The activity of holy basil leaves (Ocimum sanctum, L.) to microbia food born disease (Bacillus cereus, Staphylococcus aureus and Escherichia coli)

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Abstract. Ruku-ruku leave (Ocimum sanctum L.) is part of the plant that has a distinctive aroma with a slight sensation of spicy taste when chewed. Ruku-ruku leaves contain essential oils, tannins, flavonoids, steroids and triterpenoids, some of these chemicals can inhibit bacterial growth (bacteriostatic) or kill bacteria (bacteriocidal). This research conduct a test of antibacterial activity on ethyl acetate extract of ruku-ruku leave to mikroba food-borne disease of Bacillus cereus, Staphilococcus aureus and Escherichia coli. The results showed that yield of ethyl acetate extract 8.56 ± 0.87%, with a dark green color with shrinkage drying 22.44 ± 0.0305%, as h content 0.16 ± 0.0156%. The ethyl acetate extract of holy basil leave has an effective blocked to the concentration of 85 mg/ml to the bacteria Bacillus cereus in 15 mm, on concentration 80 mg/ml to the bacteria Strepococcus aureus on 21 mm, in diameter on concentration 75 mg/ml to the bacteria Escherichia coli on 17 mm. The inhibition of relative extract as an antibacterial compared with 1000 ppm 60.00% sodium benzoate to B. cereus, 88.24%, S. aureus, and 78.57% E.coli.

1. Introduction
Food is an essential need for every human being for growth and survival, but diseases can also arise caused by food. Food poisoning or food borne disease, mainly caused by pathogenic bacteria which is still a serious problem in many countries, including Indonesia.

In addition to being a source of nutrition for humans, foodstuffs are also a source of food for microorganisms as an intermediary or substrate for the growth of pathogenic microorganisms and disease-causing organisms. Pathogenic microorganisms are harmful microorganisms, often causing poisoning. Food poisoning is caused by an infection in which food is used as a culture medium for the growth of pathogenic bacteria to reach a sufficient number to cause infection for those consuming the food. Bacteria that can cause food poisoning are Bacillus cereus, Escherichia coli, Salmonella spp. [1].

The leaves of Holy Basil (Ocimum sanctum L.) are a type of shrub, often used in food processing. Fish processing in West Sumatra adds holy basil leaves as characteristic of Padang cuisine, especially the tamarind fish product, which is more favorite known as pangekpadeh and fish curry (pangekmasin). The use of its leaves in food processing aims to provide a distinctive aroma and taste so as to increase consumer preference [2].
Holy basil (O. sanctum L.) including plant shrubs could reach 50 cm tall. The smell and shaped is like leaves of basil that have a sensation with a little no taste spicy when are chewed. The shape of the leaves is smaller than the leaves of basil. There are colored green and somewhat purplish. Seasoning with this leaf is much found in the cuisine of Padang, as the dish goulash fish or cooking fry milk. Its fresh smell which is generated from the leaves of holy basil can reduce the fishy smell on processed fish, shrimp, beef and chicken. Usually it is used in fresh form with young leaves, then wash it clean. The use of holy basil leaves in pindang presto processing of mackerel was still good visually on the 6th day of storage and resulted in a total microbial growth of 3 x 10³ colonies/gram lower than the control [3]

Holy basil leaf refined oil contains 30% thymol. Fresh holy basil plants contain 0.8 - 1.2% essential oils with the main components of eugenol, tymol, citral, ethyl cinnamate and linalool [4]. The effectiveness of the inhibition is one of the criteria for selecting an antimicrobial compound to be applied as a food preservative. The stronger the inhibition will have the more effectively it is used. The damage caused by the antimicrobial components can be microcidal (permanent damage) or microstatic damage (temporary damage), depending on the concentration of the culture used. Substance antimicrobial would interfere with the process of formation asam folat, thus generating acid are nonfunctional and metabolism in the cells of microbes will be disturbed [5]

The mechanism of action of the inhibition of antimicrobial compounds can be caused by several factors, among others; disturbances in cell wall constituent compounds, increased cell membrane permeability which can cause loss of cell building components, inactivate enzymes and damage to the function of genetic material [6]. Base on that, the research are carried out about activity against microbial pathogen destroyer food (Bacillus cereus, Staphylococcus aureus and Escherichia coli)

2. Methodology
2.1 Materials and Tools
The research was conducted at the Laboratory Institute of Research and Standardization Industry Padang and Laboratory of National Agency of Drug and Food Control Padang. The materials used were holy basil leaves, ethylacetate, hexane, ethyl acetate, DMSO media, chloroform, nutrient Agar, Nutrient Broth, 0.9% NaCl solution, aquades, H₂SO₄, HNO₃ standart test bacteria included Bacillus cereus, Staphylococcus aureus and Escherichia coli. Equipment that is used include autoclave, equipment for microbial culture (petri dish, culture tube, incubators, spirits lamp, loop needles), equipment extraction (Vacuum rotary evaporator, funnel separator, soklet), spetkotofotometer, oven, vortex tube eppendorf 1, 5 and 2 ml, micro pipette, centrifuge, spatula, desiccator, shaker, filter paper, scales analytics, paper labels, and appliance glass

