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Antibacterial Activity of Cinnamon Extract (*Cinnamomum burmannii*) against *Staphylococcus aureus* and *Escherichia coli* In Vitro

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Abstract

Introduction

Infectious disease is one of the most common diseases in the world. *Staphylococcus aureus* and *Escherichia coli* are two common causes of infection and are resistant to many antibiotics, so the new agents are needed to overcome antibiotic resistance. Cinnamon is often used as a preservative because it has antibacterial activity. *Cinnamomum burmannii* is kind of native cinnamon from Indonesia. The antimicrobial active compounds cinnamaldehyde and eugenol are the main reasons for its antibacterial activity.

Objective

This study observed the efficacy of the cinnamon extract (*Cinnamomum burmannii*) as antibacterial against *Staphylococcus aureus* and *Escherichia coli*.

Methods

An experimental study, in vitro using Post-test Only Control Group Designed, has been done in Microbiology and Biotechnology Laboratory of Medical Faculty of Sriwijaya University. Cinnamon was extracted, then tested for its antibacterial activity using well diffusion and serial dilution to determine diameter of inhibition zone and minimum bactericidal concentration. Phytochemical tests were also conducted to determine the antibacterial compounds of cinnamon extract. Ethanol extract of cinnamon was able to inhibit the growth of *Staphylococcus aureus* with MBC 5% and inhibitory zone 6,84±0,68 mm and *Escherichia coli* with MBC 10% and inhibitory zone 5,69±0,69 mm. Cinnamon extract which has the greatest effectiveness is concentration of 40% with inhibition zone 15,69±0,80 mm (*Staphylococcus aureus*) and 9,63±0,59 mm (*Escherichia coli*). This ability is due to the antibacterial compounds as evidenced by positive results in various phytochemical tests.

Conclusion

Cinnamon extract is effective as antibacterial against *Staphylococcus aureus* and *Escherichia coli* in vitro.

Keywords: efficacy, antibacterial, *Cinnamomum burmannii*, *Staphylococcus aureus*, *Escherichia coli*

Introduction

In general, humans have a large number of microorganisms which usually do not cause disease (normal flora). But on the way, some bacteria that are important causes of disease generally originate from normal flora, resulting in an infection.¹ Based on Indonesia's health profile in 2011 about the 10 most diseases in hospitals, infectious diseases occupy several ranks, including diarrhea which is on the first rank and at rank 10 is pneumonia.² *Staphylococcus aureus* and *Escherichia coli* are the two main causes of various infections in humans, such as *bloodstream infections* (BSI).^{3,4}

Staphylococcus aureus is part of the normal flora that lives on the skin and mucosa of the human body.⁵ *Staphylococcus aureus* causes infections of the skin and soft tissue, surgery marks, infections of the bones and joints, and causes of hospital-acquired bacteraemia (HAB) and respiratory infections that are obtained from hospitals.³ *Staphylococcus aureus* is the most isolated pathogenic bacteria from patients treated in US hospitals, and is number two in patients outside the hospital.⁶

Escherichia coli, a gram negative bacterium in the form of bacilli, is a normal flora that generally colonizes the large intestine. *Escherichia coli* is the main pathogen that causes about 90% of urinary tract infections, gastrointestinal infections, and systemic infections in humans.³

The high incidence of antimicrobial resistance (AMR) due to antibiotic abuse has led to new discoveries of antibacterial agents becoming very important and are considered to be one of the pillars of modern medical science in preventing millions of premature deaths caused by infectious diseases, especially bacterial infections.⁷

Cinnamon (*Cinnamomum sp*) is one of the flavor enhancing agents that is often used in the culinary and industrial world. Cinnamon has other functions such as giving a sweet taste and a distinctive aroma to food.⁸ Cinnamon is also used in the manufacture of cosmetics and the medical industry because it has an antibacterial, antioxidant, and anti-carcinogenic effect.⁹

