

TLC Bioautography Analysis of Sappan (*Caesalpinia sappan* L.) Wood Extract Against *Propionibacterium acnes* and *Staphylococcus epidermidis* Bacteria

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Article info	Abstract
History Submission: 23-10-2023 Review: 09-11-2023 Accepted: 20-12-2023	<i>Sappan wood (Caesalpinia sappan L.) is one of the plants that is widely used in traditional medicine in Indonesia. Sappan wood is known to have several benefits, including its ability to treat several infectious diseases caused by bacteria. The aim of this research is to determine the class of active compounds that have antibacterial activity against Propionibacterium acnes and Staphylococcus epidermidis using TLC Bioautography analysis. The TLC results showed that the ethanol extract of Sappan wood contains alkaloid, flavonoid and phenol compounds. Bioautography TLC results show that the ethanol extract of Sappan wood has the potential for good antibacterial activity against the bacteria Propionibacterium acnes and Staphylococcus epidermidis with Rf values of 1: 0.92, Rf 2: 0.78, Rf 3: 0.65, Rf 4: 0.50, Rf 5: 0.38, Rf 6: 0.23 and Rf 7: 0.09 for Propionibacterium acnes. And the values of Rf 1: 0.65, Rf 2: 0.50, Rf 3: 0.38, Rf 4: 0.23 and Rf 5: 0.09 for Staphylococcus epidermidis.</i>
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I. Introduction

Traditional medicines, especially those sourced from natural ingredients, have been used to prevent, treat, and maintain human health for a long time. Most people living in developing countries use medicinal plants for primary health care and to treat many diseases (Amin *et al.*, 2018). Sappan (*Caesalpinia sappan* Linn) is a plant that has many benefits in the fields of medicine and cosmetics, and it is empirically known to have many healing properties, including its ability to treat several infectious diseases caused by bacteria.

Caesalpinia sappan L. wood shows the presence of homoisoflavonoids and phenolics such as 4-O methylsappanol, protosappanin A, protosappanin B, protosappanin E, brazilin, brazilin, caesalpin, brazilide A, neosappanone A, caesalpin P, sappanchalcone, 3-deoxysappanone, 10 7,3',4'-trihydroxy-3-benzyl-2H-chromene, etc (Vardhani, 2019). Sappan ethanol extract contains flavonoid compounds as both primary and secondary antioxidants (Febriyenti *et al.*, 2018). Sappan wood ethanol extract contains brazilin, protoSappanin A and Sappanone B. C. sappan wood extract was also reported to show better antibacterial activity against *P. acnes* compared to 39 other medicinal plants from Indonesia. Brazilin has been reported as an active compound against *P. acnes* (Nirmal & Panichayupakaranant, 2014).

Several research results state that Sappan wood extract (*Caesalpinia sappan* L.) has been proven to have antibacterial activity. Sappan wood ethanol extract can inhibit bacterial growth with a percentage reduction in *P. aeruginosa* and *S. aureus* populations of 99.88% and 96.28% respectively (Lukmayani *et al.*, 2022). Although several studies have reported the classes of compounds in Sappan wood, it is not yet known what the distribution and prospective of the compounds contained in Sappan wood are. Therefore, testing is needed that produces more accurate data through TLC Bioautography analysis of ethanol extract of Sappan wood (*Caesalpinia sappan* Linn) against the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis*.

II. Research Method

II.1 Tools

The tools used include analytical balance, Buchner funnel, vacuum rotary evaporator, petri dish (Pyrex), set of glassware (Pyrex), incubator, set of TLC tools, UV 254 and UV 366 nm lamps, Laminar Air Flow.

II.2 Materials

The materials used include Sappan wood, 96% ethanol, *n*-hexane, ethyl acetate, chloroform, methanol, distilled water, Natrium Agar (NA) media, TLC plates.



II.3 Research Procedure

II.3.1 Tools and Materials Preparation

Tools and materials are prepared according to the needs of the research to be carried out.

II.3.2 Sample Preparation

The sample of Sappan wood (*Caesalpinia sappan* L.) used in this research was taken in Bone Regency, South Sulawesi. The sample is taken, cleaned so that there is no dirt attached to the part to be taken. After the sample was cleaned, it was shaved into Sappan wood powder and dried in a drying cabinet at a temperature of $\pm 50^{\circ}\text{C}$.

II.3.3 Extract Making

A total of 200 grams of Sappan wood were macerated using 96% ethanol solvent for 3x24 hours and then re-macerated twice. The maceration process is assisted by occasional stirring so that the extraction process takes place optimally. The filtrate obtained from the maceration results was combined, then evaporated with a rotary evaporator.

II.3.4 Identification by Thin Layer Chromatography

Sappan wood ethanol extract was first tested by Thin Layer Chromatography, using a silica gel 60 F254 plate as the stationary phase, and as a mobile phase a mixture of *n*-hexane and ethyl acetate was used with a starting ratio of (1:4). After spotting and the TLC process, several stains will be obtained and the Rf value of each stain is calculated (Yemirta, 2010).

