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Allelopathic Potential of *Etlingera coccinea* (B.) Sakai & Nagam on Seed Germination and Growth of Mung Bean and Siam Weed

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ABSTRACT

Etlingera coccinea, a native Borneon Zingiberaceae are found to exert allelopathic effect on some weed species. The objective of this study is to investigate the allelopathic effects of hexane (80%), methanol (80%), ethyl acetate (80%) extracts from the dried powder of both stem and leaf of *E. coccinea* on mung bean (*Vigna radiata*) and Siam weed (*Chromolaena odorata*). The phytochemical screening of both stem and leaf crude extracts elicited saponin, tannin, flavonoid, and terpenoid, which are targeted bioactive compounds for allelopathy. The allelopathic activity was assessed by evaluating their effects on seed germination and percentage of radicle and shoot growth. The results showed that both stem and leaf extracts have a suppressive effect on the mung bean development during *in-vitro* bioassay. The methanolic extracts of both stem and leaf showed the most significant suppression (100%) than other solvent extracts. In the pot experiment, the methanolic stem extracts suppressed the Siam weed's germination by 57 ± 0.13% and the methanolic leaf extracts suppressed the growth by 46 ± 0.29%. The present study shows that *E. coccinea* methanolic extract has the potential as bio-herbicide.

Keywords: *Allelopathic potential, Etlingera coccinea, seed germination, bioherbicide*

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INTRODUCTION

Allelopathy is defined as any process of one plant that directly or indirectly affects the growth of other plants in their proximity through the production of secondary metabolites in the environment. These secondary metabolites are known as allelochemicals. They are present in all plant parts, but the concentrations are different between each plant parts [1,2]. These chemical compounds may inhibit or promote the growth of adjacent plants, weeds and animals, when released to the environment. Many studies on the screening and evaluation of allelopathic potential of plants have been conducted [3,4].

The allelopathic actions may vary from growth retardation of plant organs such as roots and shoots to the inhibition of seed germination. Cheng & Cheng in 2015 [5] asserted that allelochemicals such as phenolic compounds, tannins, terpenoids, flavonoids, and alkaloids are some of the few from a vast class of allelochemicals. These molecules are regarded as better alternatives in creating herbicides through allelopathy as they leave no toxic residues that may affect humans and the environment as the allelochemicals are biodegradable. Therefore, allelochemicals have the potential to be developed as a natural herbicide or bio-herbicide to replace common synthetic herbicide in weed management [1,6].

Etlingera coccinea (B.) Sakai & Nagam is a flowering plant from the family Zingiberaceae and subfamily Alpinioideae that is a native to Borneo, Malaysia, and Thailand. *E. coccinea* is widely consumed as pickles or salads and used in traditional medicine to cure ailment related to the gastrointestinal tract such as gastritis [7]. The *Etlingera* species are infamous for their high content of secondary metabolites such as saponins, flavonoids, tannins, alkaloids, terpenoids, and many other bioactive compounds [8,9]. These bioactive compounds are reported to exert allelopathic effect that retarded the growth of the several weed species, as conducted by Ibáñez and Blázquez [10]. These studies also revealed that the bioactive compounds released by members of the Zingiberaceae family shall have adverse effects on neighbouring plants, affecting the growth and development of other plants including crops.

The objectives of this paper are to identify the phytochemical constituents of the stem and leaf of *Etlingera coccinea* crude extracts and to evaluate their potential allelopathic effects on mung bean (*Vigna radiata*) using in-vitro bioassay and Siam weed (*Chromolaena odorata*) by pot experiment.

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EXPERIMENTAL

Plant Sample Preparation

The *E. coccinea* leaves and stems were purchased from the market in the district of Ranau, Sabah, Malaysia. The stems and leaves of *E. coccinea* were washed with running tap water to remove impurities and dirt before being cut into small pieces and dried in an oven for 3 days at 50°C until a constant weight was achieved. The plant materials were further ground to powder form using a blender and stored at 4°C until further analysis.

Extraction of E. coccinea Stems and Leaves

The extraction method used was adapted from Maimulyanti and Prihadi [11]. 10 g of powdered leaves was soaked in 100mL of 80% of 3 different solvents (hexane, ethyl acetate and methanol) each respectively, for 48 hours using cold maceration technique. The extracts was filtered using Whatman filter paper No. 1 and concentrated using vacuum rotary evaporator at 45°C. The same procedures was applied to the powdered stems of *E. coccinea*.

Phytochemical Screening

Phytochemical screening was conducted on the crude extracts by using standard qualitative methods as described by [11]. The extracts were tested for the presence of tannins, saponin, flavonoids, alkaloids and terpenoids.

