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Antibacterial and Antibiofilm of *Cinnamomum burmanii* Bark Oil (CbBO) against *Klebsiella pneumoniae* ATCC 700603: In Vitro Study

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ABSTRACT

Many cases of multidrug-resistant (MDR) bacterial infections are caused by *Klebsiella pneumoniae* (K.pn), an infectious disease bacterium. Hospital inpatients can be exposed to this occurrence; MDR has transformed 26.96% of infections at Klaten Hospital into highly infectious biofilms, while 54.49% of infections have produced biofilms. Approximately 80% of bacterial illnesses resistant to antibiotics are caused by biofilm-forming bacteria. To reduce biofilm formation, antibacterial compounds, one of which comes from natural products, are necessary. Renowned for its essential oil, *Cinnamomum burmanii* Bark Oil (CbBO) has been utilized extensively in herbal medicine to combat pathogenic bacteria such as *Pseudomonas aeruginosa, Candida albicans*, *Enterobacter spp.*, and *Staphylococcus aureus*. This study aims to evaluate CbBO's antibacterial capabilities using the microdilution method and its antibiofilm properties against *K. pneumoniae* ATCC 700603 using the MTT test. A CbBO minimum bactericidal concentration (MBC) of 0.25 mg/mL and a minimum inhibitory concentration (MIC) of 0.125 mg/mL were employed for antibacterial activity. The antibiofilm potential was determined by measuring the minimum biofilm eradication concentration (MBEC) at 0.5 mg/mL and the minimum biofilm inhibition concentration (MBIC) at 0.25 mg/mL. In conclusion, CbBO demonstrated antimicrobial and antibiofilm qualities. However, a greater concentration of CbBO was required for antibiofilm formation than for antibacterial purposes.
Keywords: Antibacterial; Antibiofilm; *Cinnamomum burmanii*; Essential Oil; *Klebsiella pneumoniae*

INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae*) is a gram-negative rod bacterium infecting the bloodstream and tissues. A recent study discovered that multidrug-resistant *K. pneumoniae* has caused community-acquired infections (Murphy & Clegg, 2012). *K. pneumoniae* belongs to the *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, and *Escherichia coli* (ESKAPEE) bacteria category. Those bacteria have been reported to be multidrug-resistant (MDR), extensively drug-resistant (XDR), and even pan-drug-resistant (PDR), identified by the World Health Organization (WHO) as a priority pathogen requiring strict management (Yu et al., 2020).

The 2019 global epidemic led to a prevalence of 50% of carbapenem-resistant *K. pneumonia* (CRKP) infections and 4.1% to 79.4% of ciprofloxacin-resistant XDR *K. pneumoniae* infections (GLAS, 2020). In Singapore, the mortality rate from *K. pneumoniae* bacteremia reached 20% to 26% (Chew et al., 2017). Meanwhile, in Indonesia, a study conducted at RSUP Dr. Soeradji Tirtonegoro Klaten disclosed that 54.49% of patients had MDR *K. pneumoniae* infection, and 85.63% of those isolates produced biofilms with a high intensity of 26.95% (Nirwati et al., 2019).

The emergence of biofilm-forming bacteria has triggered the development of antibiotic resistance and chronic diseases. Compared to non-biofilm-forming bacteria, biofilm-forming bacteria have substantially more resistance to antimicrobials (Shadkam et al., 2021). Antibiotic sensitivity of biofilm bacteria differs from that of planktonic bacteria; for example, the minimum inhibitory concentration (MIC) value of ampicillin for *Escherichia coli* ATCC 25922 is 2 μg/mL in the planktonic phase, but it escalates to 512 μg/mL in the biofilm phase (Fux et al., 2005).

