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The effectiveness of the mixture of basil and betel leaves methanol extracts to suppress *Colletotrichum acutatum*, the causal agent of anthracnose disease on postharvest cayenne chili

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Abstract. *Nasahi C, Ramadhanty S, Sudarjat S, Kurniadie D, Bari IN, Subakti-Putri SN. 2023. The effectiveness of the mixture of basil and betel leaves methanol extracts to suppress* Colletotrichum acutatum, *the causal agent of anthracnose disease on postharvest cayenne chili. Biodiversitas 24: 5513-5522.* Anthracnose, caused by *Colletotrichum acutatum*, is a major disease in the cultivation of chili plantations in Indonesia. It is usually controlled by farmers using synthetic fungicides with many adverse effects. Therefore, botanical fungicides are applied as alternative control techniques. This research aimed to examine the effect of mixed methanol extract of basil (*Ocimum basilicum*) and betel leaves (*Piper betle*) against *C. acutatum* on cayenne chili (*Capsicum annuum*). The method used was a randomized block design (RBD) with thirteen treatments and three replications. The results showed that combining basil and betel leaf methanol extracts suppressed *C. acutatum.* Furthermore, the most effective treatment was a mixture of 1.5% of each of the extracts, which suppressed the development of the disease at 62.07%. The GC-MS profiling of the basil and betel leaf methanol fraction indicated the presence of bioactive compounds that have antifungal effects against anthracnose.

Keywords: *Capsicum annuum*, *Ocimum basilicum*, phytopathogens, piper betle

INTRODUCTION

Red chili (*Capsicum annuum* L.) is an essential horticultural commodity for Indonesians. Due to its industrial applications, chili is a high-value commodity (Rukmana and Oesman 2006). According to the Central Statistics Agency of Indonesia (2020), large red chili production in Indonesia in 2018 was 1.26 million tons with a harvested area of 144,391 hectares (ha) and a productivity of 8.77 tons per hectare. The demand for cayenne pepper in the market from time to time tends to continue to increase and even be relied on as a non-oil and gas export commodity. The high demand for chilies makes some producers provide chilies in good condition, fresh, and without any damage due to pathogen infection.

Colletotrichum acutatum L., the cause of anthracnose, is one of the major problems in cultivating chillis. Semangun (2000) states that anthracnose disease in chili plants is widespread worldwide, including in Indonesia. This disease, which causes dark blotches with concentric grooves, tissue shrinkage, and necrosis, could result in a 50% decrease in chili crop yield if it is prevalent (Pakdeevaraporn et al. 2005). Using plant-based fungicides is one of the methods potentially inhibiting or eliminating fungal growth using compounds derived from plant extracts. Based on Zahara et al. (2020) stated that vegetable fungicides are derived from plants whose chemical structure is not altered during processing. Due to their antifungal and antibacterial properties, plant-derived biomolecules are the most effective substitutes for fungicides and bactericides (Abdullahi et al. 2022)

Betel leaf (*Piper betle* L.), often discovered in Southeast Asia, can be a bio fungicide. Betel leaves contain as much as 4% essential oils (chavicol, cavibetol, eugenol, methyl eugenol, carvacrol, terpenes, and sesquiterpenes), phenols, flavonoids, saponins, sugars, and starch. The compounds in betel leaves can destroy microorganisms (bacteriocides) and fungi (fungicides) (Maryani and Kristiana 2004). According to Khalifah et al. (2021), its extract concentration of 5% presents the highest average inhibition percentage of *C. acutatum* on cayenne chili, which is 72.45%. The Piper genus significantly influenced the morphology of conidia, prevented germination, and stimulated anthracnose pathogen proliferation (Piperaceae) (Janthong et al. 2021). Red chilies with anthracnose disease can be controlled by using betel leaf extract in a solution of 100 gr/L of water with a 0.31% concentration (Zulkipli et al. 2018).

Another potential bio fungicide is basil leaves (*Ocimum sanctum* L.), which contain other compounds such as flavonoids, saponins, and phenols, acting as antifungals (Wabale and Kharde 2010). Phenol, eugenol, and cineol, a component of *O. sanctum*, can damage cell membranes and cause cell permeability changes, inhibiting cell development or killing fungal cells (Ridwan 2016). The results of Berlian et al. (2016) showed that using basil leaf extract with a concentration of 10% could suppress *Fusarium*

oxysporum Schlecht disease with an emphasis of 2.46 mm.

In addition to being applied singly, bio fungicides can also be used by mixing two types of organic materials, thereby increasing efficiency and effectiveness and minimizing the occurrence of resistance (Akhtar and Isman 2013). The mixture of soursop leaf extract (*Anona muricata* L.), siam weeds leaves (*Chromolaena odorata* L.), and galangal rhizomes (*Alpinia galanga* L.) at a concentration of 1% successfully inhibit the growth of *C. acutatum* colonies in cayenne chili with an incubation period of seven days and inhibitory percentage of 66.19% (Hodiyah et al. 2017). A total of 1% betel leaf methanol extract mixed with 2% *Andrographis paniculata* 2% can suppress the development of the diameter and biomass of *Colletotrichum gloeosporioides* colonies by 91.26% (Idris and Nurmansyah 2015). Mixing 2.5% basil leaf extract and 2.5% *Chromolaena odorata* leaf extract was able to suppress the diameter of the colony on *Alternaria solani* fungus by 1.49 cm with an inhibition percentage of 76.65% (Indriani 2021).

