

Antifungal Activity of Neolignan Derivatives from *Eusideroxylon zwageri* Against Pathogenic Fungus *Microsporum gypseum*

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ABSTRACT

Introduction: Bulian wood (*Eusideroxylon zwageri*) known as iron wood. It was known as wood source which fungi and insect resistant. These effects were interconnected with secondary metabolites contained within. **Methods:** *Eusideroxylon zwageri* wood powder was macerated with methanol and fractionated with n-Hexane, dichloromethane, ethyl acetate. Eusiderin I and Compound B were isolated from n-hexane fraction, while Compound C were isolated from dichloromethane fraction. Eusiderin I, compound A and Compound B were characterized using melting point, UV spectroscopy and compared with previous data. Antifungal activity test was conducted with *Microsporum gypseum* using paper disc method. **Results:** A research on antifungal activity of Neolignan derivatives from *Eusideroxylon zwageri* against pathogenic fungus *Microsporum gypseum* had been carried out. Eusiderin I, Compound B and Compound C were isolated from wood of *Eusideroxylon zwageri*. All three compounds are white crystals with melting point in such 99-100 °C, 110-112 °C, 98-99 °C, respectively and UV spectrum data is similar to reference. The antifungal activity test of Eusiderin I, Compound B and Compound C from Bulian wood (*Eusideroxylon zwageri*) to phagogen fungus of *Microsporum gypseum* showed that with five different concentrations (5, 25, 50, 100 and 200 ppm), Eusiderin I was a potent antifungal because it had a strong activity in inhibiting the *Microsporum gypseum* growth. The 5 days incubation test result showed that 50 ppm Eusiderin I could inhibit the *Microsporum gypseum* colony growth. The 100 ppm Eusiderin I gave the most effective inhibition precentage because it could inhibit the *Microsporum gypseum* colony growth (= 93.9%). **Conclusion:** Based on this data, Eusiderin I can be indicated an antifungal candidate.

Key words: *Eusideroxylon zwageri*, *Microsporum gypseum*, Eusiderin, Antifungal.

INTRODUCTION

Bulian wood (*Eusideroxylon zwageri*) 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.¹⁻³

There are four big metabolites group produced by *Eusideroxylon zwageri*. They are alkaloid, steroid, terpenoid and phenolic compounds.⁴ Among all that, Stilbene derivative phenolic compounds have fungicide and insecticide activity.^{5,6} It was estimated that these compounds can protect bulian wood from insect and wood decay fungi.⁷⁻⁹

Earlier research found that *Eusideroxylon zwageri* have five pure compounds, three of them are neolignan and two are aporphin alkaloid and phenanthrene. Three of neolignan indentified as Eusiderin I, Compound B and Compound C.¹⁰⁻¹² Eusiderin compounds have activity as antifungal against wood decay fungu.

Indonesia has a tropical climate, this causes fungi and bacteria to proliferate easily. *Microsporum gypseum* is a fungus that causes skin diseases in

humans.³ Parts of human body that are often moist can easily be infected by pathogenic fungi. One of the most infectious pathogenic fungi is *Microsporum gypseum* which causes ringworm. To prevent this disease from spreading it is necessary to find new skin fungal drugs. This drug is expected to be more effective and safe for human and the environment. One of the natural antifungals produced by bulian wood can be used. Eusiderin from bulian wood can inhibit the growth of wood decay fungi.¹¹ So that it is expected to also be used to inhibit the growth of the *Microsporum gypseum* fungus. In this paper, the results of a study of the effectiveness of eusiderin compounds in inhibiting the growth of the *Microsporum gypseum* fungus are reported.

MATERIAL AND METHODS

Materials

Eusideroxylon zwageri wood was collected from Jambi – Indonesia in May 2017. Other materials used in this experiments are *Microsporum gypseum*, Potato Dextrose Agar (PDA), Eusiderin I, Compound B and Compound C, ethanol, methanol, aceton, DMSO, Silica gel for column chromatography, Silica gel for vacuum liquid chromatography, thin layer chromatography plate and chemicals for antifungal activity test.