In-Vitro Bioassay on Mung Bean (Vigna radiata)

In-vitro bioassay is a technique to assess the feasibility of herbicide study based on the response of the organism to the herbicide [12]. This experiment aims to observe allelopathic effects of different extracts on mung bean as initial test plant because mung beans can germinate easily. The crude extracts of the stem and leave of *E. coccinea* was used to test the phytotoxic effect on mung beans. The germination of mung beans was studied by Petri dish method as described by Sandin-Espana et al. [12]. Mung beans was purchased from the local store and only dense seeds was used in the experiment. To achieve optimum seed yield, the mung beans was first soaked for 4 hours and then sterilized using 70% ethanol.

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Water was used as the germination medium. Each petri dish with 10 seeds were placed on Whatman filter paper No. 1 which acted as the base for seeds germination. 2 mL of plant extracts were applied in each petri dish, while the control was only treated with water. Seeds were incubated in the laboratory at 26 – 27°C for 7 days. At every 2 days interval, 2mL of extracts were applied into each petri dish and water was added to the control. The germination percentage, radical length, and shoot length were recorded for 7 days. This experiment was done in triplicate. Germination percentage was calculated by the following equation [13].

$$\text{Germination (\%)} = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ tested} \text{ x } 100 \qquad (1)$$

Pot Experiment on Siam Weed (Chromolaena odorata)

Pot experiment was conducted as described by Alagesaboopathi [3]. The seeds of *C. odorata* were grown in 4 pots filled with soil to allow it for germination and growth. On the first day, the seeds were treated with 5 mL of methanolic extracts, while the control was treated with water. In-vitro bioassay showed that methanolic extracts of stem and leave of *E. coccinea* significantly inhibited seed germination and seedling growth of mung bean. Therefore, methanolic extracts were tested in the pot experiment. Syngenta Dual G960 herbicide was used as positive control. The pot was treated with 5 mL of plant extracts every 2 days interval. The germination percentage, radical length, and shoot length were recorded after 10 days. Experiment was performed in triplicate.

RESULTS AND DISCUSSION

Phytochemical Analysis

The result revealed the presence of tannins, flavonoids, saponins and terpenoids in the various crude extracts of leaf and stem parts of *E. coccinea* (Table 1). Similar composition of these compounds were detected in other *Etlingera* species [8], [14]. The absence of alkaloid in leaf and stem of *E. coccinea* was supported by [9]. Most of the compounds tested were present in methanolic stem extracts. This may be due to high extremity of methanolic dissolvable which can draw high variety of plant constituents compared to other solvents [15]. Sri Widyawati et al. in 2014 [16] asserted that ethyl acetate is a poor extracting solvent to extract secondary metabolites such as alkaloid, flavonoid, and tannin while hexane is a non-polar solvent and is usually used to extract oil and other non-polar compounds [15]. Therefore, the results indicated that methanol, the highly polar solvent, is favoured for extraction of allelochemicals.

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Table 1: Standard colour test for phytochemical screening of various crude extracts of dried leaf and stem parts of *E. coccinea*

Plant constituents	Hexane		Ethyl acetate		Methanol	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	-	-	-	-	-	-
Flavonoids	+	-	+	+	+	+
Saponins	+	+	-	-	+	+
Tannins	+	-	+	+	-	+
Terpenoids	-	+	-	+	+	+

+: present, -: absent

Seed Germination of Mung Bean (Vigna radiata) through In-Vitro Bioassay

The allelopathic potential of *E. coccinea* leaf and stem extracts on seed germination of mung bean were recorded in Figure 1. The germination percentage was found to be the highest with ethyl acetate leaf (EAL) extract (63%) after 7 days, followed by hexane stem (HS) extract (57%), ethyl acetate stem (EAS) extract (23%) and hexane leaf (HL) extract (16.7%). Both methanolic extracts of stem (MS) and leaf (ML) showed a 100% inhibitory effect on the germination of mung bean compared to water which serves as a control. It should be due to large amount of allelochemicals in methanolic extracts of *E. coccinea*. The allelopathic inhibition can involve the synergistic effect of different allelochemicals than individual compounds alone [5].

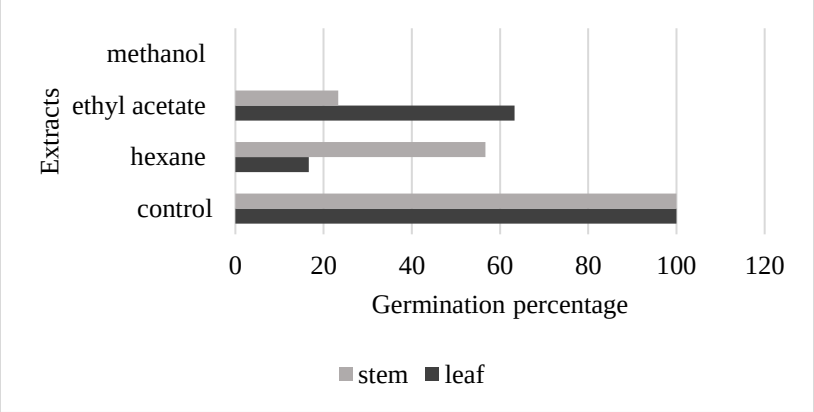

Figure 1: Effect of *E. coccinea* extracts on seed germination of mung bean after 7 days