Postharvest handling needs to be done to maintain the quality of harvested products so that they remain good in the consumers (Zam et al. 2019). In the red chili commodity, the widely used postharvest treatment is drying. Meanwhile, postharvest handling to avoid the growth of the fungus *Colletotrichum* spp. is conducted by soaking chemical or vegetable ingredients. Therefore, this research aims to study the effect of a single concentration and mixture of methanol extract from basil and betel leaves, which effectively inhibits the growth of fungus and the development of lesions of *C. acutatum*. It is anticipated that the results of this study will be utilized in chili postharvest management against *C. acutatum*.

MATERIALS AND METHODS

The experiments were conducted between February 2020 and October 2022 at the Phytopathology and Pesticide Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran, Indonesia.

Procedures

Isolation and purification

Cayenne chili samples from a chili plantation in Jatinangor, West Java, affected by anthracnose disease, were cut into several pieces and then sterilized with 1% NaCl. The fungus sample was isolated in PDA (Potato Dextrose Agar) media. Therefore, to extract one type of pathogenic microbe from the insulating medium PDA from anthracnose disease, the fungus *Colletotrichum* spp. was purified and identified based on De Silva (2019) and Widodo and Hidayat (2018).

Extraction of basil and betel leaves

Moreover, 2 kg of basil leaves were washed under running water, dredged, cut into small pieces, and mashed with a blender. The material was macerated with methanol solvent in a plastic jar until submerged in a 1:6 (w/v) ratio, then stirred with a glass stirring rod. Soaking was performed for 6 hours, after which the extract solution was filtered with a mesh cloth and put in an airtight bottle. Subsequently, the basil leaf filtrate was obtained and evaporated using a vacuum rotary evaporator at a temperature of 55-60ºC until a paste was formed. The extraction process for betel leaves was the same as for basil leaves based on the extract combination and modification of Rambun et al. (2017) and Anugrahwati et al. (2016).

The extract results were solubilized using a sterile aquadest solution dissolved with Tween 80%, obtaining four concentrations based on preliminary test results, namely 0%, 0.375%, 0.75%, 1.5%, and 3%. The 13 treatments tested and their concentrations were control of sterile aqueducts, tween Control 80 (0.1%), betel leaf methanol extract (3%), basil leaf methanol extract (3%), betel leaf methanol extract (1.5%) + basil leaf (1.5%), methanol extract of betel leaves (1.5%) + basil leaves (0.75%), betel leaf methanol extract (1.5%) + basil leaf (0.375%), betel leaf methanol extract (0.75%) + basil leaf (1.5%), betel leaf methanol extract (0.75%) + basil leaf (0.75%), betel leaf methanol extract (0.75%) + basil leaf (0.375%), betel leaf methanol extract (0.375%) + basil leaf (1.5%), betel leaf methanol extract (0.375%) + basil leaf (0.75%), and methanol extract of betel leaves (0.375%) + basil leaves (0.375%).

In vivo test

Chilli was first sterilized with 70% alcohol, dipped in sterile aquadest, immersed for 10 minutes in a mixture of betel and basil leaf extracts, and then dried at room temperature. Subsequently, it was injured by stabbing using a needle, inoculated by attaching a plug agar colony of Φ = 0.5 cm fungus *C. acutatum*, and wrapped in cotton that had been moistened using sterile aquadest. The chili was stored in a plastic container moistened with wet filter paper (Shahbazi et al. 2014). Furthermore, a plastic straw was provided so the fruit would not directly contact the filter paper. The observations include the incubation period at daily intervals (seven days), the diameter of the lesion (mm), and the rate of disease development by calculating the Area under the disease progress curve (AUDPC) using the following formula:

$$\text{AUDPC} = \sum_{i=1}^{n} \left[\frac{Y_{i+1} + Y_i}{2}\right][X_{i+1} - X_i]$$

Where:

AUDPC : Area under disease progress curve
Yi : The intensity of the disease on observation -i
Xi : time (day) on observation -i
n : Total number of observations

Gas chromatography-mass spectrometry (GC-MS) analysis

Phytochemical analysis of methanol basil and betel leaf extracts was conducted using MassHunter GC/MS Acquisition 10.0.368 (Agilent 19091-433HP). The GS-MS method was modified from Murárikova et al. (2017) and Madhumita et al. (2019). The instrument was equipped with a DB-5 MS fused silica column (5% Diphenyl/95% Dimethylpolysiloxane) film thickness of 0.25 µm, 25 µm in diameter, and 30 m in length, with the carrier gas was pure

helium (99.99%) at a constant flow rate of 1.0 mL/min. An injection of 1.0 µL was employed for every sample, and the injector was operated at 200°C (constant), which increased per minute up to 280°C, then having a total elution was 58.5 min. Finally, the phytochemicals present in the samples were identified by comparing the retention time (minute), peak area, peak height, and mass spectral patterns of the test samples with the spectral databases of real compounds kept in the National Institute of Standards and Technology (NIST) library.

