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Optimization of Extraction from Sappan Wood and Its Influence on Food Bacterial Contaminants

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Abstract

Sappan wood has already known as an ingredient of jamu, especially in Central Java. If it dissolved in hot water, it will show a red color and has been used as health drink for blood current obstruction, diarrhea, blood cough, dysentry, malaria and tetanus. The red pigment of sappan wood contained flavonoid and polyphenol. The two kind of extractions of sappan wood used in this experiment were obtained by boiling water extraction at 90^{o}C in 15 minutes and maceration in ethanol 50% overnight. The aim of this research was to find the potential of inhibiting sappan wood for presence of bacterial contaminants in food, such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* in several time storages. Microbial examination by observing visual characteristics of damage to the food model and the decrease number of food bacterial contaminants. The result showed that ethanol extract 10% of sappan wood inhibited a food bacterial contaminants in three days at cold temperatures storage (8-10^{O}C), and percent decrease population of *E. coli*, *S. aureus* and *P. aeruginosa* were 99.74%, 96.28%, and 99.88%, respectively.

Keywords : Ethanol extract, food contaminant bacterias, inhibiting, sappan wood, water extract

Optimasi Ekstraksi Kayu-Secang dan Pengaruhnya terhadap Bakteri Kontaminan Pangan

Abstrak

Kayu secang dikenal oleh masyarakat Jawa Tengah sebagai bahan pendukung jamu. Seduhannya dalam air panas berupa larutan berwarna merah yang berkhasiat sebagai minuman kesehatan untuk gangguan aliran darah, diare, batuk darah, disentri, malaria, tetanus. Zat warna merah tersebut diketahui mengandung senyawa flavonoid dan polifenol. Telah dilakukan penelitian dengan menggunakan kayu secang yang di ekstraksi dengan menggunakan dua pelarut yaitu air dengan suhu 90^{o}C selama 15 menit dan maserasi dalam etanol 50% selama 24 jam. Tujuan penelitiannya adalah untuk mendapatkan data yang menunjukkan kemampuan ekstrak kayu secang dalam menghambat pertumbuhan beberapa bakteri kontaminan pangan, yaitu *Escherichia coli*, *Staphylococcus aureus* dan *Pseudomonas aeruginosa* selama penyimpanan beberapa waktu. Pengujian mikroba dengan mengamati ciri visual kerusakan pada model pangan dan jumlah penurunan mikroba kontaminan pangan yang dikontakkan. Hasil penelitian menunjukkan bahwa ekstrak etanol 10% dapat menghambat pertumbuhan mikroba selama 3 hari penyimpanan pada suhu dingin (8-10^{o}C) dengan persen penurunan populasi *E. coli, S. aureus* dan *P. aeruginosa* berurutan adalah 99,74%; 96,28% dan 99,88%.

Kata Kunci: Ekstrak air, ekstrak etanol, hambatan, kayu secang, kontaminan pangan, mikroba

1. Introduction

Sappan wood (*Caesalpinia sappan* L) is a kind of bush plant or a small tree. Sappan wood is special because of its ability to produce red pigment when boiled in water. In Central Java, sappan wood is known as health drink because it is able to warm the body, and treat some diseases such as expedite blood circulation, dysentry, malaria, tetanus.[1]

The water extract of sappan wood with pH 7.0 has been proven to inhibit the growth of *E. coli*, *S. aureus* and *P. aeruginosa*. The 40% extract could inhibit those three bacteria with diameters 10,05; 10,75; and 13,07 mm respectively.[2] However, the amount of pigment which is able to be extracted in water depends on temperature and duration time of extraction. Extraction at 60°C for 30 minutes produce a better orange color than extraction at 90°C for 60 minutes, but the color was pale and unattractive to be used as a food pigment.[3] For obtaining a better extract of sappan wood, an advanced research using two extraction models was done in water at 90°C for 15 minutes and maceration in 50% ethanol for 24 hours.

The aim of this research was to obtain the better quality extract of sappan wood which has activity to inhibit the microbial growth of food bacterial contaminants in low concentration. The results of this research would be recommended as natural dye in food as well as a preservative if it meets the requirements of toxicity test soon.

2. Materials and Methods

2.1. Materials

Escherichia coli ATCC 8739, *Staphylococcus aureus* ITBCCB90, and *Pseudomonas aeruginosa* (Laboratorium Mikrobiologi dan Teknologi Bioproses Program Studi Teknik Kimia, Fakultas Teknik Industri, Institut Teknologi Bandung), sappan wood (SITH, ITB), *Nutrient Agar* (NA).

2.2. Procedure

Determination of Sappan wood is performed to ensure the identity of its materials. Then continued by phytochemical screening and examination of extract characteristics such as secondary metabolite properties, moisture and ethanol content.[4] Water extract was incubated 90ºC for 15 minutes and ethanol extract was treated using maceration methods with 50% ethanol.

The concentration of both extract of sappan wood were tested in present study was 5% and 10% were contacted to three representative bacterial contaminants (*E. coli, S. aureus, P. aeruginosa*) in sugar drink sample as food model to obtain the potential illustration of extract against the microbial contaminant in food.

