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Chemical Composition and Antifungal Properties of Guava Leaf Extract (*Psidium guajava* L.) Against *Pythium aphanidermatum*, Chinese Kale Damping-Off Disease Pathogen

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Abstract

Damping-off disease represents a considerable hazard to the cultivation of Chinese kale (*Brassica oleracea* var. *alboglabra*), leading to seedling death and notable economic repercussions. This study explores the efficacy of guava leaf extracts, obtained through different extraction solvents, against *Pythium aphanidermatum*, a significant pathogen causing damping-off disease in Chinese kale (Pak Kha-naa) cultivation in Thailand. Guava leaf extract was obtained using different solvents: hexane, ethyl acetate and ethanol. Subsequently, the extract was diluted to concentrations of 1,000, 1,500 and 3,000 ppm to assessment the inhibitory effects on *P. aphanidermatum* utilizing the poisoned plate technique. Results revealed that only ethanol-extracted guava leaf extract at 3,000 ppm exhibited significant inhibition of *P. aphanidermatum*, achieving complete inhibition. At 1,500 ppm, ethanol-extracted guava leaf extract demonstrated inhibition comparable to carbendazim. Greenhouse trials further confirmed the efficacy of pre-planting seeds coating with ethanolic guava leaf extract at concentrations of 1,500 and 3,000 ppm in reducing damping-off disease incidence in Chinese kale. Chemical analysis of guava extract with GC-MS revealed the presence of 58 compounds in the hexane extract, with copaene, β-caryophyllene, calamenene and caryophyllene oxide being major constituents. The ethyl acetate extract contained 53 compounds, including β-caryophyllene and squalene, while caryophyllene was a predominant component among the 40 chemicals identified in the ethanol extract. Methylparaben was exclusively present in the ethanol extract, showcasing inhibitory effects on the fungus. This research sheds light on the potential of guava leaf extracts as a sustainable solution for disease management in Chinese kale cultivation

Keywords: Damping-off disease, GC-MS, Guava, Plant extracts, Seeds coating

Introduction

Damping-off disease, caused by various fungal pathogens, poses a significant threat to Chinese kale (*Brassica oleracea* var. *alboglabra*) cultivation, resulting in seedling death and substantial economic losses [1]. This disease is historically significant, dating back to the early nineteenth century. It affects seeds and seedlings in pre- and post-emergence phases [2], leading to direct costs such as seed and seedling damage

and indirect costs including replanting and reduced yields [3,4]. Various soil-borne fungi, including *Pythium aphanidermatum*, *Fusarium* spp. and *Rhizoctonia* spp., contribute to damping-off, necessitating diverse control strategies [5]. While fungicides have traditionally been used, concerns over their environmental impact and efficacy have spurred exploration into botanical fungicides and biocontrol agents [5]. Plant extracts, rich in secondary metabolites, have shown promise in inhibiting fungal growth and enhancing plant defenses against pathogens, with extracts from cinnamon, carnation and camphor exhibiting significant antifungal activity against *P. aphanidermatum* [6].

In recent years, there has been a growing interest in natural alternatives to synthetic chemicals for disease control, aiming to mitigate the negative impacts associated with fungicide use while offering sustainable management strategies for damping-off [1]. Plant-based fungicides have emerged as promising options for sustainable disease management in agriculture, leveraging the innate defensive properties of plants to combat fungal diseases. The utilization of plant extracts for disease management aligns with the increasing interest in botanical fungicides as alternatives to chemical fungicides, which can have adverse environmental impacts [7]. Plants contain phytochemicals that offer benefits such as biodegradability and renewable sourcing. However, they suffer from drawbacks including instability, making them unsuitable for long-term storage and susceptibility to oxidation and evaporation under solar radiation, particularly UVB, which can diminish crop size, productivity and quality [8]. Plant-derived bioactive compounds offer antimicrobial properties through various mechanisms, including inhibition of microbial cell wall development and suppression of bacterial toxins [9,10]. Guava extract holds promise for the management of damping-off disease in Chinese kale and other crops, underscoring the potential of plant-derived bioactive compounds in sustainable agriculture.

Guava (*Psidium guajava* L.), a versatile tropical plant with a history of traditional usage in food and folk medicine, contains bioactive compounds in its leaves, roots, bark and fruit with medicinal properties, including antimicrobial effects [11]. Although limited research has been conducted specifically on the impact of guava extract on damping-off disease in Chinese kale, its inhibitory effects on pathogenic microorganisms suggest its potential efficacy in disease management. Studies have shown the effectiveness of plant extracts, including guava, against damping-off disease in various crops. For instance, Abdel-Monaim *et al.* [9] demonstrated that water leaf extracts of several plants, including guava, reduced damping-off and wilt diseases in lupine plants caused by *Fusarium oxysporum* f. sp. *lupini*. Additionally, Islam and Faruq [12] showed that seed treatments with various plant extracts, including guava, reduced damping-off and promoted seed germination and growth in tomato, eggplant and chili seedlings.