Data analysis

This research employed a randomized block design (RBD) analysis of variance (ANOVA) using Smart Stat software. Further analysis was conducted with the Scott Knott test at a level of 5% when the outcome was significant.

RESULTS AND DISCUSSION

Lesion diameter of *Colletotrichum acutatum*

Test results of basil and betel leaf methanol extracts against *C. acutatum* showed a significant difference with the control in suppressing symptom development. This was based on the results diameter measurement of *C. acutatum*, as presented in Table 1. The treatments differed considerably from the controls except for C (betel leaf methanol extract 3%), G (betel leaf methanol extract 1.5% + basil leaf 0.375%), L (betel leaf methanol extract 0.375% + basil leaf 0.75%), and M (betel leaf methanol extract 0.375% + basil leaf 0.375%). It implies that they could not inhibit the growth of the fungus *C.acutatum*. Meanwhile, in those significantly different from the control, the E treatment appeared to be the most effective concentration compared to others, as shown in Figure 1. This is a mixture of 1.5% each of betel and basil leaves extracts, with an average result of 0.22 cm lesion diameter observed for seven days after inoculation.

Incubation period and rate of development of the disease

Based on the experiment's results, after cayenne chillis was treated with basil and betel leaf methanol extracts, the pathogen *C. acutatum* was inoculated, with an incubation period of three days after treatment until the onset of symptoms. The application of the methanol extracts can inhibit the development of anthracnose disease. The phenomenon can be observed from the table beginning with day one after inoculation to day seven. This was performed in significant controls compared to the treatment given to the extract, as shown in Table 2.

Table 3 shows the AUDPC value of basil and betel leaves methanol extract against *C. acutatum*. It was observed that all treatments had a significantly different effect from the control, except for G (betel leaf methanol extract 1.5% + basil leaf 0.375%), L treatment (betel leaf methanol extract 0.375% + basil leaf 0.75%), M treatment (betel leaf methanol extract 0.375% + basil leaf 0.375%). However, they were significantly different and had a smaller effect than other treatments, with percentages of inhibition less than 25%. Meanwhile, both of the control treatments did not show any inhibition value. The largest value of 62.07% was obtained from E (betel leaf methanol extract 1.5% + basil leaf 1.5%), followed by H (betel leaf methanol extract 0.75% + basil leaf 1.5%), which is 60.69%.

A **B**

Figure 1. Lesion diameter comparison: E treatment (betel leaf methanol extract 1.5% + basil leaf 1.5%); (B) sterile aquadest (control)

Table 1. Effect of methanol extract of basil leaves and betel leaves on the lesion diameter of *Colletotrichum acutatum*

Code	Treatment	Lesion Diameter (mm)
A	(Control) Aquadest sterile	0.4200 [b]
B	(Control) Tween 80	0.4200 [b]
C	Betel leaf methanol extract (3%)	0.3267 [b]
D	Basil leaf methanol extract (3%)	0.3000 [a]
E	Betel leaf methanol extract (1.5%) + basil leaf (1.5%)	0.2200 [a]
F	Betel leaf methanol extract (1.5%) + basil leaf (0.75%)	0.2667 [a]
G	Betel leaf methanol extract (1.5%) + basil leaf (0.375%)	0.3467 [b]
H	Betel leaf methanol extract (0.75%) + basil leaf (1.5%)	0.2267 [a]
I	Betel leaf methanol extract (0.75%) + basil leaf (0.75%)	0.3000 [a]
J	Betel leaf methanol extract (0.75%) + basil leaf (0.375%)	0.3533 [b]
K	Betel leaf methanol extract (0.375%) + basil leaf (1.5%)	0.2733 [a]
L	Betel leaf methanol extract (0.375%) + basil leaf (0.75%)	0.3467 [b]
M	Betel leaf methanol extract (0.375%) + basil leaf (0.375%)	0.3800 [b]

Note: The average number in the same column followed by the same letter as the control shows no difference according to the Scott Knott Test at 5%

Table 2. The development of anthracnose disease in cayenne chili after seven days of treatment

