

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Leaf Essential Oil of Wild Zingiberaceae *Elettariopsis slahmong* CK Lim to Control Antrachnose Disease in Red Dragon Fruit *Hylocereus polyrhizus*

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ABSTRACT

Antrachnose caused by *Colletotricum gloesporioides* is the most destructive disease attacking red dragon fruit, *Hylocereus polyrhizus*, in Sumatra Indonesia. Throughout observations conducted in three provinces in Sumatra in the period of 2011 to 2015, it was discovered that none of the dragon fruit plantations were free from *C. gloesporioides* attack, while this plant has high economic and health values. Furthermore, control with various chemical pesticides have yet to provide expected results. The study of wild Zingiberaceae *Elettariopsis slahmong* CK Lim leaf essential oils' (EO) and its two fractions' (A/1 and C/3) against (*in-vitro*) *C. gloesporioides* in dragon fruit plant was conducted from July 2014 - May 2015, at the laboratory of plant protection of Research Station, Bogor Research Institute for Spice and Medicinal Plants in Laing Solok, West Sumatra. The research was conducted in three activities: (1) decreasing of colony diameter of *C. gloesporioides* in Potato Dextrosa Agar (PDA) medium, the treatment tested were the EO of *E. slahmong* leaves, A/1 and C/3 fractions with five concentration levels (0, 100, 250, 500 and 1000 ppm); (2) suppression of colony biomass of *C. gloesporioides* in Potato Dextrosa Broth (PDB) medium, the treatments tested were the EO of *E. slahmong* leaves, A/1 and C/3 fractions with five concentration levels (0, 100, 250, 500 and 1000 ppm); and (3) decreasing of colony diameter of *C. gloesporioides* with volatile compounds from *E. slahmong* leaves; A/1 and C/3 fractions with five test dosage (0, 0.01 ml, 0.025, 0.05 and 0.1 ml/petri dish). Experiment (1) and (2) used completely randomized design (CRD) in factorial and experiment (3) used CRD with four replicates each. At the concentration of 1000 ppm, leaf EO and A/1 fraction were able to inhibit colony diameter growth and biomass of *C. gloesporioides* up to 100%, while at the same concentration, C/3 fraction could only inhibit 55.44% of colony diameter growth and 65.05% of colony biomass. At 0.5 ml/petri dish dosage, it was discovered that volatile compounds from leaf EO and A/1 were able to inhibit colony diameter of *C. gloesporioides* up to 100%, which was significantly different from C/3 fraction with 64.05% colony diameter inhibition rate at the same concentration level. The results concluded that the EO of leaf of wild Zingiberaceae *E. slahmong* and A/1 fraction have the most potential as biopesticide.

Keywords: *Hylocereus polyrhizus*, high economic and health values, antrachnose, *Elettariopsis slahmong*, biopesticide.

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INTRODUCTION

Red dragon fruit or *Hylocereus polyrhizus* is a cactus plant which is considered to be a new promising crop because of its high economic and health values (11, 13, 14, 15, 18). It is also widely known for various other names such as pitaya or pitahaya, peer strawberries, night blooming cereus and dragon eye (15, 4, 11, 22). In West Sumatra, since the last 10 years this commodity has been widely grown in the Districts of Padang Pariaman, Dhamasraya, Solok, Pasaman and Padang. Because of its antioxidant compound, dragon fruit has a good health value and can be consumed as fresh fruit, can be made into juice or made as ingredient for cake (11, 18). However its cultivations have been constrained by a vicious pathogen attack, *Colletotricum gloeosporioides*, the cause of antrachnose disease.

