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## Test the Activity of Vegetable Oils from *Shorea sumatrana* Sym. Against Bacteria *Salmonella* sp. and Bacteria *Escherichia coli*

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**Abstract.** Tengkawang oil obtained from *Shorea sumatrana* Sym. fruit contains vegetable oil. Extraction of tengkawang oil using n-hexane organic solvents. Tengkawang oil from *Shorea sumatrana* fruit is a genus of dipterocarpacea shorea family which produces high-value vegetable oil, is used for food preservatives, cancer sores and diarrhea also as cosmetics. This study aims to determine the activity of *Salmonella* sp. bacteria and *Escherichia coli* from tengkawang oil. Tengkawang oil was obtained by soxhletation and anti-bacterial testing by diffusion method with various oil concentrations (12.5, 25, 50 b/v). Tengkawang oil mixed with bacterial supposition in NA medium and incubated at 37°C for 18-34 hours to determine the level minimum inhibition (LMI) of cultivation liquid results, incubated on SDA (Sabourraud Dextrose Agar) media. The phytochemical screening test was carried out to determine the class of compounds contained in tengkawang oil from *Shorea sumatrana* Sym. The results showed that the LMI of concentrated *Salmonella* sp. bacteria was 12.5% b/v. MIC of 21, concentration of 25% b/v. LMI of 16 and concentration of 50% b/v. The LMI is 15, the test of tengkawang oil activity from *Shorea sumatrana* Sym. fruit to *Escherichia coli* is at a concentration of 12.5% b/v. LMI of 8, concentration of 25% b/v. LMI of 19 and concentration of 50% b/v. LMI is equal to 17 by using the control as a comparison if compared with the control positive of 10 and control negative equal to 7. The extracts of the phytochemical screening test showed that tengkawang oil from *Shorea sumatrana* fruit contained penicillin, coumarin and flavonoids. The highest activity of tengkawang oil on *Salmonella* sp. bacteria was LMI at 12.5% b/v. The level of LMI was 21 activities using the larger *Escherichia coli* LMI bacteria at a concentration of 25% b/v. LMI 19, from the activity test, both bacteria were categorized as inhibiting the growth of *Salmonella* sp. and *Escherichia coli*.

### Introduction

*Shorea sumatrana* Sym. fruit can produce vegetable oil known as tengkawang oil is a high economic value forest resource. Vegetable oil from tengkawang fruit is used by local people as cooking oil and margarine to flavoring and making rice oil in Kalimantan (Hidayat et al., 2019). Most of the tengkawang *Shorea stepnotera* fruit is exported for making sweets as a substitute for butter and chocolate, for

making sweets as a substitute for butter and chocolate, for the manufacture of soap, cosmetics, medicines and animal feed. *Shorea sumatrana* is a meranti species whose fruit can be used as a source of vegetable oil producers. The spreading area includes Southeast Asia, in Thailand, Malaysia, Indonesia (Kalimantan and Sumatra), Serawak, Sabah and Phillipina. Tengkawang oil, which originates from Jambi, a yellow tree, is a genus of *Shorea sumatrana*.

Tengkawang oil is vegetable oil derived from the extraction of the *Shorea sumatrana* Sym. fruit is the shorea

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genus and the family of oil-producing plantaceous plants. Tengkwang oil is useful as a cosmetic, moisturizer that can soften the skin and inhibit wrinkles on the face. Tengkwang oil can be used as an ingredient of lipstick, wax, soap, butter, diarrhea medicine, burn medicine, flavoring and food preservatives because it is not melt quickly (Kusumaningtyas et al., 2018). Traditionally the extraction of tengkwang oil was done by pressing tengkwang oil at freezing room temperature, yellow, can last for 5 years without rancid smell.

It is tested with *Salmonella sp.* and *Escherichia coli* bacteria because tengkwang oil is traditionally used to treat diarrheal diseases and also as a medicine for burns or getting hot oil when frying can be treated using tengkwang oil. Oil derived from *Shorea sumatrana* fruit is known as tengkwang oil in international trade. Tengkwang fruit is known as the illioe nut and its fat is famous for its Borneo name talllaow or green butter. This tengkwang fruit oil is obtained from tengkwang seeds that have been dried in the sun or overlaid to dry and grown into flour and then steamed using clear and transparent cloth and pressed. Traditionally this tengkwang oil is used for cooking, as a food flavoring and preservative, and for medicinal herbs.