Code	Treatment	Day-						
		1	2	3	4	5	6	7
A	(Control) Aquadest sterile	0.000	0.000	0.090	0.107	0.120	0.130	0.163
B	(Control) Tween 80	0.000	0.000	0.090	0.107	0.117	0.133	0.160
C	Betel leaf methanol extract (3%)	0.000	0.000	0.060	0.063	0.073	0.090	0.117
D	Basil leaf methanol extract (3%)	0.000	0.000	0.050	0.053	0.063	0.073	0.103
E	Betel leaf methanol extract (1.5%) + basil leaf (1.5%)	0.000	0.000	0.033	0.037	0.043	0.053	0.067
F	Betel leaf methanol extract (1.5%) + basil leaf (0.75%)	0.000	0.000	0.040	0.050	0.053	0.063	0.083
G	Betel leaf methanol extract (1.5%) + basil leaf (0.375%)	0.000	0.000	0.050	0.067	0.097	0.120	0.153
H	Betel leaf methanol extract (0.75%) + basil leaf (1.5%)	0.000	0.000	0.040	0.043	0.047	0.050	0.060
I	Betel leaf methanol extract (0.75%) + basil leaf (0.75%)	0.000	0.000	0.040	0.047	0.060	0.070	0.090
J	Betel leaf methanol extract (0.75%) + basil leaf (0.375%)	0.000	0.000	0.040	0.050	0.057	0.97	0.113
K	Betel leaf methanol extract (0.375%) + basil leaf (1.5%)	0.000	0.000	0.030	0.040	0.050	0.060	0.073
L	Betel leaf methanol extract (0.375%) + basil leaf (0.75%)	0.000	0.000	0.047	0.067	0.087	0.120	0.137
M	Betel leaf methanol extract (0.375%) + basil leaf (0.375%)	0.000	0.000	0.047	0.057	0.087	0.130	0.153

Table 3. AUDPC value and degree of inhibition of the extract

Code	Treatment	AUDPC value	Inhibition (%)
A	(Control) Aquadest sterile	0.483 ^{c}	0.00
B	(Control) Tween 80	0.487 ^{c}	0.00
C	Betel leaf methanol extract (3%)	0.317 ^{a}	34.83
D	Basil leaf methanol extract (3%)	0.270 ^{a}	44.83
E	Betel leaf methanol extract (1.5%) + basil leaf (1.5%)	0.183 ^{a}	62.07
F	Betel leaf methanol extract (1.5%) + basil leaf (0.75%)	0.230 ^{a}	52.76
G	Betel leaf methanol extract (1.5%) + basil leaf (0.375%)	0.390 ^{b}	20.34
H	Betel leaf methanol extract (0.75%) + basil leaf (1.5%)	0.190 ^{a}	60.69
I	Betel leaf methanol extract (0.75%) + basil leaf (0.75%)	0.247 ^{a}	50.00
J	Betel leaf methanol extract (0.75%) + basil leaf (0.375%)	0.283 ^{a}	42.07
K	Betel leaf methanol extract (0.375%) + basil leaf (1.5%)	0.203 ^{a}	58.28
L	Betel leaf methanol extract (0.375%) + basil leaf (0.75%)	0.370 ^{b}	24.48
M	Betel leaf methanol extract (0.375%) + basil leaf (0.375%)	0.373 ^{b}	22.76

Table 4. GC-MS spectral analysis of a methanolic extract of basil leaves

RT (min)	Name of compound	Molecular formula	Molecular weight (g/mol)
8.547	6-Bromohexanoic acid, 3-methylphenyl ester	$C_{13}H_{17}BrO_2$	209
13.431	Camphor	$C_{10}H16_6O$	152
19.213	Geranic acid	$C_{10}H16_6O_2$	168
24.667	2-(4a,8-Dimethyl-2,3,4,5,6,7-hexahydro-1H-naphthalen-2-yl)propan-2-ol	$C_{15}H_{26}O$	222
36.464	Chloroacetic acid, dodec-9-ynyl ester	$C_{14}H_{23}ClO_2$	259
42.142	3-Oxobutan-2-yl 2-methylbutanoate	$C_9H_{16}O_3$	172
47.077	Supraene	$H_{30}H_{50}$	411
51.316	4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)-	$C_{18}H_{16}O_6$	328

