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Analysis of Metabolites from Purple Cleome Extract (*Cleome rutidosperma* Linn.) as Potential Organic Fungicides

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Abstract | One disease that often attacks chili (*Capsicum* L.) is anthracnose caused by *Colletotrichum capsici* [(Syd.) E.J. Butler & Bisby]. In the field, *C. capsici* is controlled with a chemical fungicide that harms the environment. The purple Cleome plant (Spider plant – *Cleome rutidosperma* Linn.) can be extracted and function as an organic fungicide for environment-friendly control. This research aimed to examine the type and concentration of purple cleome extract metabolites and understand their effectiveness in inhibiting *C. capsici*. Purple cleome leaf is extracted with 1:1 w/v absolute methanol and concentrated with 1:1 v/v cold absolute methanol, and then the supernatant is purified using a centrifuge. The metabolic analysis was performed using Gas Chromatography-Mass Spectrometry (GC-MS) and comparing the data with the National Center for Biotechnology Information (NCBI) database to obtain specific metabolites. The inhibition test was done with one control (P0: chemical fungicide) and two-level of treatment, i.e., the concentration of purple Cleome extract (P1: 80 % and P2: 40 %), then ANOVA and HSD test were carried out to find out the best treatment. The purple Cleome leaf extract contained three metabolite compounds as organic fungicides, namely propanoic acid 4.12 %, phenol 8.38 %, and isopropyl myristate 15.86 %. The inhibition test confirmed that 80 % (P2) purple cleome extract could suppress *C. capsici* attack up to 16.67 %, significantly higher than chemical fungicide (24.4 %).

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Introduction

Based on observations in the field, farmers often still experience difficulties growing chili (*Capsicum* L.) plants due to many pests and diseases that attack. One disease that usually attacks is anthracnose disease on chili by *Colletotrichum capsici* [(Syd.) E.J. Butler & Bisby] fungus results in crop losses reaching 65 % (Salim, 2012). According to Herwidyarti *et al.* (2013), it is stated that anthracnose disease is a salient disease that can reduce chili production by 20 % to 90 %. Anthracnose is a disease that is difficult to control because of the sudden and fatal spread, so it needs proper and environmentally friendly control.

One way to overcome these pathogens is to spray plant extract, *e.g.*, purple cleome plant (spider or bee plant - *Cleome rutidosperma* Linn.) extracts as organic fungicides (Aliyu and Prasad, 2020). Purple Cleome plant extract has a chemical compound, thiogluco-

side or glucosinolate, which can release isothiocyanate, which acts as a poison for the pest. In addition, purple Cleome plants have alkaloid and flavonoid metabolites that are potentially organic pesticides and fungicides (Panche *et al*., 2016; Stankovic *et al*., 2020; Yun and Dong, 2016). Therefore, GC-MS (Gas Chromatography-Mass Spectrometry) analyses were performed to determine the secondary metabolite compounds in purple Cleome leaves.

GC-MS is a method of separating organic compounds consisting of two methods of compound analysis, namely gas chromatography (GC) which is used to analyze compounds quantitatively, and mass spectrometry (MS) which is used to analyze the molecular structure of qualitative analytical compounds (Kalurachchi *et al*., 2017). Gas chromatography is a spectroscopic technique using the principle of separation of mixtures based on differences in the migration rates of each constituent component. Gas chromatography is used to identify a compound contained in the gas mixture and determine the compound's concentration in the gas phase. Mass spectrometry is a method for obtaining molecular weight by finding the ratio of mass to the charge of ions in which the charge is known by measuring the radius of its circular orbit in a magnetic field (Büyükköroğlu *et al*., 2018; Fiehn, 2017).

Cleome sp., *e.g*., *Cleome viscosa* L., *Cleome coluteoides* Boiss., and *Cleome isocandra* L., the extract has been found to have antioxidant and other compounds with antimicrobial properties, including antimicrobial properties antifungal activity. *C. viscosa* is well-known for its antioxidant compounds, including gallotannins, gallic acid, saponins, iridoid, and terpenoid, while *C. spinosa* contains flavonoids, phenolics, and cleomeprenols (Upadhyay, 2015). Furthermore, the study reported by Deventhiran *et al*. (2017) and Jana and Biswas (2011) showed that *C. viscosa* is used to treat infection, and lactam nonanoic acid is isolated from its root exudates has inhibitory activity on fungi due to its allelopathic and antimicrobial properties. Besides, Moghaddam *et al*. (2021) reported that *C. coluteoides* has antifungal activity against *Fusarium solani* and *Candida albicans* while Gowdra *et al*. (2019) stated that *C. isocandra* is able to inhibit the mycelial growth and spore germination of *Alternaria solani* Sorauer.