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Radicle and Shoot Length of Mung Bean (Vigna radiata)

The inhibitory effect of *E. coccinea* leaf and stem extracts on the growth of mung bean was recorded by measuring the radicle and shoot length as shown in Figure 2. The radicle and shoot mean lengths were measured in the seeds treated with extracts in comparison to seeds of the control were 1.34 cm and 8.34 cm, respectively. Out of all *E. coccinea* extracts, no seedling growth was observed in MS and ML extracts treated mung bean. This highest inhibitory effect was recorded in EAS extract (0.34 cm), followed by HL extract (0.4 cm), EAL extract (0.6 cm) and the lowest in HS extract (0.8 cm). The treatment of mung bean with *E. coccinea* extracts has decreased the radicle lengths compared to control, while in treatment with methanolic extracts, the seed germination was completely inhibited.

Meanwhile, the highest inhibitory effect of *E. coccinea* extracts on the mean shoot length was MS and ML with zero growth, followed by HL extract (2.83 cm), EAS (3.23 cm), HS extract (3.97 cm) and EAL (4.5 cm). In observation of hexane and ethyl acetate treated mung bean, the radicle growths were stunted and did not develop into roots. Moreover, the cotyledon of seeds appeared abnormal compared to root formation in the seedlings in the control. Primary growth of plants is extremely sensitive to the concentration of bioactive compounds in their surrounding [17]. The effect of allelochemicals in *E. coccinea* may have led to the metabolic impairment of mung bean which resulted decreasing formation of normal root. Similar observation was reported by Arowosegbe [18]. Abnormalities in the root will eventually lead to reduction of nutrient intake and might lead to the death of the plant.

These results indicate that all crude extracts of *E. coccinea* showed allelopathic activities. The allelopathic activity efficiency corresponds to the phytochemical constituents, where methanol having the highest content of allelochemicals showed the highest inhibitory effects on the germination of mung bean.

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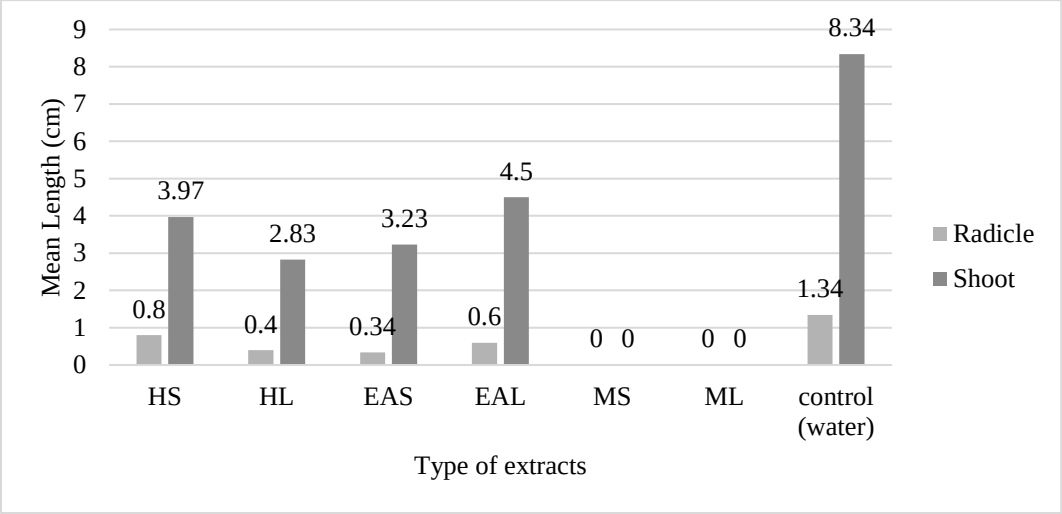

Figure 2: Mean radicle and shoot length of mung bean in different types of *E. coccinea* extracts. HS= hexane stem extract; HL=hexane leaf extract; EAS=ethyl acetate stem extracts; EAL=ethyl acetate leaf extract; MS= methanol stem extract; ML= methanol leaf extract

Pot Experiment Analysis on Siam Weed (Chromolaena odorata)

In-vitro bioassay is a method to determine the viability of herbicide tested against an organism based on the subject's response to the potential herbicide inside a laboratory environment [19]. It will be jointly tested together with a field experiment using pot method to evaluate the differences between a controlled environment (laboratory) and uncontrolled environment (field), as the uncontrolled environment may cause the phytotoxicity of the herbicide which are currently being tested, to either increase or decrease in the study [20-21]. A pot experiment was carried out to evaluate the phytotoxic effect of *E. coccinea* methanolic extracts as potential bio-herbicide on the Siam weed (*Chromolaena odorata*) in soil medium.

