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Screening for active agent to anti-diarrhea by an evaluation of antimicrobial activities from three fractions of sappan wood (*Caesalpinia sappan*. L)

Oktaf Rina[1,2], Sanusi Ibrahim[1], Abdi Dharma[1], Afrizal[1] and Chandra Utami Wirawati[2]

[1]*Department of Chemistry, Andalas University, West Sumatera, Indonesia*
[2]*Department Agriculture of Technology, The State Polytechnics of Lampung, Lampung, Indonesia*

ABSTRACT

The object of this research was to evaluate and compare the antimicrobial activities of three fractions sappan wood (Caesalpinia sappan. L.) also known as sappan wood, that was methanol, ethyl acetate, and hexane fractions. The sappan wood was collected from traditional market, minced, extracted by methanol and fractionationtreatment by n-hexane continues by ethyl acetate. These fractions were evaluated for antimicrobial activity against bacteria Escherichia coli and Staphylococcus aureus using the cup-plate agar diffusion method in Nutrient Agar medium to determine the minimum inhibitory concentration (MIC). The phytochemical of it extract was also analysis by test identification tests. The phytochemical of sappan wood proved the presence of phenolic, flavonoid, tannins and a little bit of terpenoid and saponin but no alkaloid and steroid compound. Out of the three fractions of sappan wood, the methanol fraction showed maximum average clear zone inhibition against. Antimicrobial activity from the three fraction(n-hexane, ethyl acetate, and methanol) of it wood showed that methanol fraction has the best value. The inhibition maximum concentration value of methanol extract of it to E.coli and S.aureus were 13,7± 0,6 mm and 16,3±2,1 mm, it recommended for use as agent antimi crobial to disease diarrhea earlier.

Keywords: sappan wood , antimicrobial activity, flavonoid, *E. coli, S.aureus*

INTRODUCTION

Caesalpinia sappan is a traditional medicine plant which grown in several countries like Indonesia, India, Vietnam, and Sri Lanka[1]. Several classes of chemicals compounds, such as flavonoids, diterpenes, and steroids, have been isolated from various species of genus Caesalpinia [2].*C. sappan* cultivated in Indonesia and several countries in South East Asia for the medicinal component, it is knownsappan plant and has been used curative agents for infections by microbial. Sappan wood is also knownbrazil wood and its part prepared for the produce of red dry which it contains water-soluble dyes such as brazilin[2-4].It also reported having antioxidant activity which useful for our body, beneficially [4-9].Plant-derived substances of medicinal plants are the richest bio-resources of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for syntetic drugs [10].

E. coli and *S.aureus* could live in water and air, they could infect human body e.g. digestion, diarrhea, and vomit. They infected from food, drinking, and environment. The traditional medicine treatment should suggest that boiled of guava leave (*Psidium guajava*) to eliminate the effect of they infection because it containing tannins compound. Meanwhile, if it adding with sappan wood which has antimicrobial activity that it could be better medicine. The major secondary metabolic compound in sappan wood assumption included flavonoid class that has antioxidant and antimicrobial activity. However, it metabolic compound distributed in complex cell and have a various polarity than it must be fractionation treatment to give information what fraction of sappan wood have the higher antimicrobial activity.The purpose of this research was toevaluate three fractions of it as methanol, ethyl acetate, and n-hexane.

Therefore, this investigation could be suggested to study of medicine formulation that used to superintend infection of *E.coli* and *S.aureus*, it should be a functional food.

MATERIALS AND METHODS

Materials: Materials and tools such as glassware, disc Petri, micropipette, UV-Visible Spectrophotometer Pharmaspec UV-1700 Shimadzu. A bacterial suspension of *Escherichia coli* and *Staphylococcus aureus* were taken from Microbiology Laboratory, Agriculture Institute of Bogor. Chemicals were used consist of n-hexane, ethyl acetate, methanol and DMSO (Dimethyl sulfoxide)as a solvent for samples. The antimicrobial activity was evaluated by disc diffusion in Nutrient Agar Medium.