Planktonic bacteria are the primary target of antibiotic development efforts aimed at combating multidrug-resistant pathogenic bacteria and host immunity (Bjarnsholt et al., 2013). There is still a need for effective biofilm inhibition, eradication techniques, and new antibacterial sources. Antibiofilm strategies encompass the prevention of biofilm development and the physical, chemical,

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and biological destruction of biofilms. Examples of such mechanisms include the use of chelating agents, natural plant compounds or phytochemicals, and quorum sensing inhibitors (QSI) (Borges et al., 2016). Essential oil (EO) possesses substantial antibacterial properties that enable it to prevent and manage microbial illnesses, making it a significant natural product (Tariq *et al*., 2019; Yu *et al*., 2020). Cinnamon (*Cinnamomum burmanii*) is a medical herb frequently utilized due to its abundance of beneficial compounds and essential oils, including cinnamic acid, eugenol, cinnamaldehyde, and cinnamate (Tung *et al*., 2010; Vangalapati *et al*., 2012 ; Rao & Gan, 2014). This plant is indigenous to Indonesia. Its bark extract exhibits antibacterial activity against various pathogenic pathogens, such as *Pseudomonas aeruginosa, Candida albicans,* and *Enterobacter spp*. (Silvia Novita, 2019).

Cinnamaldehyde, the primary ingredient in cinnamon oil, inhibits the same target on the plasma membrane as chlorhexidine does (Nuryastuti et al., 2009). The essential oil derived from *C. verum* leaves possesses a high eugenol content and strong antibacterial and antibiofilm properties against *K. pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, with an MIC of 0.5 mg/mL and an MBC of 1.0 mg/mL. Minimum biofilm inhibition concentrations (MBIC50) of 1.0 mg/mL and minimum biofilm eradication concentrations (MBEC) of 4.0 mg/mL were discovered. SEM revealed reduced biofilm density, cell shrinkage, and cell wall damage (Wijesinghe et al., 2021). Cinnamon oil could effectively inhibit and kill germs and has an antibiofilm effect against Methicillin-resistant *Staphylococcus aureus (*MRSA), with MIC and MBC values of 0.25 mg/mL and MBIC and MBEC values of 1.0 mg/mL (Cui et al., 2016). The literature currently lacks information on natural compounds with antibacterial properties that prevent the growth of *K. pneumoniae* biofilm, especially cinnamon oil derived from *Cinnamomum burmanii*. Accordingly, this study aims to assess the antibacterial and antibiofilm capabilities of CbBO against the standard isolate *K. pneumoniae* ATCC 700603.

MATERIALS AND METHODS

Instrument

This study utilized several instruments, encompassing mortar or pestle, round-necked flask, distillation flask, drip funnel, separating funnel, rotary evaporator (IKA ™), biosafety cabinet class II (Thermo Scientific ™), petri dish (Iwaki ™), ose needle, electric bunsen, microscope (Olympus ™), slide, incubator (Memmert ™), 96-well microplate (Iwaki ™), micropipette, 24-well polystyrene plate tip, spectrophotometer (Thermo Scientific ™), sterile test tube (Iwaki ™), sterile 96-well-flat-bottom microplate (Iwaki ™), and microtiter plate reader (Bio-rad ™).

Material

This study employed several materials, including *Cinnamomum burmanii* (Nees & T. Nees) Blume-type cinnamon bark oil and a standard isolate from the *Lauraceae*, *K. pneumoniae subsp. Pneumoniae* ATCC™ 700603 Thermo Scientific™ Culti-Loops™ 700603 *K. pneumoniae* ATCC™ R4603074, MacConkey medium (Oxoid ™), API-E identification kit (Biome Rieux ™), TSB with 1% glucose (TSBG) (Oxoid ™), phosphate buffered saline solution (PBS, pH 7.2) (Merck ™), Hucker's crystal violet of 2% (0.1% w/v) (Merck ™), sterile distilled water, acid glacial acetate (Merck ™), menadione crystallin (Sigma MCLS ™), MTT (Bio basic ™), dimethyl sulfoxide (DMSO) (Sigma ™), 2% formaldehyde (Sigma ™), BHI broth (Oxoid ™), Mueller Hinton agar (Oxoid ™), and sterile distilled water (DW).

