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Eusiderin I from *Eusideroxylon zwagery* as antifungal agent toward *Fusarium oxysporum f.sp. lycopersici*

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Abstract

A research on Eusiderin I from *Eusideroxylon zwagery* as antifungal to *Fusarium oxysporum* f.sp. *lycopersici* had been carried out. Eusiderin I, a rare benzodioxane-type neolignan was isolated as major component from *E. zwagery* which showed potent antifungal activity against *Fusarium oxysporum* f.sp. *lycopersici*. Eusiderin I was at a concentration of 5 ppm to give most effective inhibition, 49.8% on the colony growth of *Fusarium oxysporum* f.sp. *lycopersici*.

Keywords: *Eusideroxylon zwagery*, Eusiderin I, anti fungal, *Fusarium oxysporum* f.sp. *lycopersici*

1. Introduction

Bulian or iron wood (*Eusideroxylon zwagery*) is one of the timber forest with worth and high economic value. *E. zwagery* is an endemic plant in which widely distributed throughout Jambi Province. 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 *E. zwagery*, Syamsurizal et.al isolated Eusiderin I as major component from the heartwood of MeOH extract which 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

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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]. Plant disease caused by pathogen fungi such as *Fusarium oxysporum* f.sp. *lycopersici* (faded causal factor in tomato). It could upset the crop yield with catastrophic suddenness [9]. These inspired us to perform biological investigation of Eusiderin I against *Fusarium oxysporum* f.sp. *lycopersici*.

2. Material and Methods

2.1. Material

All materials were of 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* (IPB).

2.2. 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.

2.3. Isolation and Purification.

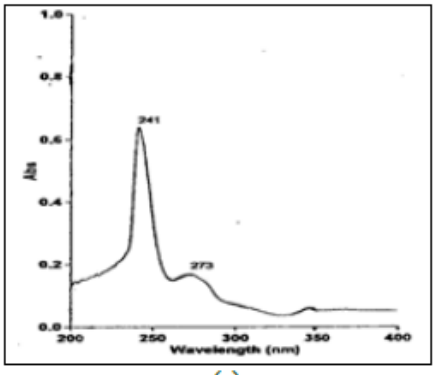
Sample of the heartwood of *E. zwagery* was collected from Senami Forest, Batanghari District, Jambi, Indonesia. The dried heartwood (8 Kg) was ground and extracted three times with MeOH at RT for 6 h and subsequently three times under reflux for 4 h. The MeOH extract (1.2 Kg) was fractionated by vacuum 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].

2.4. Bioassay [10]

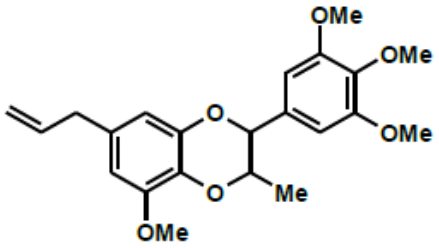
Fusarium oxysporum f.sp. *lycopersici* was obtained from Department of Plant Protection, Bogor Agriculture University (IPB). Antifungal activity of Eusiderin I was studied against *Fusarium oxysporum f.sp. lycopersici* using PDA (Potato Dextrose Agar) as testing culture media at room temperature, and were monitored for 5 days. The invitro antifungal activity was 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 were transferred into well on the prepared media. The growth inhibition was observed after five days of incubation which appeared as empty zone around the well. As solvent control chloroform was used. The growth inhibition ratio was analyzed statistically.

3. Result and Discussion

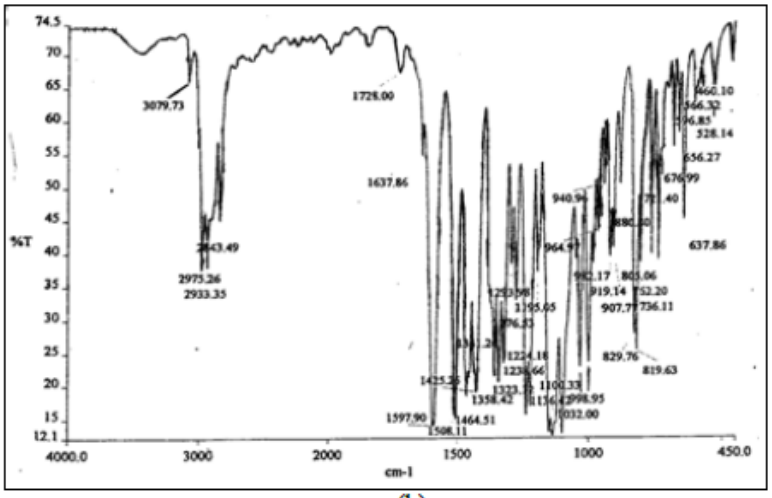
The structure of Eusiderin I was determined based on UV-Vis and IR spectroscopy data as shown in the Fig. 1, and compared with previous data [6,8]. The isolated Eusiderin I was white crystal with melting point of 99-100°C. The UV spectra in $CHCl_3$ showed absorbance at λ_{max} (log ε) 241 (4,99) and 273 (4,83). 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}.



