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The effectiveness of antibacterial essential oil of cinnamon (*Cinnamomum burmannii*) on *Staphylococcus aureus*

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ABSTRACT

Cinnamon plants (*Cinamomum burmannii*) besides being used as a spice in cooking, it also has benefits as a medicine. Essential oil is an active ingredient found in cinnamon plants (*Cinnamomum burmannii*) and can be found in parts of cinnamon bark. The content of the cinnamaldehyde and eugenol compounds contained in cinnamon essential oil is known to have antibacterial properties. *Staphylococcus aureus* is a pathogenic bacterium that can cause infectious diseases in humans and animals. The purpose of this study was to determine the antibacterial effect of essential oils of cinnamon bark (*Cinnamomum burmannii*) on *Staphylococcus aureus* isolated from the dairy cows milk. This study was an experimental laboratory study with the three repetitions, and consisted of 6 treatments with positive control of tetracycline, the negative control of 70% ethanol and essential oil concentration of 1%, 2%, 4%, and 8% on *Staphylococcus aureus* bacteria using the disc diffusion method. The finding showed that at a concentration of 2%, 4%, and 8% the essential oil of cinnamon bark (*Cinnamomum burmannii*) showed the zone of inhibition on *Staphylococcus aureus*. Based on the result, it can be concluded that the essential oil of cinnamon bark (*Cinnamomum burmannii*) has an antibacterial effect on *Staphylococcus aureus*

Key word: *Cinnamomum burmannii, Staphylococcus aureus, Antibacterial essential oil*

Introduction

Infectious disease is one of the health problems in the community that is difficult to overcome completely. This type of disease affects the most people in developing countries, including Indonesia. The term infection describes the growth or replication of microorganisms in the host body. The emergence of the disease occurs when the infection produces changes in the body's normal physiology (Pratiwi, 2008). Diseases caused by infections can be transmitted from human to human or from animal to human and can be caused by several microorganisms such as bacteria, viruses, parasites, and fungi (Jawetz *et al.*, 2005).

Staphylococcus aureus is one of the bacteria that can cause infectious diseases. *Staphylococcus aureus* is a Gram positive bacterium that attacks humans and other mammals (SNI, 2009). *Staphylococcus aureus* can cause suppurative infections in animals and humans, also often cause mastitis in cattle and goats, pyoderma in dogs and cats and can cause abscesses in all animal species including poultry (Quinn, 2002). One infectious disease that often occurs in livestock and cause harm to humans is mastitis. Cases of mastitis often begin in subclinical mastitis at lactation (Nurhayati, 2015).

The drug used to treat bacterial infections is an-

tibiotics (Mardiastuti, 2007). However, currently antibiotics are used inappropriately for a disease that does not actually require antibiotics. Causing resistance due to the high use of antibiotics. Resistance is a problem that often arises in the treatment of infectious diseases. Increased bacterial resistance to antibiotics provides a great opportunity to obtain antibacterial compounds by utilizing bioactive compounds from plant diversity in Indonesia (Nuria *et al.,* 2009). Almost all types of plants spread in Indonesia have benefits as natural medicines because there are active compounds that can be used as medicines (Fitri *et al.,* 2016). The use of herbal plants has been believed to decrease hereditaity so that the use of herbal plants as an alternative treatment can be used as a reference for future drug development (Sharif *et al.,* 2006).

One of the herbs in Indonesia is cinnamon, besides being used as spices, beverage ingredients and food preservatives, cinnamon can also be used as an antibacterial, anti-inflammatory, antioxidant, analgesic, antidiabetic, anti-thrombic and anti-tumor (Budiastuti *et al.,* 2020). Cinnamon bark (*Cinnamomum burmannii*) contains essential oils, calcium oxalate, tanners and tannins (Hariana and arief, 2008). The main content contained in the extract of cinnamon bark (*Cinnamomum burmannii*) is 1-3% essential oil consisting of cinnamaldehyde (66-75%) and eugenol (4-10%), both of which are the main components of antibacterial and antifungal methods it works by destroying bacterial and fungal cell membranes (Heinrich and Michael, 2009).

Materials and Methods

The materials used in this study include: milk samples taken from dairy cattle Kaliwaron, Jemursari, and Bendul Merisi. CMT reagent, MSA (Mannitol Salt Agar) (Merck KgaA VM739404 631 1.05404.0500), sterile distilled water, gram stain (violet crystals, safranin, lugol, 96% absolute alcohol), oil emersion, spiritus, H_2O_2 3%, blood agar , Mac Farland standard no. 1, PZ solution (0.9% Sodium Chlorida) for dilution of bacteria, cinnamon bark originating from Sulawesi, MHA (Mueller-Hinton Agar) (Merck KgaA, VM469637 243 1,05437.0500), 70% ethanol, blankdisc with brands Oxoid, 30 µg tetracycline disk antibiotics under the brand name Oxoid, a set of distillation equipment, syringes, paddle mastitis, mikeoscopes, glass objects, pertridish, test tubes, tube racks, tube covers, autoclaves, incubators, ovens, vortexes, bunsen burners, gas stoves, pan, Erlenmeyer, bent glass stirrer, ose needle, ice, styrofoam box containing ice pack, volume pipette.