Method

Preparation of *Cinnamomum burmanii* Bark Oil using Steam Distillation

Cinnamon bark (*Cinnamomum burmanii*) was ground using a mortar and pestle and then transferred into a 100 mL round-necked flask. Distillation began after adding 40 mL of water. After gathering about 20 mL of the hazy distillate, the distillation flask was filled with an additional 20 mL of water using the drip funnel. After collecting 20 mL of distillate, the procedure was stopped. Subsequently, the distillate was placed onto a separating funnel and extracted with 2 × 20 mL of diethyl ether. The fine layer was dried over anhydrous magnesium sulfate, and the mixture was filtered. The ether was removed, and the cinnamon essential oil was collected using a rotary evaporator. One kg of *Cinnamomum burmanii* bark yielded 3 mL of CbBO. Hence, a CbBO concentration of 0.3% (v/w) signifies that every 100 g of *Cinnamomum burmanii* bark contains 0.3 mL of CbBO or 3 µL/g (Prajapati et al., 2019).

Preparation of *Klebsiella pneumoniae* Biofilm Model

K. pneumoniae isolates grown statically for three hours in TSB at 37 °C were adjusted to a bacterial concentration of $1x10^{10}$ CFU/mL. Three iterations of the biofilm test were performed using 24-well polystyrene plates containing 400 µL of high glucose TSB in each well. Each well received 100 µL of bacteria, which were then statically incubated for 24 hours at 37 °C. The biofilm was

preserved with 1 mL of 2% formaldehyde for 20 minutes, rinsed three times with PBS, and then stained for 20 minutes with 0.5% crystal violet. To achieve absorbed staining, 1 mL of 95% ethanol was applied to the plates after they were properly cleaned with distilled water. A spectrophotometer read the staining at 570 nm, and the biofilm intensity was calculated using optical density (OD) and optical density control (ODc). OD ≤ ODc indicates a negative biofilm intensity; OD < 2 signifies a weak biofilm; OD < 4 implies a medium biofilm, and OD < 4 represents a strong biofilm (Vuotto et al., 2014; Alcántar-Curiel et al., 2018).

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC)

All test isolates were utilized to create standard cell suspensions in sterile BHI broth using 24-hour bacterial isolates. After that, several colonies were inoculated on 10 mL of TSB with 1% glucose (TSBG) and kept at 37 °C for the entire day. To achieve the desired concentration, each sample was diluted 1:100 in new TSB to 0.5 McFarland (~1.5 x 10^8 CFU/mL). To generate serial dilutions of CbBO (100 µL/well), 32 mg/mL of the bark oil was put into a 96-well sterile flat-bottom microtiter plate and subsequently diluted with sterile BHI broth. Then, a standard cell suspension that had been separately produced (100 µL/well) was added to the microtiter plate as an inoculant (negative control (growth control): 100 µL BHI broth + standard cell suspension). MIC was determined by inoculating 5 µL of solution from each well on a Mueller Hinton agar plate and incubating aerobically at 37 °C for another 24 hours.

Determination of Minimum Biofilm Inhibitory Concentration (MBIC50)

TSB/BHI liquid medium of 180 µL and cinnamon oil of 20 µL were added into the well of column 1. Meanwhile, the wells of column 2 and so on were filled with 100 µL of TSB/BHI liquid medium. As much as 100 µL of fluid from column 1 was put in column 2 to create serial delusions. Several wells were utilized for control: negative control (growth control, 100% growth), positive control (100 µL of liquid medium containing a certain concentration of CbBO), solvent control, and media control. Subsequently, 10 µL of 10^8 CFU/mL bacterial suspension was added. The microtiter plate was incubated for 24 to 48 hours at 37 °C, and biofilm staining was performed with MTT. The absorbance was read with an ELISA reader at a wavelength of 560 nm (400 to 700 nm). The experiment was carried out in six repetitions at two different times (Wijesinghe et al., 2021).