There are various types of cinnamon, one of which is *Cinnamomum burmannii*. Cinnamon has been developing for a long time in Indonesia, even becoming one of the main commodities of Indonesian trade since the Dutch era. The growth of *Cinnamomum burmannii* in Indonesia is supported by the availability of mountainous land that stretches along the islands of Sumatra, Java and Sulawesi with adequate rainfall. The function of cinnamon plants as medicinal plants, especially as antimicrobials against microbial pathogens in humans and plants, is now widely done. Antimicrobial activity of cinnamon has been tested on several pathogenic microorganisms including *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial activity is associated with the content of cinnamaldehyde, alkaloids, flavonoids, eugenol, coumarin, steroids, saponins, tannins, and phenols.^{9,10} The main content of cinnamon essential oil is coumarin (13.39%), eugenol (17.62%), and cinnamaldehyde (60.72%) which have antibacterial effects.¹¹

The utilization of *Cinnamomum burmannii* as an antimicrobial is quite potential to be developed in Indonesia. Therefore, further research on the effectiveness of *Cinnamomum burmannii* antibacterial against *Staphylococcus aureus* and *Escherichia coli* bacteria is necessary, so that cinnamon is expected to be one of the new antibacterial agents to overcome antimicrobial resistance

Methods

In vitro experimental research with Post-test Only Control Group designed was conducted in July 2018 to November 2018 at the Laboratory of the Faculty of Medicine, Palembang Sriwijaya University, Biotechnology Laboratory for extraction, Microbiology Laboratory for antibacterial activity testing, and Biochemical Laboratory and Medical Chemistry to carry out phytochemical tests.

The object of the research is cinnamon simplicia powder obtained from Balitro Bogor with characteristic reddish brown color. While the research sample consisted of four repetitions of the treatment group, namely four concentration of cinnamon extract concentration (5%, 10%, 20%, and 40%), the positive controls are 10 µg amoxicillin and cefotaxime 30 µg, and the negative control is aquadest. The bacteria *Staphylococcus aureus* and *Escherichia coli* used are strains that are still sensitive to empirical antibiotics.

Preparation

The obtained simplicia powder was prepared before maceration. Other tools and materials needed in the research process are certainly in good condition.

Extraction

This stage is done by maceration method. 250 grams of cinnamon powder macerated using 1 L ethanol 96% for 3x24 hours with a ratio of 1:4, then filtered to remove the liquid, then evaporated using a rotary evaporator until thickened like pasta. The ethanol extract that has been obtained is then stored in a beaker. The ethanol extract was dried using a hair dryer to get a dry extract which was later used for testing *Staphylococcus aureus* and *Escherichia coli*.

Determination of Inhibited Zone Diameter

Measuring the diameter of the inhibition zone of cinnamon extract using a diffusion method with a well with a diameter of 5 mm, the distance between wells is 24 mm, then each concentration of cinnamon extract is filled into the well filled, positive control and negative control. Then incubated 18-24 hours (35°C) and measured the diameter of the resistance.

Determination of Minimum Bactericidal Concentration (MBC)

Cinnamon is made in concentration 40%, then 100 µl of extract is put into the microwell plate of the 40% section. A total of 100 µl of mueller-hinton broth were filled into microwell plate sections 20%, 10%, and 5%. 100 µl of 40% cinnamon extract was put into 20% dosage section. Dilution starts at 20% by taking 100 µl of preparation for 20% and then moving it to 10%, and so on up to 5%. A total of 100 µl of mueller-hinton broth were put in positive and negative controls section. In positive controls antibiotics were used as controls. Then, 10 µl of bacterial colonies were added into all cinnamon extract section, positive controls, and negative controls. After incubation for 24 hours, the turbidity of each treatment group was observed visually, then inoculated on mueller-hinton agar media to see bacterial growth and incubated again to see whether there was bacterial growth in each treatment group. The MBC value is an area of the lowest concentration of cinnamon extract which is not overgrown with bacteria.

Phytochemical Test

Cinnamon extract was carried out phytochemical tests to determine the content of antibacterial compounds contained in cinnamon. Cinnamon extract was reacted with several reagents to identify the content of flavonoids, phenols, alkaloids, quinones, steroids, saponins, and tannins.

