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ANTIBACTERIAL AND ANTICANCER ACTIVITIES OF ACETONE EXTRACT *Caesalpinia sappan* L.

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Abstract

Sappan wood *(Caesalpinia sappan L.)* from the *Caesalpiniaceae* family is a plant that has been widely used as a health drink such as herbal medicine, in Sundanese called jamu or wedang secang. The drink containing *C.sappan* extract is traditionally believed to have the ability to reduce symptoms of colds, coughs, canker sores, and rheumatism, overcome fatigue and improve blood circulation. In this research, wood extract of *C. sappan L* in acetone solvent tested as antibacterial and anticancer. The antibacterial test was performed on the bacteria *Streptococcus mutans* ATCC 35668 and *Enterococcus. faecalis* ATCC 49619 with paper disc diffusion method. The microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The anticancer test is carried out *in vitro* on A549 lung cancer cells using the MTT method assay. The results showed that the wood extract of *C. sappan* L in acetone has weak antibacterial activity against S. mutans ATCC 35668 and E. faecalis ATCC 49619. The MIC is 1250 ug/mL, respectively. Aceton wood extract of *C. sappan* L. showed moderate anticancer activity with IC_{50} value 90,01µg/mL. Thus, the extract of *C. sappan* L. in acetone has the potential as a source of anticancer compounds.

INTRODUCTION

Caesalpinia sappan L. plant is known by the local names Secang (Sunda, Sumatra), Kayu Sena (Manado), Sapang (Makassar), Soga Jawa (Java), Sepang (Bugis), and Lacang (Minangkabau). The plant is also known as Buckham wood (English), Bois de sappan (French), Pau de sappan (Polish) Sappanholz (German), Su mu (Chinese), Suo (Japanese), and Fang (Thai)[1].

C. sappan L is commonly found in the tropics and grows at an altitude of 500 – 1000 m above sea level [2]. Its stem and bark are often used as natural dyes and are used in ethnomedicine. In traditional Javanese medicine. sappan wood is used as the main ingredient in making a healthy drink called wedang secang. This drink is believed to increase stamina, warm the body, and treat various ailments such as flatulence and colds [3].

In Thailand, besides being used as food coloring, Sappan wood is also used for garments and cosmetic ingredients [4]. In Asia, Sappan wood is often used for traditional medicine, especially for the treatment of tumors and cancer, as a wound medicine, as coughing up blood, antidote, anti-inflammatory, disinfectant, and as an antibacterial [5,6]. The popularity of sappan wood in traditional medicine has encouraged researchers to explore the potential of sappan wood. Several studies on the pharmacological activity of sappan wood include sappan wood extract having activity as an antioxidant [7,8,9,10], Nilesh research results et al. stated that the extract *C. Sappan* in ethanol has antimicrobial activity on several types of microbes with an inhibition zone between 14-34 mm [9]. *C. sappan* L has been extracted by various polar and protic solvents such as methanol, water, butanol, and chloroform. Extracting *C. sappan L*. by using the polar protic solvent showed able to kill several strains of cancer cells in vitro [11]. Therefore, it is necessary to conduct research by using different kinds of solvents which are aprotic and less polar, acetone. This article reports the activity of sappan wood extract in acetone as an antibacterial and

anticancer agent. The Sappan wood used in this study was from North Sumatra, Indonesia.

EXPERIMENT

Materials

A dry sample of *C. sappan L*. wood was obtained from the herbal medicine shop CV. Sempurna Medan. Mueller-Hinton Agar (MHA) (Oxoid), Mueller-Hinton Broth (MHB) (Himedia), technical acetone, and dimethyl sulfoxide (DMSO) pa were purchased from Sigma Aldrich. 0.9% NaCl, Chloramphenicol Phosphate buffered saline (PBS), Trypan Blue, Fetal Bovine Serum (FBS), Trypsin- EDTA, Trypan Blue, Roswell Park Memorial Institute Medium (RPMI), Cisplatin was used directly without any prior purification.

Instrumentations

Standard glassware in laboratories, *rotary evaporator* (Heidolph), *vortex,* incubator (Memmert), Laminar B-one V915 S, Biosafety Cabinet (BSC), Centrifuge, CO_2 Incubator, Microscope, Multimode Reader.