Regarding the use of organic approach to control *Collectotrhicum* sp., various plant extracts, *e.g*., *Curcuma longa* L., *Jasminum* L., *Ficus septica* Burm.f., *Lantana hirta* Graham, *Argemone ochroleuca* Sweet., and *Adenophyllum porophyllum* (Cav.) Hemsl. has been researched to investigate the use of organic antifungal agents *Collectothricum* sp., including *Collectothricum cocodes*, *Colletotrichum acutatum* J.H. Simmonds, and *Colletothricum gleosporioides* (Penz.) Penz. & Sacc. although the studies mostly undergo *in vitro* tests (Hernández-Ceja *et al*., 2021; Sudirga *et al*., 2014; Zaker, 2016). A review was written by Gowdra *et al*., (2019) also presented fungi toxic effects of various plant extracts even cow urine against *C. capsici*. Johnny *et al*. (2011) and Shinde and Gawai (2014) also contributed to an investigation on antifungal properties of various plant extracts towards *C. capsici*. Johnny *et al*. (2011) found that *Piper betle* L. has the most effective and the highest antifungal activities, *i.e*., inhibited 85.25 % of radial growth of *C. capsici*, amongst the fifteen tested plant extracts, while Shinde and Gawai (2014) reported that *Azadiracha indica* A.Juss. and *Ocimum sanctum* Linn. have strong inhibitory activity (63 % to 68 %) for the growth of *C. capsici* among seven tested plant extracts. So, there is very limited information about the use of *C. rutidosperma* as an antifungal or organic fungicide against *C. capsici*.

This research is intended to examine more in-depth the type and concentration of purple Cleome extract metabolites that act as organic fungicides in the pathogenic fungus *C. capsic*i and understand their effectiveness in inhibiting the pathogens that attack chili plants.

Materials and Methods

Analysis of metabolites of purple cleome extract

Extraction: The purple cleome plant was acquired as a weed with 30 cm to 50 cm height and stored as a fresh leaf for the extraction procedure. The selected purple cleome leaf sample was the fifth leaf from the shoot or mature enough to extract the secondary metabolites. Purple Cleome leaf was extracted using absolute methanol 1:1 w/v and shook overnight for 16 h at 150 rpm, 1 rpm = 1/60 Hz (Intelligent Orbital Shaker Model MS-1, China). The extractant obtained was concentrated with cold absolute methanol (4 °C ± 1 °C) at a ratio of 1:1 v/v, and the supernatant was purified by a 4 000 rpm centrifugation (Hettich EBA 12, USA) system for 5 min.

Metabolite Analysis: Metabolic analysis of purple Cleome leaf extract was done using GC-MS (Gas Chromatography-Mass Spectrometry) type QP2010S Shimadzu (Japan), semi-polar column RXi-5MS, with helium carrier gas flow rate of 0.5 mL min^{-1}, and pressure 27.4 kPa. The initial temperature of the GC 120 °C oven was increased with a speed of 5 °C min^{-1} until it reached 320 °C min^{-1} and the sample volume of 1 uL to 2 uL (Ahmed and Annadurai, 2017). The data obtained were analyzed with the NCBI (National Center for Biotechnology Information) database to obtain specific metabolites that act as organic fungicides.

Test for purple cleome as organic fungicide

Pathogen Inoculation in Chili: Pathogen inoculation of 100 g chili was done by spraying 5 mL inoculum on the surface of fresh Chili. The chili fruit extracted was put into a hollow plastic mica and stored at room temperature of 28 °C.