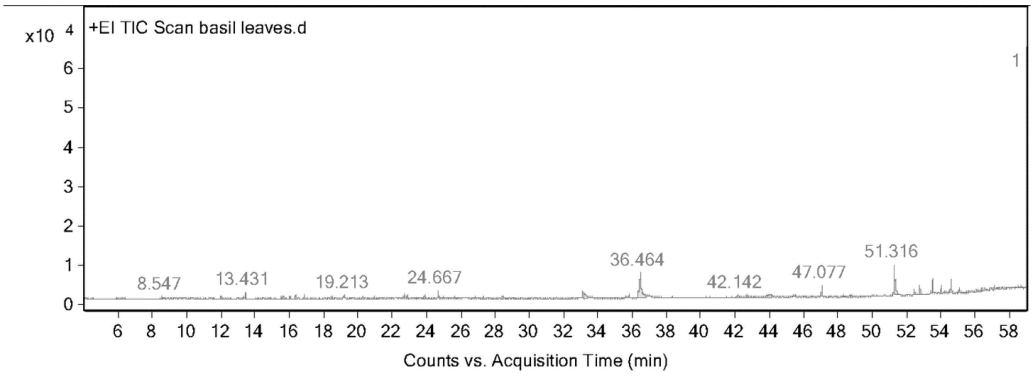


Figure 2. GC-MS chromatogram for a methanolic extract of basil leaves

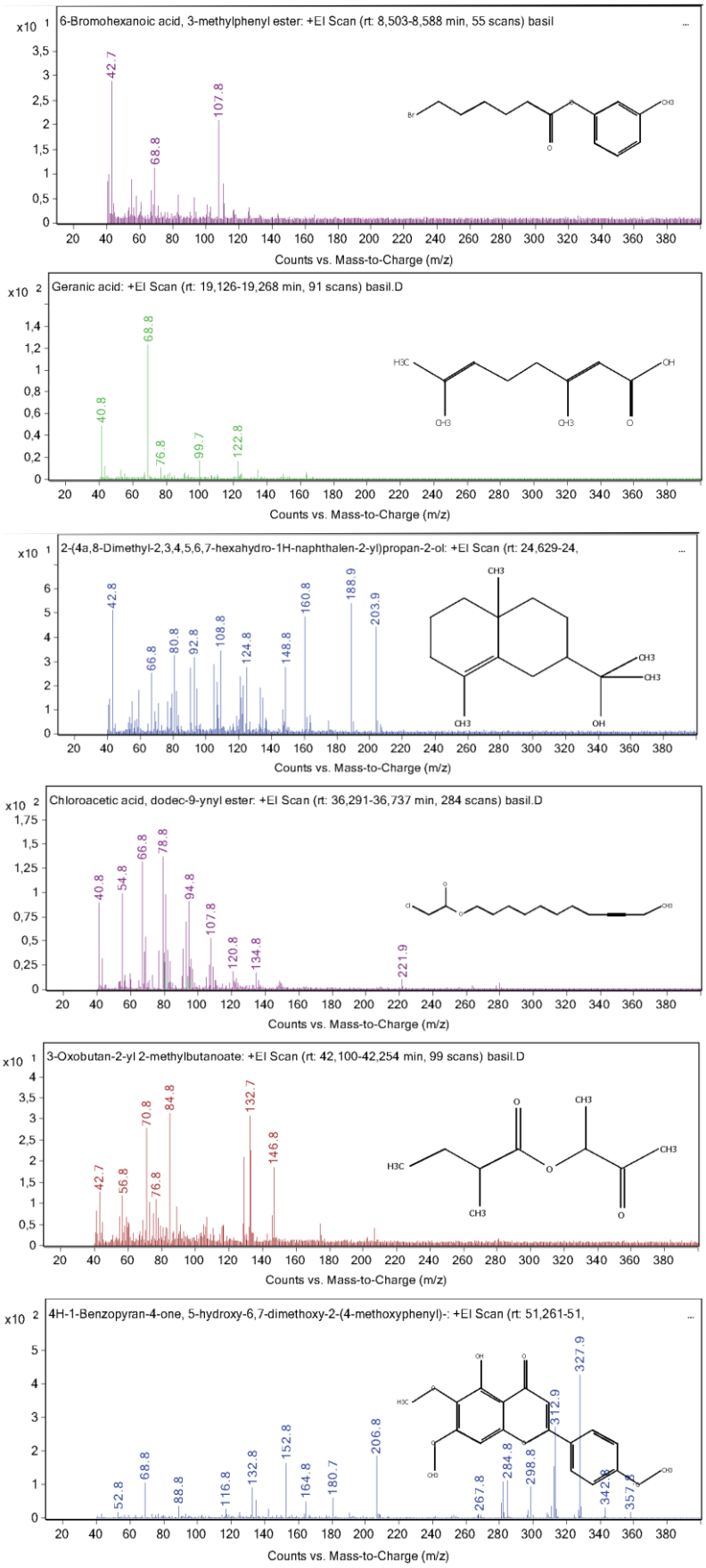


Figure 3. Mass spectra of the identified compound from methanolic extract of basil leaves.

Phytochemical screening of basil and betel leaves

Gas chromatography and mass spectrometry were used to identify the bioactive components present in the methanolic extract of basil leaves. Table 4 and Figure 2 provide the active principles and their retention times (RT), molecular formulas, and molecular weights (MW). The methanolic extract of basil leaves contains eight bioactive phytochemical components. Figure 3 shows the mass spectra of the chemicals that have been identified. Furthermore, the GC-MS analysis of the methanol extract of the basil leaves fraction showed bioactive substances, such as 6-Bromohexanoic acid, 3-methylphenyl ester, Camphor, Geranic acid, 2-(4a,8-Dimethyl-2,3,4,5,6,7-hexahydro-1H-naphthalen-2-yl)propan-2-ol, Chloroacetic ac-id, dodec-9-ynyl ester, 3-Oxobutan-2-yl 2-methylbutanoate, Supraene, and 4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)-.

The GC-MS analysis of the methanol extract of betel leaves showed 7 compounds, each with distinct phytochemical properties. The chromatogram is shown in Figure 4, while Table 5 lists the chemical components along with their retention times (RT), molecular formulas, and molecular weights (MW). Furthermore, the mass spectra of the chemicals were pointed out in Figure 5. The GC-MS analysis of the betel leaves methanol extract identified bioactive compounds such as Glycerine, 1H-Inden-5-ol, 2,3-dihydro-, Hydroxychavicol, 9,12-Octadecadienoic acid (Z,Z)-, Benzonitrile, 2-(4-benzyloxybenzylidenamino)-, Benzoic acid, 2,4,6-trimethyl-, 2,4,6-trimethylphenyl ester, and Methyl 3-bromo-1-adamantaneacetate.

