest extract concentration (50,000 ppm). Overall, we found that the higher concentration of plant extract induced the more potent inhibition of the growth of the pathogen in terms of mycelial growth and spore germination. Subsequently, the corresponding phytochemical profile responsible for this potent activity was further clarified in the following experiment, as described below.

Phytochemical profile of the Myrtaceae extracts by GC/MS and LC/MC-TOF

The 95% EEMC and 95% EECV were selected from the four Myrtaceae extracts due to their potential plant-growth stimulating effect and antifungal activities from the experiment described above. Subsequently, both 95% EEMC and 95% EECV were analyzed for non-targeted phytochemical compounds by GC/MS QTOF and LC/MS QTOF techniques. An overview of all annotated compounds of 95% EEMC and 95% EECV was given in Tables 3 and 4. These compounds were summarized along with their retention time, molecular formula, m/z, peak area and known bioactivity from the literature.

Table 3. Phytochemical compounds from the 95% ethanolic extract of Thai *M. cajuputi* (95% EEMC) analyzed by GC/MS QTOF and LC/MS QTOF

	Compound name	RT (min)	Molecular formula	m/z	Peak area (%)	Known bioactivity from literature
	By GC/MS QTOF					
Terpenoids						
1	Copaene	16.74	$C_{15}H_{24}$	204	0.20	antimicrobial (Scur et al. 2016)
2	β-Elemene	17.12	$C_{15}H_{24}$	204	0.64	anticancer (Xie et al. 2020)
3	Caryophyllene	17.87	$C_{15}H_{24}$	204	0.41	antimicrobial (Selestino Neta et al. 2017)
4	α-Muurolene	19.35	$C_{15}H_{24}$	204	0.33	antimicrobial (Marinas et al. 2021)
5	β-Selinene	19.59	$C_{15}H_{24}$	204	0.95	antifungal (Ding et al. 2017)
6	γ-Selinene	19.82	$C_{15}H_{24}$	204	0.39	antifungal (Foudah et al. 2021)
7	Elemol	21.26	$C_{15}H_{26}O$	222	0.35	antimicrobial (Noriega et al. 2020)
8	Spathulenol	22.22	$C_{15}H_{24}O$	220	0.45	antimicrobial (Fu et al. 2022)
9	Caryophyllene epoxide	22.44	$C_{15}H_{24}O$	220	2.53	antimicrobial (Selestino Neta et al. 2017)
10	Muurola-4,10(14)-dien-1β-ol	23.78	$C_{15}H_{24}O$	220	1.54	unknown
11	Caryophylla-4(12),8(13)-dien-5α-ol	24.00	$C_{15}H_{24}O$	220	1.71	antimicrobial (Selestino Neta et al. 2017)
12	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5β-ol	24.12	$C_{15}H_{24}O$	220	3.89	unknown
13	β-Eudesmol	24.39	$C_{15}H_{26}O$	222	0.27	antimicrobial (Noriega et al. 2020)
14	Selin-6-en-4α-ol	24.61	$C_{15}H_{26}O$	222	2.12	antibacterial (Cordeiro et al. 2020)
15	Aromadendrene epoxide	24.75	$C_{15}H_{24}O$	220	2.45	antimicrobial (Mulyaningsih et al. 2011)
16	8-Methoxycedrane	25.27	$C_{17}H_{28}O_2$	264	0.74	unknown
17	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	25.44	$C_{15}H_{24}O$	220	1.17	unknown
18	Pluchidiol	26.97	$C_{13}H_{20}O_2$	208	0.21	antimicrobial (Karimi et al. 2019)
19	Proximadiol	29.55	$C_{15}H_{28}O_2$	222	0.25	unknown
20	α-Eudesmol	30.29	$C_{15}H_{26}O$	222	1.40	antimicrobial (Noriega et al. 2020)
21	Squamulosone	30.90	$C_{15}H_{22}O$	218	0.78	unknown
22	6,10,14-Trimethyl-2-pentadecanone	31.46	$C_{18}H_{36}O$	268	0.43	antibacterial (Naidoo et al. 2014)
23	Corymbolone	31.55	$C_{15}H_{24}O_2$	236	0.40	antifungal (Hussein et al. 2016)
24	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	31.67	$C_{15}H_{26}O_2$	238	0.54	unknown
25	Estra-1,3,5(10)-trien-17β-ol	32.82	$C_{18}H_{24}O$	256	0.91	anticancer (Khan et al. 2022)
26	Phytol	38.60	$C_{20}H_{40}O$	296	19.14	antimicrobial (Saha and Bandyopadhyay 2020; Yusoff et al. 2020; Petpheng et al. 2023)
27	γ-Sitosterol	41.19	$C_{29}H_{50}O$	414	0.54	antibacterial (Subramaniam et al. 2014)
28	28-Norolean-17-en-3-ol	42.70	$C_{29}H_{48}O$	412	0.57	antiviral (Darshani et al. 2022)
				Terpenoids	45.31	
Phenolics						
29	Ethyl α-d-glucopyranoside	23.67	$C_8H_{16}O_6$	208	1.66	antioxidant (Dai et al. 2022)
30	2-Hydroxy-5-methoxybenzaldehyde,TMS derivative	25.82	$C_{11}H_{16}O_3Si$	224	0.26	antibacterial (Durgadevi et al. 2019)
				Phenolics	1.92	
Flavonoids						
31	Pinostrobin	32.53	$C_{11}H_{10}O_4$	206	0.32	antibacterial (Marliyana et al. 2018)
				Flavonoids	0.32	
Naphthalenes & Aromatics						
32	1,2,3,4-Tetrahydronaphthalene-1,2-diol,5,6-dimethoxy-	23.15	$C_{12}H_{16}O_4$	164	8.45	unknown
33	9-Butyl-9H-fluoren-9-ol	36.55	$C_{17}H_{18}O$	238	0.40	unknown
				Naphthalenes & Aromatics	8.85	
Other compounds						
34	2,5-Dimethoxythiophenol	15.71	$C_8H_{10}O_2S$	170	0.26	unknown
35	Butyric acid, 2,3-epoxy-, ethyl ester	21.47	$C_{11}H_{16}O$	164	0.23	unknown
36	2',3',4' Trimethoxyacetophenone	26.46	$C_{11}H_{14}O_4$	210	7.16	antibacterial (Freitas et al. 2020)

Table 3. Phytochemical compounds from the 95% ethanolic extract of Thai *M. cajuputi* (95% EEMC) analyzed by GC/MS QTOF and LC/MS QTOF (Continued)

Compound name		RT (min)	Molecular formula	m/z	Peak area (%)	Known bioactivity from literature
37	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	28.99	$C_{14}H_{22}O_3$	238	1.23	unknown
38	Octahydro-1-(2-octyldecyl)- pentalene	30.51	$C_{26}H_50$	362	3.63	unknown
39	10-Methylanthracene-9-carboxaldehyde	33.23	$C_{16}H_{12}O$	220	11.63	unknown
40	2-Isopropyl-10-methylphenanthrene	37.35	$C_{18}H_{18}$	234	5.32	unknown
41	5-Methyl-6,7,8,9-tetrahydroisothiazolo[5,4-C]isoquinolin-1(2H)-one	37.79	$C_{11}H_{12}N_2OS$	220	12.53	unknown
			Other compounds		41.99	
				Total	98.39	
	By LC/MS QTOF					
Terpenoids						
1	Ingenol	15.36	$C_{20}H_{28}O_5$	348	9.17	anti-HIV (Fujiwara et al. 1996)
				Terpenoids	9.17	
Phenolics						
2	Acetaminophen	11.99	$C_8H_9NO_2$	151	9.93	antifungal (Srikanth et al. 2005)
3	6-Gingerol	12.51	$C_{17}H_{26}O_4$	294	2.75	antifungal (Xi et al. 2022)
4	3,5-Di-tert-butyl-2-hydroxybenzaldehyde	27.74	$C_{15}H_{22}O_2$	234	6.81	antimicrobial (Zhao et al. 2020)
				Phenolics	19.49	
Flavonoids & Naphthalene						
5	Cyanidin-3-O-sophoroside	0.58	$C_{27}H_{31}O_{16}^+$	611	1.34	antibacterial (Tan et al. 2019)
6	6,2'-Dimethylflavone	15.72	$C_{17}H_{14}O_4$	282	4.21	antifungal (Mangoyi et al. 2015)
				Flavonoids & Naphthalene	5.55	
Steroids & Benzofurans						
7	5β-Androstane-3β,17β-diol	12.54	$C_{19}H_{32}O_2$	292	3.84	unknown
8	Dichlorofluorescein	14.80	$C_{20}H_{10}Cl_2O_5$	401	3.95	unknown
				Steroids & Benzofurans	7.79	
Ethylene glycol						
9	Nonaethylene glycol	0.58	$C_{18}H_{38}O_{10}$	415	10.28	antimicrobial (Shukla et al. 2012)
10	Decaethylene glycol	0.58	$C_{20}H_{42}O_{11}$	459	10.42	antimicrobial (Shukla et al. 2012)
11	Pentaethylene glycol	12.51	$C_{10}H_{22}O_6$	238	5.55	antimicrobial (Shukla et al. 2012)
				Ethylene glycol	26.25	
Vitamins						
12	R-lipoic acid	0.46	$C_8H_{14}O_2S_2$	207	6.63	PGPM[z] (Elkelish et al. 2021)
				Vitamins	6.63	
Fatty acid & Glucosides						
13	2,3-Dinor-11β-prostaglandin F2α	12.56	$C_{18}H_{30}O_5$	326	9.45	unknown
14	Harpagide	14.4	$C_{15}H_{24}O_{10}$	364	3.46	unknown
				Fatty acid & Glucosides	12.91	
Other compounds						
15	1-(1',3'-Benzodioxol-5'-yl)-2-butanamine	0.42	$C_{11}H_{15}NO_2$	193	1.03	unknown
16	Salicylic acid, valerate	0.82	$C_{12}H_{14}O_4$	222	3.70	ISR[y] (War et al. 2011; Sangpueak et al. 2021)
17	2-Acetyl-5-(tetrahydroxybutyl)imidazole	12.47	$C_9H_{14}N_2O_5$	230	1.24	unknown
18	Leukotriene B3	12.54	$C_{20}H_{34}O_4$	338	3.34	antibacterial (Serban et al. 2018)
				Other compounds	9.31	
				Total	97.1	

[z]PGPM = Plant growth promoting metabolites.

[y]ISR = Induced systemic resistance.