Anthrachnose disease has spread widely in a variety of dragon fruit plantations in Sumatra. In several production centers in the province of West Sumatra, Riau and Riau Islands, the severity level of the attack is at the moderate to severe (8, 18). From continuous field observations since 2011 to 2015, none of the dragon fruit plantations within the three provinces previously stated was free from antrachnose disease (18). Furthermore, in the province of Riau Islands, this disease has caused a decline in dragon fruit production up to 80% (24). In Padang (West Sumatra) and Bintan Island (Riau Islands), severe attacks reaching more than 60% resulted in plants rotting, becoming woody, drying and finally dying (18, 24). Malaysia, Indonesia's neighbor, reported a 32% level of attacks on its plantations (13). Other pathogens which were also detected attacking dragon fruit plant were *Fusarium*, *Phytophthora*, *Phytium*, *Sclerotium*, *Rhizoctonia* and *Acremonium* (6, 8), *Enterobacter cloacae*, *Klebsiella mobilis*, *Klebsiella oxytoca*, *Pantoea dispersa* and *Rahnella aquatilis* (14). However, from the pathogenicity test results, only attacks from *C. gloeosporioides* and *Enterobacter cloacae* showed severe disease symptoms (13,14). In Sumatra, there has been no report of *E. cloacae* attack (18).

Until today, controlling *C. gloeosporioides* in dragon fruit plant has been proven unsuccessful and there has been no research result to suggest otherwise. Common control practices among farmers could only go as far as cutting infected plant parts or using synthetic chemical fungicides. Several of these fungicides which are generally used, such as copper hydroxide, propineb, calcium chloride and difenoconazol (1, 21). However none of these chemical pesticides able to reduce *C. gloeosporioides* attack in red dragon fruit plantations in Sumatra, even some of the plantations have been ignored by the growers (18).

E. slahmong CK Lim is a Zingiberaceae group that was first discovered in Thailand by CK Lim (12). This plant a very sharp stinging odor which gave it its name of smelly leaves (23) or stink bug plant (12). In West Sumatra, *E. slahmong* can be found as wild plant in the forests of Bonjol (District of Pasaman Timur), Kinali (District of Pasaman Barat), Lembah Anai (District of Padang Pariaman), Lubuk Basung (District of Agam) and Air Hangat (District of Sijunjung) (17).

EO of *E. slahmong* and its fraction has proven to be insecticidal agents against *Trigona minangkabau* (16) and *Drosophilla melanogaster* (17), both are insect vectors of *Rashtonia solanacearum*, the bacteria causes blood disease in bananas. Nasir (19) also discovered that this specific EO was able to decrease *Phytophthora palmivora* attacks that cause cocoa fruit rot disease in West Sumatra. According to Picheansoonthon and Yupparach (23), EOs of *E. slahmong* also contains antimicrobial compounds. This study aims to determine the potential of EO of *E. slahmong* leaf and its fractions as biopesticide against *C. gloeosporioides* on red dragon fruit.

METHODOLOGY

The study was conducted from July 2014 - May 2015 in the laboratory of plant protection at Research Station of Bogor Research Institute for Spice and Medicinal Plants (BRISMP) in Laing Solok, West Sumatra. Wild Zingiberaceae of *E. slahmong* originated from forest in the Districts of Pasaman Barat, West Sumatra.

Distillation and fractionation:

There were two sources of EO used as biopesticides in this study:

1. EO from distillation of *E. slahmong* leaves only;

2. Fractionation of EO derived from the distillation of all parts of *E. slahmong*, all combined without separating EO from leaves, stems and rhizomes. The plant parts are left to wither in advance for 3-5 days before distillation process. Distillation was done through steaming, using a BRISMP boiler prototype in the the Research Station of BRISMP. Fractionation process of *E. slahmong* EO was conducted at the Laboratory of Horticulture and Floriculture, Faculty of Agriculture, Kagoshima University, Japan, in 2014 and produced seven fractions (19 *in press*). This study only used two fractions that have the highest result volume from fractionation process of 100ml pure EO of *E. slahmong*.

b. Pathogen isolates:

C. gleosporioides fungal isolates originated from the collection of the Laboratory of Phytopathology Tropical Fruits Research Institute in Solok, West Sumatra, from the isolation of dragon fruit plants which have been attacked by antrachnose disease. The isolate was propagated in Patato Dextrosa Agar (PDA) medium and was used for testing at the age of nine days.

c. Antifungal Potential Testing

c1. Decreasing Colony Diameter:

Each treatment material (leaf EO, A/1 and C/3 fractions) was mixed into sterile PDA medium according to the treatment and concentration tested, before the PDA freezes (45°C). Each mixture was then poured into a petridish and left to harden. After the treated PDA has hardened, propagated 9 days of *C. gleosporioides* was then cultured into 45°C treated PDA. Pure *C. gleosporioides* cultures were sliced using sterile corkborer into 6mm in diameter pieces, it was then placed at the center of treated PDA medium, then incubated at the temperature 28° C for nine days. The experiment was arranged in the form of completely randomized design (CRD) in a factorial with four replications each, those treatments were: leaf EO of *E. slahmong*, A/1 fraction, and C/3 as factor I, concentration levels of 0, 100, 250, 500 and 1000 ppm as factor II.

c2. Colony Biomass Supression:

This test used liquid Patato Dextrosa Broth (PDB) medium. As much as 25 ml PDB was inserted into each test tube (1.5 cm diameter and 16.5 long) then sterilized in an autoclave, then further cooled at 45°C. Essential oil test materials were then added to these test tubes according to the determined concentration. Pure *C. gleosporioides* cultures were sliced using sterile corkborer into 6mm in diameter pieces, it was then placed in the PDA medium that has been treated and then incubated at the temperature 28° C for 9 days. The experiment was arranged in the form of completely randomized design (CRD) in a factorial with four replications each. Those treatments were: leaf EO, A/1 and C/3 fraction as factor I, concentration levels of 0, 100, 250, 500 and 1000 ppm as factor II. Fungus colonies growing in the medium were extracted and dried in an oven at a temperature of 80° C for 48 hours and then the colony biomass was weighed.

c3. Volatile Compounds Testing:

EO extracted from *E. slahmong* leaf and from each fraction tested (A/1 and C/3) were spread on the inside of the coverside (cap) of the petridish by using a cotton swab, according to treatment these were 0; 0.01; 0.025; 0.05 and 0.10 ml/petridish. As much as 15ml of Patato Dextrosa Agar (PDA) was placed at the bottom (base) of the petridish. Pure *C. gleosporioides* fungus culture, measuring 6 mm in diameter, was then centered in the PDA medium. The petridish was then covered with the coverside (cap) wich has spread with EO. When the base of the petridish was covered with the cap, the position of the tested fungus in the PDA medium should be directly in front of the tested EO and fraction. All treatments were then incubated at a temperature of of 28° C for 9 days.

d. Inhibitor Potential

Inhibition of colony growth was calculated using the formula (1):

$$I = \frac{C - T}{C} \times 100\%$$

I = Inhibition of colony growth
 C = Colony diameter or biomass of control
 T = Colony diameter or biomass of treatment

RESULT AND DISCUSSION

Severe antrachnose disease attacks lead to failed crops which even end in land and property abandonment by farmers. Within two years, infected plants will look like Picture 1: rotting, decaying and becoming woody. Picture 2 shows new planting activity by farmers in the former disease infected area. However the cultivation was conducted without sanitation.



Picture.1. Disease attack on ignored red dragon fruit *Hylocereus polyrhizus* plantation in Padang, West Sumatra Indonesia in 2014 (Doc. Nasril Nasir).



Picture 2. Former disease infected area (2 ha) of red dragon fruit plantation in the District of Bintan Province, Riau Islands, 100% destroyed by *Colletotrichum* in 2010. Farmer re-planted his land with new cuttings, but without any sanitation to the trellises and lands, including ingnoring pruning diseased branch which have spread in this property (doc. Nasril Nasir 2013).

Conditions such as Picture 1 and 2, are commonly found in dragon fruit plantations in West Sumatra, Riau and Riau Islands.

The result of this research indicated that leaf EO of *E. slahmong* and the fractions are antifungal against *C. gloesporioides*, the cause of antrachnose disease in red dragon fruit plant. **The A/1 fraction** exhibited the highest inhibitor potential (41.61%). This result was not different from conclusions presented by EO extracted from only the leaves (41.26%). However, this was significantly different from results of C/3 fraction with an inhibitor rate of only 20.46% (Table 1).