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The test species and isolate used for this investigation was *Microsporum gypseum*. Identification of isolate obtained was performed according to standard methods.^{3,11}

Preparation of extracts: Extraction and partition of *Eusideroxylon zwageri* wood powder

10 Kg of *Eusideroxylon zwageri* wood powder was macerated with methanol and fractionated with n-Hexane, dichloromethane, ethyl acetate. n-Hexane and dichloromethane fractions were applied to Vacuum Liquid Chromatography, then Column Chromatography and Thin Layer Chromatography. Eusiderin I and Compound B were isolated from n-hexane fraction, while Compound C were isolated from dichloromethane fraction. The structure analysis was conducted by UV and IR Spectroscopy and compare than literature.^{11,13-16}

Minimum inhibitory concentration (MIC)

MIC determination of Eusiderin I against *Microsporum gypseum* were performed by using paper disc diffusion method. The samples were dissolved in DMSO using a certain ratio and were mixed with liquid PDA in a sterile petri dish. Petri dish were shaken until the mixture becomes homogeneous, and solidify at room temperature. Suspension of *Microsporum gypseum* then were introduced using a wire loop. All petri dishes then were incubated at 30 °C for 5 days. Colony growth of *Microsporum gypseum* were observed.^{3,11}

In vitro antifungal activity assay

Antifungal activity test was conducted with *Microsporum gypseum* as pathogen fungi using paper disc diffusion method.^{17,18} Twenty milliliter of sterilized Potato dextrose agar medium was poured into a 15 cm Petri dish. Twenty microliter of inoculum suspension of each test organism was distributed evenly over the surface. Paper disc (6 mm) was put in plate using the wide-end of a sterilized Pasteur pipette. Isolates (pure compound) of Eusiderin I, Compound B and Compound C were used in 5, 25, 50, 100 and 200 ppm concentrations. Fifty microliter of serial dilution of Eusiderin I, Compound B and Compound C and Ketoconazole were placed into paper discs. All assays were made in triplicate. The investigation were conducted by measuring colony growth diameter of *Microsporum gypseum*.^{3,11}

The plates were incubated for 5 days at 30°C. Results of the qualitative screening were recorded as the average diameter of the inhibition zone surrounding the paper discs containing the test solution. Results were compared with Ketoconazole. The percentage of mycelia inhibition was calculated as follows:

$$\text{mycelia inhibition (\%)} = \left[\frac{\text{dc} - \text{dt}}{\text{dc}} \right] \times 100$$

dc: colony diameter in control, dt: colony diameter in treatment.

Three replicate plates were used for each treatment. The MIC was regarded as the lowest concentration that produced a visible zone of inhibition.³

Statistical analyses

The comparison of average zone diameters and the evaluation of isolate antifungal effects were analyzed by SPSS 11.5 and t-test. In this experiment, P value was statistically significant ($P < 0.05$).

RESULT AND DISCUSSION

Neolignan derivative compounds from *Eusideroxylon zwageri*

There are four large groups of secondary metabolites produced by the *Eusideroxylon zwageri* T et B plant, namely groups of alkaloid

compounds, steroids, terpenoids and phenolics.^{4,10,11} Among the four groups of compounds, as is usual in other wood species, stilben derivative phenolic compounds and lignans have fungicidal and insecticidal properties. It is estimated that this class of compounds can protect bulian plants against the attack of termites and wood decay fungus. From previous studies, five pure compounds have been found in this plant, each of three neolignan derivative compounds and two aporphine and phenanthren alkaloid derivatives. Three of these neolignan compounds has been identified as eusiderin I, Compound B and Compound C.^{4,10,11} These all three neolignan compounds have antifungal activity against wood decay fungi. But how the biological role of these compounds in bulian wood against the attack of termites has never been studied. As well its bioactivity to other microorganisms. Structure Eusiderin I, Compound B and Compound C exhibited in Figure 1.

Eusiderin I, Compound B and Compound C are a benzodioxane type neolignan derivative which is biogenetically derived from the oxidation of p-allylphenol and p-propenylphenol followed by coupling the two free radicals of these compounds.^{4,5,17}

Producing number of neolignan derivative compounds in this research carried out experimentally. Total of 10 kg of dried powder of bulian wood was macerated with methanol. The results of maceration obtained concentrated methanol extract of 1.15 kg. Furthermore, the methanol extract was fractionated with n-hexane, dichloromethane and ethyl acetate solvents. After going through several processes of separation and purification of the n-hexane fraction Eusiderin I and compound B obtained, and from the dichloromethane fraction C compounds obtained.

Eusiderin I

Eusiderin I was isolated from fraction III.2 n-hexane extract. Eusiderin I was white crystals with a melting point of 99–100 °C. The UV spectrum in chloroform provides absorption at λ_{max} (nm) 241 and 273. Uptake in the area λ_{max} (nm) 241 is usually an unsaturated chromophore of substituted alkene whereas at λ_{max} (nm) 273 it is usually a chromophore of an oxygenated aromatic system.^{11,13,19-21} The complete data can be seen in Figure 2 below.