Table 4. Phytochemical compounds from the 95% ethanolic extract of Thai *C. viminalis* (95% EECV) analyzed by GC/MS QTOF and LC/MS QTOF

	Compound name	RT (min)	Molecular formula	m/z	Peak area (%)	Known bioactivity from literature
	By GC/MS QTOF					
Terpenoids						
1	α-Phellandrene	8.96	$C_{10}H_{16}$	136	2.04	antimicrobial (İşcan et al. 2012)
2	o-Cymene	9.39	$C_{10}H_{14}$	134	0.84	antimicrobial (Tadtong et al. 2016)
3	1,8-Cineole	9.52	$C_{10}H_{18}O$	154	14.0	antimicrobial (Tadtong et al. 2016; Şimşek and Duman 2017; Kim et al. 2018)
4	α-Terpinene	10.77	$C_{10}H_{16}$	136	0.19	antifungal (Marei and Abdelgaleil 2018)
5	Terpinen-4-ol	12.64	$C_{10}H_{18}O$	154	0.36	antifungal (Marei and Abdelgaleil 2018; Kamiya et al. 2024)
6	L-α-Terpineol	12.89	$C_{10}H_{18}O$	154	1.42	antifungal (Marei and Abdelgaleil 2018)
7	2-Acetoxy-1,8-cineole	15.24	$C_{12}H_{20}O_3$	212	0.26	antimicrobial (Tadtong et al. 2016)
8	Caryophyllene	17.86	$C_{15}H_{24}$	204	0.35	antimicrobial (Selestino Neta et al. 2017)
9	γ-Selinene	19.81	$C_{15}H_{24}$	204	0.20	antifungal (Foudah et al. 2021)
10	Spathulenol	22.21	$C_{15}H_{24}O$	220	0.76	antimicrobial (Fu et al. 2022)
11	Isoleptospermone	23.65	$C_{15}H_{22}O_4$	266	1.01	unknown
12	Pluchidiol	29.46	$C_{13}H_{20}O_2$	208	0.57	unknown
13	α-Phellandrene, dimer	29.82	$C_{20}H_{32}$	272	0.76	antimicrobial (İşcan et al. 2012)
14	Phytyl acetate	31.42	$C_{22}H_{42}O_2$	338	0.49	antifungal (Foudah et al. 2021)
15	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	32.82	$C_{15}H_{26}O_2$	238	0.33	unknown
16	Phytol	42.67	$C_{20}H_{40}O$	296	0.53	antimicrobial (Saha and Bandyopadhyay 2020; Yusoff et al. 2020; Petpheng et al. 2023)
17	Vitamin E	65.48	$C_{29}H_{50}O_2$	430	0.56	PGPM^z (Muñoz and Munné-Bosch 2019)
18	γ-Sitosterol	68.15	$C_{29}H_{50}O$	414	1.91	antibacterial (Subramaniam et al. 2014)
19	Lanosterol	68.76	$C_{30}H_{50}O$	426	0.52	unknown
20	28-Norolean-17-en-3-ol	69.25	$C_{29}H_{48}O$	412	1.02	antibacterial (Kim et al. 2015)
21	α-Amyrin	69.56	$C_{30}H_{50}O$	426	0.40	antibacterial (Chung et al. 2011)
22	γ-Sitostenone	70.52	$C_{29}H_{48}O$	412	0.64	antibacterial (Subramaniam et al. 2014)
23	Betulinaldehyde	73.64	$C_{30}H_{48}O_2$	440	0.88	antibacterial (Chung et al. 2011)
				Terpenoids	30.04	
Phenolics						
24	2'-Hydroxy-5'-methoxyacetophenone, 3-methylbutylether	20.33	$C_{14}H_{20}O_3$	236	1.70	unknown
25	2,6-Di-tert-butyl-4-hydroxy-4-methylcyclohexa-2,5-dien-1-one	23.24	$C_{15}H_{24}O_2$	236	1.77	unknown
				Phenolics	3.47	
Flavonoids						
26	Hesperetine	58.40	$C_{17}H_{14}O_7$	330	0.39	antimicrobial (Carevic et al. 2022)
27	Prosogerin E	60.00	$C_{18}H_{16}O_7$	332	2.06	antibacterial (Shamsudin et al. 2022)
28	Guaiacin	60.63	$C_{20}H_{24}O_4$	328	0.32	unknown
29	8-hydroxysalvigenin	64.71	$C_{18}H_{16}O_5$	312	0.71	antimicrobial (Alreshidi et al. 2020)
30	4',7-Di-O-methylnaringenin	65.01	$C_{19}H_{18}O_5$	326	1.79	antibacterial (Kozłowska et al. 2017)
				Flavonoids	5.27	
Aromatic & Fatty acids						
31	Isolongifolene	19.59	$C_{15}H_{24}$	204	0.48	unknown
32	1,2,3,4,5,6,7,8-Octahydro-2-naphthol, 4-methylene-2,5,5-trimethyl-	24.79	$C_{14}H_{22}O$	206	0.25	unknown
33	2,4,6-Octatriene, 3,4-dimethyl-	9.23	$C_{10}H_{16}$	136	0.26	unknown
34	Hexadecanoic acid, ethyl ester	37.81	$C_{18}H_{36}O_2$	284	0.30	unknown
35	Ethyl oleate	44.93	$C_{20}H_{38}O_2$	310	0.20	unknown
				Aromatic & Fatty acids	1.49	
Quinones & Steroids						
36	5-Hydroxy-2,2,6,6-tetramethyl-4-cyclohexene-1,3-dione	14.26	$C_{10}H_{14}O_3$	182	1.04	unknown
37	Androst-1-en-3-one, 17-(acetyloxy)-4,5-epoxy-,(4β,5β,17β)-	50.33	$C_{21}H_{28}O_4$	344	2.14	unknown
				Quinones & Steroids	3.18	
Other compounds						
38	6-acetyl-2,2,4,4-tetramethylcyclohexane-1,3,5-trione	16.37	$C_{12}H_{16}O_4$	224	1.59	unknown
39	2-Cyclopropene-1-carboxylic acid, 2-(1,1-dimethyl-5-oxohexyl)-, methyl ester	18.68	$C_{13}H_{20}O_3$	224	0.31	unknown

Table 4. Phytochemical compounds from the 95% ethanolic extract of Thai *C. viminalis* (95% EECV) analyzed by GC/MS QTOF and LC/MS QTOF (continued)

	Compound name	RT (min)	Molecular formula	m/z	Peak area (%)	Known bioactivity from literature
40	2-Hydroxy-5-methoxybenzaldehyde, TMS derivative	19.16	$C_{11}H_{16}O_3Si$	224	0.32	unknown
41	2,2,4-Trimethyl-4-trimethylsilylethynylcyclopentane-1,3-dione	21.01	$C_{13}H_{20}O_2Si$	236	25.0	unknown
42	1-Ethyl-2,2,4a,7,7-pentamethyl-1,2,3,4,4a,5,6,7-octahydro[1,8]naphthyridine	22.96	$C_{15}H_{28}N_2$	236	0.72	unknown
43	4a,6a-Dimethyl-2-oxo-1a,2,4a,4b,5,6,6a,7,8,9,9a,9b,10,11-tetradecahydrocyclopenta[7,8]phenanthro[1,10ab]oxiren-7-yl acetate pk2	51.49	$C_{21}H_{28}O_4$	344	7.63	unknown
44	2-Amino-3-cyano-4-methyl-4,6-dipyridin-4-ylcyclohexa-1,5-dien-1,3-dicarboxylic acid, diethyl ester	61.33	$C_{24}H_{24}N_4O_4$	432	0.32	unknown
45	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-	62.28	$C_{30}H_{50}$	410	0.76	unknown
			Other compounds		36.65	
				Total	80.1	
	By LC/MS QTOF					
Terpenoids						
1	11-Keto-β-boswellic acid	16.22	$C_{30}H_{46}O_4$	471	6.96	antimicrobial (Jaroš et al. 2022)
				Terpenoids	6.96	
Phenolics						
2	Metaproterenol	13.34	$C_{11}H_{17}NO_3$	212	5.33	unknown
				Phenolics	5.33	
Flavonoids						
3	Leiocarposide	12.63	$C_{27}H_{34}O_{16}$	653	2.41	antimicrobial (Toiu et al. 2019)
4	Peonidin cation	15.24	$C_{16}H_{13}O_6^+$	301	1.03	antifungal (Chen et al. 2023)
				Flavonoids	3.44	
Coumarins, Alkaloid & Steroids						
5	7-Methoxycoumarin	0.80	$C_{10}H_8O_3$	177	2.11	antifungal (Chen et al. 2023)
6	Quinupramine	12.12	$C_{21}H_{24}N_2$	305	1.04	antimicrobial (Caldara and Marmiroli 2018)
7	5β-Pregnan-3α,17,20β21-tetrol-11-one	12.20	$C_{21}H_{34}O_5$	366	1.39	unknown
8	γ-Muricholic acid	15.28	$C_{24}H_{35}D_5O_5$	391	1.70	antibacterial (Watanabe et al. 2017)
9	5β-Androstane-3α,17β-diol	12.07	$C_{19}H_{32}O_2$	275	1.33	unknown
			Coumarins, Alkaloid & Steroids		7.57	
Ethylene glycol						
10	Nonaethylene glycol	0.81	$C_{18}H_{38}O_{10}$	432	5.00	antimicrobial (Shukla et al. 2012)
11	Decaethylene glycol	0.81	$C_{20}H_{42}O_{11}$	459	6.10	antimicrobial (Shukla et al. 2012)
				Ethylene glycol	11.1	
Vitamins						
12	1,25-Dihydroxy vitamin D2	16.23	$C_{27}H_{44}O_3$	411	2.07	PGPM (Buchala and Pythoud 1988)
				Vitamins	2.07	
Fatty acid						
13	18-Carboxy dinor leukotriene B4	12.12	$C_{18}H_{26}O_6$	321	3.53	unknown
14	16,16-Dimethylprostaglandin E2	12.23	$C_{22}H_{36}O_5$	363	1.20	unknown
15	Tafluprost (free acid)	12.25	$C_{22}H_{28}F_2O_5$	411	8.59	unknown
				Fatty acid	13.32	
Other compounds						
16	Etidocaine	12.05	$C_{17}H_{28}N_2O$	277	2.05	unknown
17	N-Desethylamodiaquine	12.12	$C_{18}H_{18}ClN_3O$	328	2.05	unknown
18	Nigerose	12.19	$C_{12}H_{22}O_{11}$	365	5.49	PGPM (Ichimura et al. 2022)
19	Unoprostone isopropyl ester	12.33	$C_{25}H_{44}O_5$	407	1.23	unknown
20	Glu-Ile	13.49	$C_{11}H_{20}N_2O_5$	261	5.21	PGPM (Qiu et al. 2020)
21	Cinnamic acid	15.24	$C_9H_8O_2$	149	2.12	antifungal (Guo et al. 2020)
22	3β-Hydroxy-5-cholenoic acid	15.49	$C_{24}H_{38}O_3$	357	7.98	unknown
23	Octamethylcyclotetrasiloxane	15.54	$C_8H_{24}O_4Si_4$	297	1.14	unknown
			Other compounds		27.27	
				Total	77.06	

[z]PGPM = Plant growth promoting metabolites.