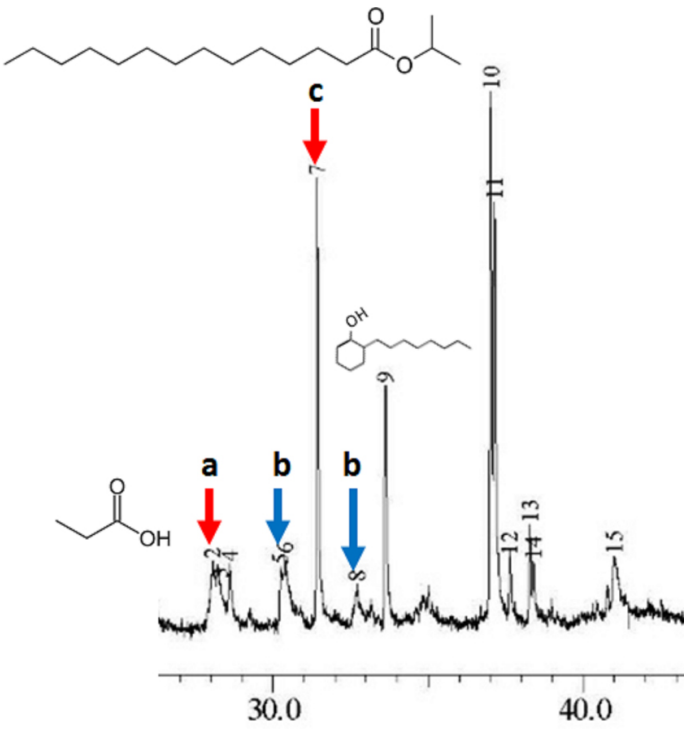

Figure 1: *Chromatogram results of GC-MS analysis of purple Cleome leaf extract, (a) Propanoic acid, (b) Phenol, and (c) Isopropyl myristate.*

Inhibition test of Purple Cleome extract on *C. capsica*: The inhibition test was carried out using a simple randomized complete trial design with 80 % (P1) and 40 % (P2) purple Cleome extract concentration with chemical fungicide (Antracol 70 WP, with the active ingredient Propineb 70 %, produced by Bayer Indonesia) (P0) control. Every 3 d, purple Cleome extract is sprayed evenly on chilies that have been inoculated with pathogens and repeated three times. Observations were made every 3 d with observational parameters: (i) diseases incidence, with Equation (1), (ii) the disease severity index, with Equation (2), and (iii) the weight loss of the fruit, with Equation (3) and measured according to Supriatna *et al.* (2017).

$$Diseases\ incidence\ (\%) = \frac{sum\ of\ fruits\ sample\ attacked}{sum\ of\ total\ fruit\ sample} \times 100 \ldots\ldots (1)$$

$$Disease\ severity\ index\ (\%) = \frac{\sum(class\ frequency \times score\ of\ rating\ class)}{total\ number\ of\ fruits \times maximal\ disease\ index} \ldots (2)$$

$$Weight\ shrinkage(\%) = \frac{initial\ weight\ of\ fruits - fruits\ weight\ at\ observed\ time}{initial\ weight\ of\ fruits} \times 100 \ldots (3)$$

The data were analyzed by ANOVA. To determine the difference in treatment, a comparative test of Tukey's honestly significance difference (HSD) 5 % was carried out if the treatment proved to be influential (Adinurani, 2016).

Results and Discussion

Analysis of purple cleome leaf extract

GC-MS analysis: GC-MS results obtained 18 metabolites, and three of them were characterized as organic fungicides, namely: propanoic acid, phenol, and isopropyl myristate, as shown in the chromatogram (Figure 1) as follows:

Analysis of metabolite as a fungicide

GC-MS analysis results obtained three metabolite compounds that act as organic pesticides, namely propanoic acid, phenol, and isopropyl myristate, with a total composition of 28.36 %, which acts as an antifungal and anti-bacterial as shown in Table 1.

The mechanism of inhibiting microorganisms by Propanoic acid varies depending on the type of microorganism. The mechanism in inhibiting microbes is competition with acetate in the acetokinase system, namely the blockage of pyruvate to acetyl coenzyme-A and B-alanine to inhibit microbes. Antifungal activity by propanoic acid compounds is due to the performance of propionyl–CoA, which inhibits glucose metabolism in certain fungal species through the accumulation of CoA derivatives (May and Sang-Keun, 2016).

Phenol acts as a toxic and corrosive antimicrobial that irritates the fungal cell wall. The mechanism

of the performance of phenol compounds is by destroying the hydrophobic bonding components of the cell membrane, such as proteins and phospholipids. Damage to the cell membrane can inhibit the activity and biosynthesis of specific enzymes that play a role in metabolism. Moreover, phenol acts as an inhibitor of essential enzymes in cells and can be used as an active antimicrobial agent against vegetative cells of bacteria, viruses, fungi, and conversely inactive against bacterial spores. Phenol can reduce the permeability of cytoplasmid membrane on fungi so that the membrane is damaged and results in stunted cell growth or even cell death (Ghosh *et al*., 2019).

Table 1: *Results of the metabolism analysis of purple Cleome extract which acts as an organic fungicide.*

No.	Compound Name (CAS)	Composition (%)	Function
1.	*Propanoic acid* $C_{11}H_{14}O_2$	4.12 %	Acts as an anti-fungal and anti-bacterial by inhibiting their growth (Yun and Dong, 2016).
2.	*Phenol* $C_{17}H_{28}O$	8.38 %	Acts as an anti-microbial that is toxic and corrosive. Phenol can damage the hydrophobic bonds that makeup cell membranes such as proteins and phospholipids.
			Acts as an inhibitor of essential enzymes and as active anti-microbial vegetative cells of bacteria, viruses, and fungi. Phenol can reduce the permeability of cytoplasmic membrane and dead fungal cells (Ariyanti *et al.*, 2012)
3.	*Isopropyl myristate* $C_{17}H_{34}0_2$	15.86 %	Act as an active ingredient of pesticides (Bessette, and Brentwood, 2007).