Preparation of simplistic, identification, extraction and fractionation: Sappan wood was collected from traditional market, minced, extracted by ethanol thanfractionation with ethyl acetate and n-hexane resulted in three fractions. Identification test of simplistic was done by qualitative phytochemical tests according to find the presence of secondary metabolic compound were carried out by standard procedure [10].Extraction and fractionation: 1,00 kg of sappan wood was extracted by 2,0 L methanol for three times, then evaporatethe solvent by rotary evaporator. The crude extract was fractionated by ethyl acetate step by step in a separateflash andthe solventwas evaporated by rotary evaporator too. The last, we get it as a fraction of methanol.

Minimum Inhibitory Concentration Test : The treatments were conducted by three fractions of sappan wood for four degree concentrations (10 ; 30 ; 50 and 70% (w/v) in DMSO. 2 µL of the suspension of bacteria was added in hole plates and the solution DMSO served as a negative control then incubated for 2 x 24 hours at 30-32^{o}C. All plates were incubated for 18 hours at 37^{o}C in incubator case. The clear zone were formed around it colony was evaluated by vernier caliper. All the samples were tested in triplicates to confirm the activity.

RESULTS AND DISCUSSION

Phytochemical investigation

Phytochemical test of dried sappan wood samples was described in Table 1.

Table 1.Phytochemical screening of sappan wood (*Caesalpinia sappan*. L)

Phytochemical tests	Results
Alkaloids	-
Flavonoid	+
Phenolics	+
Saponins	+
Terpenoid	+
Steroid	-
Tannin	+

(+) presence; (-) : absence

The secondary metabolites form by the plants, which are organic compounds for the protection agents to pests and dysfunctions in the human body, coloring agents, as the plant's own hormones and scents or attractants of that plant. *Caesalpinia sappan*.L as known sappan wood or secang wood which collected too in plant medicine garden at Department of Pharmacy, Andalas University, West Sumatera, Indonesia.

The phytochemical test was proved that sappan wood was contained flavonoid, phenolics, tannin, saponins, and terpenoid but none alkaloids and steroid. That compoundhad different polarity and activities. Then it must be fractionation treatment in several solvents with adifferent polarity so we were used methanol, ethyl acetate, and n-hexane as solvents for the separate compound in sappan wood.

Antimicrobial test of three fraction *Caesalpinia sappan*. L

The clear zone will appear around colonies of *E.coli* and *S. aureus*than measured as microbial activity value (Figure 1).

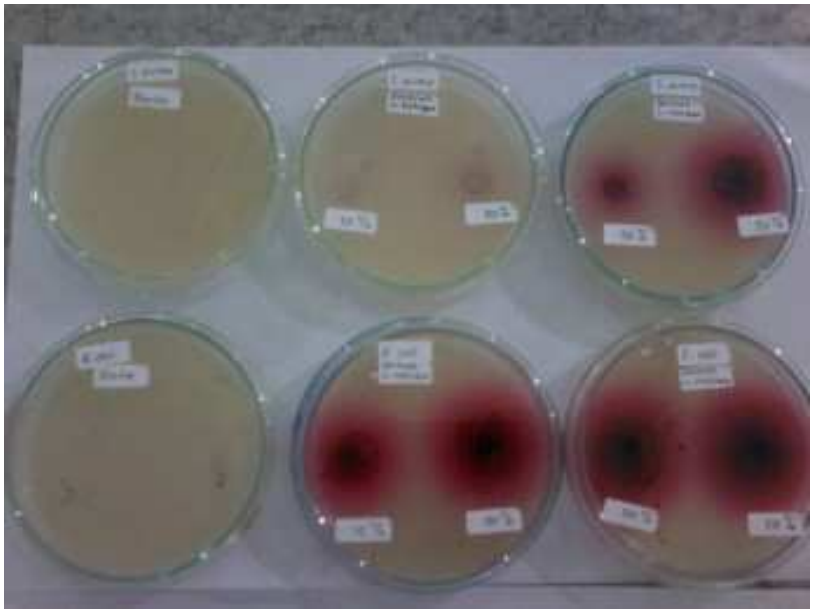

Figure 1.The clear zone of three fractions of sappan wood to *E.coli* and *S.aureus*

The sappan wood was containing a secondary metabolic compound which has antimicrobial activity. The test for screening antimicrobial activity of three fractionsit was explained in Table 2. It was done by disc diffusion essay for in vitro test to *E. coli* and *S.aureus* strains. There was three concentration of each extract as 10, 30, 50 and 70% (w/v) in DMSO. Suspensions of each were inoculated in wells of Nutrient Agar Mediumand incubated for 24 hours at incubator case for 30^{o}C.