As shown in Table 3, the analysis of the 95% EEMC by GC/MS revealed 41 components comprising 98.39% of the total extract composition, represented by 45.31% of terpenoids, 1.95% of phenolics, 0.32% of flavonoids, 8.85% of naphthalene and aromatics as well as 41.99% of other compounds. Of terpenoids containing 28 phytochemicals, phytol (14%) was the predominant component, followed by 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5β-ol (3.89%), caryophyllene epoxide (2.53%), aromadendrene epoxide (2.45%) and selin-6-en-4α-ol (2.12%). For phenolics, the compounds presented a number of components, such as ethyl α-d-glucopyranoside (1.66%) and 2-hydroxy-5-methoxybenzaldehyde, a TMS derivative (0.26%). In contrast, pinostrobin (0.32%) was the only component of flavonoids. The LC/MS analysis showed the presence of 18 chemicals comprising 97.1% of the total extract composition, represented by 9.17% of terpenoids, 19.49% of phenolics, 5.55% of flavonoids and naphthalene, 7.79% of steroids and benzofurans, 12.91% of fatty acids and glucosides, 6.63% of vitamins, 26.25% of ethylene glycol and 9.31% of other compounds. The main compounds of ethylene glycol were nonaethylene glycol (10.28%) and decaethylene glycol (10.42%). The phenolics namely, acetaminophen (9.9%), 3,5-di-tert-butyl-2-hydroxybenzaldehyde (6.81%) and 6-gingerol (2.75%) were detected. Meanwhile, terpenoids and vitamins each contained only one component namely, ingenol (9.17%) and R-lipoic acid (6.63%), respectively. Based on the 95% EEMC analysis, it was clearly shown that the highest diversity of terpenoids was found among the total identified phytochemical compounds. Our findings were in good agreement with earlier work (Al-Abd et al. 2015) showing that the major phytochemical groups in *M. cajuputi* were terpenoids, phenolics, flavonoids aromatics and fatty acids. Recently, Isah et al. (2023) compiled and summarized work showing that terpenoids, phenolics, and flavonoids were the major phytocompounds of the solvent extracts and essential oil of *M. cajuputi*.

Regarding 95% EECV, as shown in Table 4, its compositional variability was in line with those of the 95% EEMC case, showing the highest diversity of terpenoids. Based on GC/MS, the 95% EECV contained 45 intricate chemical compositions consisting of 80.1% of the total extract composition, represented by 30.04% of terpenoids [mainly 1,8-cineole (14%), α-phellandrene (2.04%), γ-sitosterol (1.91%) and L-α-terpineol (1.42%)], 3.47% of phenolics [2'-hydroxy-5'-methoxyacetophenone, 3-methylbutylether (1.7%) and 2,6-di-tert-butyl-4-hydroxy-4-methylcyclohexa-2,5-dien-1-one (1.77%)], 5.27% of flavonoids [prosogerin E (2.06%) and 4',7-di-O-methylnaringenin (1.79%)], 1.49% of aromatic and fatty acids, 3.18% quinones and steroids, including 36.65% of other compounds. Based on the LC/MS, the analysis revealed the presence of 23 phytochemicals comprising 77.06% of the total plant extract composition. A total phytochemical compounds were identified as 6.96% of terpenoids (11-keto-β-boswellic acid), 5.33% of phenolics (metaproterenol), 3.44% of flavonoids (leiocarposide and peonidin cation), 7.57% of the groups of coumarins, alkaloids and steroids, 11.1% of ethylene glycol, 2.07% of vitamins (1,25-dihydroxy vitamin D2), 13.32% of fatty acids (tafluprost, 18-carboxy dinor leukotriene B4, and 16,16-dimethylprostaglandin E2) along with 27.27% of other compounds (nigerose and glu-ile). Based on the 95% EECV analysis, our findings were in good agreement with those of the previous studies by Salem et al. (2017), Ahmad and Athar (2016) and de Oliveira et al. (2014), demonstrating the detection of terpenoids (1,8-cinole), phenolics and flavonoids in *C. viminalis.*

As shown in Tables 3 and 4, terpenoids were the main compounds in both the EEMC and EECV followed by phenolics, flavonoids and ethylene glycol. The known biological activities (such as antifungal, antibacterial and antiviral activities) of the phytochemical compounds identified in EECV and EEMC were cited and compiled in Tables 3 and 4. Regarding the antimicrobial activities, terpenoid compounds (mainly phytol, 1,8-cineole, α-terpinene, terpinene-4-ol and 11-Keto-β-boswellic acid) were documented to exert potent antifungal activity against *Candida albicans* (Tadtong et al. 2016),

Aspergillus spp. (Morcia et al. 2012; Kim et al. 2018), *Alternaria* spp. (Morcia et al. 2012; Marei and Abdelgaleil 2018), *Botrytis cinerea* (Yusoff et al. 2020), *Fusarium* spp. (Morcia et al. 2012; Marei and Abdelgaleil 2018), *Penicillium* sp. (Morcia et al. 2012; Marei and Abdelgaleil 2018), and *Rhizoctonia solani* (Marei and Abdelgaleil 2018). The large amount of terpenoids found in the 95% EEMC (55%) from this analysis tended to support our earlier result regarding the antifungal activity of both plant extracts tested via poisoned food testing, showing the maximum potential inhibition effect of 95% EEMC. Interestingly, our phytochemical analysis also confirmed the presence of vitamin E, 1,25-dihydroxy vitamin D2, nigerose and glu-ile in 95% EECV, which were cited as plant-growth-promoting metabolites (Muñoz and Munné-Bosch 2019; Qiu et al. 2020; Ichimura et al. 2022). Hence, this likely supported the positive effect (such as plant-growth-promoting activity) of EECV on the growth of lettuce in our study found during the phytotoxicity assessment.

Evaluation of EEMC and EECV as an inducer on development of *Alternaria* leaf spot and induction of defense enzymes in lettuce grown in a hydroponic system

The EEMC effect

The indirect effect of 95% EEMC at 15,000 and 50,000 ppm against *Alternaria* leaf spot in lettuce using CRD with 3 replications revealed that the use of the highest concentration (50,000 ppm) of the extract and fungicide led to the complete inhibition of leaf spot at 3 DAI (Fig. 4). In addition, disease progress developed further in all treatments. However, the 2 tested concentrations of the extract as well as the fungicide treatment still significantly ($p < 0.05$) showed a strong inhibition effect at 5 DAI. At 7 DAI, a statistically significant difference ($p < 0.05$) in the severity of *Alternaria* leaf spot was noted among the treatments. Disease severity in the extract treatments was significantly reduced ($p < 0.05$) by 50 − 70% compared to an inoculated control, while only the fungicide treatment resulted in a 40% reduction. Importantly, the high concentration (50,000 ppm) significantly ($p < 0.05$) resulted in the greatest level of disease reduction in this regard. Moreover, the activities of the induced defense enzymes of β-1,3-glucanase, chitinase and peroxidase were monitored along with the disease severity levels at 1, 3, 5 and 7 DAI. When lettuce plants were infected with a pathogen, the enzymatic activities increased with time as the disease developed. On the first day after inoculation, the strongest activity of β-1,3-glucanase at a significant ($p < 0.05$) level was noted in the 95% EEMC treatments (15,000 and 50,000 ppm) compared to an inoculated control. At 7 DAI, the highest activity at a significant level ($p < 0.05$) was shown in the 95% EEMC treatments, followed by the inoculated and fungicide control treatment, while the lowest level of activity was recorded in the healthy control. The activities of the chitinase and peroxidase enzymes showed patterns identical to that of β-1,3-glucanase. At 7 DAI, the 95% EEMC treatments resulted in significantly higher activity ($p < 0.05$) of chitinase compared to the inoculated control and the fungicide treatment, while the highest activity of peroxidase at a significant level ($p < 0.05$) was also noted in the 95% EEMC treatments (Table 5). This result confirmed that the disease severity was related to the activities of the 3 induced defense enzymes. To be precise, the highest level of enzyme activity was noted in lettuce treated with 95% EEMC showing less severity of *Alternaria* leaf spot. This implied that 95% EEMC acted as a resistance inducer.

With regard to plant parameters at harvest (21 days old), the 95% EEMC and fungicide treatments showed the highest values of both parameters (SPAD value and yield), which were statistically different ($p < 0.05$) compared to the inoculated control but not significantly different ($p < 0.05$) from the healthy control.

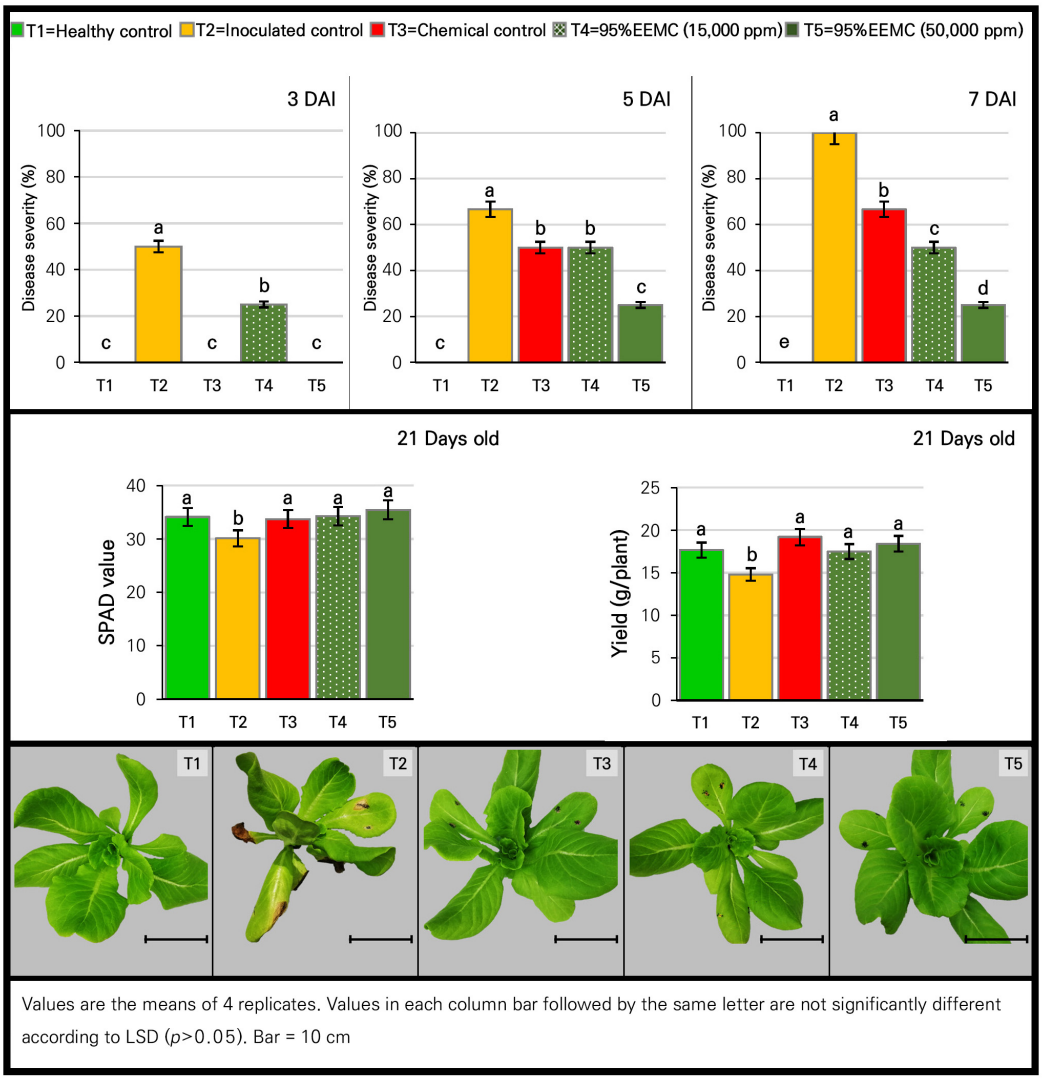

Fig. 4. Effect of 95% ethanolic extract from *Melaleuca cajuputi* leaves (95% EEMC) on *Alternaria* leaf spot (at 7 DAI) and growth of lettuce in a hydroponic system (21 days old).