In general, essential oils are obtained by distillation from plant material. Distillation is the process of separating components in the form of liquid or solid from two kinds of mixtures, based on the vapor point and this process is carried out on essential oils that are not soluble in water (Guenther, 2006). This research uses water vapor distillation.

The research sample is milk obtained from dairy farms Kaliwaron, Jemursari and Bendul Merisi. A total of 14 milk samples from the Kaliwaron farm, 9 milk samples from the Jemursari farm, 9 milk samples from the Bendul Merisi farm, and a total of 32 samples. From the 32 samples the CMT test was then performed, and 24 positive samples were obtained for the CMT test. The samples used further in this study were 24 samples. Subclinical mastitis testing is performed directly at the study site using CMT reagents. A sample of 2 mL of each udder was then put into paddle mastitis and the same amount of CMT reagent was added to each sample. After that paddle mastitis is gently rocked clockwise for a few moments and the results are then observed under direct sunlight (Puspasari *et al.,* 2018).

The steps taken to isolate and identify *Staphylococcus aureus* are preparing MSA (Mannitol Salt Agar) media and 10 mL of cow's milk sample. Followed by turning on Bunsen, then the OS that will be used is burned first until it glows. Dip the ose in the sample and then streak a zig-zag line on the MSA isolation media. Incubate the media at 37 ºC for 24 hours. Observing colonies that grow and separate, if the color of the colony is yellow, the most likely colony is *Staphylococcus aureus*. Yellow colonies taken from MSA media were then made microscopic observations by carrying out Gram staining tests. Then the catalase test is carried out, taking yellow bacterial colonies from MSA media using ose then mixed with a drop of 3% H_2O_2 on glass objects, then observe. The presence of *Staphylococcus aureus* is marked by the appearance of gas bubbles, because *Staphylococcus aureus* produces the enzyme catalase, which converts hydrogen peroxide to water and oxygen (Ramandinianto *et al.,* 2020). After the catalase test is performed, to ensure that the bacterium is *Staphylococcus aureus*, hemolysin is tested because *Staphylococcus aureus* is a bacterium capable of hemolytic erythrocyte cells so that

clear zones around the colonies can form (Yunita *et al.,* 2020). Visible clear zone around the colony after 18 hours of incubation at 37 ºC is considered a positive result of hemolysin production (Osek, 2004).

How to make a suspension of *Staphylococcus aureus* that will be used is by inserting a colony of *Staphylococcus aureus* taken from MSA media into a PZ solution then vortexing until it is homogeneous. Furthermore, the suspension is likened to turbidity with Mc Farland's number 1 standard (Tyasningsih *et al.,* 2019).

Prepare the MHA media, followed by pouring as much as 20 mL of MHA media on each petri dish and allowed to condense. Taking *Staphylococcus aureus* bacterial suspension with a sterile pipette of 0.2 mL which has been synchronized with Mc Farland number 1 with a bacterial density of 3 x 108 CFU / ml (Effendi *et al.,* 2019), then poured into petridish containing MHA media, then flattened with a bent glass mixer. Let stand for 10 minutes so that bacteria stick to the media (Tyasningsih *et al.,* 2019).

Prepare positive controls, negative controls, and essential oils with a concentration of 1%, 2%, 4%, 8% in petridish then soak paperdisc in each concentration of the essential oil. Followed by placing paper disc on the surface of the MHA media using tweezers. After that, incubate the preparations for 24 hours at 37 ºC. The following day an observation of the inhibition zone (clear zone) was formed (Ibrahim and Kuncoro, 2012).

Results and Discussion

Essential oils are active ingredients found in cinnamon plants (*Cinnamomum burmannii*) and are found in the cinnamon bark. The content of cinnamaldehyde and eugenol compounds found in cinnamon essential oil is known to have antibacterial properties (Phantong *et al.,* 2013).

One way to isolate essential oils is by distillation, there are several distillation processes, namely steam distillation, water distillation, and steam water distillation. Distillation (distillation) has a definition as the separation of the components of a mixture of several types of liquid based on differences in the boiling point of each substance (Guenther, 2006). In this study the process of distillation (distillation) of essential oils uses water vapor distillation.

Based on the results of isolation and identification carried out on 32 fresh cow milk samples per individual from 3 dairy farms in Surabaya there were 8 milk samples (25%) which were positive for *Staphylococcus aureus.*

Eight positive samples of *Staphylococcus aureus* in the bacterial identification test were then continued with the antibacterial test of cinnamon bark essential oils (*Cinnamomum burmannii*) with concentrations of 1%, 2%, 4%, and 8% conducted on MHA (Mueller Hinton Agar) media with the method disk diffusion.