Compound B

Compound B was isolated from fraction III.5 n-hexane extract. Compound B is the result of isolation in the form of white crystals with a melting point of 110–112 °C. The UV spectrum in chloroform provides absorption at λ_{max} (nm) 210, 273 and 335. This absorption area is similar to eusiderin I which has been described previously. The complete data can be seen in Figure 3 below.

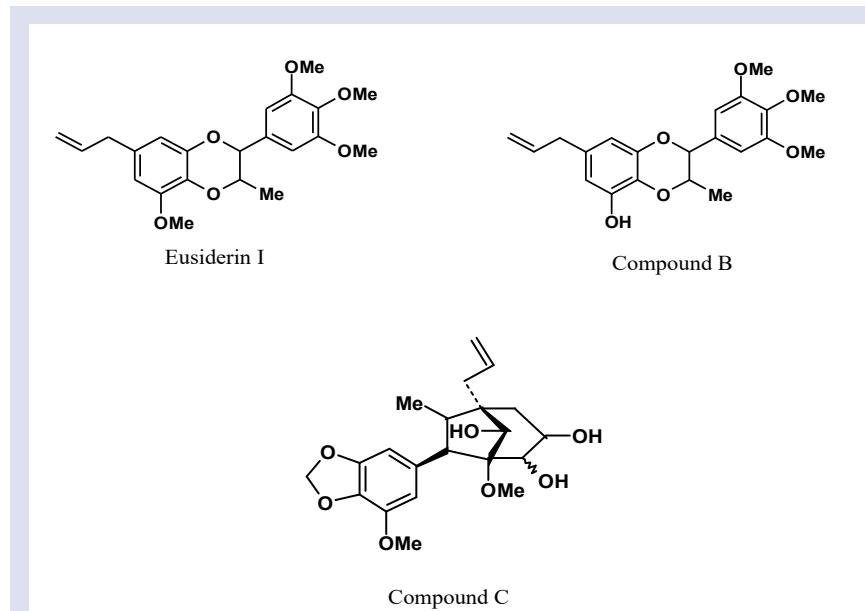
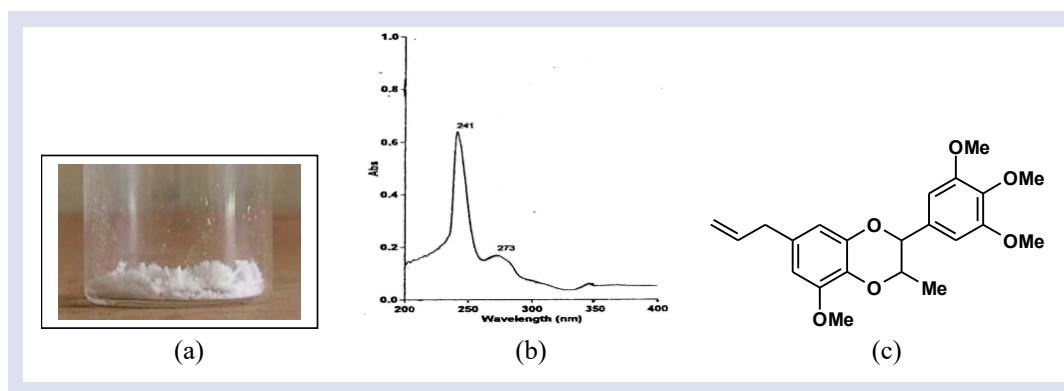
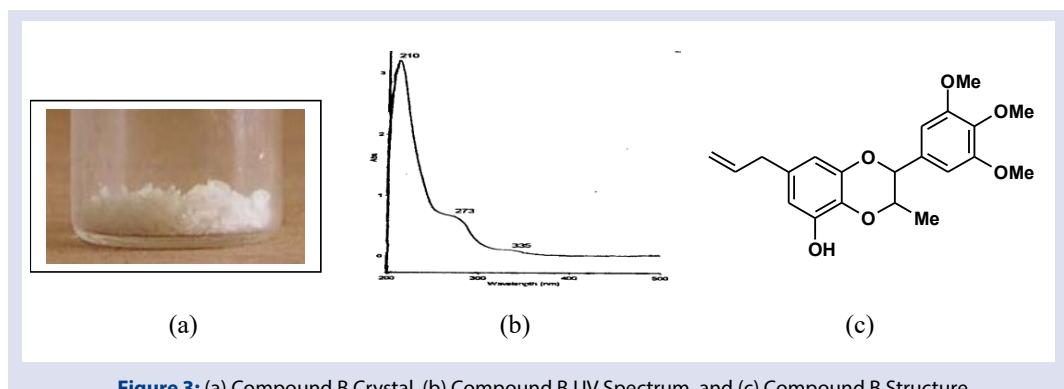
Compound C