Table 5. Activity of defense enzymes against *Alternaria* in lettuce treated with 95% EEMC

Treatment	Activity of defense enzyme											
	β-1,3-Glucanase (μmol glucose /g of fresh leaves)				Chitinase (μmol GlcNAc/g of fresh leaves)				Peroxidase (unit/ g of fresh leaves)			
	1 DAI	3 DAI	5 DAI	7 DAI	1 DAI	3 DAI	5 DAI	7 DAI	1 DAI	3 DAI	5 DAI	7 DAI
T1[z]	1.53 d[y]	3.53 d	3.60 d	3.46 d	5.50 d	5.07 d	5.97 d	5.63 e	3.38 e	4.90 e	6.52 c	8.44 c
T2	2.17 b	5.27 b	6.17 b	7.36 b	9.43 b	11.25 b	15.95 b	17.30 c	5.37 c	6.89 c	8.51 b	10.43 b
T3	2.20 c	4.63 c	5.20 c	6.20 c	8.63 c	9.17 c	11.03 c	11.33 d	4.70 d	6.22 d	7.85 b	9.76 bc
T4	2.55 a	8.62 a	7.87 a	11.66 a	13.49 a	15.29 a	19.23 a	20.24 b	7.50 b	9.02 b	10.65 a	12.56 a
T5	2.72 a	8.72 a	7.97 a	11.83 a	13.49 a	14.91 a	19.99 a	23.12 a	8.30 a	9.82 a	11.45 a	13.36 a

[z] T1 = Healthy control, T2 = Inoculated control, T3 = Chemical control + pathogen, T4 = 95% EEMC-15,000 ppm + pathogen, T5 = 95% EEMC-50,000 ppm + pathogen.

[y] Values are the means of 3 replicates. Values in each column within each enzyme followed by the same letter are not significantly different according to LSD ($p > 0.05$).

The EECV effect

The determination effect of 50% and 95% EECV at 15,000 and 50,000 ppm on disease development and plant defense enzyme induction using CRD with 3 replications revealed that all treatments except for that of the healthy control showed no significant difference with regard to disease severity compared to the inoculated control (at 3 DAI). At 5 DAI, the leaf spot progressed to lesions in all treatment cases, except at the highest concentrations of 50% and 95% EECV, which still showed static lesions. At 7 DAI, the highest concentration (50,000 ppm) of both EECV treatments led to the highest ($p < 0.05$) disease reduction (75%), followed by the lower concentration (15,000 ppm) and the fungicide control treatments in the range of 43.75 − 50%. Regarding the induced defense enzymes (Table 6), all presented a pattern similar to those in the 95% EEMC experiment; that is, the enzymatic activities increased over time, and higher levels of enzyme induction were observed in the lettuce treatments with the plant extracts (including the 50% EECV). At 7 DAI, the highest and significant ($p < 0.05$) enzymes activities of β-1,3-glucanase and chitinase were noted in all 4 EECV treatments compared to the fungicide treatment and the inoculated control. For peroxidase, the highest and significant activity ($p < 0.05$) was only found in the 95% EECV case at 50,000 ppm. Considering these results, there was a reduction in the severity of *Alternaria* leaf spot and an increase in defense-related enzymes in the 50% and 95% EECV-treated lettuce. This result was in line with the EEMC experiment implying that EECV acted as a resistance inducer as well.

For the plant growth parameters, the 50% and 95% EECV as well as the fungicide treatments gave statistically ($p < 0.05$) higher SPAD values, which were significantly different compared to the inoculated control but not significantly different from the healthy control (Fig. 5). Interestingly, low concentrations of 50% EECV, 95% EECV and the fungicide treatment gave significantly higher ($p < 0.05$) yields than that of the inoculated control. Besides, the highest concentration (50,000 ppm) of 50% and 95% EECV significantly ($p < 0.05$) led to the highest yields and strongest growth stimulation effects on lettuce (about 23.5 g/plant) compared to the other treatments (in the range of 16.04 − 19.36 g/plant).

Table 6. Activity of defense enzymes against *Alternaria* in lettuce treated with 50% and 95% EECV

Treatment	Activity of defense enzyme											
	β-1,3-Glucanase (μmol glucose /g of fresh leaves)				Chitinase (μmol GlcNAc/g of fresh leaves)				Peroxidase (unit/ g of fresh leaves)			
	1 DAI	3 DAI	5 DAI	7 DAI	1 DAI	3 DAI	5 DAI	7 DAI	1 DAI	3 DAI	5 DAI	7 DAI
T1[z]	1.53 c[y]	3.53 d	3.60 c	3.46 d	5.50 c	5.07 c	5.97 c	5.63 c	3.33 c	4.98 e	5.48 e	6.45 e
T2	2.17 b	5.27 c	9.43 b	12.86 c	11.00 b	14.30 b	18.67 b	21.96 b	6.02 b	6.47 cd	6.83 d	7.45 d
T3	2.20 b	5.37 c	9.50 b	12.43 c	10.80 b	15.07 b	18.60 b	21.66 b	6.79 a	6.54 bc	7.53 c	7.99 cd
T4	3.30 a	8.87 b	11.20 a	16.26 ab	16.00 a	18.20 a	21.63 a	26.46 a	6.48 a	7.12 a	7.50 c	8.29 cd
T5	3.27 a	9.30 a	11.60 a	15.73 b	15.50 a	17.63 a	22.03 a	26.63 a	6.52 a	6.83 ab	7.84 b	8.68 bc
T6	3.30 a	9.37 a	11.67 a	16.93 a	15.93 a	17.73 a	21.67 a	26.23 a	6.37 a	6.74 bc	6.91 d	9.52 b
T7	3.47 a	9.47 a	11.83 a	16.2 ab	15.93 a	17.27 a	21.83 a	26.53 a	6.22 b	6.18 d	8.18 a	10.97 a

[z] T1 = Healthy control, T2 = Inoculated control, T3 = Chemical control, T4 = 50% EECV-15,000 ppm + pathogen, T5 = 50% EECV-50,000 ppm + pathogen, T6 = 95% EECV-15,000 ppm + pathogen, T7 = 95% EECV-50,000 ppm + pathogen.

[y] Values are the means of three replicates. Values in each column within each enzyme followed by the same letter are not significantly different according to LSD ($p > 0.05$).

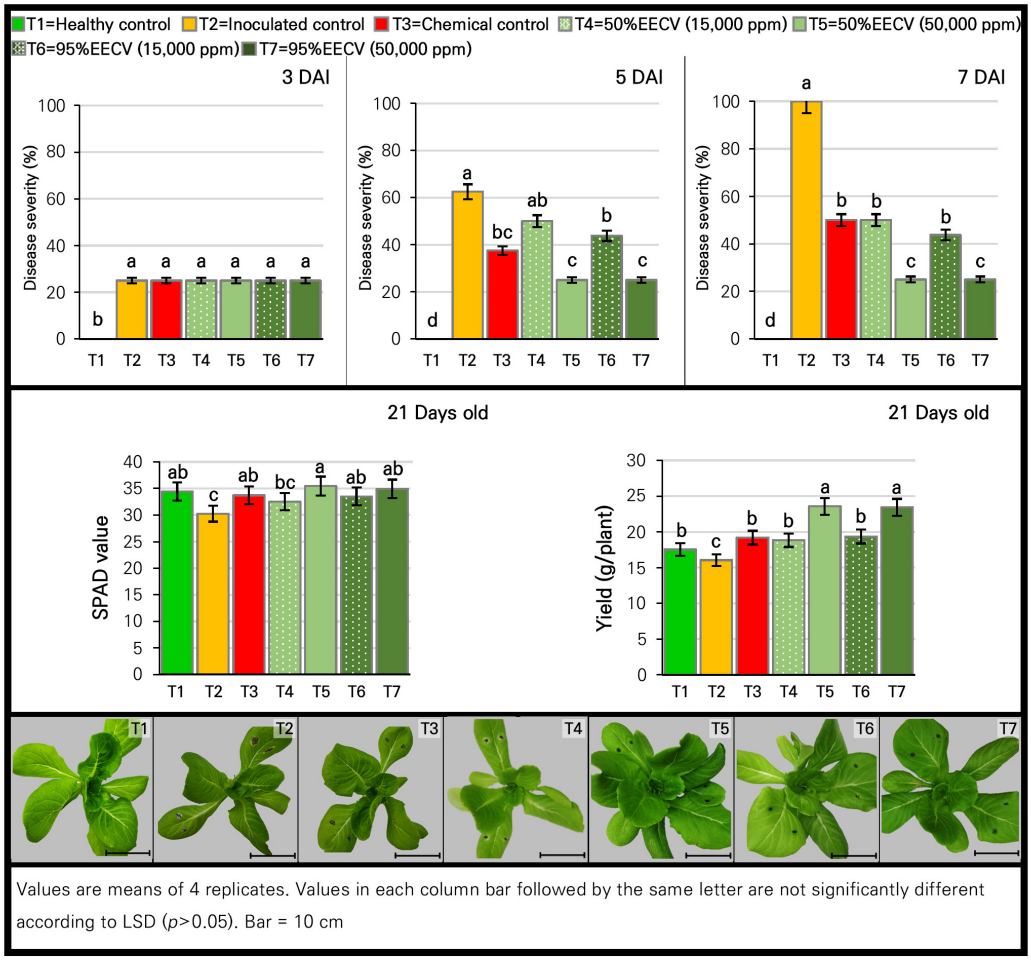

Fig. 5. Effects of 50% and 95% ethanolic extracts from *Callistemon viminalis* leaves (EECV) on *Alternaria* leaf spot (at 7 DAI) and growth of lettuce in a hydroponic system (21 days old).

