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# Eusiderin I from Eusideroxylon zwagery as Antifungal agent against Plant Pathogenic Fungus

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**Abstract :** The objective of the study was to investigate antifungal activity of Eusiderin I from *Eusideroxylon zwagery* by determining the inhibition zone of Eusiderin I against some pathogenic plant fungus. The antifungal activity were determined in a series of Eusiderin I concentration using agar well diffusion method. The antifungal activities of Eusiderin I (at 3, 4 and 5 ppm concentration) from *E. Zwagery* were tested against four plant pathogenic fungals such as *Fusarium oxysporum* f.sp. *lycopersici, Sclerotium roefsii*, *Rhizoctonia solani* and *Gliocladium fimbriatum.* Inhibition zone were compared with that of chloroform's as solvent. The results showed that the remarkable inhibition on the fungal growth was shown against the tested organisms. Eusiderin I, as major component of *E. zwagery* showed potent antifungal activity against *Fusarium oxysporum* f.sp. *lycopersici, Sclerotium roefsii* and *Rhizoctonia solani*. At 5 ppm concentration, it gave the most effective inhibition (49.80%) against the colony growth of *Fusarium oxysporum* f.sp. *lycopersici*. Whilst inhibitory activity against the growth of *Gliocladium fimbriatum* colony was not found. The result was in line with *Gliocladium fimbriatum's* nature as antagonist agent against various pathogenic plants and it is very well known as a biological control.
**Keywords:** Eusideroxylon zwagery, antifungal activity, Eusiderin I, Fusarium oxysporum f.sp. lycopersici, Sclerotium roefsii, Rhizoctonia solani, Gliocladium fimbriatum.

## Introduction

Bulian or iron wood (*Eusideroxylon zwagery*) is one of the timber forest with high economic value. *E. zwagery* is an endemic plant in which widely distributed throughout Jambi Province, Indonesia. It is a dense red-brownish durable wood, proofed to termite ubiquitous tropical wood-decayed insects and fungi[1,2,3,4]. As a consequence, the wood is widely used for construction materials such as bridge, boat, window frame, etc. It is particularly prominent resistance towards wood decayed fungi that put the wood as first class timber. Eusiderin I is a neolignan which isolated as major component from *E. zwagery*. It is found in leaf, stem, bark and root of this plant. Formation of secondary metabolites in plants related to ecological functions as the embodiment of

the plant interaction with the environment. The durability of Bulian wood (*E. zwagery*) is a manifestation of this kind of interaction[1,2,5,6].

A study on chemical potency of Eusiderin I as major component from the MeOH extract of heartwood of *E. zwagery* guided the isolation to antifeedant potency investigation[5,6,7,8]. Eusiderin I was firstly isolated by Hobbs, J.J and King, F.E in 1960[1]. However the biological activity as antifeedant was firstly reported by our research group. Eusiderin I showed potent antifeedant activity at a concentration of 0.01% against *Epilachna sparsa*[6]. In addition, it also could prevent *Etiella zinckenella* from destroying soybean, *Glicine max* at 0.5% concentration[7]. This finding leads to the reason why this plant has durable wood. It is concluded that Eusiderin I may play a role in the protection of the plant against insects and fungi[5,6,9].

The common names of plant diseases often reflect the type of symptom they cause. This fungal blight infects ornamental plants, vegetables, fruit trees, rices and shade trees worldwide. Infected shoots wilt and look blackened. On leaves, brown to black spots form and enlarge, developing concentric rings. Heavily blighted leaves dry up and die as spots grow together. Cankers usually form on woody stems and may be cracks, sunken areas, or raised areas of dead or abnormal tissue. Blights and diebacks due to cankers look quite similar. Cold-injury symptoms may look like, or lead to the development of, cankers and diebacks.This fungus attacks most hardwoods and some vines and shrubs. It is most damaging on maples. Small sunken areas appear on the bark near wounds, and small pink spore-producing structures are formed. It kills twigs and branches and may girdle young trees. Control by limiting pruning cuts and removing diseased branches[10]. Rots are diseases that decay roots, stems, wood, flowers, and fruits. Some diseases cause leaves to rot, but those symptoms tend to be described as leaf spots and blights. Rots can be soft and squishy or hard and dry. They are caused by various bacteria and fungi. Many are very active in stored fruits, roots, bulbs, or tubers. Rusts are a specific type of fungal disease. Many of them require two different plant species as hosts to complete their life cycle. Typical rust symptoms include a powdery tan to rust-colored coating[10].

