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## Quantitative Analysis of Secondary Metabolites and Solvent Optimization of Secang Wood Extract (*Caesalpinia Sappan L/BiancaeaSappan*) as Disclosing Agent

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## ABSTRACT

Disclosing solution is a material that is often used to see the presence of plaque, made of erythrosine base material. Erythrosine is one of the food coloring ingredients and can also be used for bacterial staining, can cause allergic reactions and this dye also has the potential as a carcinogen.The purpose of the study is to figure 1). qualitative of the secondary metabolites of brazilin compounds in secang wood extract, 2). quantitative of the secondary metabolites of brazilin compounds in secang wood extracts and 3). toxicity in secangwood extract using the BSLT shrimp larva toxicity test (Brine Shrimp Lethality Test).The study design is a non-experimental one that will examine quantitatively on secondary metabolites of secangwood extract and toxicity tests.The research tools used are ovens, grinders, mechanical sieves, maceration vessels, hydraulic presses, rotary evaporators, water baths, Uv-Visspectrophotometers, glass tools and materials used are secang wood and other materials used namely ethanol pro analysis, methanol pro analysis, hexane pro analysis, ethyl acetate pro analysis, aqua sterile, what man paper, FeCl3, HCl, shrimp larvae, and larval growing media. Quantitative analysis test obtained the level of secang wood brazilin which is 8.54% b / b. In toxicity tests using Uv-Vis spectrophotometers, the results of wood extracts are not toxic to living things based on toxicity tests with shrimp larva test models. Conclusion, secang wood contains brazilin which plays a role in giving red color and is not toxic to living creatures so that it is safe to use in humans.
Keywords :Secang Woods ; Disclosing Agent

## Introduction

Secang wood (Caesalpinia Sappan L / Biancaeasappan) has an attractive color so that it can be used as a natural dye. Secang wood (Caesalpinia Sappan L / Biancaeasappan) contains such phenolics, flavonoids, tannins, polyphenols, kardenolin, anthrax, sappan chalcone, caesalpin, resin, resorcin, brazilin, d-alpha phallandren, oscimenen, and essentials (Wicaksono, et al., 2008).

Some of the research on secang wood (Caesalpinia Sappan L / Biancaea sappan) that has been done by previous studies include research conducted by:
1).Nugraha,I.P.W, .W.Suwartawan,P.A.A.Prayoga, et al., (2018), Brazilein Potential of Secang Wood (Caesalpinia Sappan L) As An Agent of Skin Depigmentation In Silico, the difference with the research to be conducted is a study conducted by Nugraha et al, using a Skin Depigmentation Agent in silico on the skin using computerized, while the research will be conducted inlaboratorium with secangwood as a disclosing agent material on the surface of the teeth..

2). Rina O.,(2017), Exploration of Natural Dyes as Food Additives that Are Safe and Have Bioactivity

for Health, the difference in Rina O's research, the application of use in food additives application in food and drink while in the form of disclosing agent applied to the surface of the teeth.

3). Widyasti, R,A, Lestari A, Amri K,et al.,(2017), Development of Standardization of Natural Batik Dyes From Secang Bark (Caesalpinia Sappan L) with Spectroscopic Techniques, measurement of red secang used as natural dyes, the equation is that natural dyes of wood as different as it is done for natural dye batik on fabric while in the study to be made in the form of disclosing agent applied to the tooth surface.

4).Amin S, Yuliana A., (2016), Analysis and Test of The Stability of Secang Wood Color Substance (Caesalpinia Sappan L) Using Visible and Infrared Spectofotometeruv, the difference is to measure the stability of the color substance of the wood here which is done is to test its pH is being done for its natural color substance and made in the form of disclosing agent.

5). Nurlisa,H,L, Riyadi H,P, Romadhan.,(2015), Use of Secang Wood (Caesalpinia Sappan L) As An Alternative To Rapid In Tilapia Skin Coloring, the results of softening on tilapia skin, the difference in research is made in the form of disclosing agent and will later be given to humans.

Disclosing agent is a plaque dye that is usually used in dentistry to be able to see the effectiveness of oral hygiene measures or to see the presence of a thin layer on the surface of the teeth (biofilm). Biofilms on the surface of teeth are often referred to as dental plaque. Dental plaque is a diverse collection of microorganisms on the surface of the tooth, which are firmly attached to the host extracellular matrix and microbial polymers (Arezoo, T, Rooha, K,K, Salehi, R,r,et al., 2010).

Disclosing agent is made from the basic ingredient erythrosin, which is one of the food coloring ingredients and can also be used for bacterial staining, can cause allergic reactions and this dye also has the potential as a carcinogen. This is because erythrosin is a derivative of fluorescein with a high iodine content (Mangiri, B,S, Yani,S, Anitasari, S.,2018).