Correlation of disease severity and plant defense enzymes

The correlation between disease severity and plant defense enzymes in lettuce due to the effects of the inducers (EEMC as well as EECV) was assessed at 3, 5 and 7 DAI, as presented in Fig. 6. Moderate to high correlations (r) of −0.766, −0.575 and −0.807 were found between defense-related enzymes of β-1,3-glucanase, chitinase and peroxidase with disease severity in lettuce treated with EEMC at 7 DAI. The corresponding simple regression equation also showed that the increased levels of defense-related enzymes played a negative role in reducing the disease severity. A similar observation of a moderate to high negative correlation (r = −0.631, −0.722 and −0.708) as well as the corresponding simple regression equation outcomes were also found between defense enzymes and disease severity in the EECV lettuce at 7 DAI.

In the experiments here, EEMC and EECV significantly reduced *Alternaria* leaf spot disease, while significantly higher accumulations of the three enzymes were found in lettuce treated with both extracts. Furthermore, a moderate to high negative correlation was observed between disease severity and plant defense enzymes in lettuce treated with both extracts. These results were likely due to the induction of plant defense enzymes by the plant extracts (Yamunarani et al. 2004; Latha et al. 2009; Gupta et al. 2013; Franzener et al. 2018). These enzymes played a role in suppressing plant

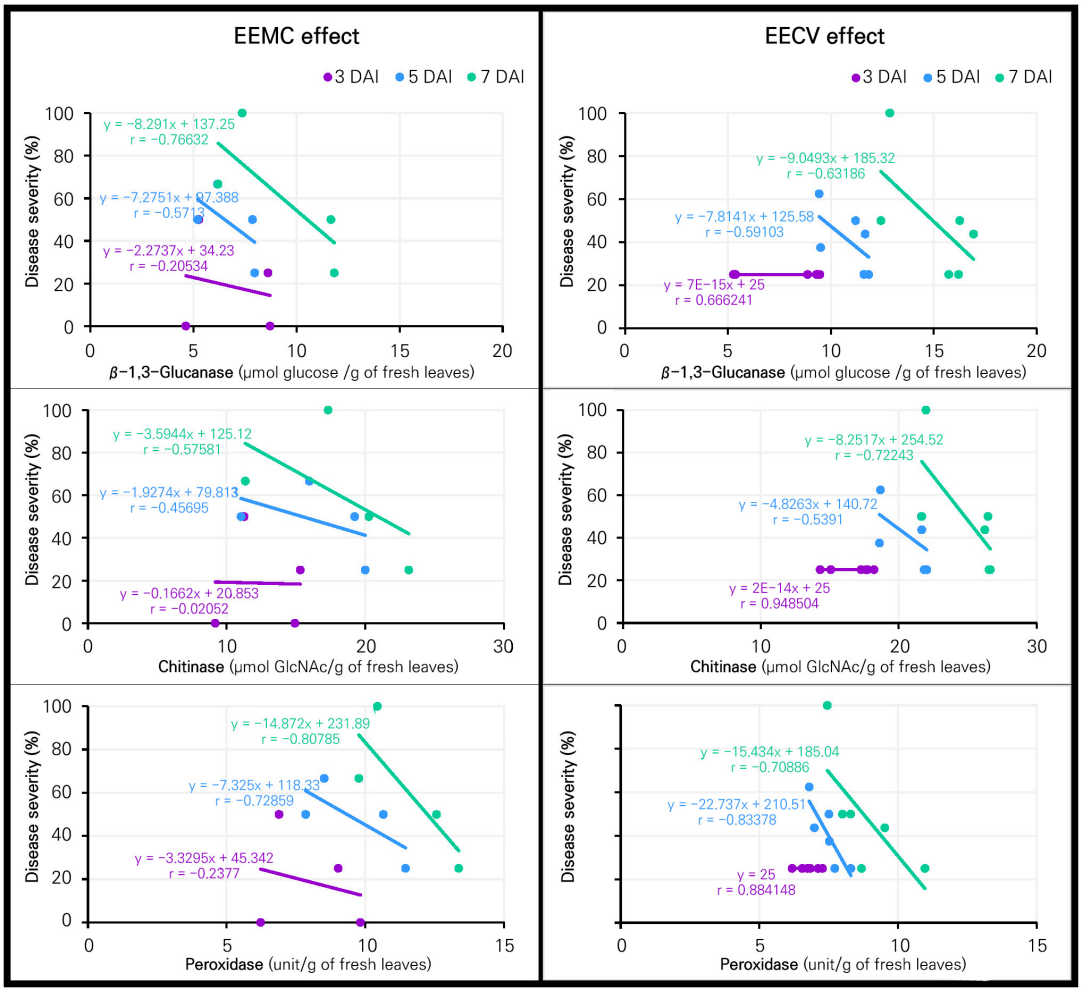

Fig. 6. Correlation of disease severity with β-1,3-glucanase, chitinase and peroxidase activities in lettuce plants treated with EEMC and EECV as an inducer against *A. brassicicola* at 3, 5 and 7 DAI.

diseases, thereby contributing significantly to better overall health and resistance of the plants (Akila et al. 2011; Arzoo et al. 2012; Franzener et al. 2018; Gholamnezhad 2019). β-1,3-glucanase and chitinase sequentially hydrolyzed cell wall components, such as β-1,3-glucans and chitins (Yamunarani et al. 2004; Gupta et al. 2013; Prasannath 2017). Meanwhile, peroxidase was associated with various defense-related processes, including the hypersensitive reactions; lignin biosynthesis; and the cross-linking of phenolics, glycoproteins, and suberin as well as the production of phytoalexins in plants (Prasannath 2017). In addition, our results were in good agreement with the reports from other Myrtaceae plant extracts demonstrating that *Corymbia citriodora* aqueous extract reduced *Fusarium* wilt and increased soluble-protein-related β-1,3-glucanase, chitinase and peroxidase levels in tomatoes under greenhouse condition (Arzoo et al. 2012). While Franzener et al. (2018) reported that a water extract of *Corymbia citriodora* (Myrtaceae plant) reduced the disease severity of *Colletotrichum lagenarium* and induced 2 enzymes (peroxidase and β-1,3-glucanse) in cucumber, our findings suggested that 95% EEMC as well as 50% and 95% EECV could act as inducers by increasing plant defense enzymes in the plants. These potential plant extracts could be developed into botanical fungicides for the management of leaf spot disease in the near future.

Conclusion

Thai indigenous Myrtaceae plants were tested here as substitutes for chemical fungicides. The 95% ethanolic extracts derived from 4 Myrtaceae leaves, namely *Callistemon viminalis* (EECV), *Melaleuca cajuputi* (EEMC), *Syzygium jambos* (EESJ), and *Syzygium malaccense* (EESM) exhibited no phytotoxic effects on lettuce. Interestingly, a plant growth stimulation effect on lettuce was detected with the 95% EECV application. Notably, all concentrations (5,000, 15,000, 30,000 ppm) of 95% EEMC significantly demonstrated the highest inhibitory effect, ranging from 85% to 100% on the mycelial growth of *A. brassicicola*, followed by 95% EECV ranging from 40% to 63%. In the spore germination test, complete inhibition was observed with the application of 95% EEMC (5,000 to 50,000 ppm) and 95% EECV (10,000 to 50,000 ppm). Among the identified phytochemical compounds, terpenoids (such as phytol, 1,8-cineole, α-terpinene, terpinene-4-ol and 11-Keto-β-boswellic acid) were the major bioactive components of both extracts (about 37% − 55%) followed by phenolics, flavonoids, ethylene glycols and vitamins. Furthermore, the applications of 95% EEMC, 95% EECV and 50% EECV as inducers reduced the severity of *Alternaria* leaf spot on lettuce by 50% to 75% but increased the levels of plant defense enzymes (β-1,3-glucanase, chitinase, and peroxidase). Our findings provide evidence of the potential use of EEMC and EECV for controlling *Alternaria* leaf spot disease in lettuce, which was likely due to their direct effect with the presence of phytochemicals responsible for antifungal activity and the plant-growth-stimulating effect as well as their indirect effects as inducer for plant defense enzymes. Therefore, these *M. cajuputi* and *C. viminalis* extracts could serve as alternative potential sources for the development of botanical fungicides in agriculture.

References

Ahmad K, Athar F (2016) Phytochemistry and pharmacology of *Callistemon viminalis* (Myrtaceae): A review. J Nat Prod 7:1-10. https://doi.org/10.2174/2210315507666161216100323

Akila R, Rajendran L, Harish S, Saveetha K, Raguchander T, Samiyappan R (2011) Combined application of botanical formulations and biocontrol agents for the management of *Fusarium oxysporum* f. sp. *cubense* (Foc) causing *Fusarium* wilt in banana. BioControl 57:175-183. https://doi.org/10.1016/j.biocontrol.2011.02.010

Al-Abd NM, Mohamed Nor Z, Mansor M, Azhar F, Hasan MS, Kassim M (2015) Antioxidant, antibacterial activity, and phytochemical characterization of *Melaleuca cajuputi* extract. BMC Complement Altern Med 15:385. https://doi.org/10.1186/s12906-015-0914-y

Alreshidi M, Noumi E, Bouslama L, Ceylan O, Veettil VN, Adnan M, Danciu C, Elkahoui S, Badraoui R, et al. (2020) Phytochemical screening, antibacterial, antifungal, antiviral, cytotoxic, and antiquorum-sensing properties of *Teucrium polium* L. aerial parts methanolic extract. Plants 9. https://doi.org/10.3390/plants9111418

Arzoo K, Biswas SK, Rajik M (2012) Biochemical evidences of defence response in tomato against *Fusarium* wilt induced by plant extracts. Plant Pathol J 11:42-50. https://doi.org/10.3923/ppj.2012.42.50

Bali AS, Batish DR, Singh HP, Kaur S, Kohli RK (2017) Phytotoxicity and weed management potential of leaf extracts of *Callistemon viminalis* against the weeds of rice. Acta Physiol Plant 39:25. https://doi.org/10.1007/s11738-0162313-5

Barros FC, Sagata É, Ferreira LCdC, Juliatti FC (2010) Indução de resistência em plantas à fitopatógenos. Biosci J 26:231-239

Benoit F, Ceustermans N (1995) Horticultural aspects of ecological soilless growing methods. *In*, Ed 396. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp 11-24. https://doi.org/10.17660/ActaHortic.1995.396.1

Bharat CS, Praveen D (2016) Evaluation of in vitro antimicrobial potential and phytochemical analysis of spruce, cajeput and jamrosa essential oil against clinical isolates. Int J Green Pharm 10:27-32