After 10 days of treatment with methanolic extracts, the germination, radicle and shoot growth were recorded and is shown in Figures 3 and 4. Results showed that ML and MS extracts greatly reduced the seed germination and seedling growth of the weed *C. odorata* when compared to the controls. The inhibitory effect was more pronounced in methanolic stem extract (57 ± 0.13%) than leaf extract (46 ± 0.29%) with reduction of radicle and shoot length. Torawene and Mokat in 2020 [22] reported that the allelochemicals could interfere the balance of different growth hormones and cell division thus affecting the seed germination and seedling growth.

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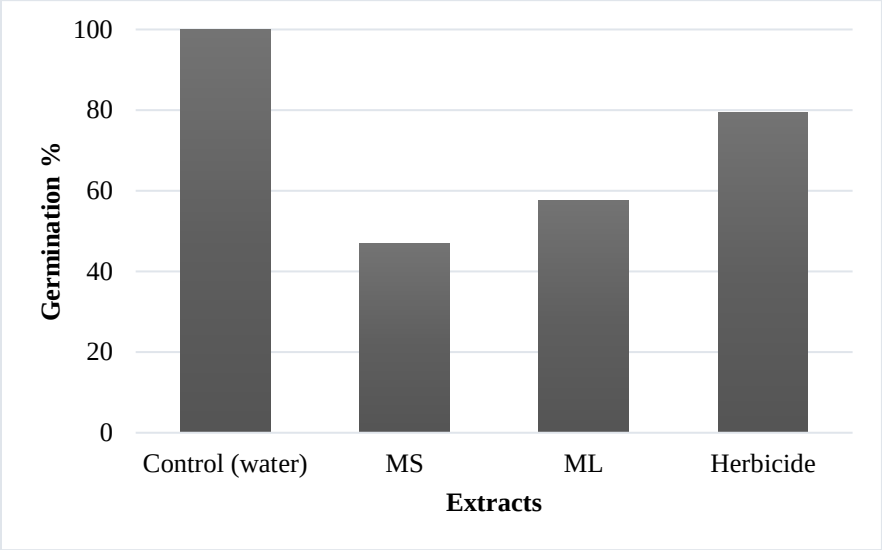

Figure 3: Germination percentage of Siam weed in ML and MS extracts after 10 days. MS= methanol stem extract; ML= methanol leaf extract

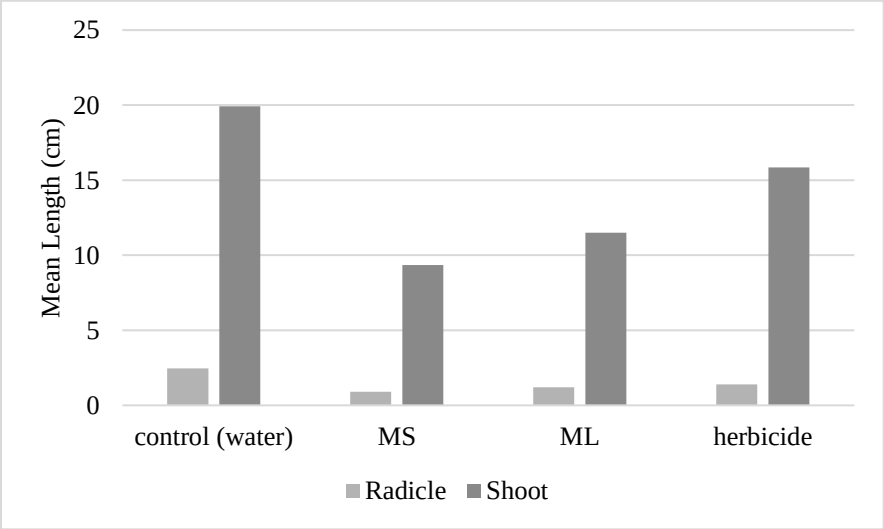

Figure 4: Mean radicle and shoot length of Siam weed in MS and ML extracts after 10 days. MS= methanol stem extract; ML= methanol leaf extract

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CONCLUSION

This preliminary study concluded that *E. coccinea* methanolic extracts have inhibitory effect to reduce the early growth of Siam weed. Methanolic stem extract had the most inhibitory effect as compared to other extracts. This provides the evidence of allelopathic potential of *E. coccinea* stem and leave parts as potential bioherbicide agent in weed management.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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