

ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS OF LEAVES, RHIZOMES OILS AND FRACTION WILD GINGER *Elettariopsis slahmong* CK Lim INHIBIT THE COLONY GROWTH OF *Sclerotium rolfsii*

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Abstract. This study aims to see the effectiveness of essential oils of leaves, rhizomes and fraction of wild ginger *Elettariopsis slahmong* CK Lim against the pathogenic fungus *Sclerotium rolfsii* which causes rot disease of the stem base of peanut plants an in vitro. The study consisted of two sub activities: (a) inhibited of colony diameter using Patato Dextrosa Agar (PDA) medium and (b) inhibited of colony biomass using Potato dextrose Broth (PDB) medium, the treatments tested were leaf essential oil and rhizome of wild ginger and fractions A1, B2, C3 and D4, with concentration levels (0, 100, 250 and 500 ppm). Experiments (a) and (b) were arranged in the form of a Completely Randomized Design (CRD) in factorial each with 4 replications. The results showed that the leaf essential oil and rhizome of wild ginger and its fractions had the potential to be used as a vegetable fungicide. The A1 fraction has the best antifungal effectiveness compared to the B2 fraction, leaf oil, rhizomes and other fractions, with the highest inhibition of diameter and biomass of *S rolfsii* colony the 49.47% and 51.46%. Essential oils of leaves and rhizome oil are not statistically significantly different, but in numerically leaf oil are better than rhizome oil. The C3 fraction showed the lowest colony diameter inhibition and biomass of 34.70% and 36.95%. The best concentration level in inhibition the growth of *S rolfsii* mushroom is 500 ppm, with inhibition of the diameter and biomass of the colony by 81.74% and 84.25%.

Keywords: essential oil; rhizomes; fraction; wild ginger; sclerotum rolfsii

1. Introduction

Wild Ginger *Elettariopsis slahmong* C.K. Lim, is a wild plant of the family zingiberaceae which was first discovered in Thailand by CK Lim this plant, it has a very strong pungent odor called the smelly leavis or stink bug plant (Picheansoonth & Yupparach, 2007). There are several other species of the genus *Elettariopsis* that have been studied are *E elan* C.K. Lim, *E exserta* (Scort) Bak, *E smithiae* Y. K. Kam, *E triloba* (Gagneb) Loes, *E manophylla* and *E wandokthong* which are new species from Thailand (Picheansoonthon & Yupparach, 2010). Wild Ginger contains essential oils from leaves, rhizomes and roots obtained by hydro destilation (Wong et al, 2006). This wild ginger plant is widely used as a spice and salad in Malaysia, Thailand and Indonesia (Magdaulih & Nasir, 2014).

These wild ginger plants can also be found in West Sumatra as wild plants in the forest of Bonjol Pasaman Regency, Kinali Regency of West Pasaman, Anai Valley Padang Pariaman Regency, Lubuak Basuang Agam Regency and Aia Angek Sijunjung Regency. Wild ginger plants

contain essential oils from leaves and rhizomes and their roots. Essential oils and volatile compounds from this plant are insecticidal against the *Drosophila melanogaster* insect which is a vector of blood diseases in banana plants (Nasir *et al.*, 2014).

Research on the effect of wild ginger essential oil on plant pathogenic fungi has not been widely tested, essential oils from leaves can control the pathogen *Colletotrichum gloeosporioides* that causes Antrachnose disease in the red dragon fruit plant *Hylocereus polyrhizus*. At the concentration level of 1000 ppm leaf oil can suppress the growth of pathogenic *C. gloeosporioides* 100% (Nasir & Nurmansyah, 2016), it is also effective against *Phytophthora palmivora* fungi (Nasir, 2017).

The main content of wild ginger leaf oil is 2-decanoic acid 48.04%, nonanoic acid 9.18%, 2-octenal 8.97%, nonanal 2.96%, octanal 1.2% and 75 other components below 1% (Nasir & Nurmansyah, 2016), The main components of rhizomes and roots are 2-Tridecenal 39.81%, 2-decanoic acid 26.3%, 2 octanal 7.56%, hexadecanoic acid 2.86%, nonanoic acid 3.85% and 70 other components below 1% (Nasir, 2016).