## Methods

Non-experimental research that will examine quantitatively on secondary metabolites of secang wood extract and toxicity tests. The research was conducted at the Betel Banjarmasin Herbal Medicine Factory which already has a certificate of How to Make Good Traditional Medicine (CPOTB) from BPOM. Implementation time June to September.

**Detection of brazilin compounds:** Fractionation that has contained the two elements above is then selected, then continued with analysis using thin layer chromatography. The fraction is dissolved in ethanol, then attached to the KLT plate. Then entered the vessel and chromatography is done using the solvent methanol: chloroform (5:5). The patches formed are then dredged, and analyzed using a UV-Vis spectrophotometer, then determined the presence of brazilin compounds through the wavelength obtained.

Extract taken as much as 100 mg then dissolved in ethanol solvent as much as 10 mL, then filtered with Whatman paper. The extract is then fractionated using solvents hexane, ethyl acetate, and aquades. The three fractions are then analyzed using 2 methods namely color reagent and chromatography.Qualitative analysis is carried out as follows: a). The existence of Phenol Groups, b). The existence of C6-C3-C6 Groups and c). Detection of Brazilin

The fractions containing Brazilin resulted from fractionation and subsequent qualitative tests were analyzed in spectrophotometer. Brazilin standards are used as a comparison. Brazilin is made in a series of concentrations of 10, 20, 30, 40, 50 ppm and then reads its absorbance at maximum wavelength using a UV-Vis spectrophotometer. Furthermore, the reading results in the form of absorption are made to plot the equation of the standard curve between absorbance versus standard levels. Samples in the form of selected fractions are dissolved in methanol solvents then at maximum wavelengths analyzed like standard compounds. The absorption result is then included in the standard curve equation, so that brazilin levels are obtained..

The eggs retrieval of Artemia salina shrimp. Leach was taken from the Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, LambungMangkurat University.

Shrimp eggs are hatched 2 days before the test. Prepare vessels for hatching shrimp eggs. Egg hatching is done by soaking as much as 2.5 mg of eggs in a container containing 250 mL of seawater under a 25 watt lamp light and equipped with an aerator. The eggs will hatch and become larvae after 24 hours. Larvae of *Artemia salina*. *Leach* is good for BSLT tests that are ± 48 hours old and

ready to be used as a target toxicity test. Because if more than 48 hours feared the death of *Artemia salina. Leach* is not due to the toxicity of the extract but rather by the limited supply of food. Shrimp larvae are separated from their eggs by being picked into beakers / vials containing seawater.

The concentrations of test solutions for BSLT are 1500, 1000, 500, 100, and 0 µg/mL (as controls). For the manufacture of stock solution, tofu is smoothed to form an extract and then weighed as much as 1000 mg, then dissolved into seawater as much as 100 mL, until the concentration of stock solution is obtained 10,000 µg / mL. From this stock solution, again made concentrations of 1500, 1000, 500, 100, and 0 µg / mL.

Test the toxicity on each sample extract. Prepared containers for testing, for each concentration of sample extract requires 4 containers and 1 container as a control. Then at each concentration of solution is inserted 10 larvae of *Artemia salina. Leach*. Observations were made over a 24-hour basis for larval death where each concentration was performed three times and compared to the controls. The standard criterion for assessing the death of shrimp larvae is when it shows no movement during observation. After observation for 24 hours then the level of toxicity is determined by calculating the number of dead larvae. LC50 values are determined by Linear Regression Analysis (Widiyatni, 2010)

## Results and Discussion

**Tabel.1.**
**Secang Wood Toxicity Test**

| Kadar (µg/ml) | Percent Mortality Replication 1 | Replication i 2 | Replication 3 |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 |
| 500 | 5 | 5 | 10 |
| 1000 | 10 | 20 | 30 |
| 1500 | 40 | 40 | 40 |
| LC50 | 2232,81 µg/ml | 2012,32 µg/ml | 1809,25 µg/ml |
| **Average LC50** | | **2018,12 µg/ml**(Not Toxic) | |

Primer Source**.**

Based on qualitative analysis with the test using detection reagents obtained positive results containing flavonoids and phenolics only in ethyl acetate frasi.

While quantitative analysis conducted on KLT tests on brazilin obtained KLT profile on Secang wood obtained Rf extract = 4 /5 = 0.80 and in the literature of Pharmacopoeia Herbal Indonesia stated the value of Rf = 0.8 so that the results are in accordance with literature.