2.2 Research Implementation
2.2.1 Preparation extract of ruku-ruku leaf. The holy basil leaf extract was made by multilevel extraction done through sokletasi using solvent hexan, the residue extracted with ethyl acetate then the ethyl acetate residue was extracted with ethanol. The extraction process by complete with characterized when the liquid on the chiffon is colorless, or the circulation has reached 20-25 times. Each filtrate is separated from the solvent by evaporation in a rotary evaporator, until no more solvent drips. The first solvent is evaporated at 40°C, the second and third solvents are evaporated at 50°C, then the evaporation is continued inwater bath until obtained a thick extract. The extract obtained will be used as a sample for analysis and antibacterial testing.

2.2.2 Preparation of the test bacterial culture. Culture test that is used is a bacteria B.cereus ATTC 11778, S. aureus ATCC 25923 and E. coli ATCC 25 922. These bacteria were obtained from the culture collection of National Drug and Food Control Agency. The cultures were stored in glycerol beads at freezing temperature.
2.3 Observation

The ethyl acetate extract of holy basil leaves was tested for its physical properties (color, appearance, texture), water content and ash content. The activity test of rulu-ruku leaf extract was carried out by determining the MIC, determining the diameter of the inhibition area and the relative resistance activity of the pathogenic microbes *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*.

2.3.1 Water content and Ash content. Water content 2 g of the extract were weighed and put into a weighing bottle with a lid which previously had known constant weight. Then the extract was put in an oven at 105 °C until a constant weight was obtained. Drying loss is calculated using the formula.

\[
\text{Water content (\%)} = \frac{W_1 - W_2}{W_1 - W} \times 100
\]

\( W \) = Weight of empty container
\( W_1 \) = Container + sample before heating
\( W_2 \) = container + sample after heating

Ash content. The sample was weighed carefully, approximately 2 g were put into the crucible which had been tared earlier. The sample is annealed to ashes and a constant weight is calculated

\[
\text{Ash content (\%)} = \frac{W_1 - W_2}{W} \times 100
\]

\( W \) = weight before burned
\( W_1 \) = container + sample after burned
\( W_2 \) = Empty container

2.3.2 MIC (Minimum Inhibitory Concentration). Determining the value of MIC d’s done by the method of Kubo \(^{[7]}\) at a concentration of 1-20 mg / ml. The extract was mixed with the tested bacterial culture in liquid media and was inoculated as much as 30 µl of the tested bacterial suspension, incubated at 37 °C for 24 and 48 hours. Observations were made by looking at changes in the media compared to controls (without additions). Then the culture was transferred to Nutrient Agar media. The MIC value (\%) was determined based on the lowest concentration of holy basil leaf extract which was able to inhibit growth (bacteriostatic).

2.3.3. Testing for antibacterial activity. Testing activity of extract ruku-ruku leaves to the bacteria used medium Nutrient agar, samples tested first dissolved in DMSO. The concentration of anti bacterial extract was tested on the tested bacteria. The bacteria to be tested are *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. Sodium benzoate is used as a comparison antimicrobial.

Activities antimicrobials such as antibacterial determined by measuring the diameter of the barriers that are formed, this area clear that formed around a ring of metal that has been spilled extract. A microbe is said to have activity that is higher against microbes when the value of the concentration of minimum resistance is low but has power resistor that is large \(^{[8]}\).

2.3.4. Determination of the diameter of the resistance area. The antimicrobial activity test was carried out on the tested bacteria, to *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. Preparation of inocula was carried out by mixing the parent suspension of the tested bacteria into NA media in order to obtain 2% inocula levels, and then prepared steril petri dish. Into each cup, 15 ml of NA medium was poured and allowed to solidify as a base layer. As much as 5 ml so that the inocula is poured onto the surface of the base layer in each plate according to the group, shaken and rotated to
form an even layer and allowed to solidify. Then 3 cylinders of stainless steel were dropped onto the surface so that the inocula was in each cup, then 0.1 ml of boiling extract at several concentrations was dropped into each cylinder. All plates were left for about 1 hour, then incubated at 35 °C- 37 °C for 18-24 hours. Observations were made on the clear zone formed after incubation, and measured average midline barrier region [8].