Sclerotium rolfsii is a pathogenic fungus that attacks various plants including peanuts, tomatoes, chili, papaya, orchids and soursop. This pathogenic fungus is also known to attack patchouli plants (Sukanto & Wahyuno, 2013), *S. rolfsii* is a soil borne pathogen such as *Botrytis cinerea*, *Pythium* sp, *Rhizoctonia solani*, *Sclerotinia minor* dan *Verticillium dahlia* (Sumartini, 2012; Thiessen & Woodward, 2012)

The yield decrease caused by *S. rolfsii* fungus attack on peanut plants can reach 44.51 percent (Buhaira & Asniwita, 2009). Each component has not been tested, therefore further research is recommended. Antifungal essential oil chemical components that are able to penetrate the fungal cell walls, thereby causing interference with the metabolic processes in the cell so that it interferes with cell growth, at certain concentrations will result in the death of the fungal cells.

Control of this pathogen can be done with leaf oil fungicide and cinnamon stick (*Cinnamomum burmanii*) with a concentration level of 500 ppm able to suppress the growth of the colony 100% (Nurmansyah, 2014) and *Piper aduncum* oil at a concentration level of 500 ppm able to control the pathogen *S. rolfsii* reached 92.77% (Nurmansyah, 2016). Extract of garlic can control the pathogen *S. rolfsii* 92.66% at concentration 5% (Supriyono, 2011). The citronella and bamboo piper oil botanical fungicides have a good enough ability to suppress the stem rot disease of peanut with an emphasis percentage of 86.38 and 93.21% at interval application one time a week (Idris *et al.*, 2020).

Based on the description above, then the use of wild ginger pesticides to control plant diseases has enough potential, considering that these plants are wild plants and will be more beneficial if they can be developed as plants that have economic value for controlling pests and

plant diseases. This study aims to look at the effectiveness of leaf oil fungicides, wild ginger rhizomes (*Ellettariopsis slahmong* CK Lim) and their fraction of *Sclerotium rolfsii* fungi that cause root rot disease in the peanut an in vitro.

2. Methods

The study was conducted from April to December 2018 in the pest and disease laboratory of the Laing Solok Experiment Garden in West Sumatra.

2.1 Distillation and fractionation

Wild ginger oil is obtained by distillation of leaves and rhizomes, which are withered for 4 days for the leaves first, while the rhizomes are dried for about 10 days, the ingredients are chopped before being distilled first. Distillation is carried out using a kettle protip Balittro system of steam, the results of the distillation of leaf oil and rhizomes are mixed and fractionated at the Faculty of Agriculture of Kogoshima University Japan (by means of multilevel distillation), seven fractions are obtained and there are quite a large volume number of four fractions namely the A1, B2, C3 and D4 fractions. Leaves, rhizomes and fractions obtained in the identification of chemical components contained using GC-MS in Andalas University Laboratory Padang.

2.2 Pathogen isolation

Sclerotium rolfsii isolates were obtained by isolating from peanut plants that were attacked by stem rot in the Payo area of Tanjung Harapan Solok sub-district. Isolates were identified and propagated Potato Dextrosa Agar (PDA) media as the source of the inoculum to be tested, isolates were used 5 days old in PDA media.

2.3 Antifungal power testing

2.3.1 PreSSION of *Sclerotium rolfsii* colony diameter

Tests are carried out by mixing until homogeneous the treatment material into a sterile PDA medium, according to the treatment and concentration tested before freezing (45°C), then poured into petridish and allow to harden, after hardening the inoculation of fungi, fungal mat from the *Sclerotium rolfsii* fungus is cut with a sterile corkbore diameter of 6 mm, placed in the middle of the treated medium, then incubated in an incubator of 28°C for 4 days. The experiment was arranged in the form of a Completely Randomized Design (CRD) in factorial each of 4 replications, the treatments were: Leaf oil and roots of wild Ginger and fractions A1, B2, C3 and D4 as factors I, concentration levels (0, 100, 250 and 500 ppm) as factor II.