Discussion

Based on the results, it is suspected that the compounds in betel and basil leave function effectively in inhibiting the growth of lesion diameters and affecting sporulation. Betel leaf and basil leaf extracts act as antifungals, which can inhibit fungi growth and metabolism. Antifungals are divided into two, namely fungicidal and fungistatic. Fungicidal is a substance that can kill the fungus, whereas fungistatic inhibits the development of the fungus but does not kill it (Putri 2013).

Single basil leaf extract is better at suppressing lesion diameter results than its single betel extract. This can also be observed by mixing basil and betel leaf extracts, where the concentration of basil leaf extract is greater than *C. acutatum*. The phenomenon suggested that 3% of basil leaf compounds are more toxic against *C. acutatum* than its 3% betel counterpart. The experiment from Rizki et al. (2021) showed that basil leaf successfully inhibits the growth of *C. gloeosporioides* both in vitro (43.13%) and in vivo (0.10 cm).

Table 5. GC-MS spectral analysis of a methanolic extract of betel leaves

RT (min)	Name of compound	Molecular formula	Molecular weight (g/mol)
11.830	Glycerin	$C_3H_8O_3$	92
16.538	1H-Inden-5-ol, 2,3-dihydro-	$C_9H_{10}O$	134
22.467	Hydroxychavicol	$C_9H_{10}O_2$	150
36.559	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280
41.191	Benzonitrile, 2-(4-benzyloxybenzylidenamino)-	C_7H_5N	103
44.182	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-trimethylphenyl ester	$C_{19}H_{22}O_2$	282
53.529	Methyl 3-bromo-1-adamantane acetate	$C_{13}H_{19}BrO_2$	287

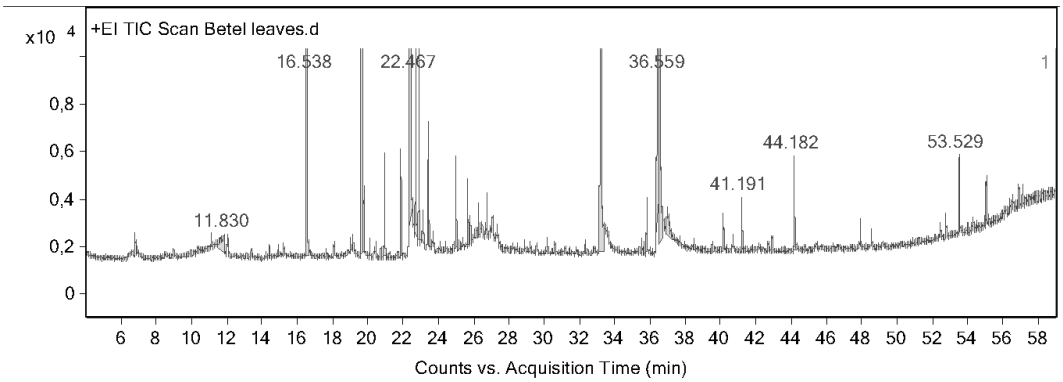
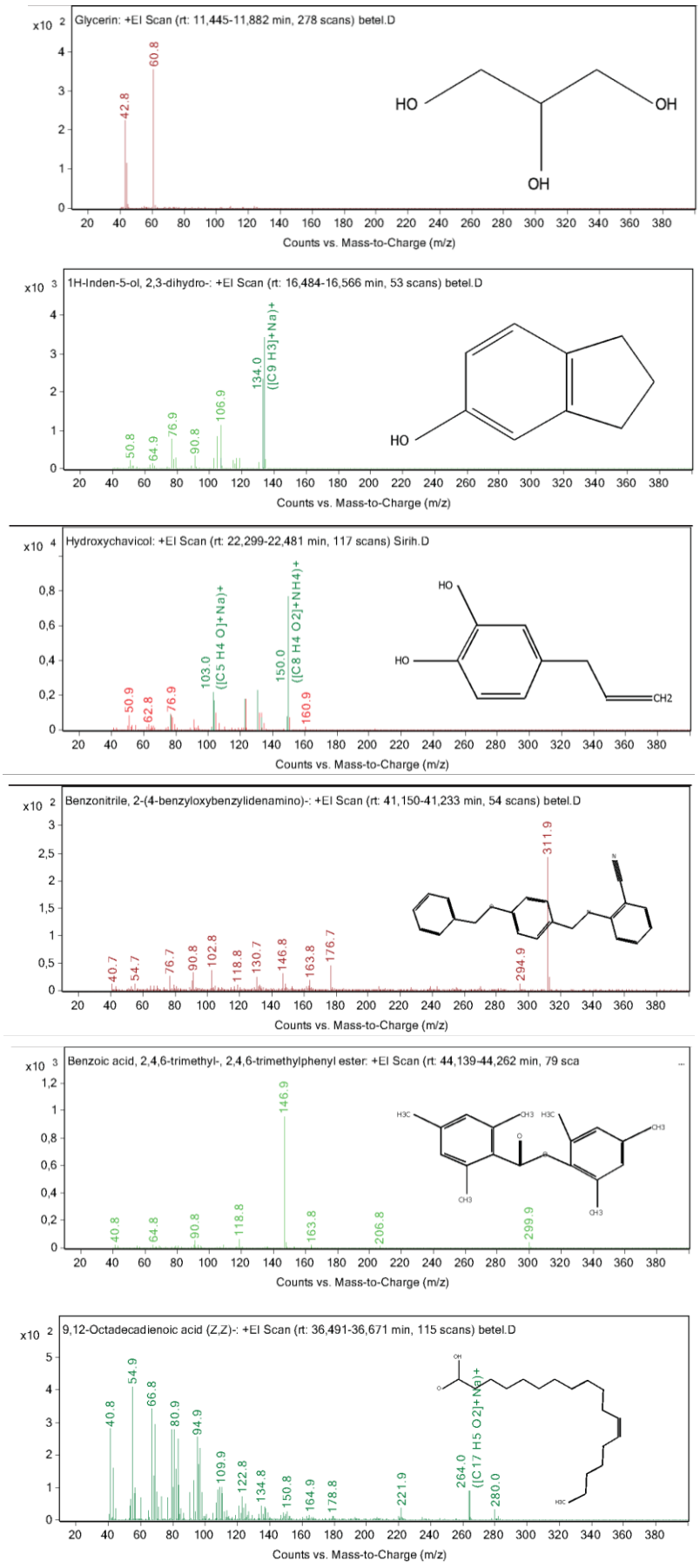


