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# Antibacterial Activity Test Of Kawista Fruit Peel Extract (Limonia Acidissima L.) From Pasinan Village, Pasuruan, Against *Salmonella Sp* Colonies In Vitro

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**Abstract.** One of the potential agricultural products from Pasinan Village, Lekok sub-district, Pasuruan, East Java Province is Kawista Fruit. Residents of Pasinan used kawista fruit for consumption, both raw and ripe. The large consumption of this kawista fruit leaves an abundance of fruit peels. Based on the results of phytochemical analysis, the extract of kawista fruit peel contains saponins, tannins, alkaloids, flavonoids, and terpenoids. Another income source of Pasinan residents comes from marine resources, that can be served in fresh fish, dried fish and other processed fish is susceptible to contaminated by microorganisms such as *Salmonella sp*. *Salmonella sp*. is pathogenic microbe lives on intestines of animals and humans. This study aims to determine the antibacterial activity of kawista rind extract on the growth of *Salmonella sp.* in-vitro and to determine the concentration of Kawista rind extract which is effective for inhibiting the growth of *Salmonella sp* using the paper disc diffusion method.

The results of this study, the diameter of the inhibitory zone for the growth of *Salmonella sp.* using variables concentrations of kawista rind extract at 12.5%;25%;50%;75%;100%, with negative control of aquadest and methanol and positive control is tetracycline. At a concentration of 100% Kawista extract, the diameter of the bacterial inhibition test was the largest and significantly different from another treatment group at concentrations of 12.5%;25%;50%;75%. However, the 100% Kawista extract showed a smaller inhibitory zone than the tetracycline treatment group, but not significantly different. In methanol treatment, the inhibition test showed that the diameter of the bacteria was greater than Kawista extract treatment group of 12.5%;25%;50%;75% but not significantly different. Based on the results of the study, it can be concluded that kawista rind extract is effective for inhibiting the growth of *Salmonella sp.* bacteria, Kawista rind extract at a concentration of 100% is most effective for inhibiting the growth of *Salmonella sp* bacteria and shows results that are not significantly different from tetracycline.

**Keywords:** Kawista Fruit Peel Extract (Limonia Acidissima L.), *Salmonella Sp.*, Antibacterial, Paper Disc Diffusion Method

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## 1 Introduction

Pasinan Village, which is one of the areas in the Lekok sub-district, Pasuruan, East Java, where part of the population depends on the agricultural sector, and the location of the area which is quite close to the sea so that some of its residents work as fishermen (Radar Bromo,

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2018). One of the potential agricultural products that is quite large from Pasinan Village is Kawista (*Limonia acidissima L*). Kawista is generally planted in coastal areas, and in dry meadows, especially near the sea and towards the mainland (Sukamto, 1999). *Limonia acidissima L.* or Kawista has a regional language, namely Kwisto, is a plant belonging to the Rutaceae family. This plant originates from India and is generally used as a medicinal plant (Panda, et al., 2013). Some residents has also used the young kawista fruit to medicated diarrhea (Rini, et al., 2017). Residents of Pasinan use kawista fruit for consumption, both raw and ripe. The large consumption of this kawista fruit leaves quite an abundance of fruit peel.

One of the way to deal with kawista rind waste is to process it into a useful material. Based on the results of the phytochemical analysis test on kawista fruit peel extract, it contains saponins, tannins, alkaloids, flavonoids, and terpenoids. One of the ingredients of kawista rind, namely alkaloids, can damages the peptidoglycan structure in the bacterial cell wall, so that the cell wall layer is not formed intact and causes cell death (Taufiq, et al, 2015).

Another income source for Pasinan residents comes from marine resources such as fresh fish, preserved fish and other processed fish are susceptible to contaminated by microorganisms such as *Salmonella sp. Salmonella sp.* Is pathogenic microbe which is lives in the intestines of animals and humans. So that the purpose of this study is: to utilize kawista fruit peel to be extracted into an antibacterial compound *Salmonella sp.* which generally contaminate marine products such as fresh fish, preserved fish and other processed fish. *Salmonella sp.* has a rod-shaped bacterium belonging to Enterobacteriaceae, namely gram-negative bacteria that is parasitic in the intestines of animals and humans (Brooks, 2005). The disease caused by this bacteria is called salmonellosis which can be transmitted through feces, food and drinks that are contaminated with these bacteria (Pui, 2011). The way to medicated the disease caused by *Salmonella* contaminationis using antibiotics. Antibiotics can causes bacterial resistance, besides that it can also have a negative effect as well as the occurrence of hypersensitivity reactions, even spinal cord depression (Wibowo, et al., 2010) and other physiological disorders in humans.