Table 1. Phytochemical Tests of Cinnamon Extract

Chemical Compound	Method
Flavonoids	+ HCl and amyl alcohol
Phenols	+ FeCl ₃ in water/ethanol
Alkaloids	Dragendorff (filter paper)
Quinones	+ NaOH
Steroids	Liebermann-Burchard
Saponins	Shuffling + HCl
Tannins	+ FeCl ₃

Results

Extraction

Cinnamon extract weighing 26.65 grams (10.66%) was obtained from macerating 250 grams of cinnamon simplicia. This extract is divided into tested antibacterial and phytochemical activities. The extract that will be used to test the antibacterial activity was made into four concentration gradients using aquadest as a solvent.

Table 2. Concentration of Cinnamon Extract

Cinnamon Concentration	Weigh of Cinnamon (gram)	Solvent Volume (ml)
5%	0,125	2,5
10%	0,25	2,5
20%	0,5	2,5
40%	1	2,5

Phytochemical Test

Phytochemical tests are carried out by reacting cinnamon extract with various different reagents to find out the content of antibacterial compounds in cinnamon.

Table 3. Phytochemical Test Results

Chemical Compound	Results
Flavonoids	Orange in the amyl alcohol layer
Phenols	Black
Alkaloids	Red on filter paper
Quinones	Red
Steroids	Blue
Saponins	1,5 cm foam after 10 minutes of HCl drops
Tannins	Black

Determination of Inhibited Zone Diameter

The results of the antibacterial activity test by the fourth dilution method of cinnamon concentration can be seen in Tables 4 and 5. In Table 4 it is known that the concentration of 40% has the largest zone of inhibition of *Staphylococcus aureus*, which is 15.69 ± 0.80 mm. While the largest zone of inhibition of *Escherichia coli* is a concentration of 40%, which is 9.63 ± 0.59 mm (table 5)



Figure 1. Inhibited Zone of Cinnamon Extract against *Staphylococcus aureus*. A. Concentration of 5%, B. Concentration of 10%, C. Concentration of 20%, D. Concentration of 40%, E. Amoxicillin, F. Aquadest

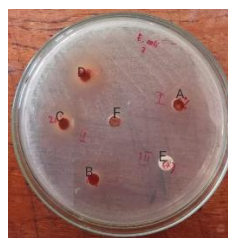


Figure 2. Inhibited Zone of Cinnamon Extract against *Escherichia coli*. A. Concentration of 5%, B. Concentration of 10%, C. Concentration of 20%, D. Concentration of 40%, E. Cefotaxime, F. Aquadest

To determine the effectiveness of cinnamon extract, *Kruskal Wallis* test was performed. The results of statistical analysis using the *Kruskal Wallis* test showed a value of $p < 0.05$. The analysis was continued using the *Kruskal Wallis post-hoc* to see the preparation of cinnamon extract which had the highest effectiveness on *Staphylococcus aureus* and *Escherichia coli* bacteria (table 6 and table 7).

Table 4. Inhibited Zone Diameter of Cinnamon Extract against *Staphylococcus aureus*

Type	Concentration	Inhibited Zone Diameter (mm)				Average ± SEM
		I	II	III	IV	
Cinnamon	5%	3,75	7,75	7	8,88	6,84±0,68
	10%	11,50	10,50	11,5	10,75	11,06±0,36
	20%	16,25	14,50	15	14,75	15,13±0,35
	40%	12	17	16,50	17,25	15,69±0,80
Amoxicillin	10 µg	26,25	28,50	28,50	25,50	27,19±0,48
Aquadest		0	0	0	0	0±0

Table 5. Inhibited Zone Diameter of Cinnamon Extract against *Escherichia coli*

Type	Concentration	Inhibited Zone Diameter (mm)				Average ± SEM
		I	II	III	IV	
Cinnamon	5%	1,25	0	0	1,50	1±0,33
	10%	4,75	3	8,75	6,25	5,69±0,69
	20%	6,25	5,75	12,75	6,50	7,81±0,76
	40%	7,38	8,50	12,88	9,75	9,63±0,59
Cefotaxime	30 µg	29,38	30,85	31,75	23,75	28,90±1,39
Aquadest		0	0	0	0	0±0

Table 6. Conformity Test of *Cinnamomum burmannii* against *Staphylococcus aureus*