The results of the antibacterial test of cinnamon bark (*Cinnamomum burmannii*) essential oil showed a clear zone (inhibitory zone) around disc paper, this shows that cinnamon bark essential oil (*Cinnamomum burmannii*) was able to inhibit the growth of *Staphyylococcus aureus*. Show that cinnamon bark essential oils with concentrations of 2%, 4% and 8% show inhibition zones against 8 *Staphylococcus aureus* isolates, whereas a concentration of 1% does not show any inhibitory zones against 8 *Staphylococcus aureus* isolates. The 1% concentration did not show inhibitory zones against 8 *Staphylococcus aureus* bacterial isolates, due to the 1% concentration of antibacterial compounds contained in cinnamon bark essential oils (*Cinnamomum burmannii*) were not enough to form inhibitory zones on Mueller Hinton Agar (MHA) media.

The inhibition zone formed is caused by antibacterial compounds contained in cinnamon bark (*Cinnamomum burmannii*) essential oils. Compounds that have been known to have antibacterial activity in cinnamon bark essential oils are cinnamaldehyde and eugenol. Each compound has a different mechanism but has a relatively similar function that can inhibit the growth of bacteria or even kill it. Sinamaldehid is a phenylpropene group that has a phenolic group. Sinamaldehid activity is better than eugenol as an antibacterial. Sinamaldehid is able to

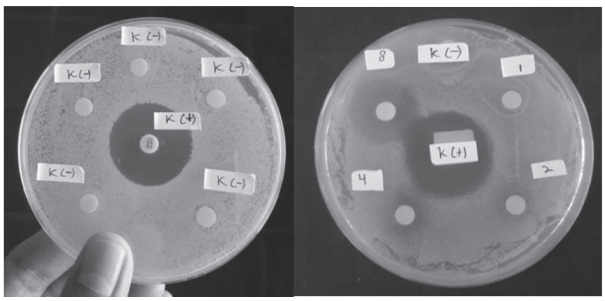

Fig. 1. Inhibition zone in the antibacterial test of cinnamon bark (*Cinnamomum burmannii*) essential oil against *Staphylococcus aureus* bacteria

enter the phospholipid bilayer in bacterial cells so that it can bind to proteins that are able to disrupt the normal function of these cells. Sinamaldehid can also inhibit bacterial growth without destroying the outer membrane or depleting intracellular ATP and being able to enter the periplasm to the inside of the cell. Based on the amount of concentration, cinnamaldehyde has three mechanisms of action: low concentrations can inhibit enzymes involved in cytokine interactions, high concentrations can inhibit ATPase, and lethal concentrations can interfere with cell membranes where sinamaldehyde is able to change the cell structure of bacterial membrane membranes (Luciano and Holley, 2009).

Eugenol can change the composition of membranes, affect ion transport and ATP, and replace the composition of fatty acids. Eugenol compounds are also capable of disrupting bacterial enzymes such as ATPase, histidine carboxylase, amylase, and protease. In the structure of fatty acids, eugenol will have the effect of increasing the amount of saturated fatty acids and decreasing the amount of unsaturated fatty acids in bacterial membranes so that they can have a direct effect on the outer membrane and enzymes used for fatty acid synthesis (Nagayama *et al.,* 2015).

Eugenol and cinnamaldehyde are able to influence protein from bacteria that can inhibit the formation of adenosine triphosphate from dextrose so that it changes the composition of cell membranes, inhibits the activity of ATPases and, inhibits ATPase activity that is present on the membrane, including the activity of transporting proteins that depend on ATP (Siahaan *et al.,* 2014). In this study, it was proven that cinnamon bark (*Cinnamomum burmannii*) essential oil has an antibacterial effect

Table 1. Isolation and identification of *Staphylococcus aureus* bacteria

No.	Sample	Isolasi and Identifikasi *Staphylococcus aureus*				
		CMT	MSA	Staining Gram	Katalase	Hemolysin
1.	K1	+	+	Coccus / purple	+	+
2.	K9	+	+	Coccus / purple	+	+
3.	J1	+	+	Coccus / purple	+	+
4.	J3	+	+	Coccus / purple	+	+
5.	J4	+	+	Coccus / purple	+	+
6.	J9	+	+	Coccus / purple	+	+
7.	B3	+	+	Coccus / ungu	+	+
8.	B5	+	+	Coccus / ungu	+	+

K : Kaliwaron + : Positive
J : Jemursari - : Negative
B : Bendul Merisi

Table 2. Inhibitory zones formed in the oil antibacterial test cinnamon bark essential oil (*Cinnamomum burmannii*)

No	Isolate	Inhibitory zones					
		Cinnamon Essential Oil				Tetrasiklin	Etanol
		1%	2%	4%	8%	30 µg	70%
1.	K1	-	+	+	+	+	-
2.	K9	-	+	+	+	+	-
3.	J1	-	+	+	+	+	-
4.	J3	-	+	+	+	+	-
5.	J4	-	+	+	+	+	-
6.	J9	-	+	+	+	+	-
7.	B3	-	+	+	+	+	-
8.	B5	-	+	+	+	+	-

Ket : K : Kaliwaron + : inhibition zone
J : Jemursari - : no inhibition zone
B : Bendul Merisi

against *Staphylococcus aureus*, known by the inhibition zone formed on the Mueller Hinton Agar (MHA) media.

Conclusion

Based on the results of research that has been done, it can be concluded that the essential oil of cinnamon bark (*Cinnamomum burmannii*) has an antibacterial effect on *Staphylococcus aureus.*

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