The principle of the test is based on the number of viable bacteria (which still survive) after contacted with the extracts which is suspected to have the potential to inhibit bacterial growth. The total population of three bacteria has been calculated before the contact with Total Plate Count (TPC). The contact between bacteria and samples was carried out at room temperature and cold temperature (8-10ºC) then the effect was observed on day 1, 3 and 5. Potential inhibition of both sappan wood extract on the growth of food bacterial contaminants is indicated by a decrease in the bacterial population.[5]

3. Results

3.1. Determination of sappan wood plant (*Caesalpinia sappan* L)

Sappan wood plant was determined as follows :

Division : Magnoliophyta
Class : Magnoliosida (Dicots)
Clade : Rosidae
Ordo : Fabales
Family : Fabaceae
Genus : *Caesalpinia*
Species : *Caesalpinia sappan* L

3.2. Dried Wood Extraction

The extraction of the dried sappan wood was carried out by two methods, extraction in water at 90°C for 15 minutes and then followed by maceration in 50% ethanol for 24 hours (Figure 1).

3.3. Phytochemical screening

Secondary metabolites of the dried

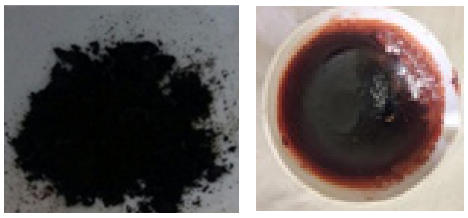


Figure 1. Water Extract (freeze-dried) and Ethanol Extract of Sappan Wood

wood, water extract and ethanol extract were tested for those existence. The extracts revealed the presence of: flavonoids, tannins, polyphenols, quinones and monoterpenes. Those contents provided an alleged potential of sappan wood extract in inhibiting the growth of the test bacteria.

3.4. Characteristics of Extracts

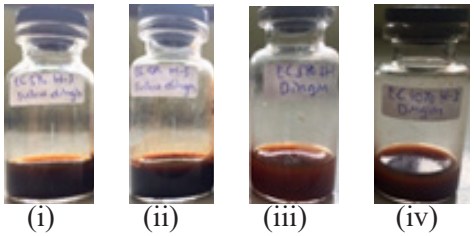
The obtained extracts were examined for some characteristic properties. The result was that the extract of water and ethanol of sappan wood have characteristics as follows:

a) Water content: water extract 6.67% and ethanol extract 4.5%
b) The specific gravity of ethanol extract is 81.33% v/v
c) Yield: water extract 1.96% and ethanol extract 8.89%.

3.5. Influence Test of Sappan Wood Extracts in Food Bacterial Contaminants

The samples which contained sugar was mixed by using water extract or ethanol extract 5% or 10%, then contacted with one type of the test bacteria (Figure 2).

3.6. Observation of food model during storage[10]



(i) (ii) (iii) (iv)

Figure 2. Sample drinks that have been contact with test bacteria: (i) sample + water extract 5%, (ii) sample + water extract 10%, (iii) sample + ethanol extract 5%, (iv) sample + ethanol extract 10%

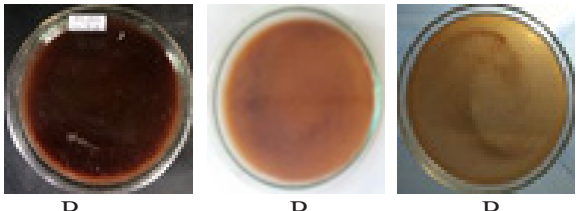
During 5 days of storage, some samples were seen as turbid solution and the bacterial growth was observed. Some other samples were seen as a clear and no signs of damage was observed. The clear sample which was odorless and showed no signs of test bacterial growth, was contained 10% ethanol extract of sappan wood. Calculation of the test bacteria population which still viable (survived) ranged between 55.53% - 97.12%. A decrease population of contaminant bacteria was higher in sample which was stored in refrigerator than at room temperature on the third day. The concentration of 10% ethanol extract was better in inhibiting the growth of test bacteria than the water extract and 5% ethanol extract. In those conditions, the percent decrease of the bacterias population were *E.coli* 97.12%; *S. aureus* 96.28% and *P. aeruginosa* 99.88% (Figure 3).

4. Discussion

The water extract of sappan wood showed no inhibitory effect against the three test bacteria, hence it is necessary to freeze-drying to eliminate some of the water content.[11]

The ethanol 50% extract of sappan wood with test bacteria sample, had a resistance for three days at room temperature, while the samples which were stored at cold temperatures could last until the fifth day. The damage of samples were indicated by the presence of bacterial growth, the solution turned into turbid and smell sour.

The potential examination of ethanol extract 50% of sappan wood showed a good result. After observation for 5 days, samples



B_1 B_2 B_3

Figure 3. Observation of viable bacteria in drinks sample containing 10% ethanol extract of sappan wood in cold temperature. *E.coli* (B1); *S. aureus* (B2) and *P. aeruginosa* (B3).

stored at cold temperatures and appear clear orange in color.

This showed that ethanol solvent can attract much more secondary metabolites of sappan wood hence that ethanol extract was able to inhibit the growth of all three test bacteria, with data percent reduction of bacteria population test almost reach 100%.

Unfortunately, the effective time of inhibition only last for 3 days, so the extract of sappan wood could only be recommended as fresh drink preservative or not stored for long time. The extract of sappan wood could be recommended as a dyes of food and preservative of healthy drinks such as jamu or herbs.

5. Conclusion

Sappan wood contained secondary metabolites such as flavonoids, polyphenols, quinones and monoterpenes. Sappan wood is able to be used as a dye food, because it's have attractives colors when extracted in water or ethanol 50%. Extraction using ethanol 50% is more efficient, because its ability to extract secondary metabolites better than using water. Besides it also capable to be used as dyes of food, sappan wood extract appropriate to be recommended as preservative because ethanol extract of sappan wood show good result in potential inhibition of bacterial contaminant in food with decrease of percent bacterial population for *E.coli, S.aureus*, *P. aeruginosa* are 97.12%, 96.28%, and 99.88% respectively.

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