Determination of Minimum Biofilm Eradication Concentration (MBEC)

The minimum biofilm eradication concentration (MBEC) was determined using the method from Ramage et al. (2001). The MBEC determination principle involves initially growing biofilm cells and subsequently subjecting them to treatment with CbBO. The steps involved growing biofilm isolate *K. pneumoniae* ATCC 700603 in a MacConkey selective medium for 48 hours at 37 °C. The supernatant and planktonic cells were carefully discharged and washed three times with 200 µL of PBS. Serial samples of CbBO were created using another 96-well plate. As much as 100 µL of liquid medium containing the concentration of CbBO was added to the plate on which the biofilms were grown. Then, it was incubated at 37 °C for 24 hours. The medium solution was then discharged and washed three times with 200 µL of PBS. A total of 100 µL of MTT solution was added to each 96-well-containing biofilm cell. The plate was closed and incubated at 37 °C for two hours. The plate cover was removed, and the solution was thrown away after the incubation. A total of 100 µL of isopropanol acid at 5% was poured and incubated for one hour at room temperature. The absorbance was determined using an ELISA reader set to 560 nm in wavelength (Ramage & Wickes, 2001).

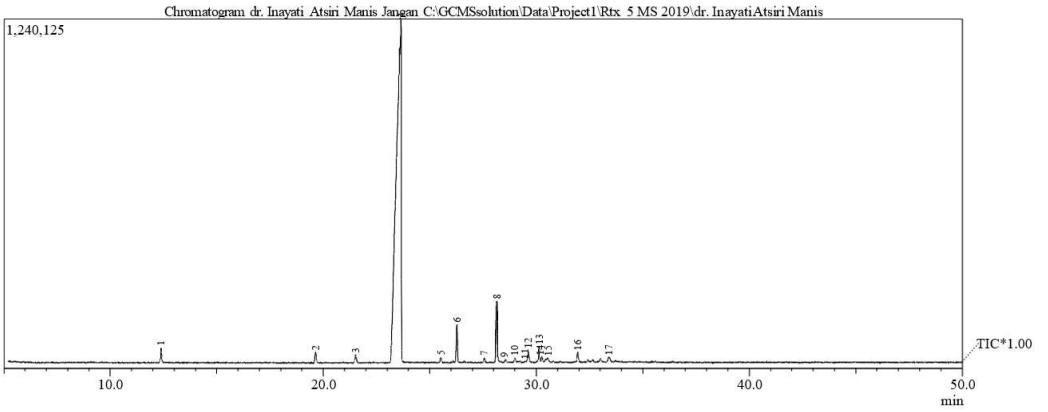
RESULTS

CbBO contains several chemicals, with cinnamaldehyde being the most prevalent (86.78%), followed by alpha copaene (2.42%). The chemical content of CbBO obtained through steam distillation using gas chromatography-mass spectrometry (GC-MS) was analyzed qualitatively, as displayed in Figure 1.

The CbBO MIC value had the least amount of CbBO concentration able to prevent the growth of the standard isolate *K. pneumoniae* ATCC 700603. Based on the OD value, as depicted in Figure 2, the MIC value of CbBO against the standard isolate *K. pneumoniae* ATCC 700603 was 0.125 mg/mL.

The minimal bactericidal concentration (MBC) was determined based on the lowest CbBO concentration not demonstrating any growth of *K. pneumoniae* colonies on the MacConkey selective agar. Figure 3 exhibits the MBC CbBO values for the standard isolate *K. pneumoniae* ATCC 700603, the negative control, and the positive control.

Antibiofilm potency of CbBO was measured using the MBIC and MBEC values. Treatment with 0.25 mg/mL CbBO inhibited the biofilm of the



3	21.519	21.433	21.642	160881	0.73	30237	2-Propenal, 3-phenyl- (CAS) Cinnamaldehyde
4	23.636	23.142	23.717	19051938	86.05	1157653	2-Propenal, 3-phenyl- (CAS) Cinnamaldehyde
5	25.525	25.433	25.592	79993	0.36	19020	EUGENOL
6	26.275	26.167	26.358	535733	2.42	136731	.alpha.-Copaene
7	27.554	27.492	27.650	66257	0.30	17299	trans-Caryophyllene
8	28.152	28.033	28.267	957648	4.33	217867	2-Propen-1-ol, 3-phenyl-, acetate (CAS) Cinnamyl acetate

