



Essential Oil Components Identification of Siam Weed Leaf (*Chromolaena odorata* L.) From West Kalimantan and Activity Tests on Rubber Tree Termite *Coptotermes curvignathus* Holmgren

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A B S T R A C T

Research on siam weed (*C. odorata*) essential oil from West Kalimantan has been conducted. This study aims to determine the components of Siam weed (*C. odorata*) essential oils and the activity of rubber tree termites, *Coptotermes curvignathus* Holmgren. Siam weed leaf essential oil was obtained by the steam distillation method for 6 hours and analyzed using the GC-MS method. The yield of Siam weed (*C. odorata*) essential oil was obtained at 0.0623% with a density of 0.901 gr / mL. The results of GC-MS analysis showed that some of the main components of the siam weed leaf (*C. odorata*) essential oil compound were geijerene (26.94%), germacrene (26.44%), trans-caryophyllene (13.29%), and α -pinene (6.44%). The activity of essential oils on termites was used antifeedant bioassay test method with concentration 2; 4; 6; 8 and 10%. The best results for mortality are in the range of 6 to 10%, with a mortality percentage of 91.33 to 100% and an LC₅₀ value of 2.338%. Reduction in feed paperweight of essential oils in concentration 2 to 4% is in the range 1,417 to 10,278%.

INTRODUCTION

Siam weed (*Chromolaena odorata*) plant is one of the plants in the Asteraceae family that has the potential to produce essential oils. Plant species are quite widely spread as wild plants that are not utilized in West Kalimantan. Besides being known as Siam weed, this plant is also known as Kirinyuh, botto-botto, kopasanda, and laruna (Saputra et al., 2017). According to Munte and Lubis (2016), Siam weed leaf extract contains secondary metabolites in the form of alkaloids, flavonoids, steroids, terpenoids, saponins, and essential oils (Owolabi et al., 2010). Essential oils found in Siam weed leaves have components the main compounds are α -pinene (20.7%), pregeijerene (14.6%), geijerene (12%), β -pinene (10.3%), and germacrene-D (9.7%) (Félicien et al., 2012). According to Kiran et al. (2006) that geijerene and

pregeijerene compounds are insecticides that can be used as insect control agents. Also, according to Siramon et al. (2009) that α -pinene compounds have anti-crawl activity.

Several studies have reported that species of *C. odorata* have been known to have acted as pest control. Hadi (2012) found that siam weed leaf extract containing secondary metabolite compounds such as terpenoids, tannins, saponins, and sesquiterpenes can cause termite deaths with an LC50 value of 2.50%. Research that has been done by Udebuani et al. (2015) that Siam weed leaf extract was toxic to cockroaches (*Periplaneta americana*).

Based on several studies of the Siam weed plant, it shows that this plant has enough potential insecticide. Therefore, researchers will characterize the components

of Siam weed leaf essential oil from West Kalimantan and conduct activity tests on the termites of the *Coptotermes curvignathus* Holmgren. Although research on this plant has been done a lot, the difference in the habitat of a plant will affect the component content of chemical compounds of a plant (Munte and Lubis, 2016). The plant's chemical compounds content is also influenced by differences in the environment in which it grows, the age of the plant when it is harvested, and the age of the leaves used (Febrianasari, 2018).

METHODS

Sample preparation

The sample used in this study was the Siam plant leaves. Samples taken came from the village of Kuala Mandor B, Kubu Raya Regency, West Kalimantan Province. The sample was determined at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak.

Isolation of essential oils by steam distillation method

A total of 26 kg of fresh siam weed leaves that have been cut into pieces are put into the sample collection kettle and gradually distilled steam. The distillation process was conducted for 6 hours. Furthermore, the essential oil which was still mixed with steam was separated by separating funnel. The essential oil was then added to anhydrous Na_2SO_4 to obtain a water-free oil. Furthermore, the percent yield of essential oils was calculated using the following equation.

$$\text{Yield \%} = \frac{\text{mass oil (g)}}{\text{mass sample (g)}} \times 100\%$$

GC-MS analysis of siam weed (*C. odorata*) essential oils

Siam weed leaf essential oil was analyzed using the GC-MS instrument at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Yogyakarta.