Guava, known as the “poor man’s apple” possesses significant medicinal value due to its rich phytochemical content. The gas chromatography-mass spectroscopy (GC-MS) analysis of guava leaf extract, as indicated by references [10,13], has revealed a spectrum of bioactive compounds renowned for their antimicrobial, antioxidant, anticancer and antitumor properties. Among these compounds are quercetin, avicularin, apigenin, gallic acid and catechin, which collectively contribute to the health benefits associated with guava leaves. Notably, guava leaf extracts are characterized by their diverse phytochemical composition, showcasing promising antimicrobial properties alongside a range of biological activities such as anticancer, antidiabetic, antioxidant, antimicrobial and hepatoprotective effects [10]. Qualitative analysis of guava leaf extracts has revealed the presence of phenolic acids, flavonoids, terpenoids, glycosides and saponins, which correlate with antimicrobial activity [14]. High-performance liquid chromatography coupled with time-of-flight mass spectrometry (HPLC-TOF–ESI/MS) analysis has confirmed the presence of gallic acid, chlorogenic acid, rutin, isoquercitrin and quercetin in fermented guava leaf extracts, which inhibit fungal cell membrane components and fungal cell growth [14]. Antibacterial and antifungal activities have been attributed to compounds such as quercetin, betulinic acid and lupeol found in guava

leaf extracts [14]. Various solvent extracts of guava leaves have demonstrated antibacterial activity, with ethyl acetate extract showing significant inhibition against pathogens such as *Enterobacter* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [14]. Methanol extract exhibited considerable activity against *Escherichia coli*, while acetone and ethyl acetate extracts showed potential activity against *Candida albicans* [14]. GC-MS analysis has identified several compounds in guava leaf extracts, including β-carotene, α-bisabolol and 2,4,6-cycloheptatrien-1-one,3,5-bis-trimethylsilyl [14]. Our study contributes to the current state of the art by comprehensively evaluating the efficacy of guava leaf extracts obtained through various solvents against *P. aphanidermatum*, a significant pathogen affecting Chinese kale cultivation in Thailand. By systematically comparing the inhibitory effects of different extraction solvents and concentrations, we provide valuable insights into the optimal utilization of guava leaf extracts for disease management. Furthermore, our investigation into the chemical composition of these extracts using GC-MS analysis sheds light on the specific compounds responsible for their antifungal properties, offering a deeper understanding of their mode of action. Overall, our research not only addresses the pressing issue of damping-off disease but also contributes novel findings to the field of natural product-based disease management strategies.

Materials and methods

Microorganisms

The causative agent of damping-off disease of Chinese kale *Pythium aphanidermatum*, was obtained from the Microbiology Laboratory, Department of Biology, Faculty of Science, Mahasarakham University, Thailand.

Preparation of plant leaf samples

Leaf samples from the Kimju guava species (*Psidium guajava* L.) were gathered from guava plantations located in Nong Bua Lamphu Province, Thailand. A quantity of 10 kg of mature leaves was collected, followed by a cleaning process to remove impurities. Subsequently, the cleaned leaves were sun-dried until adequately desiccated. Post-drying, the leaves were finely ground and carefully preserved in a sealed container to protect against moisture. These samples were stored at 28 °C until required for the extraction process.

Preparation of guava leaf extract

Preparation of guava leaf extract from prepared finely ground leaf. Ground leaf (100 g) was immersed individually in hexane, ethyl acetate and ethanol, using a ratio of 1:3 (leaf: solvent) within a 500 mL flask. The sealed flask underwent agitation in a shaker at room temperature (150 rpm) for 48 h. Subsequently, the resultant solution was filtered through cheesecloth to eliminate plant debris, followed by transfer into a container. The extraction process continued through evaporation of solvents in a rotary vacuum evaporator at 45 °C, yielding an extract [15]. The percent yield (% yield) was computed using the equation:

$$\% \text{ yield} = \frac{\text{weight remaining after rotary evaporation} \times 100}{\text{dry weight of guava leaf}} \quad (1)$$

The resulting extract was then stored in sterilized amber bottles at –20 °C, until use.

