Synthesis of Acetyl

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Synthesis of Acetyl Propylen Ester Eusiderin A and its activity againts *Tricophyton mentagrophytes*.

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ABSTRACT

Eusiderin A, a neolignan derivative which is the main secondary metabolites of *Eusideroxylon zwagery*, had been widely reported as a potent pesticide especially antifungal. Previous studies on the oxidation of terminal methylene of allylic chain in Eusiderin A produced a primary alcohol, pinacol and an aldehyde which demonstrate strong activity against plant pathogenic fungi. The current study aimed to improve the hydrophilicity of Eusiderin A via bromination of the allylic chain in order to give effect as a potent antifungal. The method for an allylic chain bromination was conducted through the mechanism of electrophilic addition reaction between bromine and allylic chain, using bromine in a glacial acetic acid. Furthermore, in order to introduce an acetyl group on the allylic part, the intermediate of 1,2-Dibromopropyl Eusiderin A through bromination addition were synthesized, then dehydrobromination by using glacial acetic acid to form the Acetyl Propylen Ester Eusiderin A. The structure of Acetyl Propylen Ester Eusiderin A was determined based on spectroscopy data and compared with references data. The invitro antifungal activity was performed by agar diffusion method against *Tricophyton mentagrophytes*. Based on UV, IR, ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{13}C HMBC and ^{1}H-^{13}C HSQC spectras and references, Acetyl Propylen Ester Eusiderin A is formed. Acetyl Propylen Ester Eusiderin A (50 ppm) has shown good antifungal activity toward *Tricophyton mentagrophytes*. It can be concluded that Acetyl Propylen Ester Eusiderin A was a candidate compound for a potent antifungal agent since it could exhibit *Tricophyton mentagrophytes* colony growth.

Keywords: Eusiderin A, bromination, dehydrobromination, antifungal, *Tricophyton mentagrophytes*

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INTRODUCTION

Bulian wood (*Eusideroxylon zwagery*) known as iron wood, is one of Lauraceae popular as furnished and kitchenettes wood. It was known as wood source which fungi and insect resistant. Its fruits used as antiinflammatory agent. These effects were interconnected with secondary metabolites contained within. There are four big metabolites group produced by *E. zwagery*. They are alkaloid, steroid, terpenoid and phenolic compounds. Among all that, Stilbene derivative phenolic compounds have fungicide and insecticide activity [3]. It was estimated that these compounds can protect bulian wood from insect and wood decay fungi [1,2,3]. Earlier research found that *Eusideroxylon zwagery* have five pure compounds, three of them are neolignan and two are aporphin alkaloid and phenantrene. One of neolignan indentified as Eusiderin A [2,4,5,6,7].

A study on chemical potency of Eusiderin A from *E. zwagery* guided the isolation to antifeedant and antifungal potency investigation [4,5,6,7,8]. Eusiderin A was firstly isolated by Hobbs and King in 1960 [2]. However the biological activity as antifeedant and antifungal were firstly reported by our research group. Eusiderin A showed potent antifeedant activity at a concentration of 0.01% against *Epilachna sparsa* [6]. In addition, it also could prevent *Fusarium oxysporum* f.sp. *lycopersici* from fading tomato at 3 ppm concentration [4,5]. This finding leads to the reason why this plant has durable wood. It is concluded that Eusiderin A may play a role in the protection of the plant against microbe, insects and fungi [5,6,8].

These results have inspired us to transform the eusiderin A into another analog (Acetyl Propylen Ester Eusiderin A) and evaluated the antimicrobial activity against *Tricophyton mentagrophytes*. Up to now, exploration and investigation of Eusiderin A from *E. zwagery* as sources of botanical microbials had been being investigated.

MATERIAL AND METHODS

Materials:

All materials were of at least reagent grade and used as received: methanol, ethanol 70%, n-hexane, H_2SO_4, Br_2, glacial acetic acid, $NaHCO_3$, Na_2SO_4, $Ce(SO_4)_2$, ketoconazole and ethyl acetate (Sigma Aldrich); PDA (Potato Dextrose Agar) (BioRad); nutrient 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 *Tricophyton mentagrophytes*.