Plant diseases caused by pathogen fungi such as *Fusarium oxysporum* f.sp. *lycopersici* (faded causal factor in tomato), *Sclerotium roefsii* (decayed stem at soybeans, green beans, and peanuts) and *Rhizoctonia solani* (leaves blight causal factor in rice). While *Gliocladium fimbriatum* is as antagonist agents of various plant pathogens is extremely useful as a biological control. It could upset the crop yield with catastrophic suddenness[9]. These inspired us to perform antifungal investigation of Eusiderin I against *Fusarium oxysporum* f.sp. *lycopersici, Sclerotium roefsii*, *Rhizoctonia solani and Gliocladium fimbriatum*.

## Material and Methods

### Material

All materials were at least reagent grade and used as received: methanol, ethanol 70%, n-hexane, $H_2SO_4$, $Ce(SO_4)_2$ and ethyl acetate (Sigma Aldrich); PDA (Potato Dextrose Agar) (BioRad); silica gel (Merck 60 $GF_{254}$ (230-400 Mesh), Silica gel G 60 (70-230 Mesh), Kieselgel $60F_{254}$ plates (0.25 mm, Merck). Bulian wood (*Eusideroxylon zwagery*) and *Fusarium oxysporum f.sp. lycopersici, Sclerotium roefsii*, *Rhizoctonia solani and Gliocladium fimbriatum* (IPB).

### General Experimental Procedure

IR spectra was measured with a Hitachi High-Technologies Co. Vacuum liquid chromatography was conducted using silica gel (Merck 60 $GF_{254}$ (230-400 Mesh) and column chromatography using Merck Silica gel G 60 (70-230 Mesh). Thin-layer chromatography (TLC) analysis was performed on precoated Kieselgel $60F_{254}$ plates (0.25 mm, Merck). The spots were monitored under UV light (254 or 365 nm) and visualized by spraying agents such as 1% $Ce(SO_4)_2$/10% $H_2SO_4$.

### Isolation and Purification.

Sample of the heartwood of *E. zwagery* was collected from Senami Forest, Batanghari District, Jambi, Indonesia. The dried heartwood (8 Kg) was ground and extracted three times with MeOH at RT for 6 h and subsequently three times under reflux for 4 h. The MeOH extract (1.2 Kg) was fractionated by vacuum liquid chromatography on silica gel using combination of n-hexane and ethyl acetate with increasing polarity as eluent

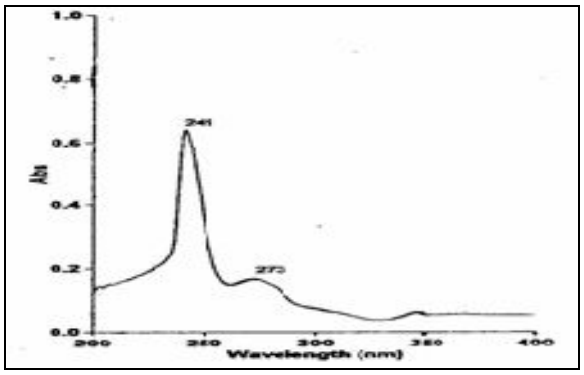
to give 6 fractions. Eusiderin I was identified on the second and third fraction then crystallized with benzene to afford Eusiderin I (1.6 gram). The structure was confirmed with UV-Vis and IR spectroscopy and compared with previous data[6,8].