II.3.5 Phytochemical Screening

Alkaloid Test

The alkaloid test can be carried out by detecting color or spots on the TLC plate that has been spotted with the extract and eluted. This test is carried out by spraying Dragendorff's reagent on the TLC plate. Positive results are indicated by the formation of an orange color on the spot (Raihan *et al.*, 2020).

Flavonoid Test

The flavonoid test can be carried out by detecting color or spots on the TLC plate that has been spotted with the extract and eluted. This test was carried out by spraying AlCl_3 reagent on the TLC plate. Positive results are indicated by the formation of a yellow color on the spot (Raihan *et al.*, 2020).

Phenolic Test

The phenol test can be carried out by detecting color or spots on the TLC plate on which the extract has been spotted and eluted. This test was carried out by spraying FeCl_3 and Folin Ciocalteu reagents on the TLC plate. Positive results are indicated by the formation of black color on the spot (Raihan *et al.*, 2020).

Steroid and Terpen Test

The phenol test can be carried out by detecting color or spots on the TLC plate on which the extract has been spotted and eluted. This test is carried out by spraying Liebermann Burchard reagent on the TLC plate. Positive results are indicated by the visible formation of blue, purplish blue or yellowish color on the spots (Raihan *et al.*, 2020).

TLC-Bioautography Test

The bioautography TLC test was carried out to detect active compounds that have antibacterial activity. On the TLC plate, ethanol extract of Sappan wood was spotted. Then it was eluted in a vessel with a mobile phase of a mixture of eluent *n* hexane: ethyl acetate (1:4). After that, the resulting TLC plate was attached to NA (Sodium agar) media which had been inoculated with 1000 μl of bacteria. Then the TLC plate was left for 30 minutes, then the TLC plate was removed. After that, observe if there are spots on the chromatogram which are marked by a clear zone that is not growing with microbes. Next, the stain that forms an inhibitory zone on the TLC plate is sprayed with a spray reagent to determine the type of compound that inhibits it (Yumita *et al.*, 2019).

III. Results and Discussion

Sappan (*Caesalpinia sappan* Linn) is a plant that has many benefits in the fields of medicine and cosmetics and Sappan is empirically known to have many healing properties and is often consumed by the public as a health drink. Phytochemical tests carried out on Sappan plants stated that the content of triterpenoid, flavonoid, phenolic and steroid compounds was positive. Phenolic compounds are found in many parts of the wood, alkaloid compounds are found in the stems and leaves, while the fruit contains a lot of tannin, namely approximately 40% (Yemirta, 2010).

The sample used in this research was ethanol extract of Sappan wood. This extract was obtained through extraction using maceration using 96% ethanol solvent. The maceration method is used because the flavonoid content is not resistant to high temperatures and the process does not occur heating like other methods, so it is hoped that the antioxidant content contained in the extract stream is not damaged.

Separation of the ethanol extract compound of Sappan wood (*Caesalpinia sappan* L.) by TLC using a mixture solvent of *n*-hexane : ethyl acetate (1:4) can be seen in Figure 1 and Table 1. From the spotting results, the spots were then seen using a UV_{254} nm. Then four stain points were obtained with an Rf value of 0.872; 0.690; 0.52 and 0.327. In Figure 2 and Table 2, on the TLC plate developed with the mobile phase *n*-hexane and ethyl acetate (1:4), it was seen that it showed a positive orange reaction for alkaloids after being sprayed with Dragendorff reagent. In testing the content of

flavonoid compounds, using the $AlCl_3$ reagent, it showed that the ethanol extract of Sappan wood probably contains flavonoid compounds. This positive result is indicated by the formation of a yellow color on the spot where the reagent has been sprayed. Testing the content of phenolic compounds, using $FeCl_3$ and Folin-Ciocalteu reagents, showed that the ethanol extract of Sappan wood probably contains phenolic compounds. This positive result is indicated by the formation of a blue-black and black color on the spot where the reagent has been sprayed. Testing the content of steroid and terpene compounds, using the Liebermann Burchard reagent, showed that the ethanol extract of Sappan wood probably does not contain steroid and terpene compounds.