Instruments:

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 1% $Ce(SO_4)_2$/10% H_2SO_4. The structure analysis was conducted by UV and IR Spectroscopy. ^1H and ^{13}C NMR spetras was measured with a Hitachi High-Technologies Co.

Methods:

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 grounded 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 A was identified on the second and third fraction then crystallized with benzene to afford Eusiderin A (1.6 gram). The structure was confirmed with UV-Vis and IR spectroscopy and compared with previous data [4,6].

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Synthesis of Acetyl Propylen Ester Eusiderin A

Eusiderin A (1 mmol) was disolved and stirred in 10 mL glacial acetic acid in a round bottom flask at room temperature then it was cooled at 0 °C. 50 µL of Br_2 in 10 mL glacial acetic acid was added to the reaction mixture and was homogenized at 0 °C for 5 h. Most of Eusiderin A was consumed at this time (monitored by TLC), the reaction mixture was concentrated under reduced pressure and the obtained solid residue was added by $NaHCO_3$ and water. The obtained residue was extracted using ethyl acetic (contain unhidrat Na_2SO_4). Column purification using flash chromatography (SiO2, 10% MeOH /CHCl3) of the crude product yielded pure. The obtained Eusiderin A dibromide solid was recrystallized from MeOH.

The Acetyl Propylen Ester Eusiderin A was synthesized by equimolar amount of Eusiderin dibromide (0.02 mmol) then it was disolved and stirred in DMSO. K_2CO_3 and glacial acetic acid were added into the reaction mixture and it was homogenized for 2 days at room temperature. The obtained product was extracted using ethyl acetate, and formed salt was dissolved by adding aqua demineralization. Furthermore, ethyl acetate extra was added anhydrous Na_2SO_4 to remove water. Acetyl Propylen Ester Eusiderin A is formed and then it was purified by column chromatography using the eluent n-hexane: ethyl acetate (8: 2 and 7: 3).

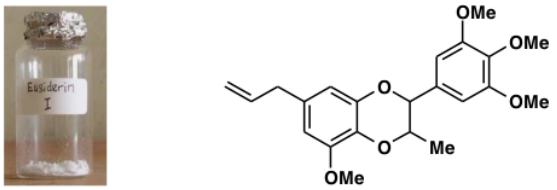
Bioassay

Tricophyton mentagrophytes was obtained from Department of Biology, Faculty of Science and Technology, University of Jambi. Antifungal activity of Acetyl Propylen Ester Eusiderin A was studied against *Tricophyton mentagrophytes* using PDA (Potato Dextrose Agar) as testing culture media at room temperature, and were monitored for 5 days. The in vitro antifungal activity was performed by paper dish method. 20 ml of sterilized medium in the presence of inoculums from *Tricophyton mentagrophytes* (cultivated for a week) was placed into petri dishes and a 20 µl of Acetyl Propylen Ester Eusiderin A solution with different concentrations were transferred into paper dish on the prepared media. The growth inhibition was observed after five days of incubation which appeared as empty zone around the paper dish. As solvent control DMSO was used.

RESULTS AND DISCUSSION

Eusiderin A

The maceration gave 1,75 kg concentrate extract (*Eusideroxylon zwagery* wood powder). The fractination then were applied into this extract and gave. Eusiderin A as white crystalline (Figure 1.a) with melting point of 99-100 °C. Eusiderin A structure exhibited in Figure 1.b.



(a) (b)

Figure 1. (a) Eusiderin I Crystal and (b) Eusiderin A structure

The UV spectra in $CHCl_3$ showed absorbance at λ_{maks} (log ε) 241 (4,99) and 273 (4,83). Absorbance at λ_{maks} 241 commonly are given by the unsaturated chromophor from substituted alkene while at λ_{maks} 273 commonly are given by the chromophor from oxygenated aromatic system. 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

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also shown in 1597 and 1508 cm^{-1} (UV and IR spectras are not displayed). The structure of Eusiderin A was determined based on spectroscopy data and compared with previous data [4,7].