2.3.2 Emphasis of colony biomass

Tests using Patato Dextrose Broth (PDB) liquid medium, as much as 25 ml of the medium are input into each test tube, then sterilized in an autoclave, after sterile is cooled and then put the treatment material to be tested according to concentration, then do inoculation of fungi test, the fungal mat of the *Sclerotium rolfsii* mushroom was cut with a sterile corkbore of 6 mm in diameter,

and inserted into the treated medium, then incubated in an incubator of 28°C for 4 days. The experiments were arranged in the form of a Completely Randomized Design (CRD) in factorial each of 4 replications. The treatments were: Leaf oil and wild ginger root fractions A1, B2, C3 and D4 as factors I, concentration levels (0, 100, 250 and 500 ppm) as factor II. Furthermore, the growth of fungal colonies was taken and dried in an oven at 80°C for 48 hours, then the biomass was weighed. Inhibition or suppression of colony diameter and biomass growth, calculated by the formula (Pandey *et al*, 1982 in (Noveriza & Miftakhurohmah, 2010)

$$x = \frac{b-a}{b} \times 100\% \quad (1)$$

X = Percentage inhibition growth of diameter/biomass colony

a = Diameter/biomass growth of treatment

b = Diameter/biomass growth of control (untreated)

3. Results and Discussion

The results showed that leaf oil, rhizome and fraction of wild ginger (*Elettariopsis slahmong*) are antifungal and can inhibit the growth of the fungus *Sclerotium rolfsii* which causes rot disease of the stem base of the peanut plant. A1 and B2 fractions showed better antifungal effectiveness compared to essential oils of leaves and rhizomes and fractions of C2 and D4 (Table 1)

Table 1. Effect of leaf, rhyzom and fraction of essential oils of *E. slahmong* and concentration level against *Sclerotium rolfsii* colony diameter growth (4 DAI)

Treatments	Colony diametre (mm)	Inhibition (%)	
Botanical pesticide			
Leaf oils	44.18	47.31	c
Rhizome oils	44.69	46.71	cd
Fraction A1	42.37	49.47	a
Fraction B2	43.19	48.50	b
Fraction C3	54.75	34.70	e
Fraction D4	45.00	46.34	d
Concentration level			
0 ppm (Control +)	81.75	2.69	d
100 ppm	53.71	36.92	c
250 ppm	32.08	61.69	b
500 ppm	15.25	81.74	a
Control (without treatments)	83.75	0.00	-
CV (%)	-	1.98	

Note. The numbers followed by the same letter are not significantly different according to DMRT. Test at 5% level. DAI (days after inoculations). Control+ (Solvents and emulsifiers)

From Table 1 it can be seen that the leaf essential oil with rhizome is not statistically significantly different, but the leaf oil rate is more fungicidal than rhizome oil, so the fraction of D4 with rhizome oil is also not statistically significantly different.

The higher of concentration level the smaller the diameter of the test mushroom colony. At a concentration of 500 ppm the suppression of *S. rolfsii* fungi colony diameter growth reached 81.74%.

The interaction of essential oils of leaves, rhizomes and fraction of wild ginger with a concentration level showed that the higher the concentration level the higher the inhibitory effect on the diameter growth of the test fungi colonies (Table 2).

Table 2. Interactions of leaf oil, rhizome and *E. slahmong* fraction with levels concentration on growth of *Sclerotium rolfii* colony diameter (4 days after inoculation)

Treatments	Colony diameter (mm)	Inhibition growth (%)	
Leaf oil s(Md)			
0 ppm	81.75	2.39	m
100 ppm	52.50	37.31	j
250 ppm	29.50	64.77	fg
500 ppm	13.00	84.78	b
Rhizome oils(Mr)			
0 ppm	81.75	2.39	m
100 ppm	53.50	36.11	jk
250 ppm	30.00	64.18	g
500 ppm	13.50	83.88	bc
Fraction A1			
0 ppm	81.75	2.39	m
100 ppm	48.75	41.78	i
250 ppm	27.50	67.16	de
500 ppm	11.50	86.26	a
Fraction B2			
0 ppm	81.75	2.39	m
100 ppm	50.00	40.59	i
250 ppm	28.50	65.97	ef
500 ppm	12.50	84.78	b
Fraction C3			
0 ppm	81.75	2.39	m
100 ppm	63.50	24.18	l
250 ppm	47.25	43.58	h
500 ppm	26.50	68.35	d
Fraction D4			
0 ppm	81.75	2.39	m
100 ppm	54.00	35.52	k
250 ppm	29.75	64.48	fg
500 ppm	14.50	82.68	c

Note. The numbers followed by the same letter are not significantly different according to DMRT. Test at 5% level.

From Table 2, it can be seen that the concentration level of 100 ppm of wild ginger essential oil and the fraction has shown an emphasis on the growth of *S. rolfii* mushroom colony diameter with growth suppression ranging from 24.18 - 41.78% and at a concentration level of 500 ppm A1 fraction has been able suppress the growth of test fungi colonies by 86.26%, while in leaf essential oil the growth of new colonies increased to 84.78% at the same concentration level.