Figure 1. Percentage of qualitative GC-MS test results of CbBO containing 86.05% 3-phenyl-(CAS) cinnamaldehyde

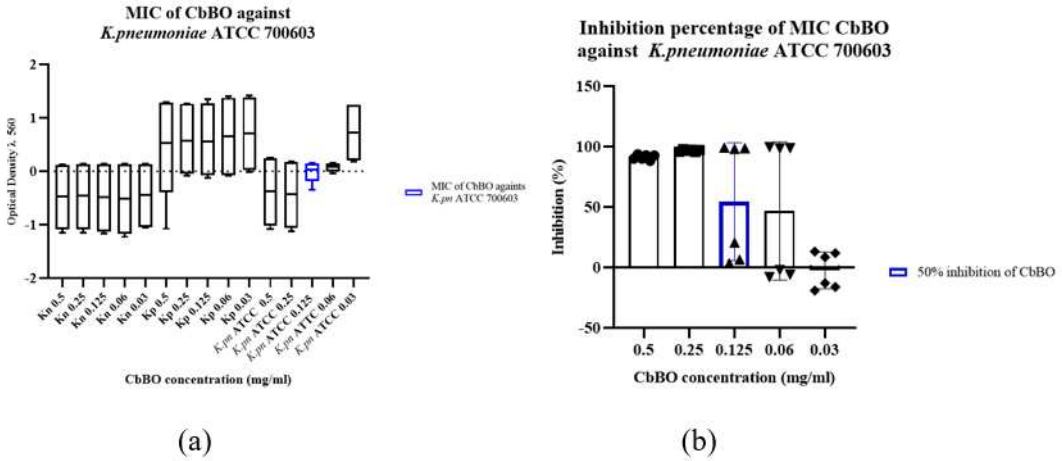


Figure 2. (a) The MIC of CbBO at different concentrations against the standard isolate *K. pneumoniae* ATCC 700603 (*K.pn* ATCC) comparisons to negative and positive controls (Kn and Kp) and (b) The CbBO's percentage inhibition MIC against the isolate *K. pneumoniae* ATCC 700603. The OD value was measured with six repetitions at two different times.

standard isolate *K. pneumoniae* ATCC 700603. Figure 4 illustrates the MBIC value of CbBO against the reference isolate *K. pneumoniae* ATCC 700603 of 0.25 mg/mL.

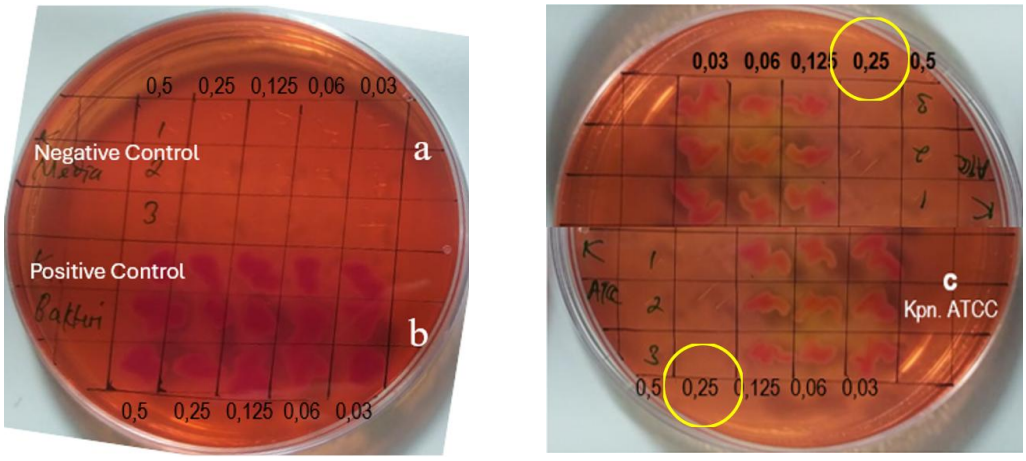
The results of measuring MBEC CbBO values at concentrations of 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, and 0.06 mg/mL against the standard isolate *K. pneumoniae* ATCC 700603 biofilm were compared with the negative control and positive control. The MBEC CbBO value against the standard isolate *K. pneumoniae* ATCC 700603 was measured based on the OD value, as displayed in Figure 5.