Table 1. Effect of leaf and fractions of EO of *E. slahmong* and concentration level against *C. gloesporioides* colony diameter growth (9 DAI)

Treatment	Colony Diameter (mm)	Inhibitor Potential (%)
Biopesticide:		
<i>E. slahmong</i> leaf EO	51,10	41,26 a
A/1 Fraction	50,80	41,61 a
C/3 Fraction	69,20	20,46 b
Concentration level:		
0 ppm(K+)	85,00	2,30 e
100 ppm	79,66	8,43 d
250 ppm	68,08	21,74 c
500 ppm	38,92	55,27 b
1000 ppm	13,50	84,48 a
Control (without treatment)	87,00	0,00 -
CV (%)	-	1,68

Note: Numbers followed by the same letters in the same column are not significantly different at 5% level DMRT.
 K+ (emulsifier and dissolven)
 DAI (days after inoculation)

The higher the level of concentration of leaf oil and the fractions (fraction A/1 and C/3), the smaller the diameter of *C. gloesporioides* colonies became. At a concentration of 1000 ppm, *C. gloesporioides* inhibition growth level has reached 84.48%.

Statistical analysis described interaction between leaf EO and the fractions with concentration level, where the increase in concentration level causes increase in inhibitory effect on the growth of fungal colony diameter tested. At 100 ppm concentration level, leaf EO has shown suppression effect on the growth of *C. gloesporioides* colonies, with inhibition rate ranging from 5.17 to 10.34%. Using the same EO and A/1 fraction, this rate reached a remarkable 100% inhibitory effect on fungal colony growth at 1000 ppm. These results are significantly different from C/3 fractions where the inhibition of colony growth only reached 53.44% at the similar concentration level (Table 2).

Table 2. Interaction between EO of *E. slahmong* leaf, the fractions and concentration level with *C. gloesporioides* colony diameter growth (9 DAI)

Treatment	Colony Diameter (mm)	Control Potential (%)
<i>E. slahmong</i> leaf EO:		
0 ppm	85,00	2,30 h
100 ppm	78,50	9,77 f
250 ppm	63,25	27,30 e
500 ppm	28,75	66,96 b
1000 ppm	0,00	100,00 a
A/1 Fraction :		
0 ppm	85,00	2,30 h
100 ppm	78,00	10,34 f
250 ppm	62,75	27,88 e
500 ppm	28,75	67,53 b
1000 ppm	0,00	100,00 a
C/3 Fraction :		
0 ppm	85,00	2,30 h
100 ppm	82,50	5,17 g
250 ppm	78,25	10,06 f
500 ppm	59,75	31,32 d
1000 ppm	40,50	53,44 c

Note: Numbers followed by the same letters in the same column are not significantly different at 5% level DMRT.
 K+ (emulsifier and dissolven)
 DAI (days after inoculation)

Results of testing the effect of *E. slahmong* leaf oil and fractions against *C. gloesporioides* colony biomass on Patato Dextrosa Broth medium, described effective suppression on biomass fungal colony growth. Colony biomass in A/1 fraction treatment showed the highest biomass suppression growth, compared to the

treatment with EO of *E. slahmong* leaf and C/3 fraction; respectively the results were 40.70 mg (55.04% inhibitory rate), 41.15 mg (55.58% inhibitory rate) and 63.35 mg (31.51% inhibitory rate).

When compared to the control, the average colony biomass at 9 DAI was 92.50 mg. The higher the concentration, the more effective the inhibitory effect on fungal growth became, where the concentration of 1000 ppm reached 88.02% inhibitory rate (Table 3).