Blagojević JD, Vukojević JB, Ivanović ŽS (2020) Occurrence and characterization of *Alternaria* species associated with leaf spot disease in rapeseed in Serbia. Plant Pathol 69:883-900. https://doi.org/10.1111/ppa.13168

Boller T, Mauch F (1988) Colorimetric assay for chitinase. *In* Methods in Enzymology, Vol 161. Academic Press, pp 430-435. https://doi.org/10.1016/0076-6879(88)61052-4

Bonaldo SM, Schwan-Estrada KRF, Stangarlin JR, Tessmann DJ, Scapim CA (2004) Fungitoxicity, phytoalexins elicitor activity and protection of cucumber against *Colletotrichum lagenarium*, by *Eucalyptus citriodora* aqueous extract. Fitopatol Bras 29:128-134. https://doi.org/10.1590/S0100-41582004000200002

Bouzada MLM, Fabri RL, Nogueira M, Konno TUP, Duarte GG, Scio E (2009) Antibacterial, cytotoxic and phytochemical screening of some traditional medicinal plants in Brazil. Pharm Biol 47:44-52. https://doi.org/10.1080/13880200802411771

Buchala A, Pythoud F (1988) Vitamin D and related compounds as plant growth substances. Physiol Plant 74:391-396

Bulhões CC, Bonaldo SM, Santos BTd, Trento RA (2012) Produtos alternativos no controle de antracnose (*Colletotrichum gloeosporioides*), cladosporiose (*Cladosporium herbarum*) e bacteriose (*Xanthomonas campestris* pv. *passiflorae*) em maracujazeiro no norte demato grosso. Rev Ciências Exatas e da Terra e Ciências Agrárias 7:12-19

Caldara M, Marmiroli N (2018) Tricyclic antidepressants inhibit *Candida albicans* growth and biofilm formation. Int J Antimicrob Agents 52:500-505. https://doi.org/10.1016/j.ijantimicag.2018.06.023

Carevic T, Kostic M, Nikolic B, Stojkovic D, Sokovic M, Ivanov M (2022) Hesperetin-Between the ability to diminish mono- and polymicrobial biofilms and toxicity. Molecules 27:6806. https://doi.org/10.3390/molecules27206806

Carmello CR, Cardoso JC (2018) Effects of plant extracts and sodium hypochlorite on lettuce germination and inhibition of *Cercospora longissima* in vitro. Sci Hortic 234:245-249. https://doi.org/10.1016/j.scienta.2018.02.056

Celoto MIB, Papa MdFS, Sacramento LVSd, Celoto FJ (2008) Atividade antifúngica de extratos de plantas a *Colletotrichum gloeosporioides* Acta Sci Agron 30:1-5. https://doi.org/10.4025/actasciagron.v30i1.1104

Chen YZ, Wang SR, Li T, Zhang GC, Yang J (2023) Antifungal activity of 6-methylcoumarin against *Valsa mali* and its possible mechanism of action. J Fungi (Basel) 9:5. https://doi.org/10.3390/jof9010005

Chung PY, Navaratnam P, Chung LY (2011) Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains. Ann Clin Microbiol Antimicrob 10:25. https://doi.org/10.1186/1476-0711-10-25

Cordeiro L, Figueiredo P, Souza H, Sousa A, Andrade-Júnior F, Medeiros D, Nóbrega J, Silva D, Martins E, et al. (2020) Terpinen-4-ol as an antibacterial and antibiofilm agent against *Staphylococcus aureus*. Int J Mol Sci 21:4531. https://doi.org/10.3390/ijms21124531

Dai J, Hu Y, Si Q, Gu Y, Xiao Z, Ge Q, Sha R (2022) Antioxidant and hypoglycemic activity of sequentially extracted fractions from pingguoli pear fermentation broth and identification of bioactive compounds. Molecules 27:6077. https://doi.org/10.3390/molecules27186077

Darshani P, Sen Sarma S, Srivastava AK, Baishya R, Kumar D (2022) Anti-viral triterpenes: A review. Phytochem Rev 21:1761-1842. https://doi.org/10.1007/s11101-022-09808-1

de Oliveira CM, Cardoso MdG, Figueiredo ACdS, de Carvalho MLM, Miranda CASFd, Marques Albuquerque LR, Lee Nelson D, Souza Gomes Md, Silva LF, et al. (2014) Chemical composition and allelopathic activity of the essential oil from *Callistemon viminalis* (Myrtaceae) blossoms on lettuce seedlings. Am J Plant Sci 5:3551-3557. https://doi.org/10.4236/ajps.2014.524371

Dethoup T, Songkumarn P, Rueangrit S, Suesa-ard S, Kaewkrajay C (2018) Fungicidal activity of Thai medicinal plant extracts against *Alternaria brassicicola* causing black spot of Chinese kale. Eur J Plant Pathol 152:157-167. https://doi.org/10.1007/s10658-018-1460-5

Dethoup T, Songkumarn P, Sirirak T, Kijjoa A (2019) Fungicidal activity of *Acorus calamus* L. extracts against plant pathogenic fungi. Agric Nat Resour 53:527-532

Ding Y, Huffaker A, Kollner TG, Weckwerth P, Robert CAM, Spencer JL, Lipka AE, Schmelz EA (2017) Selinene volatiles are essential precursors for maize defense promoting fungal pathogen resistance. Plant Physiol 175:1455-1468. https://doi.org/10.1104/pp.17.00879

Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S (2023) Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. J Exp Pharmacol 15:51-62. https://doi.org/10.2147/jep.S379805

Durgadevi R, Abirami G, Alexpandi R, Nandhini K, Kumar P, Prakash S, Veera Ravi A (2019) Explication of the potential of 2-Hydroxy-4-methoxybenzaldehyde in hampering uropathogenic *Proteus mirabilis* crystalline biofilm and virulence. Front Microbiol 10:2804. https://doi.org/10.3389/fmicb.2019.02804

El-Hefny M, Ashmawy NA, Salem MZM, Salem AZM (2017) Antibacterial activities of the phytochemicals-characterized extracts of *Callistemon viminalis*, *Eucalyptus camaldulensis* and *Conyza dioscoridis* against the growth of some phytopathogenic bacteria. Microb Pathog 113:348356. https://doi.org/10.1016/j.micpath.2017.11.004

Elkelish A, El-Mogy MM, Niedbala G, Piekutowska M, Atia MAM, Hamada MMA, Shahin M, Mukherjee S, El-Yazied AA, et al. (2021) Roles of exogenous alpha-lipoic acid and cysteine in mitigation of drought stress and restoration of grain quality in wheat. Plants (Basel) 10:2318. https://doi.org/10.3390/plants10112318

Faleiro JH, Gonçalves RC, dos Santos MNG, da Silva DP, Naves PLF, Malafaia G (2016) The chemical featuring, toxicity, and antimicrobial activity of *Psidium cattleianum* (Myrtaceae) leaves. New J Sci 2016:1-8. https://doi.org/10.1155/2016/7538613

Foudah AI, Alqarni MH, Alam A, Salkini MA, Alam P, Alkholifi FK, Yusufoglu HS (2021) Determination of chemical composition, in vitro and in silico evaluation of essential oil from leaves of *Apium graveolens* grown in Saudi Arabia. Molecules 26:7372. https://doi.org/10.3390/molecules26237372

Franzener G, Schwan-Estrada KRF, Moura GS, Kuhn OJ, Stangarlin JR (2018) Induction of defense enzymes and control of anthracnose in cucumber by *Corymbia citriodora* aqueous extract. Summa Phytopathol 44:10-16. https://doi.org/10.1590/0100-5405/2218

Freitas TS, Xavier JDC, Pereira RLS, Rocha JE, Muniz DF, da Silva PT, da Hora JP, Dos Santos HS, Bandeira PN, et al. (2020) Direct antibacterial and antibiotic resistance modulatory activity of chalcones synthesized from the natural product 2-hydroxy-3,4,6-trimethoxyacetophenone. FEMS Microbiol Lett 367:1-5. https://doi.org/10.1093/femsle/fnaa124

Fu J, Gao Y, Xing X (2022) Preliminary study on phytochemical constituents and biological activities of essential oil from *Myriactis nepalensis* Less. Molecules 27:4631. https://doi.org/10.3390/molecules27144631

Fujiwara M, Ijichi K, Tokuhisa K, Katsuura K, Shigeta S, Konno K, Wang GY, Uemura D, Yokota T, et al. (1996) Mechanism of selective inhi-

bition of human immunodeficiency virus by ingenol triacetate. Antimicrob Agents Chemother 40:271-273. https://doi.org/10.1128/aac.40.1.271

Gholamnezhad J (2019) Effect of plant extracts on activity of some defense enzymes of apple fruit in interaction with *Botrytis cinerea*. J Integr Agric 18:115-123. https://doi.org/10.1016/s2095-3119(18)62104-5

Guo Y, Lv J, Zhao Q, Dong Y, Dong K (2020) Cinnamic acid increased the incidence of *Fusarium* wilt by increasing the pathogenicity of *Fusarium oxysporum* and reducing the physiological and biochemical resistance of faba bean, which was alleviated by intercropping with wheat. Front Plant Sci 11:608389. https://doi.org/10.3389/fpls.2020.608389

Gupta P, Ravi I, Sharma V (2013) Induction of β-1,3-glucanase and chitinase activity in the defense response of *Eruca sativa* plants against the fungal pathogen *Alternaria brassicicola*. J Plant Interact 8:155-161. https://doi.org/10.1080/17429145.2012.679705

Gurjar MS, Ali S, Akhtar M, Singh KS (2012) Efficacy of plant extracts in plant disease management. Agric Sci 03:425-433. https://doi.org/10.4236/as.2012.33050

Harborne JB (1998) Phytochemical methods: A guide to modern techniques of plant analysis, Ed 2. Chapmann and Hall Publishers, London, UK

Hassan M, Bala SZ, Bashir M, Waziri PM, Musa Adam R, Umar MA, Kini P (2022) LC-MS and GCMS profiling of different fractions of *Ficus platyphylla* stem bark ethanolic extract. J Anal Methods Chem 2022:6349332. https://doi.org/10.1155/2022/6349332

Horsfield A, Wicks T, Davies K, Wilson D, Paton S (2010) Effect of fungicide use strategies on the control of early blight (*Alternaria solani*) and potato yield. Australas Plant Pathol 39:368-375. https://doi.org/10.1071/AP09090

Hussein JH, Mohammed YH, Imad HH (2016) Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrophotometer and gas chromatography - mass spectrometry. J Pharmacogn Phytother 8:60-89. https://doi.org/10.5897/jpp2015.0372

Ichimura K, Takada M, Ogawa K (2022) Effects of treatments with nigerosylmaltooligosaccharide, glucose and sucrose on the vase life of cut snapdragon flowers. Sci Hortic 291:110565. https://doi.org/10.1016/j.scienta.2021.110565