Variable	5%	10%	20%	40%	Amoxicillin	Aquadest
5%	1,000*	1,000*	0,003	0,002	0,000	1,000*
10%	1,000*	0,330*	0,330*	0,249*	0,000	0,016
20%	0,003	0,330*	1,000*	1,000*	0,141*	0,000
40%	0,002	0,249*	1,000*	0,191*	0,191*	0,000
Amoxicillin	0,000	0,000	0,141*	0,191*	0,000	0,000
Aquadest	1,000*	0,016	0,000	0,000	0,000	0,000

Description: **p* value > 0,05

Table 7. Conformity Test of *Cinnamomum burmannii* against *Escherichia coli*

Variable	5%	10%	20%	40%	Cefotaxime	Aquadest
5%	0,189*	0,189*	0,008	0,000	0,000	1,000*
10%	0,189*	1,000*	1,000*	0,556*	0,000	0,007
20%	0,008	1,000*	1,000*	1,000*	0,012	0,000
40%	0,000	0,556*	1,000*	0,381*	0,381*	0,000
Cefotaxime	0,000	0,000	0,012	0,381*	0,000	0,000
Aquadest	1,000*	0,007	0,000	0,000	0,000	0,000

Description: **p* value > 0,05

Minimum Bactericidal Concentration Determination

Solid dilution method is chosen to determine MIC. Before solid dilution is carried out, liquid dilution is carried out first to determine the minimum inhibitory concentration. Visual MBC observation using liquid dilution method is continued by solid dilution method because the level of turbidity of the preparation is difficult to assess. Liquid and solid dilution test data are found in Tables 8 and 9.

Table 8. Liquid Dilution Test Results Determination of MIC *Cinnamomum burmannii* Extract against *Staphylococcus aureus* and *Escherichia coli*

Type	Concentration		Turbidity
	<i>S. aureus</i>	<i>E. coli</i>	
Cinnamon	5%	5%	++
	10%	10%	++
	20%	20%	++
	40%	40%	++
Positive control	10 µg	30 µg	-
Negative control			-

Description

- ++ : turbid
- + : clear enough
- : clear

Table 9. Solid Dilution Test Results Determination of MBC *Cinnamomum burmannii* Extract against *Staphylococcus aureus* dan *Escherichia coli*

Type	Bacterial Growth			
	<i>S. aureus</i>		<i>E. coli</i>	
Cinnamon	5%	-	5%	+
	10%	-	10%	-
	20%	-	20%	-
	40%	-	40%	-
Positive control	10 µg	-	30 µg	-
Negative control		+		+

Description:

- + : grown
- : ungrown

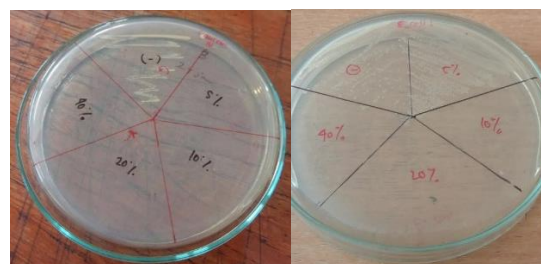


Figure 3. MBC Dilution Test of Cinnamon Extract and Controls against Bakteri *Staphylococcus aureus* and *Escherichia coli*

Table 9 presents the results of the KBM test of cinnamon extract on *Staphylococcus aureus* is concentration 5% and *Escherichia coli* is concentration 10%.

Discussion

Phytochemical tests were carried out to determine the antibacterial active compounds contained in cinnamon extract. Phytochemical is a test carried out to determine the chemical components in a plant. One of the phytochemical test principles is to detect qualitatively the presence or absence of certain chemical content in the plants used.¹²

Phytochemical test results showed that *Cinnamomum burmannii* cinnamon extract contained antibacterial, namely flavonoids, phenols. Alkaloids, quinones, steroids, saponins, and tannins. Flavonoid compounds are characterized by the presence of orange in the amyl layer of alcohol. Phenol compounds are marked by the presence of black. Alkaloid compounds are characterized by the formation of red on filter paper. Quinone compounds are characterized by the formation of red. Steroid compounds are indicated by the change in red to blue. A stable foam for 10 minutes formed as high as 1.5 cm and not lost at the addition of a drop of 2 N hydrochloric acid indicates the presence of saponin compounds. Tannin compounds are characterized by the presence of black on the addition of FeCl₃.