Table 3. Effect of *E. slahmong* leaf oil, the fractions and concentration level towards *C. gleosporioides* colony biomass (9 DAI)

Treatment	Colony Biomass (mg)	Control Potential (%)
Biopesticide:		
<i>E. slahmong</i> leaf EO	41,15	55,58 a
A/1 Fraction	40,70	56,04 a
C/3 Fraction	63,35	31,51 b
Concentration level:		
0 ppm(K+)	88,25	4,59 e
100 ppm	67,83	26,66 d
250 ppm	49,08	46,93 c
500 ppm	25,75	72,35 b
1000 ppm	11,08	88,02 a
Control (without treatment)	92,50	0,00 -
CV (%)	-	2,81

Note: Note: Numbers followed by the same letters in the same column are not significantly different at 5% level DMRT.
K+ (emulsifier and dissolven)
DAI (days after inoculation)

Table 4. Interaction between *E. slahmong* leaf oil, its fractions and concentration level towards *C. gleosporioides* colony biomass growth (9 DAI)

Treatment	Colony Biomass (mg)	Control Potential (%)
<i>E. slahmong</i> leaf EO:		
0 ppm		
100 ppm	88,25	4,59 j
250 ppm	62,50	32,43 g
500 ppm	40,50	56,48 d
1000 ppm	15,50	84,39 b
A/1 Fraction	0,00	100,00 a
0 ppm		
100 ppm	88,25	4,59 j
250 ppm	61,00	34,05 f
500 ppm	39,75	57,02 d
1000 ppm	14,25	84,55 b
C/3 Fraction	0,00	100,00 a
0 ppm		
100 ppm	88,25	4,59 j
250 ppm	80,00	13,51 i
500 ppm	67,25	27,30 h
1000 ppm	48,00	48,11 e
	32,25	65,13 c

Note: Note: Numbers followed by the same letters in the same column are not significantly different at 5% level DMRT.
K+ (emulsifier and dissolven)
DAI (days after inoculation)

From the results which were shown in Tables 1 to 4, wild Zingiberaceae *E. slahmong* leaf oil and A/1 fraction both have high antifungal against *C. gleosporioides* and were significantly different from C/3 fraction. According to Nasir *et al* (20 unpublished), the main components contained in *E. slahmong* leaf oil based on to GC-MS analysis results are 48.04% 2-decanoic acid, followed by 9.18% nonanoic acid, 8.97% octenal, 2.96% nonanal and 1.20% octanal. While the main components of A/1 fraction are 31.65% 2-decanoic acid, 17.01% 2-octenal, 12.40% decanal and 4.08% 6-tetradecene. In contrast, the dominant components in

C/3 fraction are decen-1-ol (18.82%), decanal (12.95%), decenal (10.55%), octanal (8.42%) and 6-tetradecene 3.24% (Table 5).

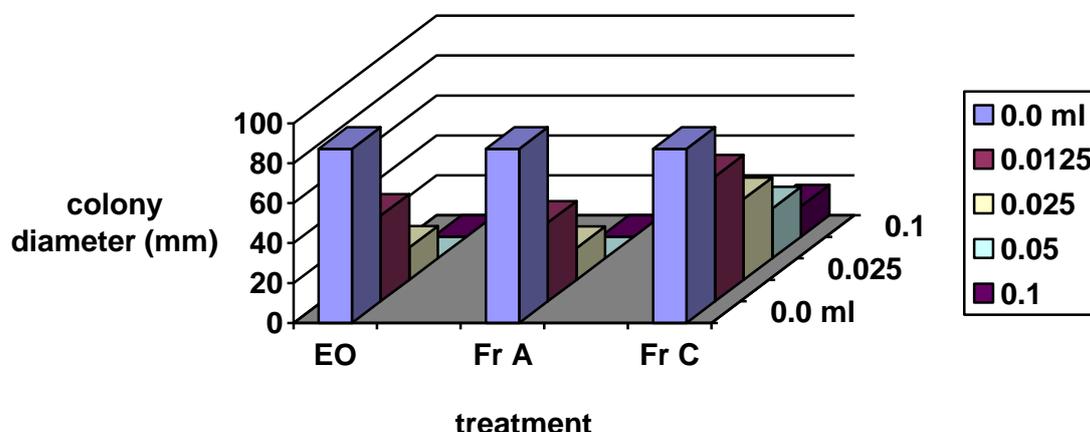
Decanoic acid (capric acid) and its esters are the best antifungal agent that has been used to control multiple pathogens in humans (10). Decanal and nonanal is an antifungal that can inhibit the growth of sclerotia from *Sclerotinia sclerotiorum* which causes canola stem rot disease and sunflower stem base rot disease (5). At a concentration level of 100 ppm, decanoic acid and nonanoic acid already have the capacity to kill *Basidiomycetes* spores which causes white and brown root disease (3). However, the role of other components such as decen-1-ol and 6-tetradecene which have high percentages in C/3 fraction, are still unknown.