There are three types of *Salmonella* species that are pathogenic, namely: *Salmonella typhi*, *Salmonella choleraesuis*, and *Salmonella enteritidis*. Human infection with *Salmonella sp.* almost all are caused by consuming food or drink contaminated with these pathogens. The purpose of this study was to determine the antibacterial activity of kawista rind extract on the growth of *Salmonella sp* in vitro and to determine the concentration of Kawista rind extract which was effective for inhibiting the growth of *Salmonella sp*.

## 2. Material and Method

### Tools and materials

The materials used were kawista fruit peels obtained from Pasinan Village, Pasuruan, East Java, *Salmonella sp.* bacterial culture, Nutrient Agar medium, methanol, tetracycline, paper

disks. erlenmeyer, measuring cup, volume pipette, stir bar, magnetic stirrer, autoclave, and vacuum rotary evaporator, calipers.

**Production of Kawista fruit peel extract**

Kawista fruit peel extract was prepared by maceration method with a ratio of powder and methanol solvent 1:7. The maceration process was carried out for 5x24 hours with occasional stirring using a magnetic stirrer. The maceration results were filtered using filter paper to obtain a filtrate. Then remaceration was carried out 1 time with the same solvent with a 1:5 ratio of powder and methanol solvent. All of the filtrate was evaporated using a vacuum rotary at 63˚C until a viscous extract was obtained, then stored in a tightly closed glass container before being used for further test.

**Phytochemical Analysis**

Phytochemical analysis aims to detect the content of secondary metabolites contained in kawista fruit peel extract :

*1. Flavonoid Test*

One ml of sample extract was mixed with 20 ml of hot water, then boiled for 5 minutes. add 0.5 gr magnesium and 10 drops of HCl then shake gently. If it turns red, orange or purple color, it indicates the presence of flavonoids (Harborne, 1987).

*2. Alkaloids Test*

Put 0.5 ml of extract into a test tube then add 1 ml of HCl 2 N and 9 ml of distilled water then heat for 2 minutes then cool and filter. 3 drops of filtrate mixed with 2 drops of Mayer's solution ($HgCl_2$ and Potassium Iodide) then a white/yellow precipitate was formed. Put 3 drops of the filtrate into the tube and then add 2 drops of Bouchardat's solution (Potassium iodide and Iodine) and then a brown or black precipitate forms. Put 3 drops of the filtrate into the tube then add 2 drops of Dragendorff (Bismuth III nitrate, concentrated nitric acid, and potassium iodide) until an orange or red color is formed. If it is positive for alkaloids, a cloudy precipitate will be form where at least 2 or 3 trials will cause a cloudy precipitate (Harborne, 1987).

*3. Terpenoids Test*

Put 1 ml of extract into the tube and add 2 ml of chloroform. Then add 10 drops of acetic anhydride and 3 drops of concentrated sulfuric acid then shake gently and let stand for a few minutes. If a red/purple color is formed, it indicates the presence of terpenoid compounds (Harborne, 1987).

*4. Tannin Test*

Put 1 ml of extract into the tube and add 12 ml of hot water and then boil for 15 minutes. Then filtered and then added 1 ml of 1% FeCl3 solution. If a dark blue/blackish green color is formed, it indicates the presence of tannins (Harborne, 1987).

*5. Saponin Test*

Put 0.5 ml of extract into the tube and add hot water, then cool and shake vigorously for 10 seconds. If foam is formed as high as 1-10 cm in less than 10 minutes and the foam does not

disappear with the addition of 2N HCl, it indicates the presence of saponin compounds (Harborne, 1987).

**Antibacterial Activity Test**

Antibacterial activity Tested of kawista fruit peel extract was carried out in-vitro using the paper disc diffusion method. The sterilized ose needle is put into a test tube containing *Salmonella sp.* then smeared on each petri dish containing Nutrient Agar (NA) media using the Streak method. Putting disc paper into a test tube containing 2 ml of kawista fruit peel extract with a concentration of 12.5%, 25%, 50%, 75% and 100% then soaked for 1 hour. Insert disc paper that has been soaked with kawista fruit peel extract into each petri dish that contains *Salmonella sp.* in NA medium. Then as a negative control put paper discs into a tube containing 2 ml of distilled water and methanol and then soaked for 1 hour. Then take the disc paper and put it in a petri dish which already contains the media and *Salmonella sp.* bacteria. Meanwhile, as a positive control, put paper discs into a test tube containing 2 ml of tetracycline and then soaked for 1 hour. Then take the disc paper and put it in a petri dish which already contains NA media and *Salmonella sp.* then incubated at 37$^{o}$C for 3x24 hours. Observing the bacterial inhibition zone by looking at the clear colored area around the disc paper and then measuring it using a caliper. Each observation was carried out with repetition for 4 times.

