CHEMICAL ENGINEERING TRANSACTIONS
VOL. 89, 2021
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Jeng Shiun Lim, Nor Alafiza Yunus, Jiří Jaromír Klemeš

ISBN 978-88-95608-87-7; **ISSN** 2283-9216
DOI: 10.3303/CET2189052

Production of Bioherbicide from Sembung Vine (*Mikania Micrantha*) Origin of Bukit Lama Palembang

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In crop control, weed is considered as a harmful plant. Weed can be controlled by applying the biochemical compound from allelopathic plants, which are environmentally friendly. In this study, a type of allelopathic plant, Sembung Vine (*Mikania micrantha)* that grow in Bukit Lama Palembang, was processed as a bioherbicide. A phytotoxicity test was conducted on the plant extract in methanol and extract mixture of n-hexane: ethyl acetate. The test was applied on the mung bean (*Vigna radiata*) seeds that were grown on a petri dish to analyze the germination, seedlings length, inhibitory effect, and biomass weight for methanol extract at 5; 10; 15; 20 % (w/v). The crude methanol extract was fractionated with the mixture of n-hexane: ethyl acetate (1:0; 9:1; 8:2; 7:3; 6:4; and 0:1). Each sample was tested at 4,000 ppm crude extract concentration to determine phytotoxicity effects. All samples were in triplicates. The methanol extract showed the highest inhibition of 99.4 % at 20 % (w/v). The fraction of *M.mircrantha* extract with n-hexane: ethyl acetate eluent showed the highest inhibition of 55.48% at 6:4 ratio.

1. Introduction

The use of herbicides as one of the techniques for weed control is widely applied nowadays, which are synthetic herbicides with high resistance in the infested soil structure. Furthermore, herbicides can have a detrimental effect on the environment if used frequently. Bio-herbicide can be an alternative to substitute synthetic herbicides (Kremer, 2019). One of the plants that can be processed into bioherbicide is the sembung vine (*Mikania micrantha*). Sembung vine (*M. micrantha*) is a tropical plant with another name bitter vine, climbing hemp vine, or American rope. Sembung vine (*M. micrantha*) proliferates in tropical locations with high temperature, humidity, high light intensity, and high fertility soils (Indriyani and Alkhadi, 2021). This plant can be found in open locations, it vines on bushes and small trees, forming a thick bush. The vines were identified as having many phytochemical compounds in the form of flavonoids and terpenoids (Raka et al., 2019). This compound is alleged to have an allelopathic effect on the affected plants. Most of these compounds are found in the leaves of *M. micrantha* as a byproduct of metabolism. The flavonoid content can be separated and utilized through extraction and fractionation processes.

Extraction is a separation process that consists of the separation process of a substance from a matrix. The solute distribution between the two phases is in equilibrium based on the partition theory, where the analyte moves from the matrix to the solvent (Navligio et al., 2019). In contrast to fractionation, extraction will take all the parts dissolved into the solvent in one unit. Fractionation is a separation process after extraction using various fractionators, resulting in an isolated compound based on its affinity.

This plant thrives in South Sumatra as a weed plant (Budiarti and Aniska, 2019) with their inhibitory mechanism in a plant community. Appropriate process variables and treatment are needed to obtain a product with a maximum repressive effect. Based on this background, this study was conducted to find a suitable chemical process to obtain *M. micrantha* extract with strong herbicide characteristics by analysing of the phytotoxicity effect of tested plants at a laboratory scale, namely mung beans (*Vigna radiata*). The goal was to identify the most active fraction of the *M. micrantha* extract, which inhibits mung beans growth.

Paper Received: 24 June 2021; Revised: 26 August 2021; Accepted: 17 November 2021
Please cite this article as: Haryati S., Lee C.T., Putri R.W., Rahmatullah ., Wardhani P.K., 2021, Production of Bioherbicide from Sembung Vine (Mikania Micrantha) Origin of Bukit Lama Palembang, Chemical Engineering Transactions, 89, 307-312 DOI:10.3303/CET2189052

2. Methods

The bioherbicides production carried out by two main process, namely process description on production and analysis product of herbicides.

2.1 Process description

The bioherbicides production was carried out by two main processes : process description and product analysis as a herbicide.

2.1.1 Feed treatment

M. micrantha was collected from the Bukit Lama area, Palembang City, South Sumatra. The plant leaves were separated from the stems and roots, then washed with clean water three times. Mung bean seeds for phytotoxicity tests were purchased from Indralaya Market. The leaves of M. micrantha were cleaned and dried in an oven at a temperature of 50 °C. The leaves were dried to a constant weight to determine the water content (%) and blended to become smooth powder. Methanol, n-hexane, ethyl acetate, and acetone were purchased from Merck Lab Chemicals.