Procedures

Plant Extract Preparation

The extraction of *C. sappan* L was carried out using the maceration method. The amount of 1 kg of *C. sappan* L wood was macerated with acetone for 3 x 24 hours, followed by the concentration of the extract using a rotary evaporator.

Antibacterial Activity Test The antibacterial

Activity test was carried out as in previous studies [12]. Preliminary tests were carried out using the paper disc method and determination of the Minimum Inhibitory Concentration (MIC) value using the CLSI-M07-A9 microdilution method [13]. The test was carried out in two repetitions. Determination of Minimum Bactericidal Concentration (MBC) follows the procedure as in previous studies [12].

An antibacterial activity test was performed on *S. mutans* ATCC 35668 and *E. faecalis* ATCC 49619. The sample concentration is 1% in 10% DMSO. Positive controls used 30 µg chloramphenicol discs (Oxoid, United Kingdom), chloramphenicol, and 500 µg/mL chloramphenicol solution in 100% DMSO.

Anticancer Activity

Anticancer activity test was carried out on lung cancer cells (A549) obtained from the Integrated Laboratory of Padjadjaran University using the MTT assay as in previous studies [14] with tetrazolium dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Live cells will form purple formazan crystals so that they can be measured using a multimode reader at 600 nm and 570 nm wavelengths.

The percentage of cell viability is calculated by the equation:

$$\%\,Viability = \frac{(Abs.\,\text{Treatment} - Abs.\ Control)}{Abs.\,Cell\ Control - Abs.\,Control} \times 100\%$$

RESULT AND DISCUSSION

Antibacterial Test

The results of a preliminary test on gram-positive bacteria *S. mutans* ATCC 35668 (Sm) and *E. faecalis* ATCC 49619 (Ef) using the paper disc method showed a clear zone around the paper disc, indicating an inhibitory activity against the bacteria. The size of the inhibition zone is summarized in **Table 1.**

Table 1. Inhibition zone data of *C. sappan L* acetone extract.

Sample	Inhibition Zona (mm)	
	Sm	Ef
chloramphenicol	26	9.3
DMSO 10 %	0	0.0
Acetone extract of *Caesalpinia sappan*	8.5	13.1

The difference used of solvents will of course affect qualitatively and quantitatively the type of secondary metabolites extracted. One reason is the difference in the polarity of each solvent. Ethanol solvents have been used to extract secondary metabolite components in *C. sappan L* and showed the ability to inhibit bacterial growth.

The results of the previous study showed that the ethanolic extract of *C. sappan L*. inhibited the growth of Staphylococcus aureus TISTR 1466, Staphylococcus epidermidis TISTR 518, Propionibacterium acne DMST 14916 with inhibition zone diameters of 20.40±1.64, 33.37±0.51, and 34.48±2.01 mm, 20.40±1.64, 33.37 ±0.51, and 34.48±2.01 mm, as well as the aqueous extract of *C. sappan* L were able to inhibit the growth of these bacteria with zones of inhibition 21.40 ± 0.17, 30.40 ± 0.00, and 57.90 ±

1.20 mm [15]. The use of solvents with different polarities such as acetone causes different types and amounts of secondary metabolites and also differs in their ability to prevent the growth of bacterial species. Table 1 shows that the extract of *C. sappan L* in acetone has an inhibition zone on *S. mutans* ATCC 35668 of 8.5 mm, smaller than the positive control (26 mm). But for the type of bacteria *E. faecalis* ATCC 49619 showed a larger inhibition zone than the positive control. When compared with the results of previous studies [15], the inhibition zone of the extract *C. sappan L* in acetone solvent is much smaller. This is most probably due to the difference in polarity between the acetone solvent and the active anti-bacterial compounds in plants *C. sappan L*, such as Brazilin and Brazilein which contains many hydroxyl groups.

The results of this study are also to the results of previous studies, which stated that the ethanolic extract of *C. sappan* L had antimicrobial activity on several types of microbes with an inhibition zone between 14-34 mm, namely against *Pseudomonas aeruginosa* (34 mm), *S. aureus* (31 mm), *Salmonella. Typhi* (24 mm), *Enterobacter aerogens* (21 mm), *Escherichia coli (*15 mm), and *Aspergillus niger* (14 mm) [9]

In general, the mechanism of action of antibiotics is to inhibit the growth of bacteria (bacteriostatic) or kill bacteria (bactericidal). The data on the results of determining the MIC and MBC of *C. sappan* L acetone extract are summarized in **Table 2**.