**Bioassay**

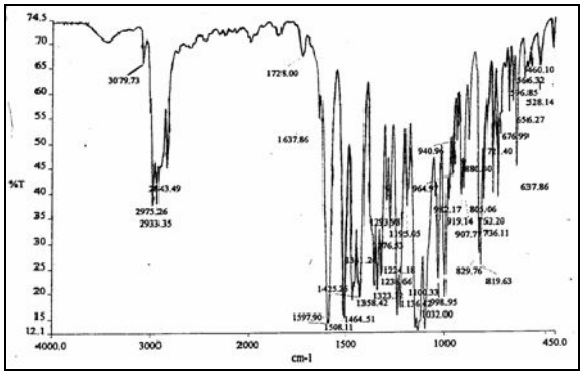
*Fusarium oxysporum* f.sp. *lycopersici, Sclerotium roefsii*, *Rhizoctonia solani and Gliocladium fimbriatum* were obtained from Department of Plant Protection, Bogor Agriculture University (IPB). Antifungal activity of Eusiderin I was studied against *Fusarium oxysporum f.sp. lycopersici, Sclerotium roefsii*, *Rhizoctonia solani and Gliocladium fimbriatum* using PDA (Potato Dextrose Agar) as testing culture media at room temperature, and were monitored for 5 days. The in vitro antifungal activity investigation were performed by agar well diffusion method. 20 ml of sterilized medium in the presence of inoculums from *Fusarium oxysporum* f.sp. *lycopersici* (cultivated for a week) were placed into petri dishes and a 20 µl of Eusiderin I solution with different concentrations (3, 4, and 5 ppm) and were transferred into well on the prepared media[11]. Control experiments were carried out under similar condition by using chloroform for antifungal activity as solvent. The growth inhibition was observed after five days of incubation which appeared as empty zone around the well. The sensitivities of the fungus species to the Eusiderin I were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against fungus[11].

## Result and Discussion

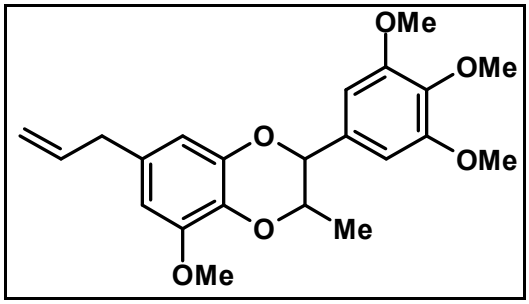
The structure of Eusiderin I was determined based on UV-Vis and IR spectroscopy data as shown in Fig. 1, and compared with previous data[6,8]. The isolated Eusiderin I was white crystal with melting point of 99-100$^{o}$C. The UV spectra in $CHCl_3$ showed absorbance at $\lambda_{max}$ (log ε) 241 (4.99) and 273 (4.83) (Fig. 1a). The infra red spectra of this compound showed the sharp aromatic C-H stretching vibration at 3079 $cm^{-1}$, aliphatic C-H stretching vibration at 2975 and 2933 $cm^{-1}$. Aromatic C-H bending vibration also shown in finger print 998, 829 and 637 $cm^{-1}$. These vibration region also indicate the substituted aromatic system. The sharp aromatic C=C stretching vibration also shown in 1597 and 1508 $cm^{-1}$ (Fig. 1b).




**(a)**




**(b)**




**(c)**

**Fig. 1. (a) UV-Vis spectra; (b) FT-IR spectra; (c) Structure of Eusiderin I**

In this study, the in vitro antifungal activity investigation were performed by agar well diffusion method with different concentrations of Eusiderin I solution (1, 2, 3, 4, and 5 ppm) against tested fungus. This investigation was to determine concentration of Eusiderin I solution for testing antifungal activity on phathogenic plant fungus. The preliminary test results on *Rhizoctonia solani* showed that proper concentration to give antifungal activity were 3, 4 and 5 ppm (Fig. 2). At concentration less than 3 ppm it has no inhibiting activity, while at more than 5 ppm would be harmful to human's health if it is used in the field[10,11]. At 3,4 and 5 ppm, Eusiderin I could inhibit *Rhizoctonia solani* colony growth as much as 0.76%, 2.30% and 21.95%, respectively (Table 1).