Imatomi M, Novaes P, Matos AP, Gualtieri SCJ, Molinillo JMG, Lacret R, Varela RM, Macías FA (2013) Phytotoxic effect of bioactive compounds isolated from *Myrcia tomentosa* (Myrtaceae) leaves. Biochem Syst Ecol 46:29-35. https://doi.org/10.1016/j.bse.2012.09.005

Iqbal E, Salim KA, Lim LBL (2015) Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. J King Saud Univ Sci 27:224-232. https://doi.org/10.1016/j.jksus.2015.02.003

Isah M, Rosdi RA, Wan Abdul Wahab WNA, Abdulla H, Sul'ain MD, Ishak WRW (2023) Phytoconstituents and biological activities of *Melaleuca cajuputi* Powell: A scoping review. J Appl Pharm Sci 13:10-23. https://doi.org/10.7324/japs.2023.130102

İşcan G, Kirimer N, Demirci F, Demirci B, Noma Y, Başer KH (2012) Biotransformation of (-)-(R)-αphellandrene: antimicrobial activity of its major metabolite. Chem Biodivers 9:1525-1532. https://doi.org/10.1002/cbdv.201100283

Isnaini I, Achmadiyah R, Awaeh G, Khatimah H, Yasmina A (2023) Antioxidant and antiproliferative activities of methanol extract from *Melaleuca cajuputi* subsp. Cumingiana [Turcz.] Fruit. Jurnal Berkala Ilmiah Sains dan Terapan Kimia 17:21. https://doi.org/10.20527/jstk.v17i1.13055

Jantasorn A, Moungsrimuangdee B, Dethoup T (2016) In vitro antifungal activity evaluation of five plant extracts against five plant pathogenic fungi causing rice and economic crop diseases. J Biopest 9:1-7. https://doi.org/10.57182/jbiopestic.9.1.01-07

Jaroš P, Timkina E, Michailidu J, Maršík D, Kulišová M, Kolouchová I, Demnerová K (2022) Boswellic acids as effective antibacterial antibiofilm agents. Molecules 27:3795. https://doi.org/10.3390/molecules27123795

Kamiya H, Haraguchi A, Mitarai H, Yuda A, Wada H, Shuxin W, Ziqing R, Weihao S, Wada N (2024) In vitro evaluation of the antimicrobial properties of terpinen-4-ol on apical periodontitis-associated bacteria. J Infect Chemother 30:306-314. https://doi.org/10.1016/j.jiac.2023.10.021

Karimi S, Farzaneh F, Asnaashari S, Parina P, Sarvari Y, Hazrati S (2019) Phytochemical analysis and anti-microbial activity of some important medicinal plants from north-west of Iran. Iran J Pharm Res 18:1871-1883. https://doi.org/10.22037/ijpr.2019.1100817

Keereedach P, Hrimpeng K, Boonbumrung K (2020) Antifungal activity of Thai cajuput oil and its effect on efflux-pump gene expression in fluconazole-resistant *Candida albicans* clinical isolates. Int J Microbiol 2020:5989206. https://doi.org/10.1155/2020/5989206

Khan FA, Khan NM, Ahmad S, Nasruddin, Aziz R, Ullah I, Almehmadi M, Allahyani M, Alsaiari AA, et al. (2022) Phytochemical profiling, antioxidant, antimicrobial and cholinesterase inhibitory effects of essential oils isolated from the leaves of *Artemisia scoparia* and *Artemisia absinthium*. Pharmaceuticals (Basel) 15:1221. https://doi.org/10.3390/ph15101221

Kim HM, Kwon H, Kim K, Lee SE (2018) Antifungal and antiaflatoxigenic activities of 1,8-cineole and t-cinnamaldehyde on *Aspergillus flavus*. Appl Sci 8:1655. https://doi.org/10.3390/app8091655

Kim S, Lee H, Lee S, Yoon Y, Choi KH (2015) Antimicrobial action of oleanolic acid on *Listeria monocytogenes*, *Enterococcus faecium*, and *Enterococcus faecalis*. PLoS One 10:e0118800. https://doi.org/10.1371/journal.pone.0118800

Kozłowska J, Potaniec B, Żarowska B, Anioł M (2017) Synthesis and biological activity of novel o-alkyl derivatives of naringenin and their oximes. Molecules 22:1485. https://doi.org/10.3390/molecules22091485

Kunasakdakul K, Suwitchayanon P (2012) Antimicrobial activities of chili and black pepper extracts on pathogens of Chinese kale. CMU J Nat Sci 11:135-141

Latha P, Anand T, Ragupathi N, Prakasam V, Samiyappan R (2009) Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. Biological Control 50:85-93.

https://doi.org/10.1016/j.biocontrol.2009.03.002

Mangoyi R, Midiwo J, Mukanganyama S (2015) Isolation and characterization of an antifungal compound 5-hydroxy-7,4'-dimethoxyflavone from *Combretum zeyheri*. BMC Complement Altern Med 15:405. https://doi.org/10.1186/s12906-015-0934-7

Marei GIK, Abdelgaleil SAM (2018) Antifungal potential and biochemical effects of monoterpenes and phenylpropenes on plant. Plant Protection Science 54:9-16. https://doi.org/10.17221/9/2017-pps

Marinas IC, Oprea E, Buleandra M, Badea IA, Tihauan BM, Marutescu L, Angheloiu M, Matei E, Chifiriuc MC (2021) Chemical composition, antipathogenic and cytotoxic activity of the essential oil extracted from *Amorpha fruticosa* fruits. Molecules 26:3146. https://doi.org/10.3390/molecules26113146

Marliyana SD, Mujahidin D, Syah YM (2018) Pinostrobin derivatives from prenylationreaction and their antibacterial activity against clinical bacteria. Mater Sci Eng 349:012057. https://doi.org/10.1088/1757-899X/349/1/012057

Mohammadi A, Bahramikia S (2019) Molecular identification and genetic variation of *Alternaria* species isolated from tomatoes using ITS1 sequencing and inter simple sequence repeat methods. Curr Med Mycol 5:1-8. https://doi.org/10.18502/cmm.5.2.1154

Mohanty S, Cock IE (2010) Bioactivity of *Syzygium jambos* methanolic extracts: Antibacterial activity and toxicity. Pharmacognosy Res 2:4-9. https://doi.org/10.4103/0974-8490.60577

Morcia C, Malnati M, Terzi V (2012) In vitro antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 29:415-422. https://doi.org/10.1080/19440049.2011.643458

Mulyaningsih S, Sporer F, Reichling J, Wink M (2011) Antibacterial activity of essential oils from *Eucalyptus* and of selected components against multidrug-resistant bacterial pathogens. Pharm Biol 49:893-899. https://doi.org/10.3109/13880209.2011.553625

Muñoz P, Munné-Bosch S (2019) Vitamin E in plants: Biosynthesis, transport, and function. Trends in Plant Science 24:1040-1051. https://doi.org/10.1016/j.tplants.2019.08.006

Murugan S, Devi P, Parameswari NK, Mani KR (2011) Antimicrobial activity of *Syzygium jambos* against selected human pathogens. Int J Pharm Pharm Sci 3:44-47

Naidoo Y, Channangihalli Thimmegowda CS, Kasim N, Nicholas A, Naidoo G (2014) Chemical composition and antimicrobial activity of the essential oil of *Ocimum obovatum* E. Mey. Ex Benth. (Lamiaceae). J Essential Oil Bearing Plants 17:142-147. https://doi.org/10.1080/0972060X.2014.884782

Noriega P, Ballesteros J, De la Cruz A, Veloz T (2020) Chemical composition and preliminary antimicrobial activity of the hydroxylated sesquiterpenes in the essential oil from *Piper barbatum* Kunth Leaves. Plants (Basel) 9:211. https://doi.org/10.3390/plants9020211

O'Neill T (2019) Diseases of lettuce crops. AHDB Horticulture, Warwickshire, UK

Patel D, Desai S, Desai A, Dave D, Meshram D (2019) Phytochemical evaluation and in-vitro thrombolytic activity of hydro alcoholic extract of *Syzygium malaccense* leaves. J Pharmacogn Phytochem 8:39163918

Pattanamahakul P, Strange RN (1999) Identification and toxicity of *Alternaria brassicicola*, the causal agent of dark leaf spot disease of Brassica species grown in Thailand. Plant Pathology 48:749-755. https://doi.org/10.1046/j.1365-3059.1999.00406.x

Pawar VC, Thaker VS (2007) Evaluation of the anti-*Fusarium oxysporum* f. sp cicer and anti-*Alternaria porri* effects of some essential oils. World J Microbiol Biotechnol 23:1099-1106. https://doi.org/10.1007/s11274-006-9339-6

Petpheng B, Mudtaleb B, Piboon W, Paichid N, Sangkharak K (2023) The extraction of phytol from *Hydrilla verticillata* using ultrasonic-assisted extraction (UAE), the analysis of antibacterial activity, and the utilization of residual extract for polyhydroxyalkanoate (PHA) production. Biomass Convers Biorefin https://doi.org/10.1007/s13399-023-04331-5

Pinto KMSP, de Melo AFR, Mondego JM, do Nascimento LC, Cortez M, Izabel Mendes Marques , Aires AAdC, dos Anjos Neto AP, Medeiros RLSd, Araujo JRG, et al. (2018) Plant extracts enhancers of defense response in ponkan mandarin Seedlings against *Alternaria alternate* f. spp. citri infection. Afr J Agric Res 13:650-656. https://doi.org/10.5897/ajar2018.13025

Prasannath K (2017) Plant defense-related enzymes against pathogens: A review. AGRIEAST J Agric Sci 11:38. https://doi.org/10.4038/agrieast.v11i1.33

Puig CG, Reigosa MJ, Valentão P, Andrade PB, Pedrol N (2018) Unravelling the bioherbicide potential of *Eucalyptus globulus* Labill: Biochemistry and effects of its aqueous extract. PLoS One 13:e0192872. https://doi.org/10.1371/journal.pone.0192872

Qiu XM, Sun YY, Ye XY, Li ZG (2020) Signaling role of glutamate in plants. Front Plant Sci 10:1743. https://doi.org/10.3389/fpls.2019.01743

Rita dCPdS, Pedro VPG, Marcelo RdS, Oscar JS, Edvan AC, and CGB-L (2017) Phytotoxicity of extracts of *Myrciaria dubia* (Kunth) McVaugh bioprocessed in vegetable crop sensitive to allelochemicals. Afr J Plant Sci 11:244-251. https://doi.org/10.5897/ajps2016.1491