The MBEC value for CbBO treatment at a concentration of 0.5 mg/mL against the standard isolate *K. pneumoniae* ATCC 700603 biofilm depicted a lower OD value than the negative control. It indicates that CbBO at a concentration of 0.5 mg/mL can eradicate the biofilm of the standard isolate *K. pneumoniae* ATCC 700603.

DISCUSSION

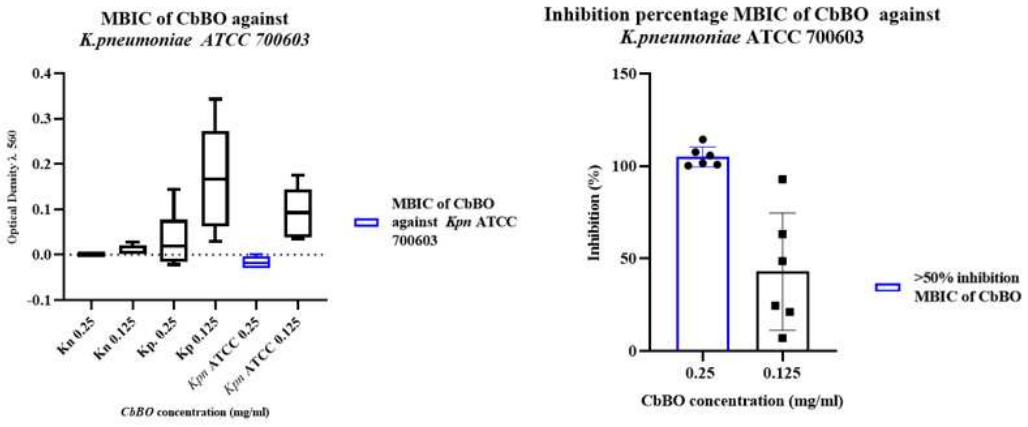
The bark of the Indonesian cinnamon tree, *Cinnamomum burmanii*, is made up of many substances. CbBO contains several chemical compounds, including cinnamaldehyde, with the highest content (86.78%) in addition to alpha copaene (2.42%). Numerous studies unveiled that cinnamon oil could inhibit germs and biofilm production through several methods.

Cinnamomum is a genus of plants containing roughly 250 species. The inner bark of many of



(I) (II)

Figure 3. CbBO MBC determination with CbBO concentrations of 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.06 mg/mL, and 0.03 mg/mL against the standard isolate of *K. pneumoniae* ATCC 700603. The MBC value test was compared with negative control (without colony growth) and positive control (with colony growth) on the MacConkey selective agar. The test was repeated six times at two different times. (I): a. Negative control (medium) = invisible colony growth of *K. pneumoniae,* b. Positive control (bacteria) = visible colony growth of *K. pneumoniae*. (II): c. CbBO treatment = visible colony growth on the standard isolate *K. pneumoniae* ATCC 700603 at CbBO concentrations of 0.03 mg/mL, 0.06 mg/mL, and 0.125 mg/mL, but invisible colony growth at CbBO concentrations of 0.25 mg/mL and 0.5 mg/mL.