Density

The specific gravity of essential oils is determined by weighing the pycnometer that has been cleaned and dried. Next, consider the pycnometer, which contains 5 ml of essential oil samples. This treatment was done three times. Specific gravity is calculated by the equation (Moeksin et al., 2011).

$$\text{Density} = \frac{W_s - W_o}{V_p}$$

W_s : Weight of the pycnometer contained

W_o : Weight of an empty pycnometer

V_p : Pycnometer volume

Preparation of the termites of the *Coptotermes curvignathus* Holmgren

The termite preparation refers to the research of Tafsir et al., (2015) the termite of *Coptotermes curvignathus* Holmgren was obtained from termite-affected trees in rubber plantations in Sungai Ambawang Village, Kubu Raya, West Kalimantan. The tree part that is attacked by termites is cut into pieces and put into a container for maintenance. Termites are covered with black cloth and carried out maintenance for one month.

Siam weed (*C. odorata*) leaf essential oil activity test on *Coptotermes curvignathus* Holmgren soil termites

The test of essential oil activity on *C. curvignathus* Holmgren's termites modified Ohmura et al. (2000) using the antifeedant bio-assay test method. Testing of essential oils against termites using test cups made of cylindrical plastic cups with a diameter of 10 cm in diameter, 8 cm in diameter and 5 cm in height. A total of 50 grams of sterile sand is put into the test glass. After that, it is stored in a plastic container that has been covered with wet cotton. The test glass scheme is shown in Figure 1.

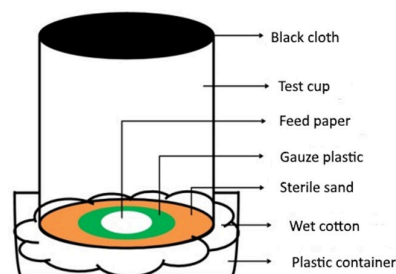


Figure 1. Termite testing scheme

Testing of essential oils using Whatman No. paper 41, which is used as bait paper. Feed paper with a diameter of 4.5 cm is dried in an oven at 105 °C for 3 hours. Once in the oven, the feed paper is stored in a desiccator for one day. The feed paper was weighed to find out its initial weight. After that, the paper feed was immersed for 1 hour in essential oils of siam weed leaves (*C. odorata*) which had been dissolved with diethyl ether at various

concentrations of succession, i.e., 0% (negative control), 2%, 4%, 6%, 8 %, 10% (v / v) and 0.25% (positive control) of the reagent brand fipronil.

The dried bait paper was placed on top of the veil in a test glass containing 50 termites consisting of 45 working termites and five soldier termites. The test beaker is then covered using a black cloth and stored in a dark room for seven days. Every day dead termites are counted and discarded. After seven days of testing, the feed paper was removed and dried in an oven at 105°C for 3 hours then stored in a desiccator for one day. The feed paper is then weighed to find out the paperweight after feeding. This test was done three times

Calculation of termite mortality and reduction in paper feed weight

Data taken from this research are termite mortality and weight loss in bait paper. Termite mortality percentage is calculated using the following equation:

$$A = M / 50 \times 100\%$$

A : Termite mortality percentage (%)

M : Number of termite deaths

The percentage weight loss of the test is calculated using the following equation:

$$KB = (Wa - Wb) / Wa \times 100\%$$

KB : Losing Weight

Wa : Weight of filter paper before being fed to termites (g).

Wb : Weight of filter paper after being smashed into termites (g).

Data obtained from this research are termite mortality and paperweight reduction. Termite mortality data was then determined by the equation of the regression line between log concentration and probit analysis to determine the value of LC₅₀ (Lethal Concentration) using the statistical IBM SPSS program 20 (Zuzani et al., 2015).

RESULTS AND DISCUSSION

Sample Determination

The determination was done aims to determine the species of the sample used. Based on the results of the

decision of Siam weed plants conducted at the Biology Laboratory of FMIPA, UNTAN stated that the plants used in this study were *Chromolaena odorata* L.

Isolation of Siam weed (*C. odorata*) essential oil by steam distillation method

Isolation of siam weed (*C. odorata*) leaf essential oil was obtained using the steam distillation method. Before the distillation process, 26 kg of Siam weed leaves, which were still fresh, were cut into small pieces. The process of cutting the sample was done so that the oil glands can open as much as possible so that essential oils can come out quickly. Distillation of Siam weed (*C. odorata*) essential oil for 6 hours produced essential oil and water to form 2 phases. Essential oils that still contain a small amount of water were dried with the addition of anhydrous sodium sulfate (Na₂SO₄) to bind the remnants of water so that free water can be obtained. The results of essential oils obtained in this study have physical characteristics in the form of bright yellow color, the typical odor of Siam weed with a density of 0.901 gr / mL. The yield of siam weed (*C. odorata*) essential oil was obtained at 0.0623%. The results of the isolation of Siam weed (*C. odorata*) essential oils by the steam distillation method can be shown in Figure 2.