Chemistry

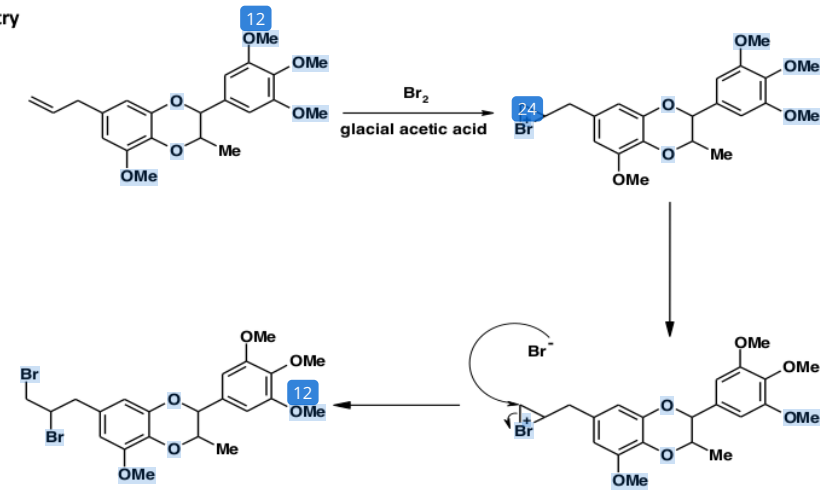

Figure 2. Synthesis of Eusiderin A dibromide

Eusiderin A dibromides were synthesized as depicted in the Scheme on Fig. 2. The Eusiderin A dibromides were synthesized by equimolar amounts of Eusiderin A (1 mmol) was disolved and stirred in glacial acetic acid. Br_2 in glacial acetic acid was added to the reaction mixture and was homogenized. The method for an allylic chain bromination is through the mechanism of electrophilic addition reaction between bromine and allylic chain, using bromine in a glacial acetic acid. The double bond breaks, and a bromine atom becomes attached to each carbon. The bromine loses its original red-brown color to give a colorless liquid. In the case of the reaction with allylic chain, Eusiderin dibromide (2) is formed (Fig. 2). Modification of allylic moiety of Eusiderin A was conducted by taking into account of the previous results that as increased hydrophobicity of allylic chain led to reduce activity of Eusiderin A. Therefore need to be calculated the clogP values in order to reduce the hydrophobicity on the contrary to enhance the hydrophilicity. ClogP is a parameter correlated to the permeability of drugs and is thus believed to be an important index in predicting the biological activity [6].