3. Results and Discussion

3.1. The yield and physico-chemical properties of ruku-ruku leaf extract

| Parameter       | Explanation          | Table 1. The yield of holy basil leaf extract (Ocimum sanctum, L) |
|-----------------|----------------------|-----------------------------------------------------------------|
| Color           | Deep green           |
| Appearance      | Viscous liquid       |
| Smell           | Typical bowing       |
| Water content (%)| 2.24 ± 0.0305        |
| Ash content (%)  | 0.16 ± 0.0156        |
| Yield (%)       | 8.56 ± 0.87          |

The results of characterization (non-specific parameters) of the extracts obtained in the form of physical properties, moisture content and ash content are shown in Table 1. Water content is a measurement of the remaining substances during the drying process after reaching a constant weight which aims to provide an overview of the compounds that are lost during the drying process. The water content of the extracts of Ocimum sanctum L is ranged from 2.24 ± 0.036 showed of substances that were lost during the drying process. Ash content useful to look at the form of internal or external mineral content came from start process until the formation of the extract. In the process of burning, organic compounds will be reduced and evaporated, leaving only mineral and inorganic elements. Extract of Ocimum sanctum L has mineral elements around 0.16 ± 0.0073.

The yield of holy basil leaves with the ethyl acetate extraction method using a vacuum rotary evaporator is 8.56 ± 0.87. Extraction with ethyl acetate using a rotary evaporator is a hot extraction by adding solvent then heated by the evaporator then the solvent will separate to produce a thick extract. The yield is the ratio of the product end of which was obtained against the material standard are used. The value of the yield that is obtained based on the weight of the dry ingredients raw. The yield of the product associated with the method of extraction that is used to separate the compounds of the chemical.

3.2. MIC (Minimum Inhibitory Concentration) extract Leaf of Ocimum Sanctum L

The minimum inhibitory power of a bioactive substance is determined of Minimum Inhibitory Concentration which is used to determine the minimum inhibitory power in inhibiting the planting of a type of tested bacteria. Minimum Inhibitory Concentration of bioactive substances against microbes is used to determine the sensitivity of microbes to bioactive substances. The more low-value MIC of an agent active then the sensitivity of the bacteria will be increasingly large. In this study the extract showed activity or zone of inhibition, the MIC value was determined by the well method at a concentration of 2; 4; 6; 8; 10; 12; 14; 16 mg / mL. Observations were made to change the extract, the smallest concentration which showed fluid lymph expressed as MIC. From tests carried out on B. cereus bacteria gave an MIC value of 8 mg / mL, while for Streptococcus aureus bacteria 12 mg / mL, and E. coli 6 mg / mL. MIC value was expressed as the lowest concentration of holy basil leaf extract which can inhibit microbial growth and incubated for 24 hours [9].

MIC results show that S. aureus is a resistant bacteria compared to B. Cereus and E. coli. Eschericia coli bacteria are sensitive bacteria with the smallest MIC. S. aureus is a gram-positive bacterium with
a cell wall composed of tetrapeptide chains and interpeptide bridges consisting of five glycine units. The muramic acid unit is substituted by a tetrapeptide connected by an interpeptide bridge with a covalent bond, which gives a strong structure. This structure is highly resistant to damage \[^{[6]}\]. The bacteria *E. coli* is a gram-negative bacterium is sensitive to extract ethyl acetate leaves of basil. This is because ethyl acetate extract can enter the periplasm of gram-negative bacterial cells through porin protein from the outer membrane of the cell \[^{[5]}\].

The structure of *S. aureus* is more resistant to semi-polar and polar leaf extracts. Tests for the antibacterial activity of kecombrang flower extract against several tested bacteria \[^{[11]}\] and betel leaf antibacterial against several tested bacteria \[^{[12]}\] and antibacterial activity of atung seed \[^{[13]}\] also showed that *S. aureus* was the most resistant bacteria.

### 3.3. Antibacterial activity

**Table 2.** Antibacterial activity test of ethyl acetate extract of holy basil leaves against *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* bacteria

| Holy basil leaf extract (concentration %) | Diameter of Resistance Area (mm) |
|-----------------------------------------|----------------------------------|
|                                         | *B. cereus* | *S. aureus* | *E. coli* |
| Holy basil leaf extract (1%)            | 6           | 6           | 6         |
| Holy basil leaf extract (5%)            | 8           | 9           | 10        |
| Holy basil leaf extract (10%)           | 13          | 13          | 16        |
| Holy basil leaf extract (15%)           | 15          | 2           | 1         |
| Control (ethyl acetate)                 | 6           | 6           | 6         |