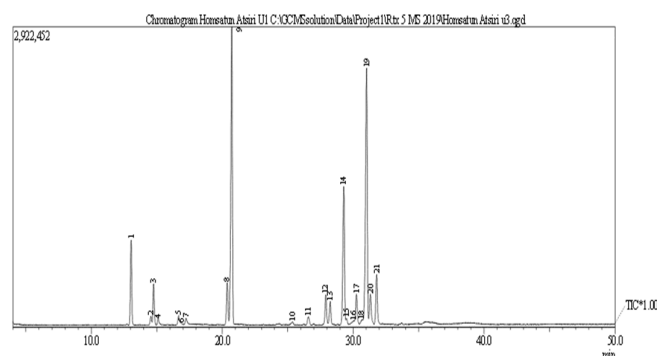
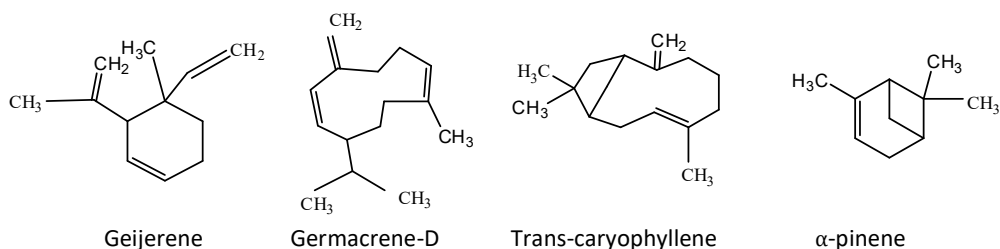


Figure 2. Siam weed leaf (*C. odorata*) essential oil

GC-MS analysis of siam weed (*C. odorata*) essential oils

The identification of the components of Siam weed (*C. odorata*) essential oil was carried out by GC-MS analysis. Chromatograms of siam weed (*C. odorata*) essential oil can be shown in Figure 3.

Based on the results of chromatograms, there were 21 peaks of compounds contained in Siam weed essential oils from West Kalimantan. The results of the GC-MS analysis of the components of Siam weed leaf essential oil compounds are shown in Table 1.

Figure 3. Siam weed essential oil chromatogram (*C. odorata*)Figure 4. Structure of the main compounds of Siam weed (*C. odorata*) essential oilsTable 1. Components of Siam weed (*C. odorata*) essential oils from West Kalimantan

Top	Retention Time	% Area	SI	Compound
1	13.036	6.44	93	α-Pinene
2	14.542	0.71	92	Sabinene
3	14.775	3.18	95	β-pinene
4	15.111	0.52	91	Myrcene
5	16.673	0.80	94	Cyclohexene, 1-methyl-4-(1-methylethenyl)
6	16.873	0.13	60	Cyclopropene, 1-methyl-3-(2-methylcyclopropyl)
7	17.226	0.54	91	Trans-ocimene
8	20.378	3.63	92	Pregeijerene
9	20.731	26.94	93	Geijerene
10	25.360	0.17	82	3,5-decadiyne, 2,2-dimethyl
11	26.582	0.86	89	Bicyclo (3.1.0) hexene, 6-isopropylidene-1-methyl
12	27.912	2.58	90	α-copaene
13	28.243	2.00	93	2,4-Diisopropenyl-1-Methyl-1-Vinyl-Cyclohexene
14	29.281	13.29	93	Trans-Caryophyllene
15	29.492	0.50	80	3,5-dodecadiyne, 2-methyl
16	30.008	0.16	70	α-cubebene
17	30.247	2.68	94	α-humalene
18	30.625	0.60	76	6,10,11,11-tetramethyl-tricyclo
19	31.032	26.44	89	Germacrene-D
20	31.323	3.18	90	Bicyclogermacrene
21	31.794	4.64	86	1H-cyclopropana naphthalene

Based on Table 1, it is known that 4 component compounds have % area with a large enough abundance. These results show the existence of 4 main compounds contained in Siam weed (*C. odorata*) essential oils from West Kalimantan. The main compounds contained in the essential oils of siam weed leaves (*C. odorata*) are geijerene (26.94%), germacrene (26.44%), trans-caryophyllene (13.29%), and α -pinene (6.44%). The structure of the main compound components of Siam weed (*C. odorata*) essential oils can be shown in Figure 4.