Test results on *Sclerotium rolfii* fungi biomass on Patato Dextrosa Broth media showed that fungal biomass growth suppression was quite effective, colony biomass in the treatment of A1 fraction showed the highest suppression of colony biomass growth compared to B2, leaf oil,

rhizome, and D4 fractions, C3 fraction showed effective suppression of biomass colonies in A1 treatment. The Suppression of the lowest colony biomass growth (Table 3).

Table 3. Effects of essential oils of leaves, rhizomes and *E. slahmong* fraction as well as levels concentration of *Sclerotium rolfsii* colony biomass growth (4 DAI)

Treatments	Colony biomass (mm)	Inhibition growth (%)	
Botanical Pesticide			
Leaf oils	79.62	49.76	c
Rhizome oils	80.75	49.17	cd
Fraction A1	76.94	51.46	a
Fraction B2	78.19	50.67	b
Fraction C3	99.87	36.95	e
Fraction D4	80.93	48.93	d
Concentration level			
0 ppm (Control +)	153.25	3.31	d
100 ppm	94.70	40.29	c
250 ppm	58.04	63.43	b
500 ppm	24.87	84.25	a
Control (<i>without treatments</i>)	158.50	0.00	
CV (%)	-	1.79	

Note. The numbers followed by the same letter are not significantly different according to DMRT. Test at 5% level. CV (coefficient variation). Control+ (Solvents and emulsifiers)

The interaction of essential oils of leaves, rhizomes and wild ginger fraction with the concentration level, showed that the higher the concentration level, the higher the inhibitory power of the biomass colony of the test fungus. At the concentration level of 500 ppm A1 and B2 fractions, no significant difference was seen in suppressing the growth of *S.rolfsii* fungi colony biomass, with growth suppression reaching 88.10% and 87.22%, but significantly different from leaf oil, rhizomes and other fractions . Leaf oil, rhizome and D4 fraction also did not show a statistical difference, but significantly different from the C3 fraction with the lowest growth suppression of 72.55% at the same concentration level (Table 4).

From Table 1-4 shows that the fraction A1, A2, leaf oil and wild ginger rhizome have a high antifungal power against the *S rolfsii* fungi significantly different from the C3 fraction and the D4 fraction. The main components contained in wild ginger leaf oil are 2-Decanoic acid (48.04%), nonanoic acid (9.18%), 2-octenal (8.97%), hexanoic acid (2.46%), 6-tetradecene (2.35%), nonanal (2.06), ascorbic acid (1.66%), heptanoic acid (1.61%) and octanal (1.20%). In the main content of rhizome oil are 2-Tridecenal (39.81%), 2-decanoic acid (26.39%), 2-octenal (7.56%), hexadecanoic acid (2.86%), nonanoic acid (3, 85), 2-deconyl acetate (2.31%), Eucalyptol (2.13%). In the A1 component, the main components are 2-Decanoic acid (27.24%), 2-octenal (17.01%), decenal (12.40%) and nonanoic acid (1.37%). The main content of B2 fraction was Decanal (38.31%), octanal (8.42%), 6-tetradecene (3.24%), 2-octenoic acid 2.03%), and octenal (1.56%). C3 fraction containing 2-Tridecenal (26.57%), decanal (25.65%), 2-octenal (7.75%), 2-propenoic acid (5.11%), 2-decenyl acetate (5) , 44%), benzaldehyde (3.87%), and dedecenal

(2.35%) and the D4 fraction of the uatam content was 2-Tridecenal (19.41%), 2-dimethyl (3chloropropyl) sililoxymethyltetra (16.99%) , 2-octenal (16.08%), 1-ethyl-1- (undec-10-enyl) oxy-1-silacyclopenta (15.99%), decanal (2.87%), octanal (2.84%), 6-tridecene 2.79% (Nasir & Nurmansyah, 2016; Nasir, 2016).. Each component has not been tested, therefore further research is recommended. Antifungal essential oil chemical components that are able to penetrate the fungal cell walls, thereby causing interference with the metabolic processes in the cell so that it interferes with cell growth, at certain concentrations will result in the death of the fungal cells.