Minimum Inhibitory Concentration Test

Table 2.Inhibitory activity of sappan wood extracts to *E.coli* and *S.aureus* strains

Fractions	Clear zoneof inhibition (mm)							
	E.coli				*S.aureus*			
	10%	30%	50%	70%	10%	30%	50%	70%
methanol	13,7±0,3	19,5±0,1	23,4±0,09	22,2±1,1	16,3±0,2	19,2±0,5	20,7±1,1	23,9±0,3
ethyl acetate	17,3±0,5	19,6±0,3	21,8±0,1	21,4±0,9	8,8±0,9	11±0,5	13,9±1,3	16,3±2,1
n-hexane	R	R	1,00±0,5	1,27±0,3	R	R	10,0±0,5	12,3 ±0,2

Experiments were done in triplicates; SD for standard deviation; R:resistance and no activity

Bacteria can be found in everywhere including of the air, soil, and water, most of them in nature pathogenic to human being. The minimum of inhibition for it potential was observed for symptom fever by infection of *E.coli* and *S.aureus*. Both of them have the ability for infection in intestines with the result that sorption of it solution could increase that feces become liquid, it calls diarrhea infection. The result wasan explanation that ethanol and ethyl acetate fractions were activity inhibition by 10% about 13,7 mm and it increased for higher concentration until 70% at 22,2 mm. Saravana *et al*. [8] reported that antibacterial of an extract of sappan wood for *E coli* with inhibition 10 mm for added 50 µl and it proved that sappanwoodhas a good antimicrobial activity. Meanwhile, fraction n-hexane has not shown inhibition for 30%and began for 50% given inhibition activity. That explained that non-polar component in sappan wood have a little inhibition activity for that microbe cause its containing lipid compounds and terpenoids. Several of alkaloid compounds have antimicrobial too but screening phytochemical of sappan wood given that it have no alkaloid compound.

Sappanwood as tropic plant and distribute in South countries like Indonesia, it has been a long time used as medicine and have abundant of secondary metabolite compounds (Table 3). Sappan plant is also known as sappan wood or secang wood. Antimicrobial of it can beused and suggest to get formulation of drug e.g. antidiarrhea. Fractionation of sappan wood have three fractions and it showed that methanol fraction has higher antimicrobial activity than ethyl acetate fraction but none in hexane fraction. It could state that methanol fraction has much of polar compound e.g. flavonoid, phenolic, and tannin that have antimicrobial activities. In compliance with several researchers that reported antimicrobial compound in sappan wood [8].

Table3.Chemical compound isolated from sappan plant (*Caesalpinia sappan*. L)

No.	Compounds	Activities reported
1.	Neoflavonoid : brazilin	collagen receptor [14]
2.	Neoflavonoid : brazilein	anthemintic[6]
3.	Flavonoid	anti-influenza[12]
4.	Phenolic	anticancer [15,16]
5.	Flavonoid	protection for DNA damage[7]
6.	Flavonoid	antimicrobial[8,9,13]
7.	Flavonoid : brazilein	immunosuppresion component[17]
8.	Phenolic	hepatoprotective[4,20-21]
9.	Homoisoflavanonesappanone A	inhibition of melanogenesis[19]
10.	Phenolic	anti-inflammatory[18]
11.	Phenolic	antioxidant [4,6,7,11]

Antimicrobial from natural compound usually flavonoid and phenolic compound. Sappan wood was indicated has both of it (Table 3).Sheng *et al*., (2012) reported that brazilin and brazilein as a major compound that constitutes of flavonoid compound (neoflavonoid) and has a red color in the water.

CONCLUSION

The extracts of sappan wood have phenolic, flavonoid, tannins and little bit of saponin, but none alkaloid and steroids. The methanol fraction for 10% (w/v) has done activity antimicrobial by inhibitory minimum concentration(MIC) to *E.coli* and *S.aureus* were 13,7 mm and 16,3 mm. It was potential for use it as a component in functional food what given fever preventive cause infection of microbial like diarrhea. Future investigated could befocussed to establish them as an agent as anti-diarrhea by*in vitro* and *in vivo*.

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