The efficacy of guava leaf extract to inhibit the growth of *P. aphanidermatum* using poisoned plate technique

The initial step involved diluting the prepared stock solution of crude guava leaf extract with 10 % dimethyl sulfoxide (DMSO). Subsequently, 1 mL of each concentration was pipetted into 14 mL of potato dextrose agar (PDA) medium, which had been melted at approximately 45 °C. The mixture was thoroughly mixed and poured into Petri dishes and then left to dry overnight. This process resulted in PDA medium supplemented with guava leaf extract at concentrations of 1,000, 1,500 and 3,000 ppm [16]. The control consisted of PDA medium supplemented with the fungicide carbendazim dissolved in dH_2O at a concentration of 1,500 ppm. *P. aphanidermatum* was cultured on PDA plates and subsequently incubated at 30 ± 2 °C for a duration of 3 days prior to experimentation. Fungal hyphae were excised from the colony edges and using a needle, agar pieces containing the hyphae were transferred onto the prepared PDA plates mixed with various concentrations of plant extract. There were 3 treatments. (1) PDA medium without any additional supplementation, (2) PDA medium supplemented with carbendazim at a concentration of 1,500 ppm and (3) PDA medium supplemented with guava leaf extract at concentrations of 1,000, 1,500 and 3,000 ppm.

The fungal culture plates were then incubated at a temperature of 30 ± 2 °C for a period ranging from 5 to 7 days. The experiment was replicated 3 times per extract concentration. Following the incubation period, the diameter of fungal colonies was measured. These measured values were subsequently utilized to calculate the percentage of growth inhibition using the designated formula [17].

$$\text{Percent inhibition of radial growth (\% PIRG)} = \frac{R1 - R2}{R1} \times 100 \quad (2)$$

R1: mean diameter of fungal colonies on control PDA plate
R2: mean diameter of fungal colonies on PDA mudium containing guava leaf extract.

The efficacy of guava leaf extract for controlling of *P. aphanidermatum*

P. aphanidermatum was mixed with peat moss that had been sterilized at 121 °C for 30 min, 2 times, 24 h interval. *P. aphanidermatum* that was 48 - 72 h old was placed in peat moss in a ratio of 1:100 (1 dish of fungus: 100 g of peat moss), thoroughly mixed and subsequently incubated at room temperature for a duration of 72 h. The resulting fungal growth was then transferred to 5 cm diameter cups and containing 20 g of infected peat moss.

For the subsequent phase of the experiment, Chinese kale seeds were subjected to surface sterilization by immersion in a 10 % Clorox solution for 3 min, followed by rinsing with distilled water twice, each for a duration of 1 min. Surface sterilized seeds were dried under sterile conditions. Ninety Chinese kale seeds were then mixed with guava leaf extracts, obtained via ethanol extraction at concentrations of 1,500 and 3,000 ppm, along with a positive control of 1,500 ppm carbendazim and a negative control of distilled water. These treated seeds were transplanted into soil pre-mixed with *P. aphanidermatum*. The experiment was designed for 3 replications, with each treatment comprising 3 cups and each cup containing 10 seeds.

The evaluation of the efficacy of guava leaf extract concentrations (1,500 and 3,000 ppm) in inhibiting the growth of *P. aphanidermatum* was conducted by coating Chinese kale seeds with these extracts and subsequently planting them in plastic cups containing soil infected with *P. aphanidermatum*. The experimental design adopted was a Completely Randomized Design (CRD). Regular watering was administered and the results were assessed by recording the percentage of germination, survival rate and disease incidence percentage [15].

1) Calculation of germination percentage as in the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seedlings} \times 100}{\text{Number of seeds planted}} \quad (3)$$

2) Calculation of the survival rate as in the following formula:

$$\text{Survival (\%)} = \frac{\text{Number of seedlings remaining} \times 1{,}000}{\text{Number of starting seeds}} \quad (4)$$

3) Calculation of the percentage of disease incidences as in the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased seedlings} \times 100}{\text{Total number of seeds used for testing}} \quad (5)$$

Gas chromatography mass spectrometry (GC-MS)

The protocol of GC-MS analysis was adapted from the previous study of Kumari *et al.* [16]. Helium was used as carrier gas at a flow rate of 0.8 mL/min. The injector was set at 250 °C and performed by split mode with a split ratio of 1:5 (in 2 µL). A fused silica capillary BRUKER 450GC column (column: Rtx-5MS capillary column (30 m×0.25 mm film thickness) was used. The oven temperature was adjusted to rise to 280 °C at a rate of 200 °C per minute after being held at 60 °C for 3 min. The column was then maintained at 280 °C for 10 min. At 280 °C, the temperature of the transfer tube heater was set. A mass spectrum with a full scan range of 45 - 500 amu was determined. For mass spectrometry electron impact ionization was performed at electron energy of 70 eV. Chemical compounds contained in the extracts were identified by computer matching retention time frames and mass spectrum data with standards from the National Institute of Standards and Technology (NIST).