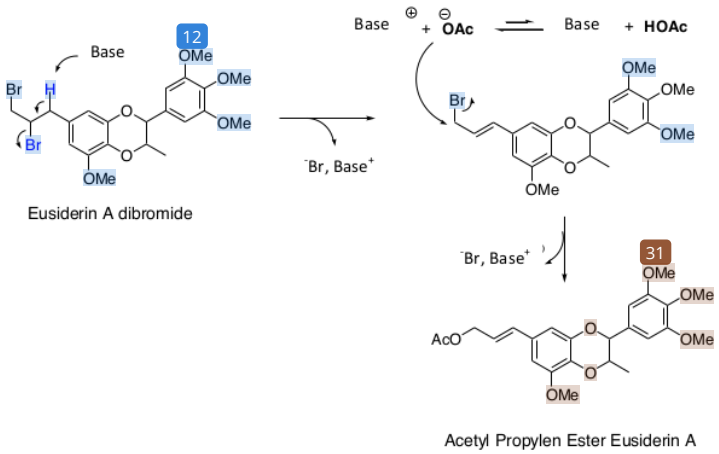

Figure 3. Synthesis of Acetyl Propylen Ester Eusiderin A

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Acetyl Propylen Ester Eusiderin A was synthesized as depicted in the Scheme on Fig. 3. The Acetyl Propylen Ester Eusiderin A were synthesized by equimolar amounts of Eusiderin A dibromide (0.02 mmol) was disolved and stirred in DMSO. K_2CO_3 and glacial acetic acid was added to the reaction mixture and was homogenized for 2 days at room temperature. The obtained product was then extracted using ethyl acetate, and the formed salt was dissolved by adding aqua demineralization. Furthermore, the ethyl acetate extract was added anhydrous Na_2SO_4 to remove water. Acetyl Propylen Ester Eusiderin A is formed and then purified by column chromatography using the eluent n-hexane: ethyl acetate (8: 2 and 7: 3). The spectral data (^{1}H NMR and ^{13}C NMR) of the synthesized compound was in complete agreement with the proposed structure. The structure of Acetyl Propylen Ester Eusiderin A was determined based on ^{1}H NMR and ^{13}C NMR spectroscopy data as shown in the Fig. 4 and Table 1. Modifications at allylic moiety of Eusiderin A was crucial point to enhance the antimicrobial potency. Modification of allylic moiety of Eusiderin A was intended to reduce the clogP value in order to increase the hydrophilicity. By promoting the hydrophilicity is prepared to enhance accumulation of Acetyl Propylen Ester Eusiderin A in the site target of microbes [6,7].

Spectroscopy Analysis of Reaction Product (Acetyl Propylen Ester Eusiderin A)

The resulting pattern of thin layer chromatography showed a single stain patterns. This indicates that an obtained compound is a pure compound (its chromatogram is not displayed). The UV spectra in $CHCl_3$ showed absorbance at λ_{maks} (log ε) 245 (4,92) and 275 (4,88). Absorbance at λ_{maks} 245 commonly are given by the unsaturated chromophor from substituted alkene while at λ_{maks} 275 commonly are given by the chromophor from oxygenated aromatic system. The infra red spectra of this compound showed the sharp aromatic C-H stretching vibration at 3071 cm^{-1}, aliphatic C-H stretching vibration at 2970 and 2938 cm^{-1}. Aromatic C-H bending vibration also shown in finger print 985, 795 and 622 cm^{-1}. These vibration region also indicate the substituted aromatic system. The sharp aromatic C=C stretching vibration also shown in 1587 and 1511 cm^{-1} (UV and IR spectras are not displayed).This compound can be identified using spectroscopy ^{1}H-NMR and ^{13}C-NMR, Spectroscopy ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation (HMBC) and Spectroscopy ^{1}H-^{13}C Heteronuclear Single Quantum Coherence (HSQC).

Analysis of ^{1}H NMR and ^{13}C NMR spectroscopy

Proton nuclear magnetic resonance spectroscopy (^{1}H NMR) provides information about hydrogen atoms making up an organic molecule. The information was obtained from this form of type of proton spectroscopy, proton environment and number of protons in a molecule. Carbon nuclear magnetic resonance spectroscopy (^{13}C NMR) provides information on position of carbon atoms in an organic molecule. The chemical shift of the spectrum occured in the range that is wider than range of ^{1}H NMR chemical shifts. ^{13}C chemical shifts are expressed in units (δ), but unusual about 0-200 ppm below the terrain TMS. A wide range of chemical shift that is likely to simplify the ^{13}C spectrum relative to ^{1}H. ^{13}C abundance is so low that spin-spin splitting is not visible.

The correlations is at benzodioxane ring of Acetyl Propylen Ester Eusiderin A. It show that H-4 and H-8 and H-3' and H-5' have the same correlations (Fig. 5). At benzodioxane ring of Acetyl Propylen Ester Eusiderin A (C-3") is correlated with H-3 and H-2 at allylic chain of Acetyl Propylen Ester Eusiderin A (Fig. 6 and 7). Based on all data analysis and reference, Acetyl Propylen Ester Eusiderin A (3) is formed. The structure of Acetyl Propylen Ester Eusiderin A was determined based on spectroscopy data and compared with references data [5,6,7].