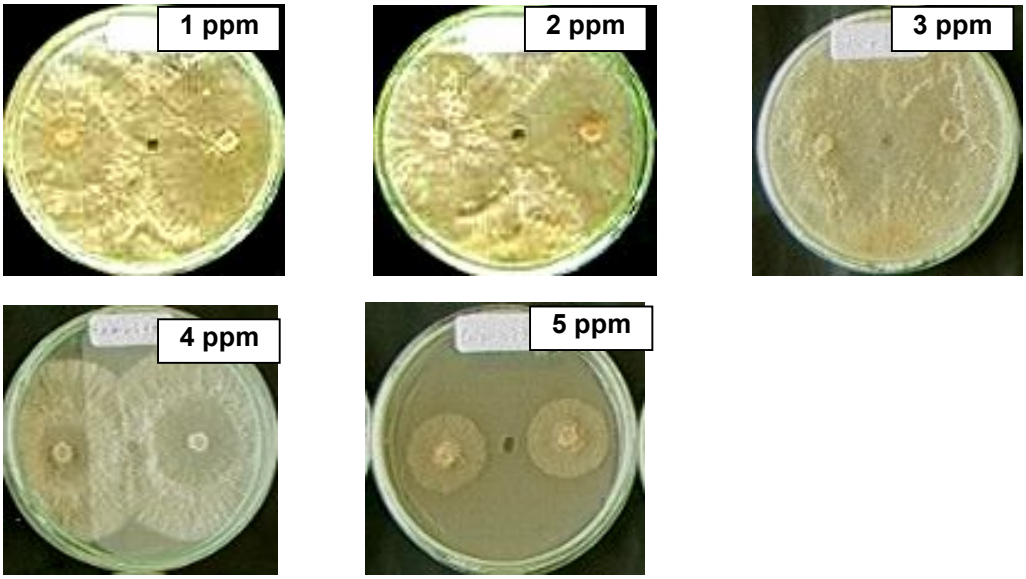



**Fig. 2. Antifungal activity of Eusiderin I with different concentration against *Rhizoctonia solani* (determination of proper concentration for antifungal activity test)**

**Table 1. Antifungal Activity of Eusiderin I against *Rhizoctonia solani***

| Fungi | Inhibition precentation mean of fungi colony growth ( r (%), n = 5) | | | | |
|---|---|---|---|---|---|
| | Concentration of Eusiderin I (ppm) | | | | |
| | 1 | 2 | 3 | 4 | 5 |
| *Rhizoctonia solani* | 0.00 | 0.00 | 1.00 | 2.50 | 22.50 |
| | 0.00 | 0.00 | 1.00 | 2.50 | 21.75 |
| | 0.00 | 0.00 | 0.60 | 2.00 | 22.00 |
| | 0.00 | 0.00 | 0.60 | 2.00 | 21.50 |
| | 0.00 | 0.00 | 0.60 | 2.50 | 22.00 |
| Mean | 0.00 | 0.00 | 0.76 | 2.30 | 21.95 |

Investigation on antifungal activity of Eusiderin I against *Fusarium oxysporum* f.sp. *lycopersici*, *Sclerotium roefsii*, *Rhizoctonia solani and Gliocladium fimbriatum* were conducted by measuring the colony growth radius of fungus after five days of incubation. Afterward, data were converted to growth inhibition ratio. The results showed that at three different concentrations (3, 4 and 5 ppm), Eusiderin I was a potent antifungal agent because it had strong activity in inhibiting the *Fusarium oxysporum* f.sp. *lycopersici* (Fig. 3), *Sclerotium roefsii* (Fig. 4) and *Rhizoctonia solani* (Fig. 5) growth.