Table 2: *Disease incidence of C. capsici fungus on chili.*

Treatment	1st observation	2nd observation	3rd observation
P0 (chemical pesticide)	20.83a	23.81a	24.4a
P1 (80 % purple Cleome extract)	13.89a	15.56b	16.67b
P2 (40 % purple Cleome extract)	16.86a	18.02ab	19.77ab

Note: *numbers followed by the same letter indicate no significant difference according to the LSD test level of 5 %*

Isopropyl myristate acts as a catalyst for active ingredients of pesticides. For example, Besette (2006) states that isopropyl myristate in pesticides containing pyrethrum can kill insects.

Thus the results of GC-MS analysis of purple Cleome extracts identified specific metabolites in the form of propanoic acid (4.12 %), phenol (8.38 %), and isopropyl myristate (15.86 %), which act as organic fungicides.

In Vitro Fungicide Metabolite Test

The percentage of *C. capsici* fungi, it appears that the 80 % extract of purple Cleome (P1) containing propanoic acid, phenol, and isopropyl myristate metabolites with concentrations reaching 28.63 % can inhibit fungal attacks more effectively than chemical fungicide (P0) (Table 2). This shows that organic pesticides of 80 % purple Cleome extract can exceed the ability of chemical pesticides to inhibit the fungus *Colletotrichum capsici*. According to Patil *et al*. (2012), purple Cleome has a broad spectrum antimicrobial content against several types of fungi and bacterial pathogens and can inhibit the growth of pathogens more effectively.

The severity of the *C. capsici* fungus attack showed no significant difference between the use of chemical fungicide with purple Cleome leaf extracts (Table 3). This indicates that the effectiveness of using purple Cleome leaf extract as an organic fungicide is equivalent to chemical fungicides in suppressing the attack of the fungus *C. capsici*. Furthermore, according to Silva *et al*. (2016), purple Cleome metabolites have phytochemicals (flavonoids, terpenoids, and saponins), which act as active ingredients to inhibit pathogenic microbial attacks equivalent to artificial chemicals.

Table 3: *Severity index of disease in Chili.*

Treatment	Severity level (%)		
	1st observation	2nd observation	3rd observation
P0 (chemical pesticide)	20.83 a	34.82 a	29.27 a
P1 (80 % purple Cleome extract)	13.89 a	24.89 a	29.44 a
P2 (40 % purple Cleome extract)	16.86 a	30.23 a	32.56 a

Note: *numbers followed by the same letter indicate no significant difference according to the LSD test level of 5 %*

There was no significant difference between chemical fungicides with purple Cleome leaf extract (Table

4). This shows that the effectiveness of using purple Cleome leaf extract as an organic fungicide is equivalent to chemical fungicides in maintaining the quality of Chili due to the attack of the fungus *C. capsici*.

Table 4: *Percentage of shrinkage weights in Chili.*

Treatment	Weight shrinkage (%)
P0 (chemical pesticide)	21.25 a
P1 (80 % purple Cleome extract)	22.00 a
P2 (40 % purple Cleome extract)	27.75 a

Note: *numbers followed by the same letter indicate no significant difference according to the LSD test level of 5 %*

Conclusions and Recommendations

The results of GC-MS analysis identified three metabolite compounds in purple Cleome that act as organic fungicides, namely propanoic acid 4.12 %, phenol 8.38 %, and isopropyl myristate 15.86 %. These metabolites can reduce *Colletotrichum capsici* attack rates to 16.67 % and are more effective than chemical fungicides.

Novelty Statement

Previous studies on the use of plant extracts as organic fungicides against *Colletotrichum capsici* have been widely conducted, but the use of *C. rutidosperma* has not been published yet. Furthermore, the application of the antifungal is still limited on the inhibitory activity against *C. capsici* on the agar medium. Still, its application on the fruit has not been much conducted. However, based on the results of this study, *C. rutidosperma* is highly potential as antifungal with the presence of specific metabolites, *i.e.*, propanoic acid, phenol, and isopropyl myristate, which act as organic fungicides and its ability to suppress the *C. capsici* attack.

Author's Contribution

AI: Conceptualized and designed research, elaborated intellectual content, statistical data analysis, prepared manuscript and revised.
DI: Carried out experimental studies and reviewed manuscript.
FH: Reviewed manuscript and revised manuscript.
MFMA and II: Elaborated the intellectual content, explored literature search, data acquisition, reviewed manuscript and guarantor.
All authors read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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