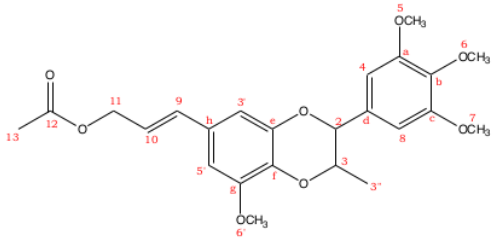

Figure 4. Structure of Acetyl Propylen Ester Eusiderin A

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Table 1. ^{1}H NMR and ^{13}C NMR structural analysis of Acetyl Propylen Ester Eusiderin A

Position	δC (ppm)	δH (p16) (m, l, J_{Hz})
2	81,4	4,67 (d, 1H, *J* = 7,5 Hz)
3	74,3	4,09 (m, 1H)
4	104,5	6,61 (s, 1H)
8	104,5	19 1 (s, 1H)
9	132,8	20 2 (d, 1H, *J* = 15,5 Hz)
10	124,5	20 7(dt, 1H, *J* = 6,5; 13 Hz)
11	65,0	4,76 (d, 2H, *J* = 6,5 Hz)
12	170,8 (C=O)	
13	21,0	2,12 (s, 3H)
3’	101,9	6,75 (brs, 1H)
5’	101,9	6,75 (brs, 1H)
3”	17,1	1,31 (d, 3H, *J* = 6 Hz)
5-OCH_3	56,2	3,88 (s, 3H)
6-OCH_3	56,2	3,92 (s, 3H)
7-OCH_3	56,2	3,88 (s, 3H)
6’-OCH_3	60,9	3,86 (s, 3H)
a	153,5	
b	138,4	
c	153,5	
d	133,8	
e	141,4	
f	131,7	
g	147,9	
h	128,3	

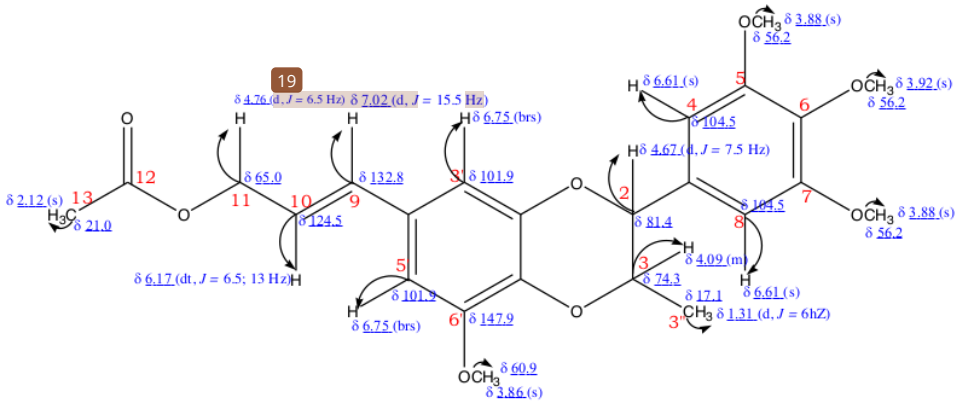

Figure 5. HSQC correlations of Acetyl Propylen Ester Eusiderin A

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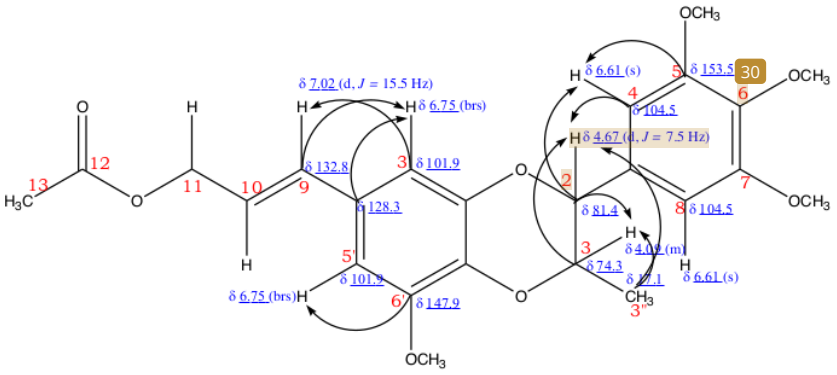