Results and discussion

Preparation of guava leaf extract

Extraction of guava leaves using 3 distinct solvents (hexane, ethyl acetate and ethanol), yielded extracts exhibiting a characteristic dark green color and viscous substances, indicating the extracts had varying yields and characteristics. Ethanol yielded the highest percentage yield of guava leaf extract (27.56 %), followed by ethyl acetate (3.3 %) and hexane (2.5 %). This variation in yield may be attributed to the solubility of different phytochemicals present in the guava leaves in these solvents [19].

The efficacy of guava leaf extract to inhibit the growth of *P. aphanidermatum* using poisoned plate technique

The efficacy of guava leaf extracts, that were extracted with solvents ethanol, ethyl acetate, hexane, was evaluated at a concentration of 1,000, 1,500 and 3,000 ppm to assess their inhibitory effects on the growth of *P. aphanidermatum*. The poisoned plate technique was employed for this purpose, as detailed in **Table 1**. Notably, ethanolic guava leaf extract demonstrated significant inhibition of *P. aphanidermatum* mycelium growth at a concentration of 3,000 ppm, achieving a remarkable 100.00 % inhibition. This was followed by the guava leaf extract extracted with ethanol solvent at a concentration of 1,500 ppm, which exhibited inhibition at a rate of 38.89 %. Additionally, the positive control, carbendazim at 1,500 ppm concentration, showed inhibition at a rate of 33.69 % (**Figure 1**).

In terms of antifungal activity against *P. aphanidermatum*, the ethanolic guava leaf extract obtained demonstrated the most significant inhibitory effect, achieving 100.00 % inhibition at a concentration of 3,000 ppm. This suggests that ethanol extraction was particularly effective in extracting bioactive compounds with strong antifungal properties. The extract obtained with ethanol at a concentration of 1,500 ppm also showed notable inhibition (38.89 %), further highlighting its effectiveness in controlling *P. aphanidermatum* growth. These findings are consistent with previous research demonstrating the antifungal activity of guava leaf extracts against various pathogens [20,21]. Comparatively, the positive control, carbendazim, exhibited inhibition at a rate of 33.69 % at a concentration of 1,500 ppm. This indicates that the guava leaf extract obtained with ethanol at 3,000 ppm concentration surpassed the efficacy of carbendazim, a commonly used synthetic fungicide. This is significant as it suggests the potential of guava leaf extract as a natural alternative to synthetic fungicides, aligning with the growing interest in eco-friendly and sustainable agricultural practices [22].

Table 1 Efficiency of guava leaf extract on inhibition mycelium growth of *P. aphanidermatum* using the poisoned plate technique.

Solvents	**Concentration (ppm)**	**Inhibition mycelium growth (%)**
Ethanol	1,000	$0.00 \pm 0.00^{d1/}$
	1,500	38.89 ± 1.56^{b}
	3,000	100.00 ± 0.00^{a}
Ethyl Acetate	1,000	0.00 ± 0.00^{d}
	1,500	0.00 ± 0.00^{d}
	3,000	0.00 ± 0.00^{d}
Hexane	1,000	8.15 ± 3.60^{cd}
	1,500	10.37 ± 4.08^{bcd}
	3,000	13.73 ± 7.07^{bcd}
PDA	0	0.00 ± 0.00^{d}
Carbendazim	1,500	33.69 ± 3.42^{bc}

[1/] The means followed by the same letter were not significantly different according to Ducan's new Multiple-Range Test (DMRT), $p < 0.05$.

