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ANTIBACTERIAL ACTIVITY OF *CINNAMOMUM BURMANII* STEM BARK EXTRACT AGAINST STAPHYLOCOCCUS *XYLOSUS*

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ABSTRACT

Objective: this study was aimed to evaluate the antibacterial activity of *Cinnamomum Burmanii* stem bark ethanolic extract against *Staphylococcus xylosus* ATCC 3342. **Methods:** The *Cinnamomum* ethanolic extract was obtained from a maceration process using 70% ethanol as the solvent. The thick extract then analyzed to detect secondary metabolites using the standard method. The antibacterial activity was done by the agar diffusion method and compared to tetracycline HCl as the antibiotic standard. Followed by the determination of minimum inhibitory concentration of the extract using the macrodilution method. **Results:** The phytochemical analysis of the *C. Burmanii* stem bark extract revealed that it contained of alkaloids, tannins, flavonoids, saponins and steroids. The antibacterial activity of the extract on *S. xylosus* was stronger as the increasing of the extract concentration. However, in comparison with tetracycline HCl, the antibacterial activity of the extract was lower. The MIC value of the extract against *S. xylosus* was 0.875 <x<0.938 %w/v. **Conclusion:** *C. Burmanii* stem bark extract provides its potential to be an antibacterial agent for infections caused by *S. xylosus.*

KEYWORDS: *Cinnamomum Burmanii*, stem, extract, antibacterial, *Staphylococcus xylosus.*

INTRODUCTION

Staphylococcus xylosus is a Gram positive, coagulase negative, novobiocin resistant and xylose fermenter.[1] Some strains of *S. xylosus* are opportunistic pathogens. It was mentioned

that this bacterium is the causing agent of urinary tract infections (UTI) in women[2] and cutaneous lesion when injected in mice[3] due to possessing several virulence factors like hemolysin[4], urease[5], proteases[6], and lipase.[7] They have been isolated from nosocomial infections, and described as multi-resistant to diverse antibiotics.[8]

Since the introduction of antibiotics there has been tremendous increase in the resistance of diverse bacterial pathogens.[9,10] Exceptionless for tetracycline as the old antibiotic that has been used for more than 60 years, have the same potential to cause the spread of acquired - specific resistance mechanisms, especially among clinically important bacterial pathogens.[11] The tet (M) and tet (O) genes are the most widely reported genes responsible for bacterial resistance to tetracycline antibiotics (e.g., Enterobacteriaceae, Bacteroides spp.) and Gram-positive bacteria (e.g., Streptococcus spp., Enterococcus spp., Staphylococcus spp.).[12] This shift in susceptibility greatly affects the ability to successfully treat patients empirically.

Plant derived products have been used for medicinal purposes for centuries. These benefits are due to their big content of bioactive compounds.[13] *C. burmanii* is a native tree from Indonesia and Southeast Asia.[14] The bark of this plant is used for traditional medicine empirically and for the flavor industry.[15] One of the bioactive substances in *C. burmannii* that has been identified is Trans-cinnamaldehyde.[16] Another study reported that the extracts of *C. burmannii* bark detected phenolics at high levels and reported a good antibacterial activity which is the inhibitory effects were higher against the Gram positive bacteria as compared to the Gram negative bacteria. The study hypothesized that the antibacterial effect of the *C. burmannii* bark extracts were closely related to their phenolic compound.[17] Therefore, it can be assumed that *C. burmannii* bark extracts have potent antibacterial activity and would be prospective to be a natural medicine.

MATERIALS AND METHODS

Materials

The growth media used in this study were Mueller-Hinton Agar (MHA-Oxoid) and Mueller-Hinton Broth (MHB-Oxoid). The chemicals used are normal saline solution, barium chloride solution (Merck), sulfuric acid solution (Merck), tetracycline standard, tetracycline commercial, hydrochloric acid and distilled water. *S. xylosus* ATCC 3342 was used as tested bacterium, obtained from Laboratory of Microbiology, Faculty of Pharmacy, Padjadjaran University. C. *burmannii* barks were collected from herbal center in Bandung, West Java, Indonesia. The barks material has been identified in the Plant Taxonomy Laboratory,

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Bandung Sumedang Km 21 Jatinangor Sumedang, West Java, Indonesia.

Extraction and Phytochemical Screening Analysis

A weight of I Kg g C. *burmannii* barks were macerated with ethanol 70% as the solvent and the macerates were collected every 24 h for 3 d. The collected maserates were evaporated by a rotary evaporator in 40°C untill the thick extract achieved in a constantly weigh. The extracts were screened using Fansworth method to detect the content of secondary metabolites such as alkaloids, polyphenols, flavonoids, tannins, quinone, triterpenoid, monoterpenoid, sesquiterpenoid, steroid, and saponins.[18]

Preparation of Bacterial Suspension

Preparation of *S. xylosus* was conducted by taking one Ose of *S. xylosus* colony from slant agar, then suspended into sterile physiological saline. Bacterial turbidity was measured using a spectrophotometer and compared with a standard 0.5 Mc Farland.