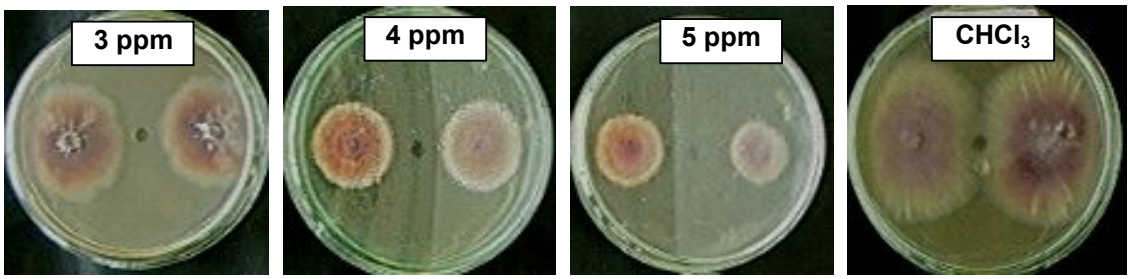



**Fig. 3. Antifungal activity of Eusiderin I against *Fusarium oxysporum* f.sp. *lycopersici***

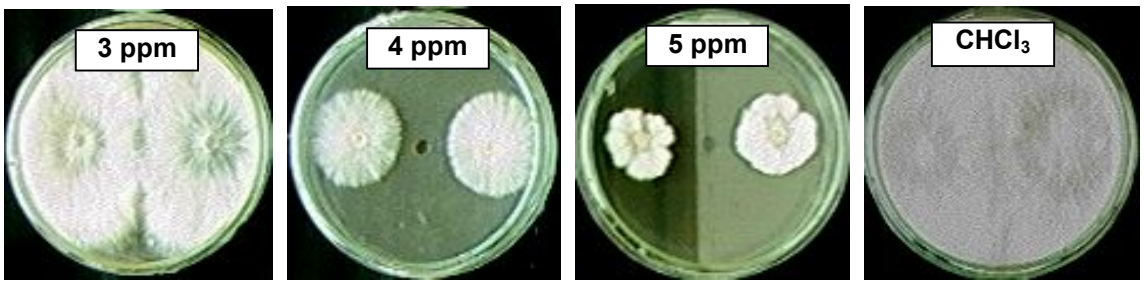



**Fig. 4. Antifungal activity of Eusiderin I against *Sclerotium roefsii***

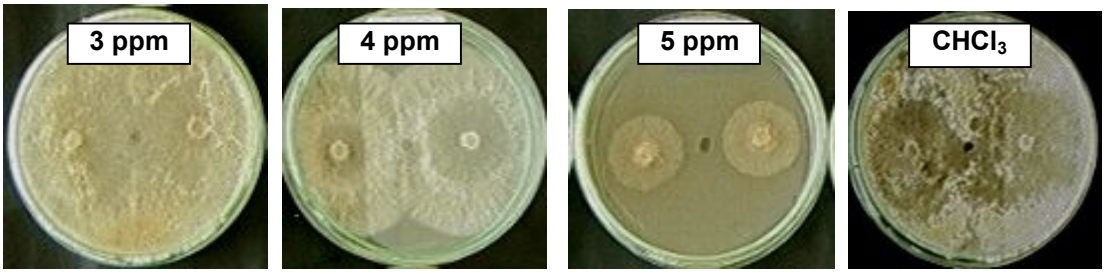



**Fig. 5. Antifungal activity of Eusiderin I against *Rhizoctonia solani***

The 5 days incubation test result showed that at 3 ppm, Eusiderin I could inhibit *Fusarium oxysporum* f.sp. *lycopersici,* while it could not inhibit *Sclerotium roefsii* and *Rhizoctonia solani* colony growth. At 5 ppm, Eusiderin I gave the most effective inhibition precentation because it could inhibit all the sample; *Fusarium oxysporum* f.sp. *lycopersici, Sclerotium roefsii* and *Rhizoctonia solani* colony growth as much as 49.80%, 49.55% and 21.95%, respectively (Table 2). It can be concluded that antifungal activity of Eusiderin I on *Fusarium oxysporum* f.sp. *lycopersici, Sclerotium roefsii* and *Rhizoctonia solani* were strongly influenced by concentrations.