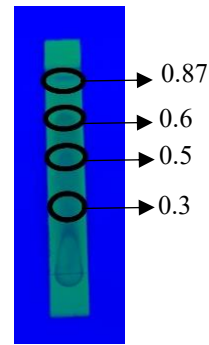


Figure 1. TLC profile from Identification of Sappan wood Ethanol Extract

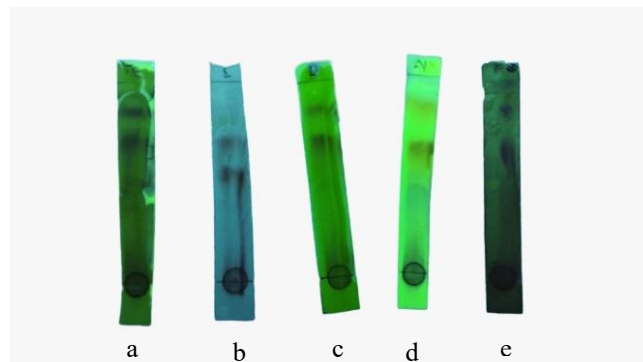


Figure 2. Phytochemical screening using specific reagents (a) $FeCl_3$, (b) Liebermann Burchard, (c) Dragendorff, (d) $AlCl_3$, (e) Folin Ciocalteu

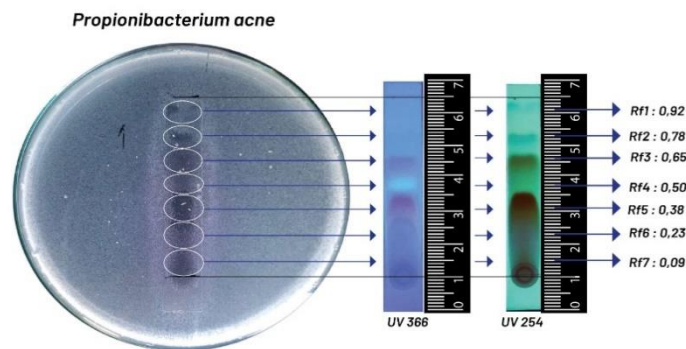


Figure 3. Bioautography TLC profile test results of ethanol extract of Sappan wood against *Propionibacterium acne* bacteria

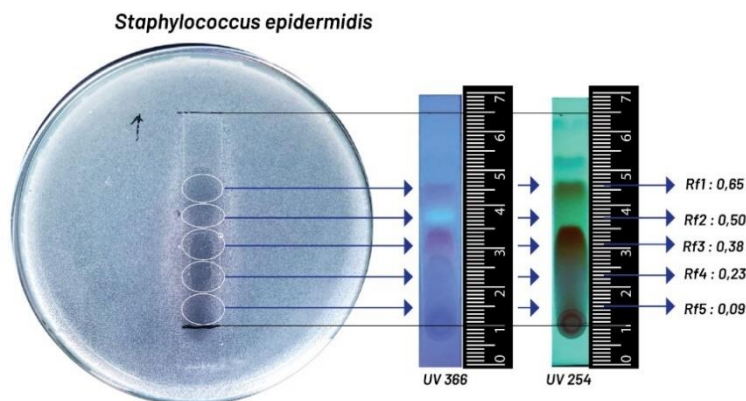


Figure 4. Bioautography TLC profile test results of ethanol extract of Sappan wood against *Staphylococcus epidermidis* bacteria

Table 1. TLC profile results of ethanol extract of Sappan wood (*Caesalpinia sappan* L.) with eluent: (*n*-Hexane : ethyl acetate (1:4))

UV Light	Spot	Rf
254	1	0.872
	2	0.690
	3	0.52
	4	0.327

Table 2. Phytochemical Screening Results using specific reagents

No	Group of Chemical compound	Reagen	Result
A	Phenol	FeCl ₃	+
B	Steroid and Terpen	Lieberman Buchard	-
C	Alkaloid	Dragendorf	+
D	Flavonoid	AlCl ₃	+
E	Phenol	Folin Ciocalteu	+

Bioautography is a detection method for finding an antimicrobial compound that has not been identified by localizing the antimicrobial activity in a chromatogram resulting from thin layer chromatography (TLC) (Yumita *et al.*, 2019). The results of TLC-Bioautography of the ethanol extract of Sappan wood showed in Figure 3 gave positive results in inhibiting the growth of *Propionibacterium acne* and *Staphylococcus epidermidis* bacteria. Bioautography TLC results show that the ethanol extract of Sappan wood has the potential for good antibacterial activity against the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* with Rf values of 1: 0.92, Rf 2: 0.78, Rf 3: 0.65, Rf 4: 0.50, Rf 5: 0.38, Rf 6: 0.23 and Rf 7: 0.09 for *Propionibacterium acnes*. And the values of Rf 1: 0.65, Rf 2: 0.50, Rf 3: 0.38, Rf 4: 0.23 and Rf 5: 0.09 for *Staphylococcus epidermidis*.

IV. Conclusions

The TLC results showed that the ethanol extract of Sappan wood contains alkaloid, flavonoid and phenol compounds. Bioautography TLC results

show that the ethanol extract of Sappan wood has the potential for good antibacterial activity against the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* with Rf values of 1: 0.92, Rf 2: 0.78, Rf 3: 0.65, Rf 4: 0.50, Rf 5: 0.38, Rf 6: 0.23 and Rf 7: 0.09 for *Propionibacterium acnes*. And the values of Rf 1: 0.65, Rf 2: 0.50, Rf 3: 0.38, Rf 4: 0.23 and Rf 5: 0.09 for *Staphylococcus epidermidis*.

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