In the food industry, tengkwang oil is used as a substitute for chocolate fat, pharmaceutical and cosmetic ingredients. Tengkwang oil is also used in making candles, soap, margarine, lubricants and also as a base for lipstick. From the results of the empirical search that in the countryside tengkwang oil is also used for cancer sores and diarrhea and overcome aches after working in the field. From the results of the library survey and the results of the study on tengkwang oil (Kusumaningtyas et al., 2012) which was recovered using n-hexane on the bakery, the tengkwang oil has not been examined from *Shorea sumatrana* and then the test from *Shorea sumatrana* fruit pulp was tested for microbial food. MIC *E.coli* with a concentration of 4% showed the inhibitory diameter of 16 mm, *S. aureus* at a concentration of 4% the inhibition diameter was 14 mm and the *A.niger* food microbes were 21% the inhibition diameter was 14 mm which showed oil from *Shorea sumatrana* seed pulp active against the three microbes from the optimum preservation results for wet noodles were at a concentration of 18% providing durability for 3 (three) days at room temperature of 25°C (Kusumaningtyas et al., 2012). Before to be made a tengkwang oil lipstick product tested against gram negative bacteria, namely *Salmonella sp.* and *E. coli* bacteria. *Salmonella* bacteria is a pathogenic germ that causes typhoid fever, which is a systematic infection with a picture of fever that lasts so

long, the presence of *Salmonella sp.* bacteria accompanied by inflammation that can damage the intestines with the liver.

This *Salmonella sp.* bacteria is one of the most pathogenic bacteria reported as a cause of systemic infectious diseases described by fever that lasts a long time, the presence of these bacteria accompanied by inflammation that can damage the intestines and organs of the liver. Typhoid fever is a contagious disease spread throughout the world and is still the biggest health problem occurring in developing countries and at tropical temperatures such as in Southeast Asia, Africa and Latin America. The incidence of this disease is still very high and an estimated 21 million cases with more than 700 cases end in death.

*Salmonella sp.* is one of the most common pathogenic bacteria reported as a cause of foodborne disease (Bintsis, 2017). This bacterium has been known to be the cause of disease for more than 100 years ago, first discovered from pig by Dr. Daniel E. Salmone (Yatnita 2011). Practically, *E. coli* is always present in the digestive tract of animals and humans because naturally *E. coli* is one of the inhabitants of the body. The spread of *E. coli* can occur by direct contact (touching, shaking hands and so on) then passed by mouth, but *E. coli* can also be found scattered in the natural environment around us. Passive spread can occur through food or drink.

Tengkwang oil will make the basic ingredients for cosmetics, for the basic ingredients of lipstick, then tested first on bacteria related to the skin or in the human body, and because of the phytochemical content that tengkwang oil contains phenolic compounds, alkaloid, steroids and fatty acids. Testers use the disc diffusion method against salmonella bacteria and *E. coli* bacteria. Taking tengkwang oil using n-hexane organic solvent. To support the benefits of tengkwang oil as a cosmetic ingredient, namely lipstick, as well as an anti-bacterial source, the initial testing of the presence of antibacterial compounds by looking at the anti-bacterial activity of tengkwang oil from shorea sumatrana fruit extract using a common procedure based on the ability to inhibit test bacteria

## Experimental

### Material and Methods

*Shorea sumatrana Sym.* fruit is obtained from the alternating village of Rantau Panjang Tabir District, Merangin Regency, in 2017. 2 kg of *Shorea sumatrana Sym.* fruit is removed from the skin of the ariac and then dried to dry and pounded using grinder tools, the powder obtained is brown. *Shorea sumatrana Sym.* 500 mg fruit powder was added to the soxhletasi wrapped in filter paper, using n-

hexane organic solvents, oil obtained from 2 kg of *Shorea sumatrana* Sym. weighing 857,350 kg white. The materials used in this study are: organic n-hexane solvents, filter paper, cotton, *Salmonella sp.* bacteria and *E. coli* bacteria, Nutrient Agar (NA), DMSO aquades, tetracycline, 70% alcohol nutrient broth, buffered peptone water, paper discs/Whatman, spectrophotometer. The equipment used is pH meter, laminar air flow, autoclave, and micrometer screw. Test tube, stirring rod, Erlenmeyer. Incubator, clamp, sprider, brunsen burner, needle. Anti-bacterial power testing was carried out by paper disc diffusion method. The research was conducted in the integrated laboratory of Jambi University.