2.1.2 Extraction *of M. micrantha* leaves

The dried and mashed leaves were soaked in methanol with a ratio of leaves and methanol about 1:10 (w:v) for 24 h. After the soaking process, the maceration results were filtered using a four-layer filter cloth, and the dregs were separated in different places. The dregs were then re-macerated using the same ratio of methanol. Maceration and re-maceration were carried out at an ambient temperature and dark bottles. The extraction was carried out ten times. The extract solution was dried in a rotary evaporator (Laborta 4000, Heidolph Instruments, Germany) at 40 °C under vacuum to obtain a concentrated extract. The concentrated extract was placed in a container and allowed to dry and solidified. The solid extract was then stored in a sealed bottle and placed in a dark room until further use. The solid extract was analysed for the components using Thin Layer Chromatography (TLC) by dissolving it in acetone using n-hexane as the eluent

2.1.3 Fractionation of *M. micrantha* extracts

10 g of the crude extract were dissolved in acetone and filtered using Whatman filter paper. The extract solution in acetone was put into a rotary evaporator and mixed with silica gel chromatography from Merck. The rotary evaporator was operated at 40 °C until all acetone was removed. The impregnated silica gel chromatography was removed and dried in an open container overnight. 100 g of new silica gel chromatography were also prepared in Vacuum Column Chromatography (VCC) and washed using n-hexane several times in the column under vacuum using a vacuum pump. The impregnated silica gel was placed on gel chromatography and fractionated using 200 mL eluent n-hexane: ethyl acetate at 1: 0; 9: 1; 8: 2; 7: 3; 6: 4; and 0: 1. Each eluent fraction was labelled as fraction F01, F02, F03, F04, F05 and F06.

2.2 Analysis

2.2.1 Phytotoxicity effect test

The methanol extract of *M. micrantha* leaves was tested for phytotoxicity effects (Aslani et al., 2013). The methanol extract was dissolved in distilled water at 5, 10, 15, and 20 % w/v. Mung bean seeds were used as indicators of the phytotoxicity effect. This test is performed on a laboratory scale. The Whatman filter paper was placed in the petri dish and moistened with 5 mL of the test solution. Each treatment was filled with 15 seeds and replicated three times.

The crude extract fraction was also tested for phytotoxicity effect using a similar method for methanol extract. The concentration used for the test was 4,000 ppm and assisted by 0.25 % v/v polysorbate 80 (Tween 80) surfactant, to determine the phytotoxicity activity of the fraction. The phytotoxicity effect test was carried out at room temperature with sunlight for 12 h and moistened with 1 mL distilled water per petri dish every day to keep it moist.

After six days, the number of sprouts was counted, the length of sprouts, inhibition and dry biomass weight were measured. Inhibition is calculated based on the Eq(1):.

$$\%_{inhibition} = \frac{C - A}{C} \times 100\ \% \quad (1)$$

% inhibition is the % of inhibition; C is the average radicle-hypocotyl length of the three replicates of the control; and A is the average radicle-hypocotyl length of the aquous extract (Aslani et al., 2013). The control variable is used as a comparison for inhibition.

2.2.2 Statistical Analysis

The research data were collected, and statistical analysis was carried out on the number of sprouts, the length of the sprouts, and the weight of wet and dry biomass. To determine the significance level between one treatment and another, a one-way analysis of variance (One Way ANOVA) was carried out at a probability level of 0.05. ANOVA was performed using IBM SPSS V23. The Levene test was performed to determine the homogeneity of the data. If the p-value is <0.05, the data have a different variance (non-homogeneous). The Welch's test could be used in this case, and the Games Howell post-hoc test would be required. On the other hand, if the p-value is > 0.05, the data is homogeneous, and the Tukey HSD (Honestly Significant Difference) post-hoc test is performed (Ostertagová et al., 2013).

3. Result and Discussion

3.1 Phototoxicity effect of *M. micrantha* crude extract on mung bean sprout

Figure 1 showed a decreasing trend in the number of sprouts as the methanol extract concentration increased. The average number of sprouts at 20 % was 3.33, while the highest sprouts (14.67) were achieved at the lowest extract concentration (5 %). Based on Figure 1, the downward trend in the number of sprouts shows that germination is a negative function of giving the extract concentration treatment. It shows a negative value, which means that there is a negative effect on the addition of extract per unit volume of water used on mung bean germination. Statistics represent the significance of each variation on the effect of increasing the amount of crude extract in water, except in control with 5 % w/v. The phenolic compounds in the methanol extract are predicted to be the cause of inhibition in the germination phase through oxidase inhibition and due to the antioxidant properties of phenol compounds in the crude extract (Tigre et al., 2015).