Antibacterial Activity

The antibacterial activity of both C. *burmannii* barks and tetracycline HCl was examined using the agar diffusion method using a perforation technique. The ethanolic extract and tetracycline HCl was diluted to achieve graded test concentration as follows: 50 µg/mL, 25 µg/mL, and 12.5 µg/mL. A total of 20µL bacterial suspension was poured into a sterile petri dish, then a volume of 20 mL MHA was poured into the petri dish. The medium was homogenized, allowed to solidify and drilled using perforator aseptically. Each hole is then filled with the extract and tetracycline in accordance with the variations of concentration in 50 uL. All the medium test was incubated at 37 ° C for 18-24 h. The diameter of inhibition value was measured by a caliper.

MIC Determination

Determination of minimum inhibitory concentration of C. *burmannii* barks ethanol extract was done using microdilution method. The extract was solubilized in dimethyl sulfoxide (DMSO) and then serially two-fold diluted MHB medium to obtain a concentration range of 0.234-7.5 %w/v. The bacterial strains were suspended in sterile normal saline (0.9%) under aseptic conditions, homogenized and adjusted to an optical density of 0.05 at 530 NM (equivalent to 1 X 10^6 CFU/ml). Volume of 10 µL standardized cell bacterial suspensions was put into each tested concentration. The tested media, then were incubated for 20 h with

temperature at 37^0C. MIC was determined from the minimal concentration of the extract which did not show any turbidity in the medium.

RESULTS AND DISCUSSION

Phytochemical Analysis Result

The phytochemical analysis of the *C. Burmanii* stem bark extract revealed that it contained of alkaloids, tannins, flavonoids, saponins and steroids, can be seen in Table 1. The presence of these various secondary metabolites which might be responsible for their medicinal attributes, such as antibacterial.[19] The presence of those metabolites has been reported to have antibacterial activities.[20] Several studies reported that flavonoids, tannins, and steroid of their extract are the responsible metabolites for the anti-staphylococcal activities.[21-23]

Table 1: Phytochemical screening.

Compounds	Results
Alkaloids	+
Flavonoids	+
Tannins	+
Polyphenolics	+
Monoterpenoids and Sesquiterpenoids	-
Steroids	+
Triterpenoids	-
Quinones	-
Saponins	+

Notes: (+) = presence; (-) = absence

Antibacterial Activity Result

The antibacterial activity of the extract on *S. xylosus* was stronger as the increasing of the extract concentration. However, in comparison with tetracycline HCl, the antibacterial activity of the extract was lower, presented in Figure 1 and Table 2. The sensitivity of *S. xylosus* gradually increased with the concentration increasing of the extract. There was a significant relation between the extract concentrations and the inhibitory diameters. The ability of C. *burmannii* barks to inhibit the *S. xylosus* growth indicated an antibacterial potential which may be applied in the treatment of Staphylococcal infections.

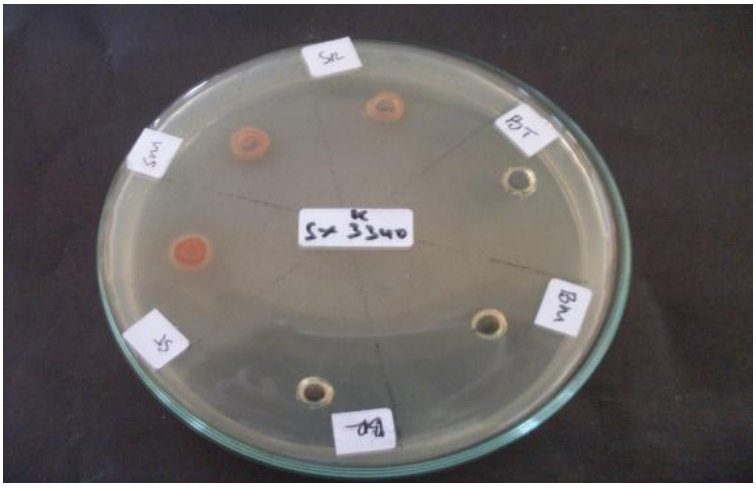

Figure 1: Inhibitory Zone of the extract and tetracycline HCl.

Table 2: Diameter of Zone Inhibition.

Antibiotic concentration (µg/mL)	Diameter of Inhibition (mm)	
	C. burmannii barks extract	Tetracycline HCl
25	15.00±0.00	34.50±0.01
50	17.00±0.00	37.00±0.00
100	20.00±0.02	41.00±0.02

Note: Perforator diameter = 9 mm

MIC Result

The lowest concentration of the tested extracts, requisite for inhibiting the growth of *S. xylosus* was considered as the MIC. The MIC value determined by broth dilution methods indicated that significant antibacterial activity of the ethanol extracts of 0.234 to 7.5 %w/v against the tested bacterial was presented in Table 3. The MIC value of the extract against *S. xylosus* was 0.875 <x<0.938 %w/v. The antibacterial activity of this extract can add scientific data about herbs that can overcome *S. xylosus*. Considering that there have not been many studies studying extracts that can inhibit the growth of these bacteria.

Table 3: MIC Result.

Extract Concentration (%w/v)	Turbidity
0.234	+
0.468	+
0.938	+
1.875	-
3.75	-
7.5	-

Notes: (+) = Turbid; (–) = Clear

CONCLUSION

This ethanolic extract of *C. Burmanii* stem bark was evidenced to demonstrate the valuable antibacterial activity against *S. xylosus.*

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