Tabel 2. Value of MIC and MBC acetone extract of *C. sappan* L.

Test Bacteria	*C.sappan L*		chloramphenicol	
Gram-Positive Bacteria	MIC µg/mL	MBC µg/mL	MIC µg/mL	MBC µg/mL
S. mutans ATCC 35668	1250	5000	0,97	125
E. faecalis ATCC 49619	1250	1250	7,8	250

MIC and MBC values of *C. sappan* L acetone extract against *E. faecalis* ATCC 49619 are 1250 µg/mL. Thus, *C. sappan* L acetone extract is bactericidal against these bacteria, while the bacteria *S. mutans* ATCC 35668 tends to be bacteriostatic. This is indicated by the concentration needed to kill the bacteria four times the inhibitory concentration.

Based on the criteria for the activity of an extract as antibacterial, if the MIC value of an extract is less than 100 µg/mL, it is categorized as active, 100 < MIC < 625 ug/mL is classified as moderate, and inactive if the MIC value is > 625 g/mL [16]. The acetone extract of C. *sappan* L from North Sumatra was categorized as inactive as a source of antibacterial compounds. The results of this measurement are different from the results of research by Mohan et al., namely methanol and water extracts of *C. sappan L* have activity against gram-positive bacteria, including *S. aureus* and *B. subtillis* with MIC values of 140 - 820 ug/ml and gram-negative bacteria against *K. pneumonia*, E. *coli*, *P. vulgaris* with MIC values of 220 - 860 ug/ml. This difference may be due to the different extraction solvents used and different growth sites for the samples [17].

Activity Test Results Against Lung Cancer Cells (Cell A549)

In Asia, especially in Indonesia, *C. Sappan* is often used in traditional medicine for the treatment of tumors and cancer. The anticancer effect of the extract can be seen from the inhibition of cell growth by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), living cells will form colored formazan crystals purple, so the rate of cell growth can be measured with a spectrometer.

The results of the measurement of absorbance of *C. Sappan* against lung cancer cells are summarized in **Table 3**.

Based on the data in **Table 3**, a linear regression graph was made, and the equation y = 0.7641x – 18.779 which can be shown in **Figure 1**.

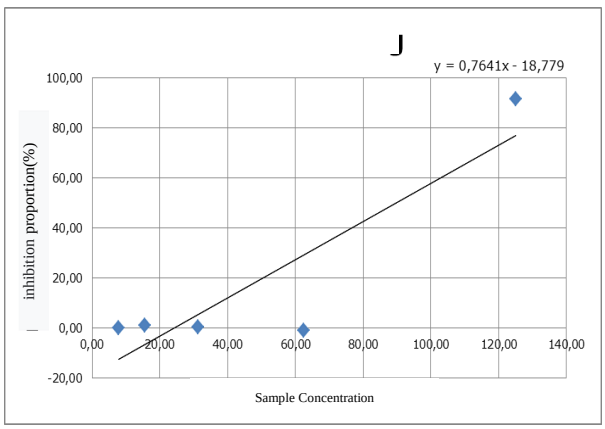


Figure 1. The curve of the cup extract test results against A549 cells.

Table 3. The results of the *C. Sappan* extract absorbance test on A54 cells.

Wavelength /nm	Media	Media cell	Cisplatin	DMSO 2,30%	Sample Concentration (µg/mL)							
					1000,00	500,00	250,00	125,00	62,50	31,25	15,63	7,81
570	0,4898	0,7985	0,6403	0,8231	0,5812	0,5137	0,5303	0,5425	0,8351	0,8116	0,7951	0,8107
	0,4866	0,8141	0,6306	0,8298	0,5670	0,5341	0,5279	0,5513	0,8366	0,8071	0,8168	0,8014
600	0,6277	0,1738	0,4902	0,1808	0,7139	0,6510	0,6375	0,6179	0,1968	0,1806	0,1765	0,1772
	0,6216	0,1773	0,5118	0,1836	0,6997	0,6766	0,6411	0,6148	0,1957	0,1791	0,1870	0,1738
Corrected Absorbance	-0,1364	0,7611	0,2864	0,7788	0,0036	-0,0008	0,0292	0,0610	0,7747	0,7674	0,7550	0,7699
		0,7732	0,2552	0,7826	0,0037	-0,0062	0,0232	0,0729	0,7773	0,7644	0,7661	0,7640

