THE EFFECT OF C*innamomum burmannii* WATER EXTRACTION AGAINST S*taphylococcus aureus, Enterobacter spp., Pseudomonas aeruginosa,* AND *Candida albicans*: IN VITRO STUDY

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ABSTRACT

Indonesian cinnamon (Cinnamomum burmannii) is a native plant of Indonesia that has a lot of potential. The most consumed part is the bark. This study aims to examine the antimicrobial effect of Cinnamomum burmannii bark extract on various types of pathogenic microbes, namely Staphylococcus aureus, Enterobacter spp., Pseudomonas aeruginosa, and Candida albicans. An experimental study using a water extract (infusion) of Cinnamomum burmannii bark and a microbial test obtained from the Faculty of Pharmacy, Widya Mandala Catholic University, Surabaya. The antimicrobial effect test was carried out by the microdilution method in 96-well-microplate to determine the Minimum Inhibitory Level (MIC) and implantation on solid media to determine the Minimum Kill Rate (KBM). The MIC and KBM against Staphylococcus aureus were 625-1,250 ppm and 1,250-2,500 ppm, respectively. MIC and KBM for Enterobacter spp., Pseudomonas aeruginosa, and Candida albicans were not found at the highest concentrations tested at 10,000 ppm. Cinnamomum burmannii extract can be used as a potential ingredient with antimicrobial effects, especially against Gram-positive bacteria. Future studies should pay attention to the quality of simplicia, particle size, and the most effective extraction methods extracting antimicrobial substances from simplicia.

***Keywords**: Antibacterial activity; water extraction of Cinnamomum burmannii; Staphylococcus aureus; Pseudomonas aeruginosa; Enterobacter spp.; Candida albicans*

ABSTRAK

Kayu manis Indonesia (Cinnamomum burmannii) adalah tanaman asli Indonesia yang memiliki banyak potensi. Bagian yang paling banyak dikonsumsi adalah kulit kayunya. Penelitian ini bertujuan untuk menguji efek antimikroba ekstrak kulit kayu Cinnamomum burmannii terhadap berbagai jenis mikroba patogen, yaitu Staphylococcus aureus, Enterobacter spp., Pseudomonas aeruginosa, dan Candida albicans. Penelitian eksperimental menggunakan ekstrak air (infus) kulit kayu Cinnamomum burmannii dan mikroba uji yang didapat dari Fakultas Farmasi Universitas Katolik Widya Mandala Surabaya. Uji efek antimikroba dilakukan dengan metode mikrodilusi dalam 96-well-microplate untuk menentukan Kadar Hambat Minimum (KHM) dan penanaman pada media padat untuk menentukan Kadar Bunuh Minimum (KBM). KHM dan KBM terhadap Staphylococcus aureus berturut-turut adalah 625-1.250 ppm dan 1.250-2.500 ppm. KHM dan KBM untuk Enterobacter spp., Pseudomonas aeruginosa, dan Candida albicans tidak didapatkan pada konsentrasi tertinggi yang diuji yaitu 10.000 ppm. Ekstrak Cinnamomum burmannii dapat dijadikan salah satu bahan potensial dengan efek antimikroba, khususnya terhadap bakteri Gram positif. Penelitian selanjutnya hendaknya memperhatikan mutu simplisia, ukuran partikel, dan cara ekstraksi yang paling efektif menyarikan zat antimikroba dari simplisia.