Compound C was isolated from fraction II.1 dichloromethane extract. Compound C from isolation is white crystal with a melting point of 98–99 °C. The UV spectrum provides absorption at λ_{x} (nm) 217 and 272 [11]. The complete data can be seen in Figure 4 below.

Based on melting point and UV spectra, compared to literature it is concluded that the isolated Bulian wood powder (*Eusideroxylon zwageri*) was truly Eusiderin I, Compound B and Compound C (Table 1).

Antifungal activity

To determine the minimum inhibitory concentration (MIC) of Eusiderin I against *Microsporum gypseum*, antifungal activity were studied at different concentrations (0.625, 1.25, 2.5, 5 and 10 ppm) of samples. Minimum Inhibition Concentration (MIC) value was observed as lowest concentration effective in inhibition of fungal

**Figure 1:** Structure of Eusiderin I, Compound B and Compound C.**Figure 2:** (a) Eusiderin I Crystals, (b) Eusiderin I UV Spectrum, and (c) Eusiderin I Structure.**Figure 3:** (a) Compound B Crystal, (b) Compound B UV Spectrum, and (c) Compound B Structure.**Table 1: Previous Data and Research Data.**

Compound	Melting point (mp) ($^{\circ}\text{C}$)		λ_{max} UV Spectra (nm)	
	Previous Data	Research Data	Previous Data	Research Data
Eusiderin I	99-100	99-100	241, 274	241, 273
B	110-113	110-112	209, 273, 335	210, 273, 335
C	98-101	98-99	219, 272	217, 272

growth. The results showed that MIC value of Eusiderin I against *Microsporum gypseum* was 5 ppm (Table 2).

Ketoconazole have been used a positive control, due to its activity as broad-spectrum antifungal agent having fungistatic and fungicidal effects. The negative control (DMSO) had no activity (data not shown). The results suggested that Eusiderin I 50 ppm was the IC₅₀ value in which the concentration capable to inhibit more than 50% of *Microsporum gypseum* growth. Therefore, Eusiderin I 50 ppm was assumed as the ideal concentration of which antifungal activity had potently inhibited the colony growth of *Microsporum gypseum*.

The antifungal activity test of Eusiderin I, Compound B and Compound C from Bulian wood (*Eusideroxylon zwageri*) to phatogen fungi *Microsporum gypseum* showed that with three different concentrations (5, 25, 50, 100 and 200 ppm), Eusiderin I was a potent antifungal because it had a strong activity in inhibiting the *Microsporum gypseum* growth (Table 3). The 5 days incubation test result showed that 50 ppm Eusiderin I could inhibit the *Microsporum gypseum* colony growth. The 100 ppm Eusiderin I gave the most effective inhibition percentage because it could inhibit the *Microsporum gypseum* colony growth (= 93.9%).

The results of antifungal activity test of Eusiderin I from Bulian wood (*Eusideroxylon zwageri*) exhibited in Figure 5.

The inhibitory effect into colony growth of *Microsporum gypseum* caused by Eusiderin I in triplicate (n = 3) shown in Table 3. It can be concluded that Eusiderin I in 100 ppm had the most effective inhibitory precentage against *Microsporum gypseum* colony (= 93.9%). These results indicated that allylic moiety group play an important role to enhance the antifungal activity. Meanwhile dioxane ring is necessary to maintain the conformation and stability.^{3,10}

In this experiment, P value was statistically significant (P < 0.05). Therefore, such results of a significant value that confirms the therapeutic potency of some compounds used in traditional medicine. The effectiveness of inhibition is one of the criteria for selecting an antimicrobial compound for anti fungi. Damage caused by antimicrobial components can be mycosidal (permanent damage) and micostatic (temporary damage that can return). A component is mycosidal or micostatic depending on the concentration and culture used.³ Phenolic compounds interact with cell membrane proteins through an adsorption process involving hydrogen bonds by binding to the hydrophilic part of the cell membrane. Phenolic protein complexes are formed with weak bonds, so that they will immediately experience decomposition then followed by penetration of phenolic compounds into cells which cause precipitation and denaturation of cell membrane proteins. Damage to the cell membrane causes changes in permeability in the membrane, rsulting in the lysis of the fungal cell membrane.³