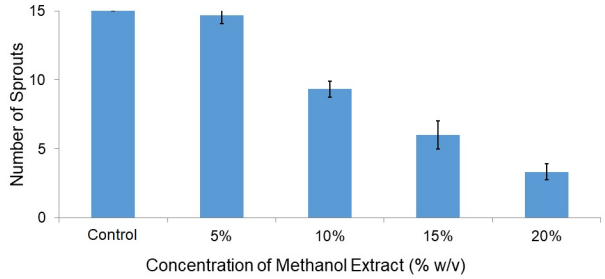

Figure 1: The effect of methanol extract concentration on mung bean sprouts

3.2 The effect of eluent on the product of *M. micrantha* crude extract fraction on the number of mung bean sprouts

The use of n-hexane was used as an eluent for polarity-based separation in column chromatography due to its low polarity to obtain non-polar compounds. This non-polar characteristic indicates by its elution strength from thin layer chromatography analysis, which can attract compounds that are also non-polar. Figure 2 shows the effect of eluent fraction on mung bean germination.

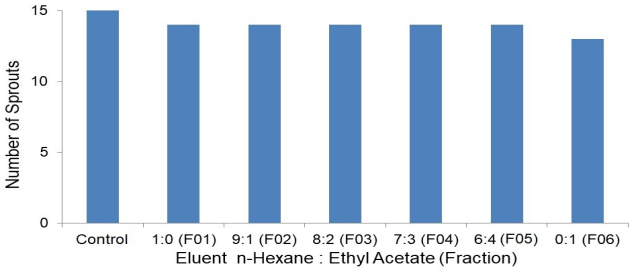

Figure 2: The effect of eluent fraction on mung bean germination

Based on Figure 2, all fractions (F01 to F06) similarly a0ffect on mung bean germination. F01 is the fraction with 1:0 n-hexane eluent, which has the weakest elution strength. The F01 is thought to have terpene with a smaller number of carbon atoms, such as monoterpenes. It was likely that n-hexane would attract terpene due to its non-polarity (Tanzi et al., 2012). This result agreed with the TLC data, where the F01 extract gave a reddish colour in the TLC plate (Jiang et al., 2016). Terpenes have a higher composition of carbon atoms composition that would be carried along with the eluents of higher polarity on F06 due to their higher elusive ability than the

pure n-hexane. The most polar fraction (F06) leads to higher inhibition that is influenced by the terpene compound. Terpenes have a composition of 15 carbon atoms, they can be readily carried away by the polar compound such as ethyl acetate or a mixture of n-hexane and ethyl acetate (Jiang et al., 2016).
The presence of non-polar compounds such as n-hexane did not significantly affect the inhibition in mung beans. Mung beans as dicots are quite resistant to non-polar compounds in all fractions studied. This result agrees that non-polar compounds such as monoterpenes had little effect on germination (Kordali et al., 2007).

3.3 Effect of crude extract concentration *M. micrantha* and eluent of crude extract concentration on mung bean sprouts length

5% crude extract did not have a significant effect on the mung bean sprout's length. A significant effect occurred among controls with 10 %, 15 %, and 20 % crude extracts compared to the control. It shows the slight effect in application of 10 % and 15% extract concentration. The most visible effect is between the 20% crude extract and the control, where the most significant inhibition occurs. In Figure 3, it can be seen the significant effect between the concentration of the crude extract and the average length of the sprouts. The higher concentration of crude extract leads to the smaller length of the sprouts. The crude extract will decrease the average length of the sprouts. The lowest average length of sprouts is at 20 % (0.06 cm).