### Preparation of Bacterial Suppression

Bacterial colonies on 24-hour nutrient culture media were taken using ose needles and suspended into 5 ml Mc Farland test tubes. Stereological NaCl solution/sterile BPW suspension was incubated at 37.5 OC for 20-24 hours,

### Test Extract Preparation

100 mg/ml extract of tengkawang oil sample was diluted with DMSO and distilled water so that the concentrations were 50%, 25% and 12.5% and then each concentration was put into a test tube as much as three sterile empty cakram paper inserted into each test tube let stand for 10 minutes, then with cakram paper tweezers drained on the edge of the tube for 10 minutes (Bintsis, 2017).

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### Procedure

A total of 20 ml of sterile nutrient is poured into a petri dish and let it solidify at room temperature. A total of 0.5-1 ml of bacterial suspension is poured on the surface of the nutrient so that it uses micropipette. Flatten the bacterial soup with spreaders and leave for 15 minutes Place the disc paper that has been saturated with tengkawang oil using tweezers. As blank/control is also placed cakram

paper, which has been saturated with fisological NaCl/BPW as a comparison used cakram tetracycline antibiotics 30 µg / disk The cup containing disc paper was then incubated at 37 OC for 18-24 hours in the inverted cup position. Anti-bacterial power testing was carried out by measuring the diameter (mm) of the resistance area (DDH) of bacterial growth formed around the disc paper with a micrometer.

## Results and Discussion

### Antibacterial Activity

Antibacterial testing was carried out by anti-bacterial activity of tengkawang oil extracted using soxhletation using technical n-hexane organic solvents. White tengkawang oil produced by disc diffusion method using cakram paper. The paper disc method is to erase paper discs that have been destroyed in solvents on solid media that have been inoculated with test bacteria. Anti-bacterial testing was done on *E. coli* and *Salmonella sp.* bacteria using tengkawang oil. With each concentration of 12.5%, 25% and 50%. Anti-bacterial activity was determined by measuring the inhibition zone of each tengkawang oil concentration.

Testing the antibacterial activity of tengkawang oil against *Salmonella sp.* bacteria and *E. coli* bacteria by using inhibitory zone classification substances is saline as a positive concomitant and negative control of DMSO solution, shown in table 1 the test results of tengkawang oil against antibacterial and in Figure 1 *Salmonella sp.* and Figure 2 *E. Coli* bacteria.



Figure 1. *Escheria coli*

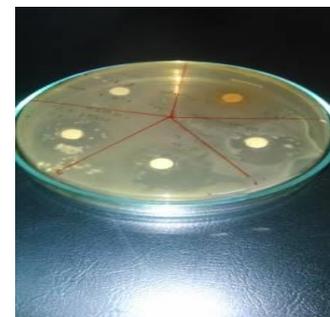


Figure 2. *Salmonella sp.*

Table 1. The results of the tengkawang oil test against anti-bacterial

No	Parameter test Anti-bacteri	Results analysis (mm)	
		<i>Salmonella</i>	<i>E.coli</i>
1	Concentration control (+)	10	23
2	Concentration control (-)	7	9
3	Concentration 50%	15	17
4	Concentration 25%	16	19
5	Consentration 12,5%	21	8

**Table 2.** Classification diameter inhibition (Yatnita 2011)

Diameter of inhibition zone (mm)	Response to growth barriers
≥ 20	Very strong
11 – 20	Strong
6 – 10	Is being
≤ 5	Weak

Figure 1 and Figure 2 shows the maximum inhibitory power of the antibacterial test results against *E. coli* and *Salmonella sp.*

Table 1 and Table 2 is a classification of the diameter of the inhibition zone for each bacterium treated in each tengkawang oil sample containing antibacterial compounds. The minimal inhibitory concentration of tengkawang oil from the results of soxhletation using n-hexane solvents against *E. coli* and salmonella bacteria can be seen in Table 1.

From the table with concentrations ranging from 50% having anti-bacterial power to *E. coli* bacteria with a diameter of 15 mm, a concentration of 25% in diameter 16 mm, a concentration of 12.5% with a diameter of 21 mm that tengkawang oil activity of inhibitory diameter of *E. coli* bacteria is still said to be strong compared to the classification of the diameter of the inhibitory zone Davis and Stout (1971). At the lowest concentration the strength/zone of antibacterial inhibition in *E. coli* bacteria while the negative control had a inhibitory power of 7 mm and positive control had an inhibition zone of 10 mm, from the results of research that tengkawang oil very strong at a concentration of 12.5% the zone of inhibition of the clear area is 21 mm. From the results of the study (Kusumaningtyas et al., 2012). In the same study tengkawang oil was 40% (From the results of previous studies that tengkawang oil contains saturated fatty acids, namely stearic, palmitic acid has activity as an antibacterial that is by absorbing nutrients present in bacteria and it can inhibit water and hinder the bacterial enzyme system. The test results showed that the decrease in the concentration of tengkawang oil could increase the antibacterial power of *Salmonella sp.* indicated by increasing the diameter of the inhibitory zone. The test results showed a concentration of 12.5%. 21% and 50% of tengkawang oil have antibacterial activity against *Salmonella sp.*, with the formation of inhibitory zones in each concentration of tengkawang oil, the clearer the diameter of the inhibition zone, the greater the