Table 5. Main components of EO of *E. slahmong* leaf and fraction from GCMS analysis results

Wild Zingiberaceae oil/ fraction	Main Components
<i>E. slahmong</i> leaf EO	2-decanoic acid (48,04%), nonanoic acid (9,18%), 2-octenal (8,97%), nonanal 2,96%, octanal 1,20% and 75 other components, each of which are under 1%.
A/1 Fraction	2-decanoic acid (31,65%), 2-octenal (17,01%), decenal (12,40%), 6-tetradecene (4,08%), nonanoic acid (1,37%), and 51 other components, each of which are under 1%.
C/3 Fraction	decen-1-ol (18,82%), decanal (12,95%), decenal (10,55%), octanal (8,42%), 6-tetradecene (3,24%), octenal (1,56%) and 66 other components, each of which are under 1%.

This analysis displays different components compared to results presented by Picheansoonthon and Yupparach (23), who reported *E. slahmong* essential oil components are 2-octenal (46%), 2-decenal (29%) and terpenoids. Other researchers discovered 32 compounds of *E. slahmong* essential oil which is dominated by aldehyds (25). The difference in these findings are linked to the probability of the difference of secondary metabolites formation due to different agro-ecological environments, where secondary metabolites in plants are strongly influenced by environmental factors, such as growth area, climate and rainfall. Another probability is the increasing use of sensitive tools.

Volatile compounds produced by *E. slahmong* leaf oil and the fractions (A/1 and C/3 fraction), was also able to inhibit the diameter growth of *C. gleosporioides* pathogenic fungi. Until 9 DAI, at the dose of 0.0125 ml/petridish, *E. slahmong* leaf oil can suppress the growth of fungal colony diameter tested to up to 50.14%. At the same dose, A/1 fraction reached 57.88% inhibitory rate and, C/3 fraction showed the lowest inhibitory rate of only 25.52% (Picture 3). Knobloch *et al* (9) and Jay *et al* (7) stated that the antifungal chemical component of volatile oil is capable of penetrating the cell walls of the fungus and affecting the cell's metabolic process which interferes with cell growth; at certain concentrations, resulting in fungal cell death. According to Chairgulprasert *et al* (2) and Picheansoonthon and Yupparach (23), *Elettariopsis* sp. group contains antimicrobial compounds.



Picture 3. The effect of volatile compound in *E. slahmong* leaf oil and the fractions towards *C. gloesporioides* colony diameter growth at 9 DAI (EO = Essential Oil of *E. slahmong* leaf, Fr A=fraction A/1, Fr C=fraction C/3).

At the dose of 0.05ml/petridish, *E. slahmong* leaf oil and A/1 fraction were able to suppress the diameter growth of *C. gloesporioides* colonies to 100%. While at the same concentration, C/3 fraction could only reach 71.34% inhibitory potential.

CONCLUSION

Wild Zingiberaceae *E. slahmong* leaf oil and A/1 fraction has a high potential as biopesticide:

1. At the concentration level of 1000 ppm, *E. slahmong* leaf EO and A/1 fractions were able to completely inhibit *C. gloesporioides* colony diameter and biomass growth (100%).
2. At the dose of 0.5 ml/petridish, volatile compounds of *E. slahmong* leaf oil and A/1 fraction have the highest capacity to inhibit the diameter growth of *C. gloesporioides* to up to 100%.

ACKNOWLEDGMENT

The authors would like to thank Mr. Budi P., owner of the red dragon fruit farm in Lubuk Minturun, Padang, West Sumatra, Indonesia for his willingness in letting us conduct research on his farm and his cooperation in discussions.

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