Analysis of the antibacterial activity of holy basil leaf extract against bacteria *B. cereus, S. aureus* and *E. coli* showed an inhibition by the ethyl acetate extract of holy basil leaves. At a concentration of 1% extract provided a low resistance area with a diameter of 6 mm (weak) for the three tested bacteria. The test was continued with a 5% concentration of rukuruku ethyl acetate extract. Antibacterial activity of the extract of ethyl acetate 5% showed *B. cereus* with an inhibitory diameter of 8 mm is greater than that of a *S. aureus* of 9 mm. Antibacterial activity at 10% holy basil ethyl acetate extract concentration showed strong inhibition (10-20 mm diameter) for *B. cereus* (13 mm), *S. aureus* (13 mm), and *E. coli* (16 mm) and whereas there were extract concentrations of 15% *B. cereus* (15 mm), *S. aureus* (21 mm), and *E. coli* (17 mm). The antibacterial activity test was indicated by the resulting clear zone, where the larger the clear zone produced, the stronger the antibacterial activity. Antibacterial activity was categorized as very strong in the clear zone diameter with ≥ 20 mm, strong (10-20 mm), moderate (5-10 mm) and weak (≤ 5 mm) (Davis Stout). This is different from the antibacterial activity test of kecombrang flower extract against several tested bacteria which showed that *E. coli* has a high inhibition diameter (19 mm at a concentration of 30% w/v) compared to *S. aureus* \[^{[11]}\].

*E. coli* is a gram-negative rod with a size of 1.1-1.5 µm, a normal flora found in the digestive tract of humans and animals, generally found in meat, fish and their preparations. The cell structure in gram-negative bacteria has a cell wall component of 5-10% peptidoglycan, the rest consists of protein, lipoprotein and lipopolysaccharide \[^{[14]}\].

The effectiveness of antimicrobials in preserving foodstuffs is by relying on the growth of microorganisms. The ability of an antimicrobial compound to inhibit or kill microbes is largely determined by the concentration of the antimicrobial compound, the type, number, age and background of microorganism life, temperature and time of contact as well as the physical and chemical properties of the media. \[^{[15]}\]

*S. aureus* is a gram-positive bacteria with a cell wall composed of a tetrapeptide chain consisting of (L alanil-Disoglutaminil L lysis D alanine) and an interpeptide bridge consisting of five glycine
units. The muramic acid unit is substituted by a tetrapeptide connected by an interpeptide bridge with covalent bonds, which gives a strong structure. This structure is highly resistant to damage \[^{10}\]

3.4. Relative resistance
Relative resistance is used to see the extract activity in inhibiting bacterial growth compared to food preservatives. In determining the relative resistance test of O. sanctum L extract using sodium benzoate 1000 ppm as a comparison as shown in Table 3.

| Concentration of Test Material | B. cereus | S. aureus | E. coli |
|-------------------------------|-----------|-----------|--------|
| 1%                            | 0.00      | 0.00      | 0.00   |
| 5%                            | 13.33     | 17.65     | 28.57  |
| 10%                           | 46.67     | 47.06     | 71.43  |
| 15%                           | 60.00     | 88.24     | 78.57  |
| 1000 ppm sodium benzoate      | 100       | 100       |        |

Relative inhibition is used to see the extract activity in inhibiting bacterial growth compared to synthetic food preservatives. In determining the relative resistance test of O. sanctum L ethyl acetate extract using 1000 ppm sodium benzoate as a comparison as shown in Table 3.

The results of the holy basil leaf relative resistance test showed that the rukuruku leaf extract was 5% against B. cereus with a relative resistance to sodium benzoate 13.33%. There was an extract of 10% with a relative resistance to sodium benzoate 46.67%, and at 15% with a relative resistance of 60%. The increase in the percentage of the extract indicated an increase in resistance relative to sodium benzoate. The higher the extract concentration used, the higher the resistance produced. Relative resistance of extracts holy basil against S aureus in comparison Sodium benzoate in 5%, 10% and 15% respectively 17.65%; 47.06%;88.24%. While the relative resistance of holy basil extract to E. coli was 5%, 10% and 15% respectively 28.57%; 71.43%; 78.57%.

The inhibition of antibacterial relative to sodium benzoate was different in the tested bacteria. Relatively high barriers on extract ethyl acetate to 15% with test bacteria S. aureus 88.24%. This shows that the use of extracts of ethyl acetate to 15% with a relative obstacle 88.24% of the sodium benzoate in bacteria S. aureus

4. Conclusion
The results showed that the ethyl acetate extract of holy basil leaves could inhibit the growth of pathogenic bacteria (Bacillus cereus, Staphylococcus aureus and Escherichia coli). Antibacterial activity of rukuruku leaf ethyl acetate extract can inhibit bacterial growth with MIC against B. cereus 8 mg / mL S. aureus 12 mg / mL and E. coli 6 mg / mL. E. coli is a sensitive bacteria compared to B. cereus and S. aureus with a relative resistance of 78.57% compared to Sodium benzoate.

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