Figure 4. GC-MS chromatogram for a methanolic extract of betel leaves


Glycerin: +EI Scan (rt: 11,445-11,882 min, 278 scans) betel.D
60.8
42.8
HO
OH
OH
Counts vs. Mass-to-Charge (m/z)
1H-Inden-5-ol, 2,3-dihydro-: +EI Scan (rt: 16,484-16,566 min, 53 scans) betel.D
134.0 ([C9 H3]+Na)+
106.9
76.9
90.8
50.8
64.9
HO
Counts vs. Mass-to-Charge (m/z)
Hydroxychavicol: +EI Scan (rt: 22,299-22,481 min, 117 scans) Sirih.D
150.0 ([C8 H4 O2]+NH4)+
103.0 ([C5 H4 O]+Na)+
50.9
62.8
76.9
160.9
HO
HO
CH2
Counts vs. Mass-to-Charge (m/z)
Benzonitrile, 2-(4-benzyloxybenzylidenamino)-: +EI Scan (rt: 41,150-41,233 min, 54 scans) betel.D
311.9
294.9
40.7
54.7
76.7
90.8
102.8
118.8
130.7
146.8
163.8
176.7
Counts vs. Mass-to-Charge (m/z)
Benzoic acid, 2,4,6-trimethyl-, 2,4,6-trimethylphenyl ester: +EI Scan (rt: 44,139-44,262 min, 79 sca
146.9
299.9
40.8
64.8
90.8
118.8
163.8
206.8
Counts vs. Mass-to-Charge (m/z)
9,12-Octadecadienoic acid (Z,Z)-: +EI Scan (rt: 36,491-36,671 min, 115 scans) betel.D
264.0 ([C17 H5 O2]+Na)+
280.0
54.9
66.8
80.9
94.9
40.8
109.9
122.8
134.8
150.8
164.9
178.8
221.9
Counts vs. Mass-to-Charge (m/z)

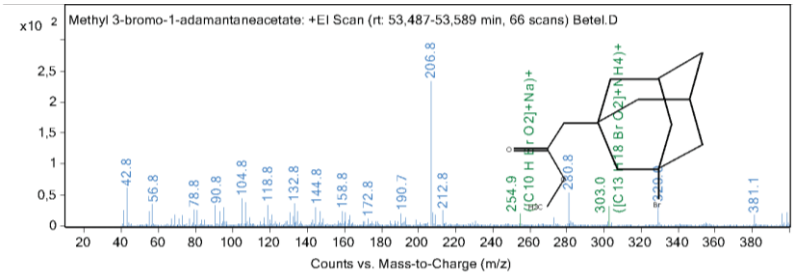


Figure 5. Mass spectra of the identified compound from methanolic extract of betel leaves

Based on the research, the application of basil and betel leaf extracts has no noticeable effect on the incubation period of the pathogen. It is assumed that the incubation period on the pathogen appearance of visible disease symptoms can be delayed when infection occurs late in several conditions, such as host maturity and abiotic factors (Leclerc et al. 2014). Other research supported the statement that the fungus *C. acutatum* causes anthracnose (Ratulangi et al. 2012). After inoculation, the symptoms of infection with the disease begin on the third day (Ratulangi et al. 2012). Britto et al. (2012) stated that if combining two categories of plant-based ingredients increases their efficacy, the combination is synergistic; if combining two types of plant-based ingredients decreases their efficacy, the combination is antagonistic. These properties depend on the structure of the secondary metabolites in the mixed plants.