Saha M, Bandyopadhyay PK (2020) In vivo and in vitro antimicrobial activity of phytol, a diterpene molecule, isolated and characterized from *Adhatoda vasica* Nees. (Acanthaceae), to control severe bacterial disease of ornamental fish, *Carassius auratus*, caused by *Bacillus licheniformis* PKBMS16. Microb Pathog 141:103977. https://doi.org/10.1016/j.micpath.2020.103977

Sakander H, Koteshwara AR, Akhilesh B (2015) Evaluation of antifungal potential of selected medicinal plants against human pathogenic fungi. Int J Green Pharmacy 9:110-117. https://doi.org/10.4103/0973-8258.155058

Salem MZM, El-Hefny M, Nasser RA, Ali HM, El-Shanhorey NA, Elansary HO (2017) Medicinal and biological values of *Callistemon viminalis* extracts: History, current situation and prospects. Asian Pac J Trop Med 10:229-237. https://doi.org/10.1016/j.apjtm.2017.03.015

Sangpueak R, Phansak P, Thumanu K, Siriwong S, Wongkaew S, Buensanteai N (2021) Effect of salicylic acid formulations on induced plant defense against cassava anthracnose disease. Plant Pathol J 37:356-364. https://doi.org/10.5423/PPJ.OA.02.2021.0015

Scur MC, Pinto FG, Pandini JA, Costa WF, Leite CW, Temponi LG (2016) Antimicrobial and antioxidant activity of essential oil and different plant extracts of *Psidium cattleianum* Sabine. Braz J Biol 76:101-108. https://doi.org/10.1590/1519-6984.13714

Selestino Neta MC, Vittorazzi C, Guimaraes AC, Martins JD, Fronza M, Endringer DC, Scherer R (2017) Effects of beta-caryophyllene and *Murraya paniculata* essential oil in the murine hepatoma cells and in the bacteria and fungi 24-h time-kill curve studies. Pharm Biol 55:190-197. https://doi.org/10.1080/13880209.2016.1254251

Selvaraj T, Ambalavanan S (2013) Induction of defense-related enzymes in anthurium by application of fungal and bacterial biocontrol agents against *Colletotrichum gloeosporioides*. Int J Curr Microbiol App Sci 2:661-670

Serban G, Stanasel O, Serban E, Bota S (2018) 2-Amino-1,3,4-thiadiazole as a potential scaffold for promising antimicrobial agents. Drug Des Devel Ther 12:1545-1566. https://doi.org/10.2147/DDDT.S155958

Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhtar S, Alsharif MA, Parveen H, Zakaria ZA (2022) Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation. Molecules 27:1149. https://doi.org/10.3390/molecules27041149

Sharif ZM, Kamal AF, Jalil NJ (2019) Chemical composition of *Melaleuca Cajuputi* Powell. Int J Eng Adv Technol 9:3479-3483. https://doi.org/10.35940/ijeat.A2668.109119

Shukla P, Walia S, Ahluwalia V, Parmar BS, Nair MG (2012) Activity of alkanediol alkanoates against pathogenic plant fungi *Rhizoctonia solani* and *Sclerotium rolfsii*. Nat Prod Commun 7:1219-1222

Siddique S, Mazhar S, Firdaus-e-Bareen, Parveen Z (2018) Chemical characterization, antioxidant and antimicrobial activities of essential oil from *Melaleuca quinquenervia* leaves. Indian J Exp Biol 56:686-693

Şimşek M, Duman R (2017) Investigation of effect of 1,8-cineole on antimicrobial activity of chlorhexidine gluconate. Pharmacognosy Res 9:234-237. https://doi.org/10.4103/0974-8490.210329

Somnuek S, Jaenaksorn T, Laosinwattana C (2020) Effect of crude ethanolic extracts from bottle brush (*Callistemon viminalis*) against leaf spot fungi and their phytotoxicity on lettuce (*Lactuca sativa* L.). Curr Appl Sci Technol 20:1-14. https://doi.org/10.14456/cast.1477.1

Somnuek S, Thipmanee K, Jaenaksorn T (2021) In vitro effect of *Callistemon viminalis* and *Melaleuca cajuputi* ethanolic extracts as botanical fungicide and insecticide. Int J Agric Technol 17:2363-2374

Srikanth CV, Chakraborti AK, Bachhawat AK (2005) Acetaminophen toxicity and resistance in the yeast *Saccharomyces cerevisiae*. Microbiology (Reading) 151:99-111. https://doi.org/10.1099/mic.0.27374-0

Subramaniam S, Keerthiraja M, Sivasubramanian A (2014) Synergistic antibacterial action of βsitosterol-d-glucopyranoside isolated from *Desmostachya bipinnata* leaves with antibiotics against common human pathogens. Rev Bras Farmacogn 24:44-50. https://doi.org/10.1590/0102695x20142413348

Sukdee S (2023) Antifungal activity of plant extracts against *Colletotrichum capsici* causal agent of chili anthracnose. Rattanakosin J Sci Technol 5:1-8

Tadtong S, Puengseangdee C, Prasertthanawut S, Hongratanaworakit T (2016) Antimicrobial constituents and effects of blended eucalyptus, rosemary, patchouli, pine, and cajuput essential oils. Nat Prod Commun 11:267-270. https://doi.org/10.1177/1934578X1601100234

Tan J, Li Y, Hou DX, Wu S (2019) The effects and mechanisms of cyanidin-3-glucoside and its phenolic metabolites in maintaining intestinal integrity. Antioxidants (Basel) 8:479. https://doi.org/10.3390/antiox8100479

Teixeira MFF, Pinheiro DT, Junior HCS, Alves EC, Barros TTV, Freitas MAMd, Dias DCFdS (2018) Allelopathic influence of some fruit tree leaf extracts on germination and seedling development of different weeds and vegetable crops. Aust J Crop Sci 12:726-730. https://doi.org/10.21475/ajcs.18.12.05.PNE839

Thanaboripat D (2011) Control of aflatoxins in agricultural products using plant extracts. KMITL Sci Technol J 11:35-42

Thanaboripat D, Sarutipaisan C, Puangtong C, Chatpongsatorn P, Suvatti Y, Sukonthamut S, Charoensettasilp S (2016) Effects of four essential oils on the growth of aflatoxin producing fungi. KMITL Sci Technol J 16:104-111

Thanaboripat D, Suvathi Y, Srilohasin P, Sripakdee S, Patthanawanitchai O, Charoensettasilp S (2007) Inhibitory effect of essential oils on the growth of *Aspergillus flavus*. KMITL Sci Technol J 7:1-7

Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H (2011) Phytochemical screening and extraction: A review. Int Pharm Sci 1:98-106

Toiu A, Vlase L, Vodnar DC, Gheldiu AM, Oniga I (2019) *Solidago graminifolia* L. Salisb. (Asteraceae) as a valuable source of bioactive polyphenols: HPLC profile, in vitro antioxidant and antimicrobial potential. Molecules 24:2666. https://doi.org/10.3390/molecules24142666

Tzortzakis N, Proestos C (2024) Natural products and essential oils biocidal activities and innovative applications. Molecules

Ukit U, Widiana A, Rahmawati E, Hasby RM (2019) Antibacterial activities test of cajuput leaf waste extract (*Melaleuca cajuputi* Powell) on pathogenic bacteria. J Phys Conf Ser 1402:033030. https://doi.org/10.1088/1742-6596/1402/3/033030

Vasconcelos LC, Carrijo TT, Venancio AN, Alves TA, Tuler AC, Hollunder RK, Garbin ML, Menini L, Praca-Fontes MM (2022) Phytochemical screening and phytocytotoxic effects of the tropical *Myrcia vittoriana* (Myrtaceae). An Acad Bras Cienc 94:e20210820. https://doi.org/10.1590/0001376520222021082O

Verburg JG, Huynh QK (1991) Purification and characterization of an antifungal chitinase from *Arabidopsis thaliana*. Plant Physiol 95:450-455. https://doi.org/10.1104/pp.95.2.450

Wamba BEN, Nayim P, Mbaveng AT, Voukeng IK, Dzotam JK, Ngalani OJT, Kuete V (2018) *Syzygium jambos* displayed antibacterial and antibiotic-modulating activities against resistant phenotypes. Evid Based Complement Alternat Med 2018:5124735. https://doi.org/10.1155/2018/5124735

Wang H, Huang Y, Wang J, Chen X, Wei K, Wang M, Shang S (2016) Activities of azoxystrobin and difenoconazole against *Alternaria alternata* and their control efficacy. Crop Protection 90:54-58. https://doi.org/10.1016/j.cropro.2016.08.022

War AR, Paulraj MG, War MY, Ignacimuthu S (2011) Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L.). Plant Signal Behav 6:1787-1792. https://doi.org/10.4161/psb.6.11.17685

Watanabe M, Fukiya S, Yokota A (2017) Comprehensive evaluation of the bactericidal activities of free bile acids in the large intestine of humans and rodents. J Lipid Res 58:1143-1152. https://doi.org/10.1194/jlr.M075143

Wu PH, Chang HX, Shen YM (2023) Effects of synthetic and environmentally friendly fungicides on powdery mildew management and the phyllosphere microbiome of cucumber. PLoS One 18:e0282809. https://doi.org/10.1371/journal.pone.0282809

Xi KY, Xiong SJ, Li G, Guo CQ, Zhou J, Ma JW, Yin JL, Liu YQ, Zhu YX (2022) Antifungal activity of ginger rhizome extract against *Fusarium solani*. Horticulturae 8:983. https://doi.org/10.3390/horticulturae8110983

Xie Q, Li F, Fang L, Liu W, Gu C (2020) The antitumor efficacy of beta-elemene by changing tumor inflammatory environment and tumor microenvironment. Biomed Res Int 2020:6892961. https://doi.org/10.1155/2020/6892961

Yamunarani K, Jaganathan R, Bhaskaran R, Govindaraju P, Velazhahan R (2004) Induction of early blight resistance in tomato by *Quercus infectoria* gall extract in association with accumulation of phenolics and defense-related enzymes. Acta Physiol Plant 26:281-290. https://doi.org/10.1007/s11738-004-0018-7

Yang LN, He MH, Ouyang HB, Zhu W, Pan ZC, Sui QJ, Shang LP, Zhan J (2019) Cross-resistance of the pathogenic fungus *Alternaria alternata* to fungicides with different modes of action. BMC Microbiology 19:205. https://doi.org/10.1186/s12866-019-1574-8

Yusoff SF, Haron FF, Tengku Muda Mohamed M, Asib N, Sakimin SZ, Abu Kassim F, Ismail SI (2020) Antifungal activity and phytochemical screening of *Vernonia amygdalina* extract against *Botrytis cinerea* causing gray mold disease on tomato fruits. Biology (Basel) 9:286. https://doi.org/10.3390/biology9090286

Zhao F, Wang P, Lucardi RD, Su Z, Li S (2020) Natural sources and bioactivities of 2,4-di-tertbutylphenol and its analogs. Toxins (Basel) 12:35. https://doi.org/10.3390/toxins12010035