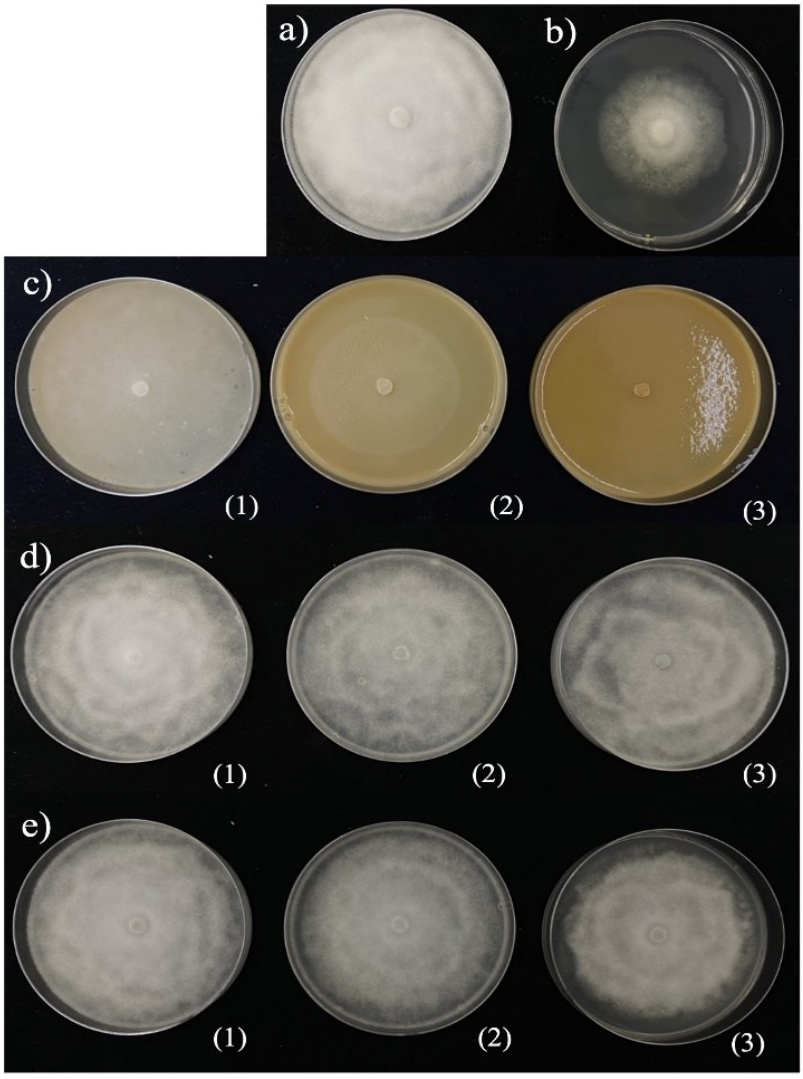

Figure 1 Efficiency of guava leaf extract on inhibiting the growth of *P. aphanidermatum* using the poisoned plate technique, 4 days after incubation. a) control, b) carbendazim 1,500 ppm c) ethanol extract, d) ethyl acetate extract, e) haxane extract, (1) 1,000 ppm, (2) 1,500 ppm and (3) 3,000 ppm.

The efficacy of guava leaf extract to controlling of *P. aphanidermatum*, the cause of damping - off disease of Chinese kale

Coating Chinese kale seeds with guava leaf extract, specifically extracted with ethanol solvent at a concentration of 3,000 ppm, resulted in the lowest percentage of damping-off disease incidence and the highest percentage of kale seedling survival. However, this outcome was not significantly different from seed coating with ethanolic extract at a concentration of 1,500 ppm, nor from the chemical carbendazim at a concentration of 1,500 ppm. The observed disease incidences were 16.67, 30.00 and 26.6 %, respectively, with corresponding survival percentages of 83.33, 70.00 and 73.33 %, respectively (**Table 2**). Overall, the results highlight the promising antifungal activity of guava leaf extract, particularly when extracted with ethanol. Further research could inquire into the identification and characterization of specific bioactive compounds responsible for this antifungal activity and explore mechanisms of action.

Table 2 Effectiveness of plant extracts in controlling *P. aphanidermatum*, the causal agent of damping-off disease in Chinese kale: Data on Disease incidence (%) and Survival rate (%).

Concentration	Disease incidence (%)	Survival rate (%)
0 ppm Ethanol extract	$86.67 \pm 6.67^{a1/}$	13.33 ± 6.67^{b}
1,500 ppm Ethanol extract	30.00 ± 11.55^{b}	70.00 ± 11.55^{a}
3,000 ppm Ethanol extract	16.67 ± 3.33^{b}	83.33 ± 3.33^{a}
1,500 ppm Carbendazim	26.67 ± 6.67^{b}	73.33 ± 6.67^{a}

[1/] The means followed by the same letter were not significantly different according to Ducan's new Multiple-Range Test (DMRT), $p < 0.05$.