The parameter for testing the cytotoxic activity of a sample as an anticancer is based on the IC_{50}. IC_{50} describes the concentration level of inhibiting the growth of cancer cells as much as 50%. From this equation, the IC_{50} = 90.01µg/mL value is obtained. The National Cancer Institute (NCI) has established criteria for anticancer activity. A plant extract is said to be moderately active if the IC_{50} value is 30 µg/mL - IC_{50}< 100 g/mL and greater than 100 µg/ml are categorized as inactive [18]. Thus, the acetone extract of sappan wood has moderate anticancer activity. This study's results align with Bukke et al.'s research. The sample of sappan wood from India extracted with chloroform at a concentration of 50 mg/ml showed anticancer activity with IC_{50} = 87.27± 0.03, aqueous extract 86.87±0.13, and methanol extract 101.52 ±0.00. Likewise, at a concentration of 450 mg/ml showed the same results, namely chloroform extract had an IC_{50} value of = 67.07 ±0.00, aqueous extract 97.48±0.00, and methanol extract 84.23±0.02 showed moderate anticancer activity [19].

Figure 2 shows the results of cell morphology observations. Although the IC_{50} value = 90.01µg/mL at a concentration of 62.50 µg/mL many A549 cells were dead and at a concentration of 125 µg/mL almost all A549 cells die. This can be seen from the cell nucleus which does not glow and the cell membrane looks broken. On the other side, the morphology of living cells can be seen in that the cell nucleus shines brilliantly and the membrane boundaries with the media are visible. Thus extract *C.sappan* L can be used as an alternative ingredient for the prevention or reduction of risk affected by cancer [20, 21, 22].

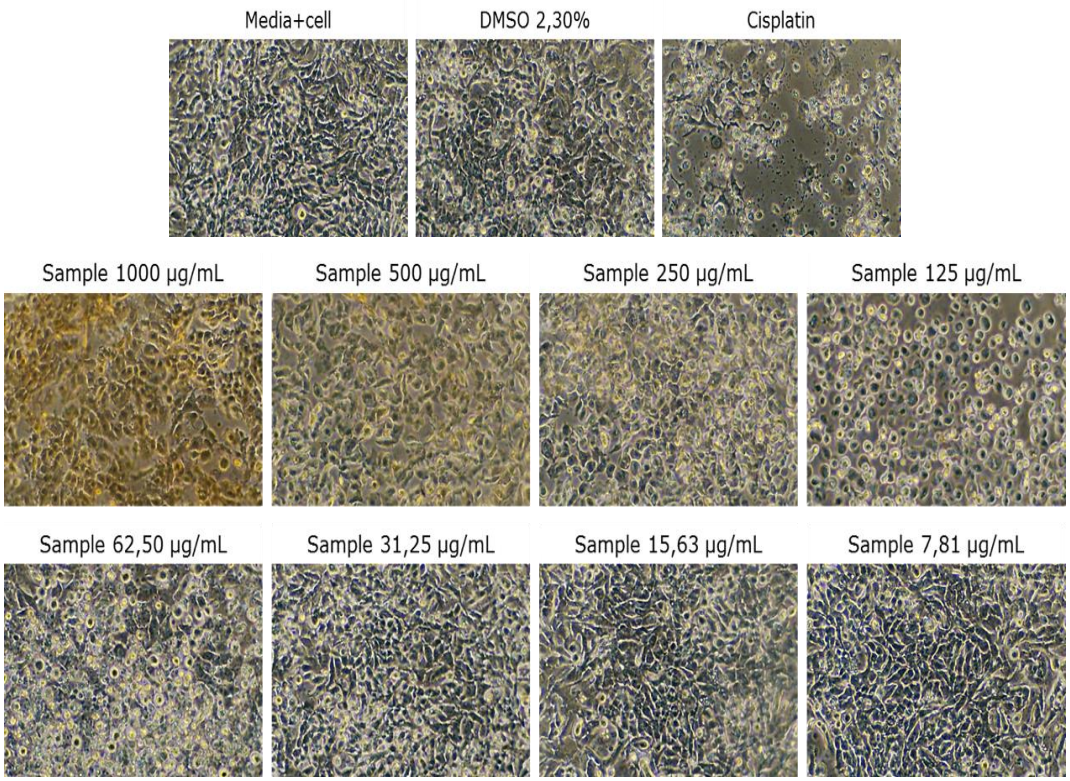
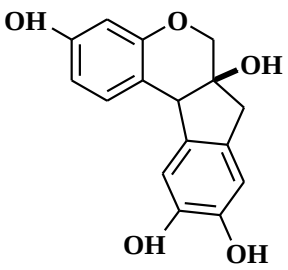