Table 4. Interactions of leaf oil, rhizome and *E. slahmong* fraction with concentration levels on growth of *Sclerotium rolfsii* colony biomass (4 days after inoculation)

Treatments	Colony biomass (mm)	Inhibition growth (%)	
Leaf oils (Md)			
0 ppm	153.25	3.31	l
100 ppm	90.00	43.21	i
250 ppm	53.50	66.24	f
500 ppm	21.75	86.28	bc
Rhizome oils (Mt)			
0 ppm	153.25	0.31	l
100 ppm	93.00	41.63	j
250 ppm	54.25	65.93	f
500 ppm	22.50	85.81	c
Fraction A1			
0 ppm	153.25	3.31	l
100 ppm	86.50	45.42	g
250 ppm	49.00	69.08	e
500 ppm	19.00	88.01	a
Fraction B2			
0 ppm	153.25	3,31	l
100 ppm	88.75	44.00	hi
250 ppm	50.50	68.13	e
500 ppm	20.25	87.22	ab
Fraction C3			
0 ppm	153.25	3.31	l
100 ppm	116.25	26.65	k
250 ppm	87.00	45.27	gh
500 ppm	43.00	72.55	d
Fraction D4			
0 ppm	153.25	3.31	l
100 ppm	93.75	40.85	j
250 ppm	54.00	65.93	f
500 ppm	22.75	85.65	c

Note. The numbers followed by the same letter are not significantly different according to DMRT. Test at 5% level.

From the above results it is clear that decanoic acid is more fungicidal than decanal, (Kumar *et al*, 2011), reporting that decanoic acid (capric acid) and its esters have been used in medical science and are the best antifungal. The decanal and nonanal are antifungal components that can inhibit the growth of sclerotia of *Sclerotinia sclerotiorum* that causes stem rot disease

from the canola and stem rot from the sunflower. Decanoic acid and nonanoic acid at a concentration level of 100 ppm have been able to kill spores from the fungus basidiomycetes that cause brown root disease, pentanoic acid and hexanoic acid are effective against test fungi at a concentration level of 1000 ppm (Schmidt, 1984 in Clausen *et al*, 2010). Essential oils from wild Ginger leaves can control the pathogen *Colletotrichum gloeosporioides* that causes Antrachnose disease in the red dragon fruit plant *Hylocereus polyrhizus*. At the concentration level of 1000 ppm leaf oil can suppress the growth of pathogenic *C. gloeosporioides* fungal colonies 100% (Nasir & Nurmansyah, 2016).

According to Lim in (Picheansoonth & Yupparach, 2007), stated that the results of the *Elettariopsis* spp oil analysis of the components inside are almost the same as slight differences such as *E. elan*, the main components are monoterpenes, geraniol 71.6%, comphane and phellandrena. *E. smithiae*, is the main component of monoterpenes, geraniol 38.10%, neral 29.10%, comphane and fancyacetate. *E. triloba* main components are 16,16% citral, limonane, phellandrena and terpene acetate. The active compounds contained in *E. slahmong* oil according to CK Lim are terpenoid components, (E)-2-octenal (46,3%) and (E)-2-decenal 36,8% rhizomes and roots with the main content (E)-2-decenal 79.4% (Wong *et al*, 2006).

Botanical fungicides that have been tested and are effective against *S. rolfsii* fungi are essential oils from Casia vera (*Cinnamomum burmanii*) leaves and twigs with a concentration level of 500 ppm which can suppress the growth of *S. rolfsii* colony 100% (Nurmansyah, 2014) and essential oils from several wild sirih such as sirih hantu, sirih cambai, sirih kaduak and sirih are quite effective in suppressing the growth of this fungus (Nurmansyah, 2012). Bamboo piper essential oil (*Piper aduncum*) at a concentration level of 500 ppm is able to control the pathogen *S. rolfsii* reaching 92.77% (Nurmansyah, 2016). This wild ginger fungicide has the bioprospek to be developed because the results are not significantly different from the bamboo piper and cinnamon fungicides.

4. Conclusions

The results of the study concluded that leaf oil and rhizome of wild ginger *Elettariopsis slahmomong* and their fractions have the potential to be used as botanical fungicides. The A1 fraction has the best antifungal effectiveness compared to leaf oil, rhizome and other fractions, leaf oil and rhizome oil are not statistically significantly different, but in terms of leaf oil is better than rhizome oil. The best concentration level in this study was 500 ppm with the highest emphasis on diameter and biomass growth of 81.74% and 84.25%.

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