GC-MS analysis of *Psidium guajava* L extracts

The phytochemical profiles of *P.guajava* extracts determined by GC-MS are shown in **Table 3** and **Figure 2**. For the hexane extract (HexE), the major compounds included β-caryophyllene (21.59 %), caryophyllene oxide (13.48 %), copaene (10.07 %) and tetracyclo [6.3.2.0(2,5).0(1,8)] tridecan-9-ol (5.49 %). It shows significant levels of sesquiterpenes, which might contribute to its antifungal activity. Sesquiterpenes are organic compounds found in plants and insects, composed of 3 isoprene units. They exhibit structural diversity and are known for their various biological activities. In the context of our study, sesquiterpenes present in the hexane extract, such as β-caryophyllene, caryophyllene oxide, copaene and tetracyclo [6.3.2.0(2,5).0(1,8)] tridecan-9-ol, are likely contributors to its potential antifungal activity. These compounds have been reported to possess antimicrobial properties and may play a role in inhibiting the growth of fungal pathogens like *P. aphanidermatum*.

For the ethyl acetate extract (EtOAcE) the major compounds included β-caryophyllene (10.74 %), caryophyllene oxide (9.46 %) and copaene (3.71 %). It also contained significant levels of sesquiterpenes, albeit lower than the hexane extract. While the ethyl acetate extract did not exhibit as high levels of sesquiterpenes as the hexane extract, it still contained notable compounds such as β-caryophyllene, caryophyllene oxide and copaene. In our study, the ethyl acetate extract demonstrated moderate antifungal activity against *P. aphanidermatum*, suggesting its potential efficacy in controlling this fungal pathogen. Although the activity may not be as potent as that observed with the hexane extract, it is still significant and warrants further investigation.

In the ethanol Extract (EtOHE) the major compounds included caryophyllene (17.58 %), α-caryophyllene (5.17 %) and caryophyllene oxide (3.42 %). This extract had a higher diversity of sesquiterpenes, with caryophyllene being the most abundant. Additionally, it contained significant levels of other compounds such as nerolidol, veridiflorol and dl-α-tocopherol.

Overall, all extracts contained a variety of compounds, with sesquiterpenes being prominent. Sequiterpenes are a class of terpenes characterized by the presence of fifteen carbon atoms arranged in 3 isoprene units. They are known for their diverse biological activities and are commonly found in various plant species, including guava. In our study, the sequiterpenes identified in the guava leaf extracts include copaene, β-caryophyllene, calamenene and caryophyllene oxide. These compounds have been extensively studied for their antimicrobial, antioxidant and anti-inflammatory properties [23,24]. For instance, β-caryophyllene, a prominent sequiterpene in our ethanol extract, has been reported to exhibit significant antifungal activity against various pathogens, which could contribute to the observed inhibition of *P. aphanidermatum* in our study.

The list of identified compounds by GC-MS analysis is shown in **Table 3**. The hexane extract contained 58 chemical compounds. The main phytochemicals identified in HE was copaene, β-caryophyllene, calamenene and caryophyllene oxide. Fifty-three compounds including β-caryophyllene and squalene were found from ethly acetate extract. Caryophyllene was one of the main constituents identified in the 40 chemicals found in the ethanol extract. There have been reports of several biological activities associated with β-caryophyllene and sesquiterpene derivatives, including antibacterial, antioxidant, gastroprotective, anxiolytic and anti-inflammatory properties [25]. Furthermore, antibacterial and anticancer properties of *P. guajava* leaf extracts were assessed by Alam *et al.* [26]. They revealed that limonene (38.01 %) and β-caryophyllene (27.98 %) were detected. The study found that the essential oil derived from *P. guajava* leaves exhibited significant antibacterial activity. Strains of bacteria such as *Streptococcus mutans* and *Candida albicans* were evaluated in the study to see if they were responsive to the essential oil. Caryophyllene, a sesquiterpene, has garnered considerable attention in recent years due to its diverse biological activities, as outlined in our study and supported by previous research. Its reported antibacterial, antioxidant and anti-inflammatory properties underscore its importance in medicinal and therapeutic applications. In the conducted experiment, it was observed that solely guava leaf extract with ethanol concentrations of 3,000 ppm and 1,500 ppm exhibited inhibitory effects on the fungus *P. aphanidermatim*. Despite the hexane extract containing higher amounts of β-caryophyllene, the ethanol extract exhibited superior antifungal activity against *P. aphanidermatim*. This could be due to synergistic effects of other compounds in the ethanol extract, which further investigation. Subsequent chemical analysis with GC-MS revealed the presence of methylparaben within the ethanol extract, which displayed antifungal properties. Conversely, methylparaben was absent in both hexane and ethylacetate extracts. This finding resonates with the investigative endeavors of Neves *et al.* [27], wherein the antifungal efficacy of methyl and propyl paraben amalgamations at varying concentrations was assessed. Neves *et al.* [27]. employed *Cladosporium* species and *Penicillium corylophilum* as fungal specimens in their trials. The outcomes underscored that a blend comprising 0.5 % methyl paraben and 1 % propyl paraben, dissolved in an 85 % ethanolic solution, represented the minimal concentration requisite for eliciting a potent antifungal response.