(a)



(c)



(b)

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

Investigation on anti fungal activity of Eusiderin I against *Fusarium oxysporum* f.sp. *lycopersici*, was conducted by measuring the colony growth radius of fungi for five days of incubation. Afterward, data was converted to growth inhibition ratio. The antifungal activity test of Eusiderin I from Bulian wood

(*Eusideroxylon zwagery*) to phatogen fungi of *Fusarium oxysporum* f.sp. *lycopersici* showed that at three different concentrations (3, 4 and 5 ppm), Eusiderin I was a potent antifungal agent because it had a strong activity in inhibiting the *Fusarium oxysporum* f.sp. *lycopersici* growth. The 5 days incubation test result showed that 3 ppm Eusiderin I could inhibit the *Fusarium oxysporum* f.sp. *lycopersici* colony growth. The 5 ppm Eusiderin I gave the most effective inhibition precentation because it could inhibit the *Fusarium oxysporum* f.sp. *lycopersici* colony growth until 49,8%. Activity of antifungal Eusiderin I on *Fusarium oxysporum* f.sp. *lycopersici* were strongly influenced by concentrations. They showed strong anti fungal activity as shown in Fig.2. It can be concluded that Eusiderin I was a candidate compound for a potent antifungal agent since it could exhibit *Fusarium oxysporum* f.sp. *lycopersici* colony growth.

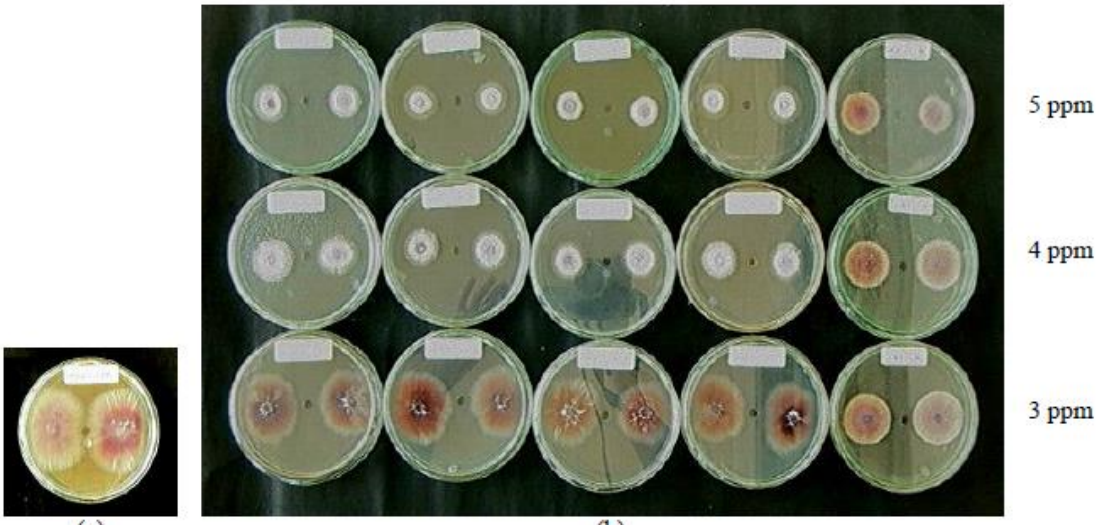


Fig. 2. Anti fungal investigation of Eusiderin I on *Fusarium oxysporum* f.sp. *lycopersici*, (a) Chloroform and (b) Eusiderin I in Chloroform

After five days incubation, at 3 ppm it could inhibit proliferation of *Fusarium oxysporum* f.sp. *lycopersici* colony. At 5 ppm concentration, Eusiderin I gave the most effective inhibition into *Fusarium oxysporum* f.sp. *lycopersici* because it gave the growth inhibition ratio as much as 49.8%. The data of colony growth inhibition for three variation concentrations (3, 4 and 5 ppm) of of Eusiderin I was shown in Table 1.

Table 1. Anti fungal investigation of Eusiderin I against *Fusarium oxysporum* f.sp. *lycopersici*

Compound	Inhibition precentation mean of *Fusarium oxysporum* f.sp. *lycopersici* colony growth (r (%), n = 5)		
	Concentration (ppm)		
	3	4	5
Eusiderin I	23.5	35.5	50.5
	24.6	35.75	50.5
	24.25	36	48.75
	25	38	49.5
	25	37.5	49.75
Mean	24.47	36.55	49.8

4. Conclusion

Eusiderin I, a rare benzodioxane-type neolignan was isolated as major component from *Eusideroxylon zwagery* which showed potent antifungal activity against *Fusarium oxysporum* f.sp. *Lycopersici*. Activity of antifungal Eusiderin I on *Fusarium oxysporum* f.sp. *lycopersici* were strongly influenced by concentrations. Eusiderin I was a candidate compound for a potent antifungal agent since it could inhibit *Fusarium oxysporum* f.sp. *lycopersici* colony growth. These results may represent as prominent resistance of *E. zwagery* against fungi.

Acknowledgements

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