Toxicity test begins with the preparation of shrimp larvae. Shrimp larvae are used as models of living things in toxicity testing. The sample is made into five concentration series i.e. 0; 100; 500; 1000; and 1500 µg/ml. Prepared five bottles each bottle filled with 20 shrimp larvae, then inserted a sample solution that has been made.

Observed for 24 hours, then calculated the number of shrimp larvae that died from mixing with the sample solution. The dead shrimp larvae are divided by the number of shrimp larvae first, then multiplied by 100%. The toxicity test of shrimp larvae obtained LC50 values calculated on average, which is 2018.12 µg / ml. Based on lc50 value literature over 1000 µg/ml indicates the sample is not toxic.

Qualitative analysis uses the tube method by adding specific reagents. Qualitative tests were conducted on phenolics and flavonoids. Qualitative test results showed ethyl acetate fraction of wood containing phenolics and flavonoids.

Flavonoids have an important role in pollination of plants by attracting animals that help the spread of seeds (Rachmawaty, 2016). Where flavonoids have the ability to form complex compounds with proteins and antiviral properties, and can inhibit the function of bacterial cytoplasmic membranes (Wahyuningtyas, 2008).
Phenol compounds are compounds that have the characteristics of the presence of aromatic rings with the presence of a hydroxyl group in it. Phenol compounds are active substances that can provide

antibacterial effects and potentially as natural dyes (Fadillah & Alfiarty, 2015). It has the ability to form complex compounds with proteins through hydrogen bonding so that it can damage bacterial cell membranes, so it can be used to fight bacteria in the mouth or teeth.

Quantitative testing uses the UV-Vis Spectrophotometry Method. The principle of UV-Visible spectrophotometry is that radiation or white light is passed through a colored solution then radiation of a certain wavelength will be selectively absorbed (absorbed) and other radiation will be passed (transmission) Brazilin levels in herbal samples can be determined by various methods. Brazilin levels were calculated as the total brasilin levels in the sample. This calculation is based on lambert-beer law which shows a straight relationship between absorption and analyte levels. Brazilin belongs to the flavonoid group can be analyzed by the UV-Vis spectrophotometry method because flavonoids contain a conjugate aromatic system which is a chromophore group and has an auxchrome group such as -OH. (Neldawati et al., 2013).

The chemical content of Secang wood is Brazilin. Brazilin is a group of compounds that give red color to wood with a structure of C6H14O5 in crystal form. Brazilin has anti-inflammatory and anti-bacterial effects (Staphylococcus aureus and Escherichia coli) (Wahyuddin, M., S. R Pakadang& A. Aprilyani). 2017.). Brazilin will quickly form this red color due to the formation of brazilein. Brazilin if oxidized will produce brazilein compounds that are brownish red and water soluble..

Toxicity test begins with the preparation of shrimp larvae. Shrimp larvae are used as models of living things in toxicity testing One of the earliest methods for cytotoxic testing is the Brine Shrimp Lethality Test (BSLT). BSLT is one of the most widely used methods for the search for new anticancer compounds derived from plants. The BSLT method has been shown to have a correlation with anticancer activity. In addition, this method is also easy to work with, cheap, fast, and quite accurate (Meyer et al., 1982).

This method uses the larvae of *artemia salina leach* as a tried animal. This BSLT toxicity test is an acute toxicity test in which the toxic effects of a compound are determined in a short period of time, i.e. a span of 24 hours after the test dose. The procedure is to determine the LC50 value of the active component activity of the plant against the larvae of *Artemia salina Leach*. An extract is said to be toxic based on the BSLT method if the price of LC < 1000 µg / ml (Carballo, 2002).

Testing using BSLT is applied by finding a Lethal Concentration value of 50% (LC50) after 24 hours of treatment. Lc50 is a number that indicates the concentration of a cause of death of 50% of the number of animals tried. Toxicity of compounds contained in plants namely phenolics, flavonoids and tannins, where at certain levels have the potential for toxicity and can cause the death of larvae. The mechanism of larval death is related to the function of phenolic compounds, flavonoids and tannins in soyogic leaves that can inhibit the feeding power of larvae (antifedants)..

The results showed that Secang wood extract is very potential as a disclosing agent with the content of phenolic compounds and flavonoids that specifically contain brazin. In addition, Secang wood is not toxic for use on living things. It is safe to use in humans..

## Conclusion

Qualitative analysis of Secangwood contains flavonoids and phenolics.Quantitative analysis shows brazilin chemical content, Rf Extract=4/5=0.80.Toxicity tests showed the Secang was not toxic to use, LC 50=2018,12 µg/ml.


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5. Head of Pharmacology Laboratory of Lambung Mangkurat University


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