antibacterial activity (Ningtyas 2010). According to Jenie and Kuswanto (1994) in (Ancela et al., 2015). Stating that the effectiveness of an anti-bacterial agent in inhibiting growth depends on the nature of the test bacteria, concentration and duration of contact. The results of the inhibition zone diameter of tengkawang oil against *E. coli* bacteria inhibitory diameter at a concentration of 25% 19 mm inhibition zone, and 50% the inhibition zone was 17 mm, and the inhibition zone decreased at a concentration of 12.5%. In contrast to *Salmonella sp.* bacteria at a concentration of 12.5%, the 21 mm inhibition zone is very strong compared to *E. coli* only 8 mm, *Escherichia coli* 4% 16 mm from the results of research (Kusumaningtyas et al., 2012) *S. aureus* while *A. niger* is 21%. The inhibitory diameter from the results of the antimicrobial measurements of tengkawang fruit emulsion from the *Shorea sumatrana Sym.* fruit plant. Because all this time, tengkawang oil is a natural preservative and tengkawang oil is not rancid for long periods of storage, for five years. This tengkawang oil is a preservative of natural food from tengkawang seed pulp (*Shorea sumatrana Sym.*) (Kusumaningtyas et al., 2018). Because many have more hydroxy groups.

Preservative or antibacterial and antimicrobial components are components that can inhibit bacterial or mold growth (bacteriostatic or fungistatic) or need bacteria or mold (bactericidal or fungicidal). The active substances contained in various types of plant extracts are known to inhibit some bacteria or pathogenic microbes and food destroyers. The active substance can come from plant parts such as seeds or fruit, rhizomes, stems or skin, leaves and tubers. From the results of the research test, the activity of *Salmonella sp.* and *E. coli* bacteria strongly inhibits the growth of these bacteria. This is because tengkawang oil contains stearic, palmitic, diethyl phthalate, oleic acid and tengkawang oil from shorea sumatrana fruit are antioxidants. According to Kuete et al., (2015) that gram-negative bacterial cells have a multilayered structure and relatively high-fat content (11-12%), thus making it more resistant to environmental changes caused by chemicals.

The results of testing the anti-bacterial activity of tengkawang oil from *Shorea sumatrana Sym.* fruit extract using n-hexane solvents against pathogenic bacteria, namely *Salmonella bacteria* and *E. coli* have very strong inhibitory power with an average MIC value above 11 mm both gram-negative bacteria or pathogenic bacteria namely *Salmonella sp.* and *E. coli* bacteria. Concentrations of tengkawang oil 12.5% inhibition power of 23 mm for *Salmonella sp.* and 25% inhibitory power of 19 mm for *E. coli* bacteria, a very strong inhibitory power for *Salmonella sp.* bacteria and strong for *E. coli* bacteria. Because

tengkawang oil from *Shorea sumatrana* Sym. fruit contains secondary metabolic phenolic, steroid and alkaloid, contains the same metabolic as fat in etyl pthalate, palmitic acid, oleic acid, stearic acid, fat content of tengkawang oil from *Shorea sumatrana* Sym is 88.87%.

## Conclusion

From the results of testing the anti-bacterial activity of tengkawang oil in *Shorea sumatrana* Sym. on *Salmonella* sp. bacteria and *E. coli*, it can be concluded that the anti-bacterial activity of *Salmonella* sp. bacteria from tengkawang oil with maximum inhibitory power at 12.5% tengkawang oil concentration, 21 mm was very strong, and bacteria *E. coli* with a concentration of 25% KHM tengkawang oil is 19 mm, based on the classification of inhibition zones above the classification of strong bacterial inhibition zones. Tengkawang oil from n-hexane extract of *Shorea sumatrana* Sym. fruit is categorized as containing anti-bacterial compounds.

## Conflict of Interest

The authors disclose no conflicts.

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