Table 3 Phytochemical profiles of *Psidium guajava* L extracts determined by GC-MS.

No.	Retention time	Compounds	% Relative peak areas		
			HexE	EtOAcE	EtOHE
1	7.529	Eucalyptol	0.09	0.07	0.39
2	14.151	α-Terpineol	0.02	-	-
3	19.785	Elixene	0.05	0.05	0.03
4	20.240	Bicycloelemene	0.51	0.49	0.18
5	22.148	Copaene	10.07	3.71	4.80
6	23.746	Caryophyllene	-	-	17.58
7	24.202	β-Caryophyllene	21.59	10.74	-
8	24.277	β-Cubebene	0.19	-	-
9	24.380	β-Gurjunene	0.16	0.10	-
10	24.836	(+)-Aromadendrene	4.37	2.16	2.76
11	25.237	α-Cubebene	2.36	2.18	2.55
12	25.358	α-Caryophyllene	1.43	1.55	5.17

No.	Retention time	Compounds	% Relative peak areas		
			HexE	EtOAcE	EtOHE
13	25.626	AlloAromadendrene	1.43	0.89	3.17
14	26.194	γ-Muurolene	0.80	0.40	0.47
15	26.577	β-Selinene	0.33	0.21	-
16	26.791	(+)-Epi-bicyclosesquiphellandrene	0.48	0.42	-
17	27.008	Bicyclogermacrene	1.17	0.99	-
18	27.150	α-Muurolene	0.71	0.24	-
19	27.232	Methylparaben	-	-	1.50
20	27.459	β-Bisabolene	0.40	0.20	-
21	27.705	gamma-Cadinene	0.33	0.19	-
22	28.296	Calamenene	6.77	4.35	4.63
23	28.860	1,1,6-Trimethyl-1,2-dihydronaphthalene	0.22	0.19	-
24	29.884	Epiglobulol	0.38	0.43	0.46
25	30.165	Nerolidol	1.55	1.60	2.25
26	30.843	Caryophyllene oxide	13.48	9.46	3.42
25	31.018	Veridiflorol	0.24	1.75	2.05
27	31.380	Ledol	0.61	0.67	0.76
28	31.614	Humulene oxide II	0.42	0.91	-
29	32.330	Cubenol	0.50	1.48	1.48
30	32.906	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	5.49	7.17	6.31
31	33.111	delta-Cadinol	0.75	0.86	0.97
32	33.388	α-Cadinol	0.32	0.42	0.35
33	34.183	Isoaromadendrene epoxide	1.62	2.14	1.53
34	34.480	Lauryl acrylate	0.16	1.46	3.21
35	39.414	Phytol acetate	0.22	3.26	1.98
36	40.830	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.11	1.03	0.77
37	41.091	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	0.11	1.03	0.97
38	43.610	Dibutyl phthalate	0.04	-	-
39	44.476	n-Hexadecanoic acid	0.13	0.53	1.41
40	47.970	Phytol	0.20	0.88	2.17
41	49.565	Dodecanamide	0.04	0.48	-
42	51.742	4,8,12,16-Tetramethylheptadecan-4-olide	0.11	-	-
43	51.977	9-Octadecenamide, (Z)-	0.13	3.59	0.73
44	53.255	2,5-Bis(1,1-dimethylbutyl)-4-methoxyphenol	0.65	1.40	0.41
45	53.821	2-Cyclohexen-1-one, 3-methoxy-2-(2,4,5-trimethoxyphenyl)-	1.56	1.71	0.92
46	53.956	Anthracene, 9,10-dibutyl-	0.49	0.47	0.34
47	54.182	Pinostrobin chalcone	0.27	0.77	0.49
48	54.313	1,3-Cyclohexanedione, 2,2'-methylenebis[5,5-dimethyl	0.64	0.97	0.62