Siam weed (*C. odorata*) Essential Oil Activity Test Against *Coptotermes curvignathus* Holmgren Soil Termites

The termite of the *C. curvignathus* Holmgren was taken from a termite-affected tree in the rubber plantation in Sungai Ambawang Village, Kubu Raya Regency, West Kalimantan. The termites obtained are stored in a plastic container and covered with a black cloth. According to Nandika et al. (2003), termites have cryptobiotic properties that do not like light, so that in this treatment termites are made with dark environmental conditions. *C. curvignathus* Holmgren termites are maintained for one month so that the termites can adapt to the new environment so that healthy, still active termites are obtained. During one month of maintenance, it appears that the termites are still in a healthy condition and actively moving. In these conditions, the termites are ready to be used as test animals.

Testing the activity of siam weed (*C. odorata*) essential oil on the termites of *C. curvignathus* Holmgren carried out variations in the concentration of 0% (negative control), 2%, 4%, 6%, 8%, 10%, and 0.25% (positive control). The positive control used is the fipronil regen brand. This treatment lasted for seven days of feeding and was carried out three times. Activities that can be seen from this test are termite mortality and weight reduction in bait paper.

Termite Mortality

The parameter used in the antipyretic activity test of Siam weed essential oil is termite mortality, which was calculated every day. Termite mortality data can be seen in the following table.

Table 2. Mortality of the termites of the *Coptotermes curvignathus* Holmgren

Treatment	Termite Mortality (%)
Control (-) dietil eter	12%
essential oil 2%	41,33%
essential oil 4%	76%
essential oil 6%	91,33 %
essential oil 8%	100%
essential oil 10%	100%
Control (+) fipronil 0,25%	100%

The observation of termite mortality for seven days of view showed that termite mortality increased with increasing concentrations of essential oils. It is supported by Wibaldus et al. (2016) that showed the higher the concentration of essential oil is given, the more termites that die. Siam weed leaf (*C. odorata*) essential oils at concentrations of 8% and 10% have the highest activity level, which causes termite mortality by 100%. At the same time, the concentration of 2% had a moderate activity level with termite mortality of 41.33%. But on the other hand, in the negative control, termite mortality only reached 12% for seven days of observation. The level of termite death activity that occurs in negative controls appears to be quite low. In this case, it shows that the termite's resistance in this condition is quite high.

The results of this study indicate that fipronil activity is more robust when compared to the essential oils of siam weed leaf (*C. odorata*). It proves that Siam weed leaf essential oil caused 100% death at a concentration of 8%, while positive control at a concentration of 0.25%, already caused 100% of termite deaths. According to Nandika et al. (2003), Fipronil has a mechanism to disrupt the central nervous system, especially interference with the exchange of chloride ions through Gamma Amino Butyric Acid (GABA) in insects.

Data analysis was performed using the IBM Statistics 20 probit analysis program to determine the LC₅₀ value. The results of the Siam weed leaf (*C. odorata*) essential oil activity on the *C. curvignathus* Holmgren soil termite showed an LC₅₀ amount of 2.338% with an R² value of 0.997. The results of observing the death of the termite *C. curvignathus* Holmgren can be seen in Figure 5.



Figure 5. Death of the termites of the *Coptotermes curvignathus* Holmgren

Termite death is thought to be due to the presence of toxic compounds in the essential oils of Siam weed leaves, which can kill the symbiotic protozoa in the termite's intestine through disruption of enzyme activity. Cellulose enzyme is an enzyme released from the symbiotic protozoa to decompose cellulose into simpler substances so that it is easily digested so that it will obtain a useful energy source for its development and growth. Thus, if the protozoa in the termite's intestine die, then the enzyme activity will be disrupted so that it will result in the termite not having the ability to decompose the feed paper that has been eaten. As a result, termites do not get energy so that it will cause termite death (Syafii, 2000). Another possibility is that termite death is caused by the content of toxic compounds that can damage the nervous system of insects, causing termite death (Hadi, 2012).