Figure 2. Morphology of cell A549 assay results Sappan wood extract.

Secondary metabolites are compounds that are not directly involved in organisms' growth, development, or reproduction. Without secondary metabolites, the organism will suffer damage or decrease its survival ability. The function of secondary metabolites in an organism is to survive predators and to support the reproductive. process. Humans use secondary metabolites, among others, as drugs. Sappan wood contains various secondary metabolites of flavonoids, terpenoids, tannins, and saponins [23]. **Figure 3** shows the molecular strucuture of the main compounds, Brazilin and Brasilein, both of which belong to the flavonoid group, Fangininoksi A and Fanginin A, while Protosappanin D and E are phenolic of biphenyl dimer, tannins.

Flavonoids from *C. sappan* are known to have antioxidant and anti-inflammatory effects. Anti-tumor and antiviral [24]. Brazilin, brazilein, and the content of other *C. sappan* compounds such as caesalpiniaphenol can affect proteins that play a role in apoptosis. Flavonoids also exhibit antibacterial activity [25].

Terpenoids that have been isolated from *C. sappan*, namely Fangininoksi A and Fanginin A, are diterpene terpenoids [26]. Based on the prediction of the difference in polarity between acetone and the compounds Fangininoksi A and Fanginin A, these compounds are more easily withdrawn by acetone solvent than the flavonoid compounds. So it can be understood that the extraction of *C.sappan* in acetone has more potential as an anti-cancer.



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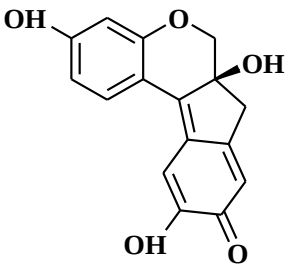

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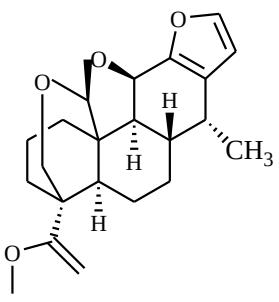

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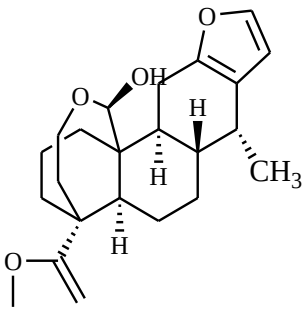

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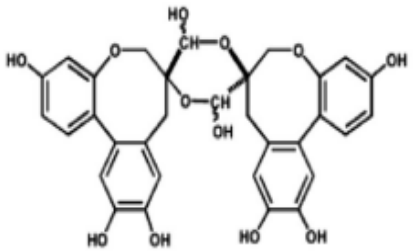

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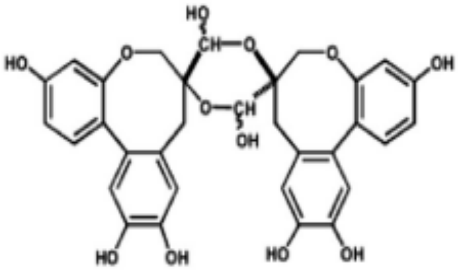

(6)

Figure 3. Molecular structure of Brazilin (1), Brasilein (2), Fangininoksi A (3), Fanginin A (4), Protosappanin D (5), Protosappanin (6) from *C. sappan* L.

CONCLUSION

The acetone extract of *C. sappan* is not prospective as an antibacterial but has good prospects as a source of lung anticancer compounds.

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