Figure 6. HMBC correlations of Acetyl Propylen Ester Eusiderin A

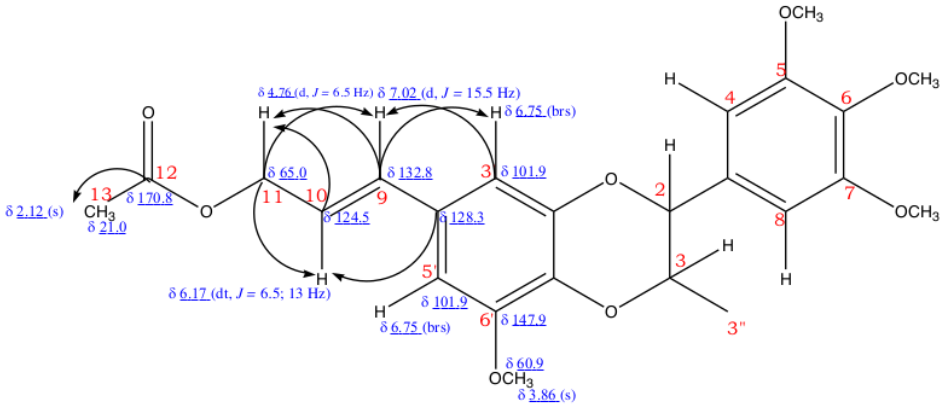

Figure 7. HMBC correlations of Acetyl Propylen Ester Eusiderin A

Antifungal activity

In present study, investigation on antifungal activity of Acetyl Propylen Ester Eusiderin A against *Tricophyton mentagrophytes* were conducted by measuring the colony growth radius of fungal after five days of incubation. Afterward, data was converted to growth inhibition ratio. The results showed that at five different concentrations (5, 25, 50, 100 and 200 ppm), Acetyl Propylen Ester Eusiderin A was a potent antifungal agent because it had a strong activity in inhibiting the *Tricophyton mentagrophytes* growth. The 5 days incubation test result showed that at 50 ppm, Acetyl Propylen Ester Eusiderin A could inhibit the *Tricophyton mentagrophytes* colony growth. The 50 ppm Acetyl Propylen Ester Eusiderin A gave an effective inhibition precentation because it could inhibit the *Tricophyton mentagrophytes* colony growth until 54.71%. Antifungal activity of Acetyl Propylen Ester Eusiderin A on *Tricophyton mentagrophytes* were strongly influenced by concentrations as shown in Fig. 8. It can be concluded that Acetyl Propylen Ester Eusiderin A is a candidate compound for a potent antifungal agent, especially for *Tricophyton mentagrophytes* since it could exhibit this fungal colony growth. These results indicated that acetyl group play an important role to enhance the antifungal activity. Meanwhile dioxane ring is necessary to maintain the conformation and stability. However aliphatic elongation on allylic moiety tends to reduce the activity [7].

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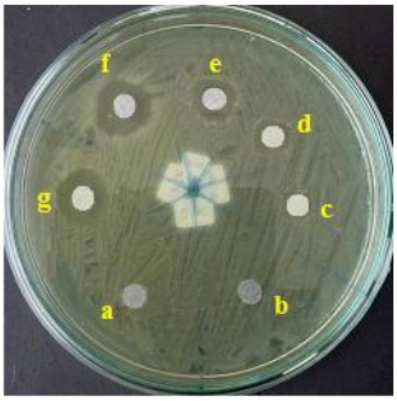

Figure 8. Antifungal investigation of Acetyl Propylen Ester Eusiderin A on *Tricophyton mentagrophytes*, (a) DMSO; (b - f) Acetyl Propylen Ester Eusiderin A in DMSO 5, 25, 50, 100, 200 ppm; and (g) Ketoconazole 100 ppm against *Tricophyton mentagrophytes*

CONCLUSIONS

In conclusion, Acetyl Propylen Ester Eusiderin A can be synthesized using Eusiderin A, Br_2, Eusiderin A dibromide and Base in glacial acetic acid. Acetyl Propylen Ester Eusiderin A has shown good antifungal activity, especially on *Tricophyton mentagrophytes.* The antifungal activity was found to be good in the 50 ppm of Acetyl Propylen Ester Eusiderin A.

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