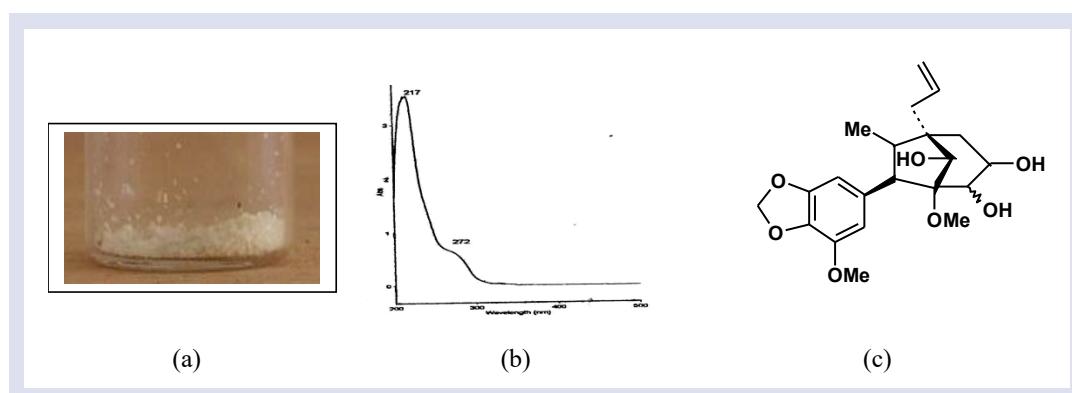


Figure 4: (a) Compound C Crystal, (b) Compound C UV Spectrum, and (c) Compound C Structure.

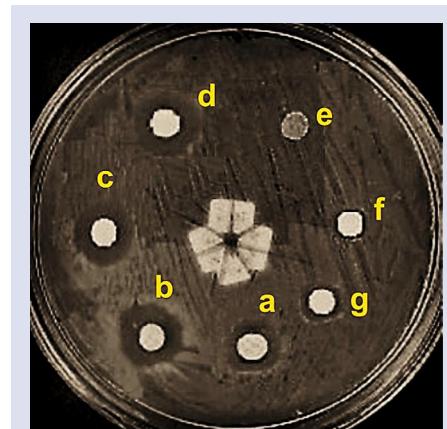


Figure 5: Antifungal activity of Eusiderin I, Compound B and Compound C from Bulian wood (*Eusideroxylon zwageri*) into *Microsporum gypseum* after 5 days of incubations. (Eusiderin I 50 ppm; b. Eusiderin I 100 ppm; c. Eusiderin I 200 ppm; d. Ketoconazole 200 ppm; e. DMSO; f. Compound B 100 ppm; and g. Compound C 100 ppm).

Table 2: MIC of Eusiderin I against *Microsporum gypseum*.

Sub culture	Concentration (ppm)	Result
1	Media	-
2	Media + Sample	-
3	10	-
4	5	-
5	2.5	+
6	1.25	+
7	0.625	+
8	DMSO + <i>Microsporum gypseum</i>	+
9	<i>Microsporum gypseum</i>	+

Annotation:

(-) : no colony growth were observed

(+) : colony growth were observed

Table 3: Percentage of Colony Inhibitory Effect of Eusiderin I, Compound B and Compound C against *Microsporum gypseum*.

Compound	Mean of Percentage of Colony Inhibitory <i>Microsporum gypseum</i> (r (%), n = 3)				
	Concentration (ppm)				
	5	25	50	100	200
Eusiderin I	18.6	38.8	68.5	95.5	94.3
	19.8	38.5	69.1	93.2	94.2
	17.7	37.9	67.9	92.9	94.7
Compound B	18.7	38.4	68.5	93.9	94.4
	6.7	11.3	20.1	43.6	57.4
	8.1	12.1	19.8	43.2	58.3
Compound C	7.4	11.7	20.4	42.8	57.2
	7.4	11.7	20.1	43.2	57.6
	11.6	20.6	41.6	60.2	73.8
Mean	11.4	21.2	42.8	60.8	72.4
	11.2	22.1	41.3	61.4	73.2
	11.4	21.3	41.9	60.8	73.1

The mechanism of inhibiting microorganisms by antimicrobial compounds can be caused by several factors, including (1) interference with the constituent cells of the cell, (2) increased permeability of cell membranes that can cause loss of cell constituent components, (3) inactivate enzymes and (4) destruction or damage to the function of genetic material.¹¹

CONCLUSION

The research had successfully tested antifungal activity of Eusiderin I, Compound B and Compound C from Bulian wood powder (*Eusideroxylon zwageri*) against *Microsporum gypseum*. The 100 ppm Eusiderin I gave the most effective inhibition percentage because it could inhibit the *Microsporum gypseum* colony growth (= 93.9%). Eusiderin I can be indicated an antifungal candidate.