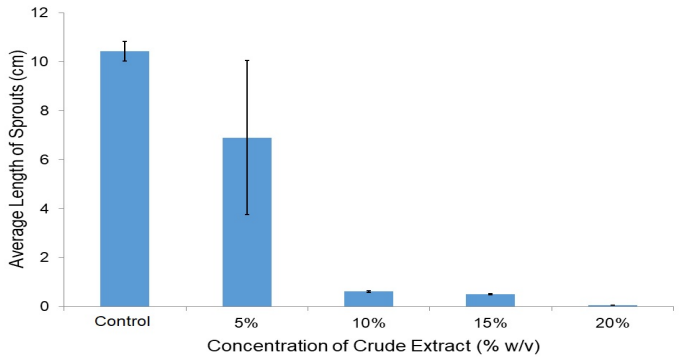

Figure 3: The effect of concentration of crude extract on length of mung bean sprouts

The inhibition process of Sembung's herbicides is also determining the length of sprouts. Figure 4 shows the average length of sprouts decreasing from F01 fraction at 1: 0 n-hexane: ethyl acetate to F06 fraction at 0: 1 n-hexane: ethyl acetate. Figure 4 shows the lowest average value is at 6: 4 n-hexane: ethyl acetate with a value of 0.45 cm. There was an increase in the F06 fraction at 0: 1 n-hexane: ethyl acetate to a value of 0.58. This increase is likely due to terpenes' which helps the growth after germination (Jiang et al., 2016). This effect results from the synergistic effect of terpenes, which is more polar in the swelling, but the inhibitory mechanism is still not recognized (Bakkali et al., 2008).

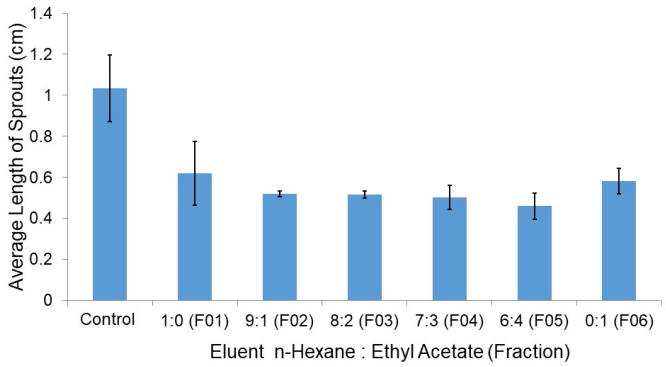

Figure 4: The effect of length of eluent fraction on mung bean sprouts

3.4 Effect of crude extract (*M. micrantha*) concentration on inhibition of mung bean sprouts

Figure 5 shows the relationship between inhibition of mung bean sprouts with the raw concentration extract (M. *micrantha*). The inhibition at 5 % shows the lowest *M. micrantha* toxicity. The addition of the extract concentration will increase the inhibition. The inhibition value was still below 50 % at 5 % treatment, but showed

a significant effect once treated at 10 %. This increase until up to 60 % provides an inhibitory effect at 50 % was achieved with 10 % and 5 % crude extract. The inhibition still increased in the following two treatments but tended to be transient. Inhibition of more than 99 % at 20 % extract per unit volume shows a high phytotoxicity effect. The value at 20 % of the crude extract is superior to nearly 100 %. The toxic effect of *M. micrantha* is considered potent because of its superior inhibiting effect. Methanol extract as the initial preparation shows that it can give a nearly complete effect in the presence of surfactant addition. The compounds in the methanol extract can change the growth through the distortion of the catalase enzyme.

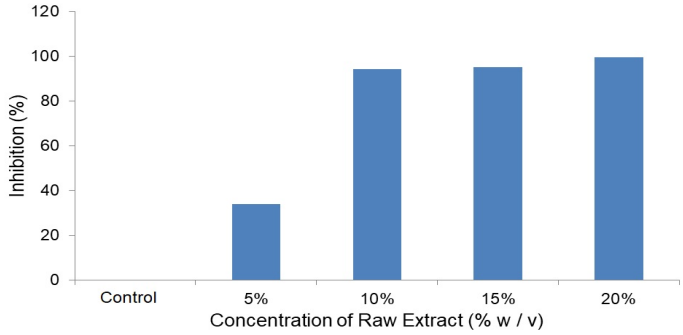

Figure 5: The effect of extract concetration on inhibition of mung beans

3.5 Effect of concentration of cracked Sembung raw extract (*m. micrantha*) on biomass weight of mung beans (*vigna radiata*)

The changes in the inhibition of the F01-F06 fraction was represented in Figure 6. Based on the inhibition data processing, the highest inhibition value was found in the F05 fraction. Data processing was carried out without ANOVA but using the average of each treatment on the length of the resulting sprouts. The inhibition value in the F06 fraction showed a decrease in the inhibition to 43.6 % from 55.5 % in F05. The increase in the inhibition of the F01 fraction on the control occurred significantly. As much as 40 % inhibition was found in the F01 fraction and an increase of 9.67 % in the F02 fraction. Inhibition reaches above 50 % at the eluent value of 9: 1 n-hexane: ethyl acetate. 7: 3 n-hexane: ethyl acetate, and 6: 4 n-hexane: ethyl acetate. Inhibition decrease below 50 % at 0: 1 n-hexane: ethyl acetate. Inhibition shows an optimum point at 6: 4 n-hexane: ethyl acetate, it is not certain whether or not there is a higher inhibition between 6: 4 n-hexane: ethyl acetate and 0: 1 n-hexane: ethyl acetate.