***Kata kunci**: Aktivitas antibakteri; ekstraksi air Cinnamomum burmannii; Staphylococcus aureus; Pseudomonas aeruginosa; Enterobacter spp.; Candida albicans*

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INTRODUCTION

Nowadays, the world faces the problem of microbial resistance to antibiotics. Therefore, this situation encourages experts to find safe and effective antimicrobial ingredients, especially from herbs (Vangalapati et al 2012, Ferry 2013). The Infectious Diseases Society of America adds *Staphylococcus aureus* (*S. aureus*), Klebsiella spp., *Enterobacter spp.*, and *Pseudomonas aeruginosa* (*P. aeruginosa*) into mnemonic ESKAPE group (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P aeruginosa*, and *Enterobacter spp.*), a group of bacteria that develop resistance to

antimicrobial agents, and cause serious health problems related to pathogenesis and transmission (Aubert, Flannagan & Valvano 2008, Forsythe et al 2015). Candida spp. is one of the microbes reported to be the most common cause of nosocomial infections in the United States in 2009-2010 and Europe in the year 2010-2011. Among other Candida species, *Candida albicans* (*C. albicans*) is the species that most often causes health problems (Groisman 2001, Peterson 2017). Due to these medical interests, the four microbes were used in this study.

Cinnamomum burmannii (*C. burmannii*), is an Indonesian native plant that has long been used as a cooking spice, natural preservative, cosmetics and traditional medicine. All parts of the cinnamon such bark, branches, twigs and leaves, contain useful phytochemicals (Nabavi et al 2015). However, the bark's part is widely and commonly used. Several studies on *C. burmannii* concluded that essential oils and extracts from the bark (cortex) of *C. burmannii* has antimicrobial effects (Ulbricht et al 2015, Zhang et al 2015). Cinnamon extract has an effect on the growth of *Staphylococcus mutans* which is carriogenic bacteria (Waty et al 2018).

This study aim was to examine the antibacterial activity of *C. burmanii* water extraction against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Enterobacter spp, and *Candida albicans* in various IC50 value in a range of 625 to 10.000 µg/mL.

MATERIALS AND METHODS

Water extraction

Cinnamomum burmannii (*C. burmannii*) was extracted by using (H2O) water extraction methods. 10 grams of crushed *C. burmannii* bark was extrated in 100 mL of distilled water infusion at 90 ° C for 20 minutes. The results obtained are freeze-dry to evaporate the water content(Ervina, Nawu and Esar 2016).

The Minimum Inhibition Concentration is the lowest concentration of *C. burmannii* water extraction inhibits microbial growth. It was seen in the results of micro delusion culture on Mueller Hinton Broth (MHB) and Sabouraud-Dextrose Broth (SDB) with absorbance 90% approach negative control (Nester et al 2013, Public Health England 2014). The Minimum Bactericidal Concentration (MBC) is the minimum concentration of *C. burmannii* H2O extract that has ability to kill microbes. It was shown in Mueller Hinton Agar (MHA) and Sabouraud-Dextrose Agar (SDA) culture with a reduction of 99.9% of the amount inoculated bacteria (Nester et al 2013, Public Health England 2014). Muller-Hinton Broth (MHB) were used in antibacterial assay for culturing the bacteria while for antifungal assay, Sabouraud-Dextrose Agar (SDA) and Sabouraud-Dextrose Broth (SDB) were used, and both of the agar plates and broth were stored at 4°C (Fadhlina et al 2013). The test microbes used for this study were *Staphylococcus aureus* (*S. aureus*), *Enterobacter spp.*, *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Candida albicans* (*C. albicans*). All microbial species are stored isolates obtained from the laboratory of the Faculty of Pharmacy, Widya Mandala Catholic University, Surabaya.

Preparation of inoculums

Stock culture for each of the tested microorganisms was sub-cultured to obtain single colonies. Three well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop and transferred into a falcon tube containing 10 mL of MHB and SDB for bacteria and fungi. Both bacteria and fungi were incubated at 37 °C for 24 hours. The optical densities (OD) of the incubated bacterial and fungal inoculums were measured using spectrophotometry. The bacteria and fungus turbidity were measured at 595 nm (Fadhlina et al 2013, Ervina et al 2016, Waty et al 2018).