No.	Retention time	Compounds	% Relative peak areas		
			HexE	EtOAcE	EtOHE
49	56.417	Squalene	3.99	5.21	3.37
50	57.600	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	0.35	-	-
51	59.683	gamma-Tocopherol	0.20	0.52	0.37
52	60.561	Stigmastan-3,5-diene	0.30	2.19	3.67
53	61.569	dl-α-Tocopherol	6.31	8.75	7.33
54	66.175	gamma-Sitosterol	1.91	3.54	2.97
55	66.449	(E)-24-Propylidenecholesterol	0.10	-	-
56	68.192	Cycloartenol acetate	0.51	0.74	0.69
57	69.815	Chol-4-en-24-oic acid, 12-hydroxy-3-oxo-,methyl ester	0.59	0.55	-
58	70.247	Vitamin E	0.49	0.69	-
59	71.822	Retandrol	1.37	1.35	-
60	76.886	Propanoic acid, 3,3'-thiobis-, didodecyl ester	-	1.86	2.89
61	78.183	Urs-12-en-28-al	0.18	0.81	-
		Total	100	100	100

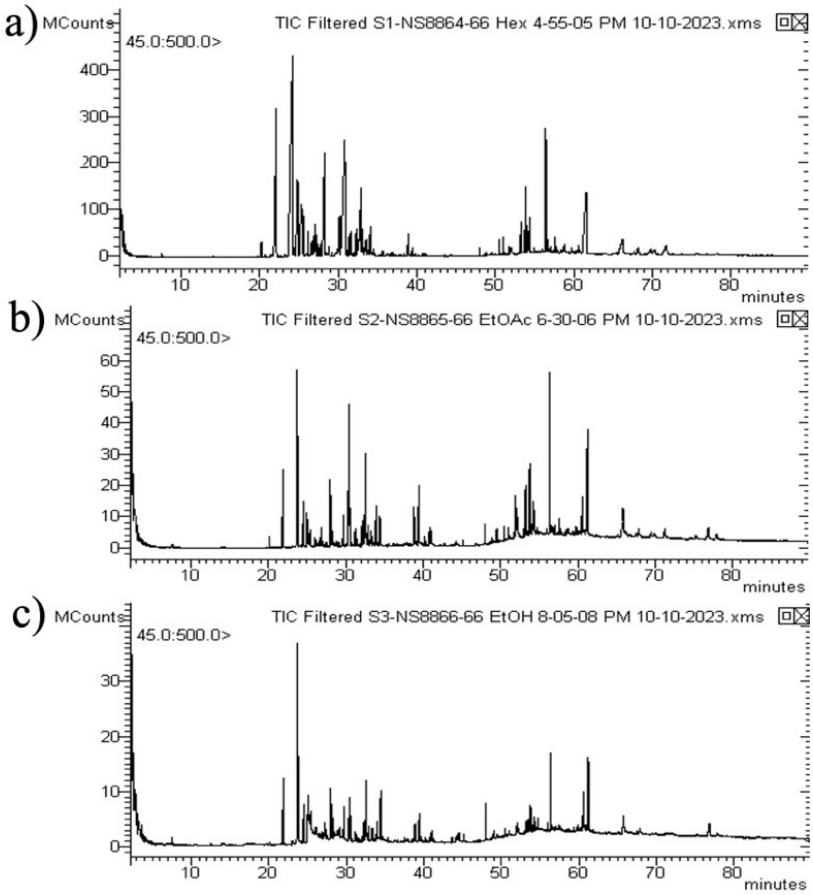

Figure 2 GC-MS chromatogram of *Psidium guajava* L extracts; a) Hexane extract b) ethyl acetate extract c) ethanol extract.

Conclusions

In conclusion, our study highlights the potential of ethanolic guava leaf extract as a promising biofungicide against *P. aphanidermatum*, the causal agent of damping-off disease in Chinese kale cultivation. The significant inhibition of *P. aphanidermatum* growth, particularly at higher concentrations, underscores the efficacy of guava leaf extract in disease management. Additionally, greenhouse trials demonstrated the practical applicability of seed coating with ethanol-extracted guava leaf extract in reducing disease incidence in Chinese kale crops. Chemical analysis revealed the exclusive presence of methylparaben in the ethanol extract, suggesting its potential role in inhibiting fungal growth. However, it is important to note that the ethanol extract also contains significant levels of Caryophyllene, a major compound with reported antifungal properties. The synergistic effects of multiple compounds, including Caryophyllene, may contribute to the observed antifungal activity of the guava leaf extract. These findings contribute to the advancement of sustainable and eco-friendly strategies for managing damping-off disease, thereby enhancing the productivity and economic viability of Chinese kale cultivation in Thailand. Further research is warranted to comprehensively explore the antifungal mechanisms of guava leaf extract and optimize its application in integrated pest management programs for sustainable agriculture.

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