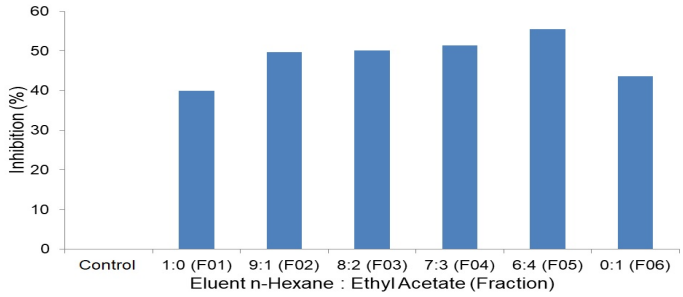

Figure 6: The effect of mung beans inhibition vs. eluent fraction

The inhibitory mechanism can explain the increase in inhibition by blocking the absorption of water and the oily compounds such as terpenes, assisted by the flavonoids included in the fraction. Terpenes are components of essential oil compounds in *M. micrantha*. Adding this compound to water will cause the viscosity to increase the surface tension, making it more difficult for the water to be absorbed. This is reinforced by the effect of the non-ionic surfactant Tween 80 which causes emulsion and participates in causing damage to plant cells (Chotsaeng et al., 2017).

3.6 Thin layer chromatography (TLC) analysis of crude methanol extract and fraction

The results of TLC in Figure 7a showed the most satisfactory component separation results in eluent n-hexane: ethyl acetate 7: 3. The analyte result of TLC at 6: 4 was too polar to reach the top of the plate boundary, while at 1: 0 it was too non-polar so that there were no visible components in the crude extract. There were at least

five large spots under UV light: after adding ceric sulfate, it only gave three blemishes. The results of TLC suggest that further separation is needed due to the stain that is still too thick. In Figure 7b, the TLC of crude extract fraction at 7 : 3 eluent shows a different TLC pattern because it is separated based on polarity. Each compound is also separated through this polarity, but many thick stains are still found, which indicates that the separation is not optimal. The stain produced by ceric sulfate showed the presence of terpenoids in the test.

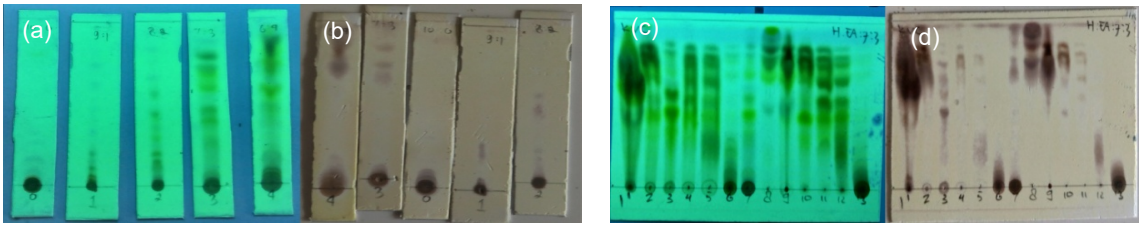

Figure 7: (a) Results of TLC analysis eluent n-hexane under UV, (b) *TLC analysis eluent n-hexane after cerric sulphate addition, (c)* TLC of Crude extract fraction at 7: 3 eluen*t under UV, (d) TLC of Crude extract fraction at 7: 3 eluent after cerric sulphate addition*

4. Conclusions

In this study, the dry weight of *M.micrantha* has a value of 10.4 % wet weight, while the total crude extract is 21.7 % dry weight in the ratio of dry leaves: methanol solvent of 1:10 w/v. The significant effect of *M. micrantha* crude extract concentration was at a concentration of 10 % w/v with the inhibitor achieving more than 90 % for the phytotoxicity test of mung beans compared to the control. The highest effect of eluent on the fraction of the *M.micrantha* extract was found in the 6: 4 n-hexane: ethyl acetate eluent composition for the phytotoxicity test at 4000 ppm against mung beans when compared to the control. In addition, the most potent *M. micrantha* extract was at a concentration of 20 % w/v, while the most active fraction is in the eluent composition of 6: 4 n-hexane: ethyl acetate.

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