While the research findings indicate that a combination of methanol-extracted betel leaves and basil leaf extracts within codes G, L, and M exhibited a relatively lower inhibitory efficacy when contrasted with individual extracts (codes C and D), it is noteworthy that a majority of the mixed extracts (codes E, F, H, I, J, and K) demonstrated statistically significant variations in their inhibitory effects, with some displaying a significant distinctions. A mixture of betel and basil leaf extracts appears to have a more effective and synergistic effect than the single form and can suppress the disease's development (Britto et al. 2012). It was proven by Suriawati et al. (2018) and Harlina et al. (2022) researched the combination of betel and basil leaf extract able to decrease the growth of *Staphylococcus aureus*, *Vibrio harveyi*, and *V. alginolyticus*. It is stated to be antagonistic when it decreases the suppression power (Britto et al. 2012). Therefore, it can be interpreted that the active compounds in the two extracts work synergistically to increase the effectiveness in suppressing the fungus *C. acutatum*. The effectiveness of the mixture of methanol extracts from basil and betel leaves is due to secondary metabolite compounds.

Secondary metabolites are compounds produced by organisms after active development but do not play a role in growth, development, and reproduction, such as their primary counterpart. They play a role in ecological function and inhibit the development of pathogenic microorganisms (Demain and Fanq 2000). Meanwhile, the single treatment showed a slight emphasis compared to the mixed treatment. Glucose factors in the extract cause this and can lead to fungi stimulation (Fiori et al. 2000).

Among the phytocompounds identified on betel leaves, camphor has been shown to have an antifungal activity that has been proven in several other phytopathogens such as *Fusarium oxysporum*, *F. solani*, *F. verticillioides*, and *F. graminearum* (Kong et al. 2022). 6-Bromohexanoic acid and 3-methyl phenyl ester are identified in the methanolic extract of *Coscinium fenestratum*, while Supraene compounds are also discovered in *Bryophyllum pinnatum*, with both having an antioxidant activity (Karthika et al. 2019; Mbachu et al. 2019). Meanwhile, geranic acid can reduce antibiotic resistance to MDR bacteria strains (Brunel et al. 2013).

According to the mass spectra of compounds present on betel leaves, glycerin is often used as an active fungicidal and bactericidal for the prevention and control of plant pathogens consisting of *Plasmopora viticola*, *Phytophthora infestans*, *Venturia inequalis*, *Oidium* spp., *Fusarium* spp., *Botrytis* spp., *Penicilium* spp., *Septoria* spp., *Rusts* spp., *Cercospora* spp., *Taphrinia* spp., and *Erwinia* spp. which has been patented since 2001 (Linser 2001). Hydroxychavicol exhibits fungistatic and fungicidal effects against *Candida albicans* (Ali et al. 2010), *Trichophyton mentagrophytes* and *Candida parapsilosis* (Ali et al. 2016), *T. rubrum* (Ridzuan 2018), *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *F. oxysporum* f. sp. cubense, *Sphaceloma ampelinum*, *Alternaria* spp., *C. brassicicola*, and *Pyricularia oryzae* (Singburaudom 2015). 9,12-Octadecadienoic acid (Z,Z)- has been reported to be present in *Chenopodium album*, which reduces the activity of soil-borne fungal pathogen *Sclerotium rolfsii* (Ali et al. 2017). Its compound isolated from quinoa and *Jatropha curcas* extracts showed antifungal and antimicrobial activity (Rahman et al. 2014; Khan and Javaid 2020). Furthermore, benzoic acid has been reportedly fungistatic on *Magnaporthe oryzae*, *P. infestans*, *Puccinia recondita*, *C. albicans*, *Eutypa lata*, *Cochliobolus lunatus*, *Aspergillus niger*, and *Pleurotus ostreatus* (Yoon et al. 2012; Lima et al. 2018; Amborabé et al. 2002; Berne et al. 2015).

These plants contain antifungal compounds, such as phenol, which can diffuse on fungal cell membranes and disrupt metabolic pathways, including synthesizing ergosterol, glucan, chitin, protein, and glucosamine, inhibiting the growth of fungal (Omidpanah et al. 2015). Furthermore, other secondary metabolite compounds in basil leaves are flavonoids, terpenoids, steroids, and

saponins. The betel leaves contain alkaloids, diastase enzymes, sugars, and tannins (Shahrajabian et al. 2020).

Phenols are antifungal secondary metabolite compounds present in betel leaves and basil leaves. As an antifungal, phenol can damage cell membranes, leading to alterations in cell permeability that can inhibit cell growth (Fardiaz 1992). Additionally, phenol compounds can denature cell proteins and reduce cell walls to lyse fungal cells (Cowan 1999). According to Omidpanah (2015), phenol compounds can diffuse in fungal cell membranes and disrupt metabolic pathways such as synthesizing ergosterol, glucan, chitin, protein, and glucosamine in mushrooms. The binding of phenol compounds to ergosterol forms a pore in the cell membrane. The formation of a pore will result in the release of fungal cell components such as amino acids, carboxylic acids, inorganic phosphates, and phosphate esters, leading to the demise of the fungal cells (Suryana 2004).

Based on the research on cayenne chili, basil and betel leaf extracts are essential in reducing *Colletotrichum* lesions, influencing the disease's incubation and development rates, indicating that several chemical compounds are responsible for the resulting test. Therefore, this research formulates suggestions for pure testing each chemical compound against *C. acutatum*.

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