**Data analysis**

Analysis of the research data used One-Way Anova (one-way analysis of variance) using SPSS (Statistical Program for Social Science). The ANOVA test is used to determine whether the differences of the results after treatment or the average of each treatment has different results or not.

## 3. Results And Analysis

Based on the results of the phytochemical analysis of the kawista fruit peel extract, it showed that the kawista fruit peel extract contained alkaloids, flavonoids, terpenoids, tannins, and saponins. Based on the results of observations of the antibacterial activity test on kawista fruit peel extract against *Salmonella sp* in vitro showed the following results

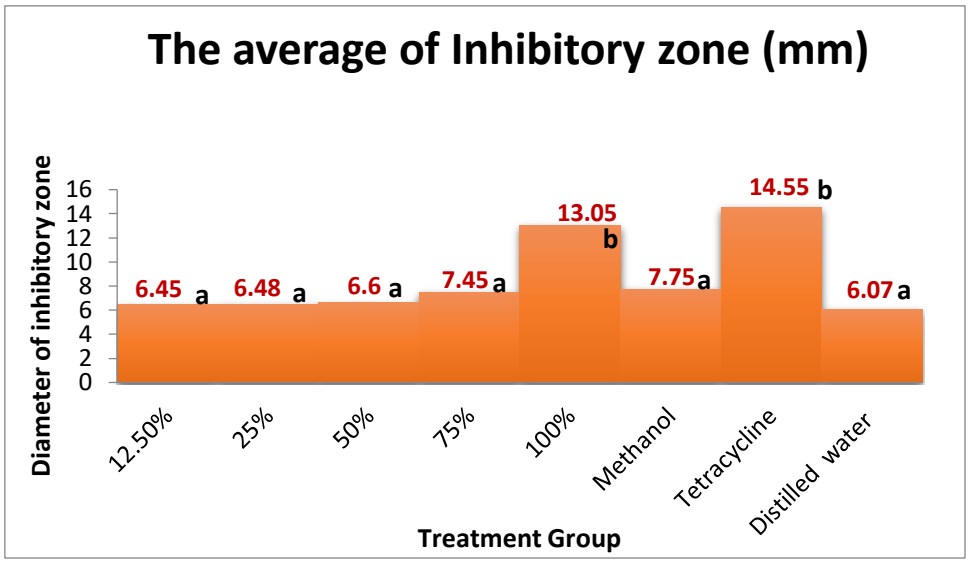


**Figure 1.** Diagram of diameter of the inhibitory zone for *Salmonella sp.* colonies (mm)
*note : a/b showed the significant difference of diameter inhibitory zone

The results of this study based on the data, the diameter of the inhibitory zone for the growth of *Salmonella sp.* using Kawista rind extract in a variable concentration of 12.5%; 25%; 50%; 75%; 100%, with negative control is distilled water and methanol, then positive control is tetracycline. At a concentration of 100% Kawista extract, the diameter of the inhibitory zone was the largest and significantly different from the treatment group with concentrations of 12.5%;25%;50%;75%. At a concentration of 100% Kawista extract, the diameter of the bacterial inhibitory zone was smaller than the tetracycline treatment group, but not significantly different. At concentrations of 12.5%;25%;50%;75% Kawista extract, the diameter of the inhibitory zone increasing as the concentration of the extract increased (directly proportional), but each treatment did not show a significant difference. In the methanol treatment, the inhibition test showed that the inhibitory zone was greater than Kawista extract treatment group of 12.5%;25%;50%;75% but not significantly different.