Minimum Inhibitory Concentration (MIC) Assay

The MIC of *C. burmanii* water extraction was determined by the broth micro-dilution method. A 96-well plate was used for this assay whereby each of the wells plate was loaded with the inoculums grown to an exponential phase containing 107 and 104 CFU/mL of bacteria and fungi. 10.000 µg/mL of *C. burmanii* water extraction was the highest concentration in this study. It transferred into the inoculated well (270 µL). Two-fold serial dilution was performed by transferring 100µL from the highest concentration of treated culture into the next well so that the final volume of each well was 200 µL. Finally, all of the tested plates were incubated. The standard antibiotics were tested in the same manner as the stated steps, and done in five repetitions.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration

(MFC) assay is treated cultures containing concentrations equal to and higher than the MIC value were swabbed onto the agar plate. The concentration that gave zero subculture growth on the agar after the incubation was considered as MBC. MFC for selected fungi applied the same procedures as MBC.

RESULTS

The microplate was filled with 156,25-10.000 µg/mL of *C. burmanii* water extraction (5 repetitions) and *S. aureus* suspension. As control, the other part of microplate was also filled with 0.375-1,5 µg/mL of penicillin and *S. aureus* suspension, and also sterile broth and 0.9% NaCl. The microplate was incubated in 37 0C for 24 hours. Using a microplate reader for OD, the absorbance was read in a wavelength of 595 nm. The MIC of *C. burmanii* water extraction against *Staphylococcus aureus* was found at the concentration of 625-1.250 µg/mL (Table 1). Moreover, the MBC was at the concentration of 1,250-2.500 µg/mL (Fig. 1).

However, *C. burmannii* water extract in the concentration of 156,25 to 10.000 µg/mL had no antibacterial effect against *Pseudomonas aeruginosa*, and *Enterobacter spp*, and nor antifungal against *Candida albicans* (Table 1).

Table 1. Minimum-Inhibitory Concentrations (µg/mL) and Minimum-Bactericidal Concentrations (µg/mL) of *Cinnamomum burmanii* water extraction

Bacteria/Fungi	MIC	MBC
Staphylococcus aureus	625	1250
Pseudomonas aeruginosa	-	-
Enterobacter spp	-	-
Candida albicans	-	-

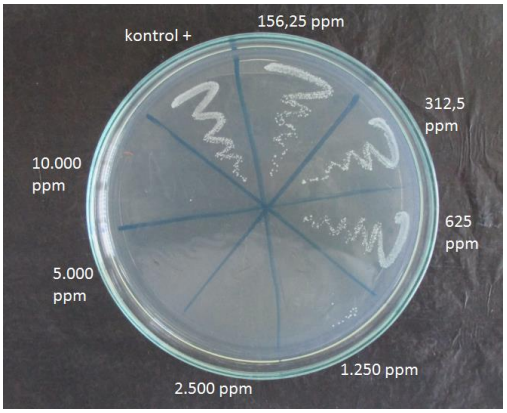

Fig. 1. Minimum-Bactericidal Concentrations (µg/mL) of *Cinnamomum burmanii* water extraction for *Staphyloccus aureus*.

DISCUSSION

Prescribing and using irrational antibiotic has reached a critical stage. Microbial resistance to antibiotics must be prevented. Rational use of antibiotics and discovery of nature products that have potential as antibacterial need to be encouraged to overcome the resistant problems (Vangalapati et al 2012, Nabavi et al 2015, Zhang et al 2015).

Cinnamomum burmannii (*C. burmannii*) is an Indonesian native plant that has been widely used as a traditional medicine. It is known to have antimicrobial effects (Zhang et al 2015, Waty et al 2018), due to its component of cinnamaldehyde, that water soluble (Vangalapati et al 2012) and non-volatile component, including condensed tannins (proanthocyanidin and catechins), that less water soluble(Lekha and Lonsane, 1997). There are several theories for the antibacterial mechanism of *C. burmannii*. The active part of the extract component binds and penetrates the surface of microbial cells, through double phospholipid layer on the cytoplasmic membrane, and through membrane-bound enzymes. Therefore, this results barriers to its proton motive force, respiratory chain (creb's cycles) and ionic transfer, as well as becoming barriers to substrate oxidation. Furthermore, phosphorylation oxidative occurs, disruption to the active transport, reduced of metabolic products, and impaired synthesis of DNA, RNA, proteins, lipids and polysaccharides (Vangalapati et al 2012).