The antibacterial compounds obtained in this study were similar to those in 2011 which showed that cinnamon ethanol extract contained alkaloid compounds, flavonoids, steroids, tannins, and quinones. In the phytochemical test *Cinnamomum zeylanicum* cinnamon obtained positive results of phenols, alkaloids, steroids, and tannins.¹⁰ Other studies also mention the results of phytochemicals of *Cinnamomum burmannii* cinnamon having the main content of cinnamaldehyde (60-77%), *Cinnamomum zeylanicum* whose main content is eugenol 65-89%.¹³

In this study cinnamon extract was able to provide inhibitory effects on *Staphylococcus aureus* and *Escherichia coli* bacteria which were seen through the diameter of the inhibitory zone formed. In Tables 4 and 5 it is known that all the concentration gradients of cinnamon extract showed antibacterial activity, in harmony with several previous studies which showed antibacterial activity of cinnamon to *Staphylococcus aureus* and *Escherichia coli*.⁹

The largest diameter of the inhibition zone was seen at a concentration of 40%, which was 15.69 ± 0.80 mm (*Staphylococcus aureus*) and 9.63 ± 0.59 mm (*Escherichia coli*). Increasing the concentration of cinnamon extract is accompanied by the diameter of the inhibition zone which is increasingly various compounds.

This result is supported by a study using volatile oil from cinnamon carried out in India where the diameter of the inhibition zone was formed according to the concentration of cinnamon used.¹⁴ Research in 2015 showed the effect of 10% cinnamon extract on *Staphylococcus aureus* had the diameter of the inhibition zone was 11.9 mm, not much different from the 10% ethanol extract carried out in this study, which was 11.06 mm.¹⁵

The smallest concentration that still forms the inhibition zone is a concentration of 5%, which is 6.84 ± 0.68 mm (*Staphylococcus aureus*) and 1 ± 0.33 mm (*Escherichia coli*), so that the diffusion method can be estimated at a concentration of 5% as a minimum inhibitory concentration Cinnamon extract (*Cinnamomum burmannii*) against *Staphylococcus aureus* and *Escherichia coli* bacteria. The estimated minimum inhibitory concentration using this diffusion method is then proven by the dilution method.

The diameter of the inhibitory zone in this study was adjusted to the available inhibition zone diameter categories.¹⁶ The inhibitory response of cinnamon extract (*Cinnamomum burmannii*) to *Staphylococcus aureus* bacteria in this study was included in the medium and strong category, ie 5% concentrations including medium, and 10% concentration, 20%, and 40% including strong. Positive control in the form of amoxicillin is included in the very strong category. While the inhibitory response of cinnamon extract (*Cinnamomum burmannii*) to *Escherichia coli* bacteria is included in the category of weak and moderate, namely the concentration of 5% including weak, and concentrations of 10%, 20%, and 40% including medium. Positive control in the form of cefotaxime belongs to the very strong category.

KBM test results on the solid dilution method found no growth of *Staphylococcus aureus* bacteria at concentrations of 5%, 10%, 20%, and 40%, there was no growth of *Escherichia coli* bacteria in cinnamon extract concentrations of 10%, 20%, and 40%. Therefore, based on the solid dilution method, it can be concluded that the minimum concentration of cinnamon extract (*Cinnamomum burmannii*) against *Staphylococcus aureus* is a concentration of 5% and that of *Escherichia coli* is a concentration of 10%.

The minimum difference in concentration in the diffusion and dilution methods is due to differences in the dose used and the effects assessed. In the diffusion process which is assessed is the ability of cinnamon extract to inhibit bacteria, while dilution assesses the bactericidal effect of extracts. Determining the diameter of the inhibitory zone using the media to require the time the extract is absorbed into the agar before giving effect, while the dilution method shows an effect 24 hours after incubation.

Conclusions

Statistically cinnamon extract (*Cinnamomum burmannii*) is effective as an antibacterial against *Staphylococcus aureus* and *Escherichia coli* in vitro. MBC cinnamon extract (*Cinnamomum burmannii*) against *Staphylococcus aureus* is 5% with moderate zone inhibition zones. MBC cinnamon extract (*Cinnamomum burmannii*) to *Escherichia coli* is 10% with moderate zone inhibition zones.

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