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CONFLICTS OF INTEREST

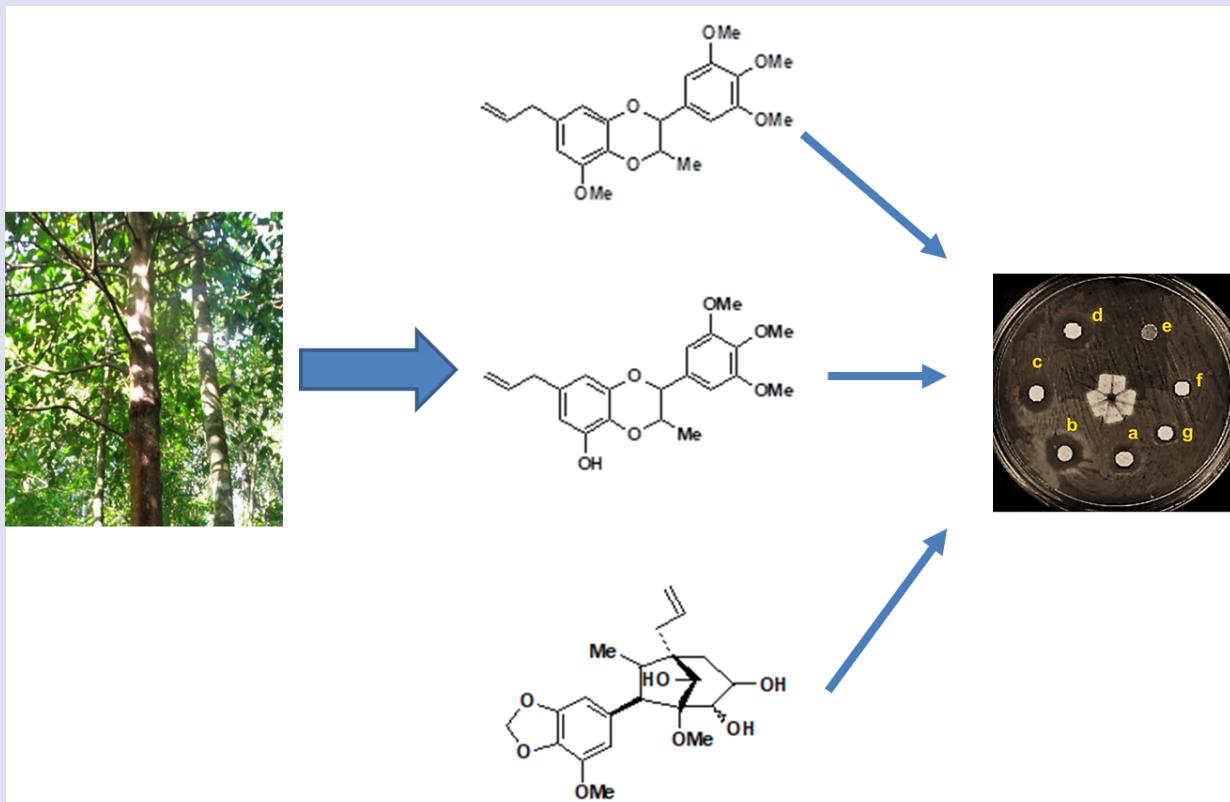
No conflicts of interest is associated with this work.

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GRAPHICAL ABSTRACT



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Anis Yohana Chaerunisaa: Associate Professor at Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Indonesia. She currently work on many research in the area of Pharmaceutical Technology and drug delivery including solid, liquid and semisolid dosage forms as well as developing polymer and other excipients for pharmaceutical dosage forms. She wrote dissertation on release adjustment of drug combined in one dosage form. She also has many experiences on drug discovery from herbals either as active compound or alternative for adjuvant therapy. She wrote many publications about her research on pharmaceutical dosage forms in many journals, as well as some books and book chapter. She is also pursuing interests on development of nanoparticles from synthesis and herbal active compounds.



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