In this study, in vitro testing of the antibacterial and antifungi effect of *C. burmanii* water extraction was conducted. It shown in table 1 that from the bark of *C. burmannii* showed that it only had antibacterial against *Staphylococcus aureus*, MIC of 625-1,250 µg/mL, and MBC of 1,250-2,500 µg/mL. This result support data from previous study about *C. burmanii* against *Staphylococcus aureus* (Fadhlina et al 2013, Khakzadihe et al 2014, Zhang et al 2015, Waty et al 2018). *C. burmanii* is sensitive against bacteria gram positive growth.

Enterobacter spp., *Pseudomonas aeruginosa*, and *Escherichia coli* are bacteria gram negative that often use in *C. burmanii* studies. However, *C. burmanii* water extraction had no effects on inhibiting nor killing *Enterobacter spp.*, *Pseudomonas aeruginosa*, and *Candida albicans*, even in the highest concentration of 10,000 µg/mL, and this result against data that shown that the oil of *C. burmanii* has ability in inhibiting *Candida albicans* and *Escherichia coli* growth (Fadhlina et al 2013).

The main difference of bacteria gram positive and bacteria gram negative is in the constituent components of the cell. The wall of gram positive's cell has a skeleton that gives shape to bacteria, a thicker peptidoglycan layer, and richness of teicoic acid (Hett &

Rubin 2008, Hogg 2013). This structure allows cinnamaldehyde, the main component of *C. burmanii* that has antibacterial effect, entering into the cell (Vangalapati et al 2012). Bacteria gram negative's wall consists of outer membrane, thin peptidoglycan layer, and the periplasmic cavity. The outer membrane of bacteria gram negative consists double lipopoly-saccharide layer and containing a lot of porin. The presence of a double lipopolysaccharide layer gives a negative charge to the surface of the bacteria, therefore lipophilic material is not allowed to enter (Groisman 2001, Rohilla 2010). Porin affects the bacterial escape mechanism against antibiotics or other antibacterial substances. Periplasmic cavity contains a layer of murein gel, which functions to capture nutrients from the environment, as well as enzymes for the degradation of macromolecules and detoxification of hazardous materials that successfully penetrate the outer membrane and peptidoglycan layer, such as antibiotics or substances with antimicrobial effects. Therefore, bacteria gram negative is more easily to be antibiotic resistant than bacteria gram positive (Rohilla 2010, Zhang et al 2015). The presence of gram negative outer membrane creates obstacles for antibacterial molecules to penetrate. Even if an antibiotic molecule is successful, the enzymes in the periplasmic cavity will damage the antibacterial before it reach its workplace.

Moreover, water extraction method may affect cinna-maldehyde, and condensed tannins (proanthocyanidin and catechins) production. Cinnamaldehyde is water solube however it is hard to enter the outer membrane of gram negative. The condensed tanins, that less water soluble, may less produce using water extraction method.

CONCLUSION

Cinnamomum burmannii water extraction has an antibacterial effect against *Staphylococcus aureus* with MIC of 625-1,250 µg/mL and MBC of 1,250-2,500 µg/mL. Therefore, *C. burmanii* with various ways of extraction has a potential effect for bacteria gram positive. However water extraction method was not suitable to enhance the *C. burmanii* antibacterial effect against *Enterobacter spp.*, and *Pseudomonas aeruginosa*. Also has no effect against *Candida albicans* at the concentration of 10,000 µg/mL.

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