**Table 2. Antifungal Activity of Eusiderin I against pathogenic plant fungus**

| Fungi | Inhibition precentation mean of fungi colony growth ( r (%), n = 5) | | |
|---|---|---|---|
| | Concentration of Eusiderin I (ppm) | | |
| | 3 | 4 | 5 |
| *Fusarium oxysporum* f.sp. *lycopersici* | 23.50<br>24.60<br>24.25<br>25.00<br>25.00 | 35.50<br>35.75<br>36.00<br>38.00<br>37.50 | 50.50<br>50.50<br>48.75<br>49.50<br>49.75 |
| Mean | 24.47 | 36.55 | 49.80 |
| *Sclerotium roefsii* | 7.00<br>7.00<br>7.50<br>7.00<br>7.50 | 22.50<br>21.10<br>20.50<br>21.50<br>22.00 | 49.50<br>50.50<br>48.75<br>49.50<br>49.50 |
| Mean | 7.20 | 21.52 | 49.55 |
| *Rhizoctonia solani* | 1.00<br>1.00<br>0.60<br>0.60<br>0.60 | 2.50<br>2.50<br>2.00<br>2.00<br>2.50 | 22.50<br>21.75<br>22.00<br>21.50<br>22.00 |
| Mean | 0.76 | 2.30 | 21.95 |
| *Gliocladium fimbriatum* | 0.60<br>0.60<br>0.00<br>0.00<br>0.60 | 2.00<br>2.50<br>2.00<br>2.00<br>2.50 | 4.50<br>5.00<br>5.00<br>4.50<br>5.50 |
| Mean | 0.36 | 2.20 | 4.90 |

In this study, antifungal activity test also showed that eusiderin I had no inhibitory activity against the growth of *Gliocladium fimbriatum* colony (Fig. 6). It is very well known as antagonist agent for various pathogenic plants which are very useful as a biological control. Then the existence of this research means compatible.

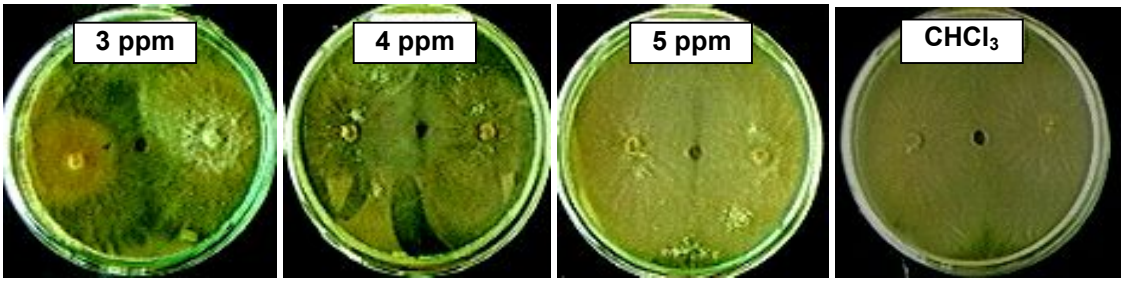



**Fig. 6. Antifungal activity of Eusiderin I on *Gliocladium fimbriatum***

In all experiment, the antifungal activity of Eusiderin I always been compared with solvent used, to confirm that activity was not from the solvent (chloroform or $CHCl_3$).

Recent studies have shown that the antifungal activity of fungicidal plants might be due to the presence and synergistic activity of bioactive metabolite. Eusiderin I is a neolignan derivative. The chemical structure of Eusiderin I has methyl group, methoxyl groups, allylic moiety, aromatic ring and dioxane ring. These results indicated that allylic moiety of Eusiderin I was the crucial point to give the antifungal activity.

## Conclusion :

Eusiderin I, was isolated as major component from *Eusideroxylon zwagery.* It showed potent antifungal activity against *Fusarium oxysporum* f.sp. *Lycopersici, Sclerotium roefsii* and *Rhizoctonia solani*. Antifungal activity of Eusiderin I on phathogenic plant fungus were strongly influenced by concentrations. Eusiderin I is a potent candidate for antifungal agent. These results may represent as prominent resistance of *E. zwagery* against fungi.

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