The main compounds in the essential oils of Siam weed leaves (*C. odorata*) are geijerene, and germacrene-D, trans-caryophyllene, and α -pinene. These compounds are thought to have a toxic power against the termites of the *C. curvignathus* Holmgren. Geijerene compound is one of the most abundant compound components in the Siam weed (*C. odorata*) leaf. According to Kiran et al. (2006) that geijerene compounds are insecticides that can be used as insect control agents. Besides, germacrene-D and trans-caryophyllene compounds are also the main compounds in Siam weed leaf essential oil. Barakat (2011) once reported that germacrene-D and β -caryophyllene compounds have insecticidal properties. The three compounds are sesquiterpenes. Sesquiterpenes can damage the nervous system in termites that can cause termite death (Hadi, 2012). Whereas on the other hand, the α -pinene compound is a monoterpene compound that is thought to have a toxic power against termites, as reported by Siramon et al. (2009) that α -pinene compounds have activities as anti-crawl. Thus, termite deaths are caused by the presence of active compounds

contained in essential oils of Siam weed leaves (*C. odorata*). Some flavonoids such as quercetin, taxifolin, 3-hydroxyflavones, and 3-hydroxyflavanones have been reported as antifeedant activity against the termite *Coptotermes formosanus* Shiraki (Ohmura et al., 2000).

Reduction in feed paperweight

Another parameter used in anti-crawl testing is to calculate the reduction in feed paperweight after seven days of feeding. Calculation of reduced paper feed weight is carried out to determine the toxicity of the assessment of feed paper consumption rate by termites (Aslamiyah, 2017). The reduction in the weight of the feed paper can be shown in Table 3.

Table 3. Reduction in feed paperweight

Concentration (%)	Reduction in feed paperweight
Control (-) 0%	19,145%
2 %	10,278%
4 %	8,786%
6 %	7,543%
8 %	5,01%
10 %	1,417%
Control (+) fiproni 0,25%	0%

Based on Table 3 shows that the weight of the paper feed decreases with increasing concentration. At a concentration of 10%, it causes the smallest percentage of paperweight reduction with an average of 1,417%. Whereas at the concentration of 0% (negative control), it caused the most significant proportion of paperweight reduction with an average of 19.145%. The negative control has the most significant percentage reduction in paperweight compared to all treatment concentrations containing Siam weed leaves (*C. odorata*) essential oils. It shows that the essential oil of Siam weed leaves (*C. odorata*) has an active role in inhibiting the feeding activity of *C. curvignathus* Holmgren soil termites. The reduction of feed paper after seven days of feeding to *C. curvignathus* Holmgren termites can be seen in Figure 6.

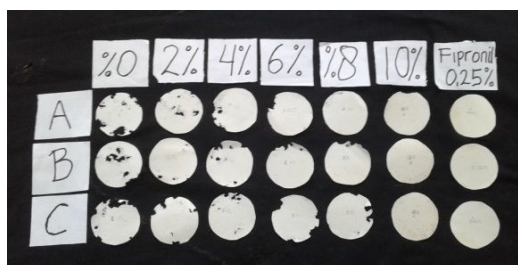


Figure 6. Reduction in feed paperweight

Figure 6. shows that the activity of eating termites decreases with increasing concentrations of Siam weed (*C. odorata*) essential oils. Thus, the higher the concentration of essential oil given to the paper feed, the reduction in the weight of the paper feed is smaller while termite mortality is increasing. It shows that the toxicity of Siam weed leaves (*C. odorata*) essential oils on the feed paper is expanding along with the increasing concentration of siam weed leaves (*C. odorata*) essential oils.

CONCLUSIONS

Based on the results of this study, it can be concluded that the yield of Siam weed (*C. odorata*) leaf essential oil from West Kalimantan is 0.0623%, which has a bright yellow color and a distinctive odor of Siam weed with a specific gravity of 0.901 gr / mL. Based on the results of GC-MS analysis of Siam weed leaf (*C. odorata*) essential oil, the main compound components are gerjerene (26.94%), germacrene-D (26.44%) and trans-caryophyllene (13.29%), α -pinene (6.44%). The activity of Siam weed (*C. odorata*) essential oil on the rubber tree termite, *Coptotermes curvignathus* Holmgren, showed an LC₅₀ value of 2.338% with an R² value of 0.997.

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