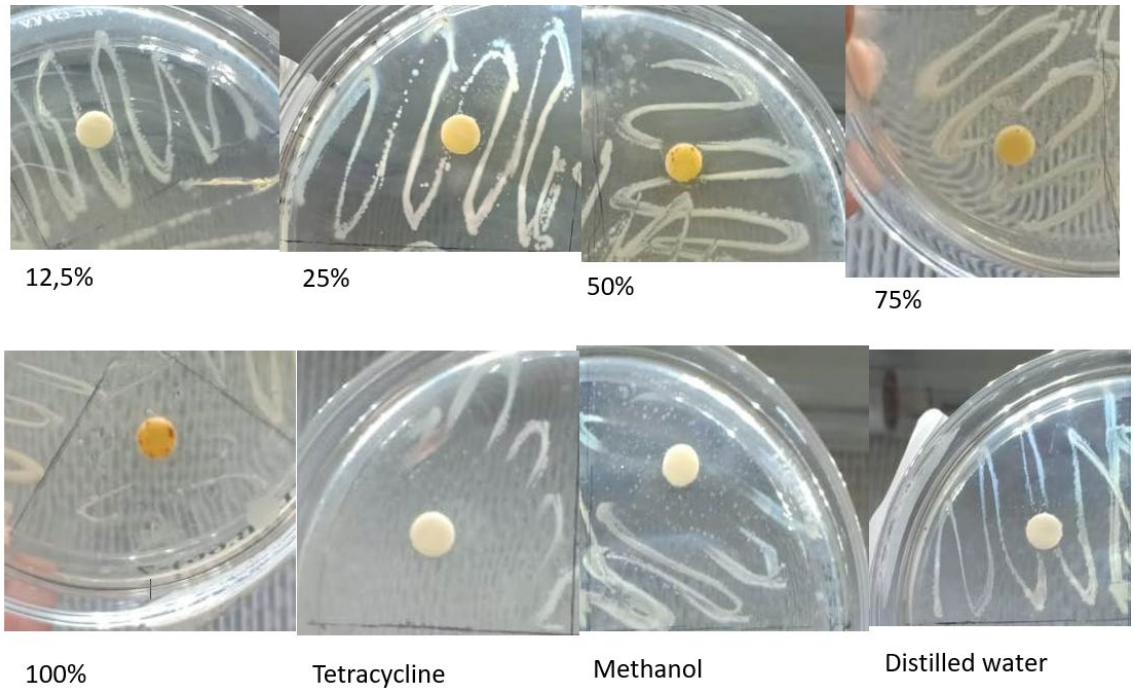


**Figure 2**. *Salmonella sp.* inhibitory zone in various concentrations of Kawista Peel Extract, tetracycline, methanol, distilled water.

Clear zone showed the inhibitory zone is formed because the extract on the paper disk diffuses into the agar and prevents the growth of *Salmonella sp.* colonies. Based on the results of the phytochemical analysis test showed that Kawista fruit peel extract contains alkaloid flavonoids, terpenoids, tannins, and saponins. These compounds are responsible for inhibiting the growth of bacteria. Flavonoids are phenolic compounds that can denature proteins in enzymes thereby disrupting cell metabolic activities. Flavanoids also have the ability to form bonds with cell wall proteins, so that bacteria are unable to attach and invade cells (Susanti, 2016). Flavonoids are also able to release transduction energy in the bacterial cell membrane thereby inhibiting bacterial motility (Manik et al., 2016). In addition, flavonoids can also damage the bacterial cell wall by inhibiting the incorporation of glycan and peptidoglycan chains in the cell wall, caused the cell wall structure to become weak (Sulatstrianah, et al., 2014). Flavonoid activity can inhibit bacterial growth by inhibiting the synthesis of macromolecules in cell membranes in bacterial cells (Dzoyem et al., 2013).

Alkaloid which is also found in kawista rind have the ability to inhibit bacterial growth (Pfoze et al., 2011), by inhibiting the synthesis of reverse transcriptase enzymes and DNA synthesis (Schmeller et al., 1997). In addition, alkaloids release lipoteichoic acid compounds from the cell surface (Sun et al., 1988) which can disrupt membrane permeability and cause lysis of the bacterial cell wall layer. The terpenoid compounds contained in the rind of the kawista fruit have the ability to inhibit bacterial growth (Mariajancyrani et al., 2013). Its activity in inhibiting bacterial growth is not fully understood, but is thought to involve lipophilic compounds that disrupt bacterial cell membranes (Cowan, 1999).

Tannin have a spasmolytic effect which can reduce intestinal peristalsis, and can shrink the bacterial cell walls, causing disturbances in the permeability of bacterial cells. Tannin compounds can also inhibit the formation of reverse transcriptase enzymes and DNA topoisomerase which play a role in cell division so that bacteria are unable to replicate (Mukhriani, et al., 2014).

The ability of saponins as antibacterial as a chemical barrier in the plant defense system to deal with pathogenic organisms. Saponins can cause protein leakage and damage to certain enzymes in bacterial cells (Ravi, et al., 2016). Apart from being able to inhibit bacterial growth, saponins are also anti-fungal (Yuliana, et al., 2015). The way saponins work as antibacterial is by damaging the cell membrane and reducing the permeability of the cell membrane (Sulastrianah, et al., 2014). In addition, saponins can inhibit bacterial growth by reducing the efficiency of glucose utilization, affecting proliferation, interfering with enzyme activity in physiological metabolism and suppressing protein synthesis in bacterial cells, which can cause cell death (Zhi-hui et al., 2013).

## 4. Conclusion

Based on the results of the study, it can be concluded that kawista rind extract is effective for inhibiting the growth of *Salmonella sp.* Colonies. Kawista rind extract at a concentration of 100% is most effective for inhibiting the growth of *Salmonella sp* and shows results that are not significantly different from tetracycline.

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