(a) (b)

Figure 4. (a) The MBIC value of CbBO against the standard isolate *K. pneumoniae* ATCC 700603 based on the OD value at λ 560 was compared with the negative control and positive control. (b) Percentage of CbBO inhibition against the standard isolate *K. pneumoniae* ATCC 700603. Kn= negative control, Kp= positive control, ATCC= standard isolation of *K. pneumoniae* ATCC 700603 with CbBO treatment. The test was carried out in six repetitions at two different times.

these trees has been utilized to manufacture cinnamon spice (Shreaz et al., 2016; Friedman, 2017; Vasconcelos et al., 2018). The two most prevalent species are *C. verum* (also called *C. zeylanicum*, often referred to as true cinnamon) and *C. cassia* (Chinese cinnamon, commonly referred to as Cassia). A study reported cinnamaldehyde content of 85.3% and 90.5% in these two species (Shreaz et al., 2016). The cinnamon species of *C. burmanii, C. camphora, C. cassia, C. osmophloeum, C. verum, and C. zeylanicum* yield the most significant volatile oils (Burt, 2004). *Trans-cinnamaldehyde* is the main ingredient responsible for many of the spice's claimed antibacterial qualities (Visvalingam et al., 2013; Vasconcelos et al., 2018 ; Firmino et al., 2018).

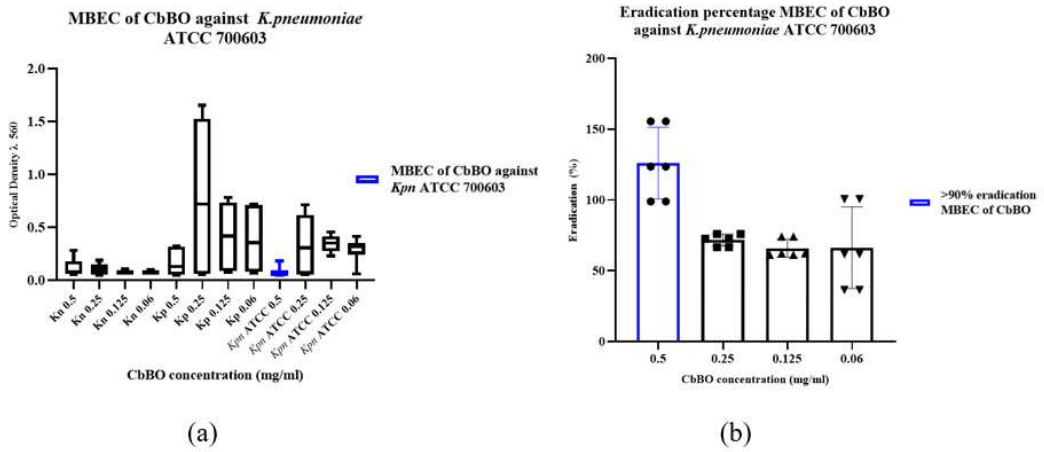


Figure 5. (a) The MBEC CbBO against the standard isolate *K. pneumoniae* ATCC 700603 was based on the OD value, compared with the negative control and positive control. (b) Percentage of MBEC CbBO eradication against the standard isolate *K. pneumoniae* ATCC 700603 biofilm. The test was carried out six times at two different times.

The growth of certain microorganisms, including molds, bacteria, and yeasts, could be effectively inhibited by isolated cinnamaldehyde. It also inhibits bacteria from producing poisons (Gill & Holley, 2006 ; (Visvalingam et al., 2013).

Cinnamon has been utilized to treat diseases because its bark and leaves have been widely employed as antifungal, antibacterial, antidiabetic (Vasconcelos et al., 2018), nematicide (Gill & Holley, 2004), anticancer (Firmino et al., 2018), and insecticide (Amalaradjou et al., 2010; Baskaran et al., 2010). The antibacterial activity of meropenem and colistin against the MDR-*Pseudomonas aeruginosa* strain was greater in cinnamon bark oil and cinnamaldehyde (Gill & Holley, 2006). According to theory, cinnamon bark oil acts on bacterial membranes by removing the potassium cation (K+) gradient, damaging the permeability barrier and resulting in membrane damage and cell death (Bouhdid et al., 2010). These findings provide proof that the number of bacterial cells has decreased. Leakage of cellular substances and cell death might result from the accumulation of different chemicals in the cytoplasm and disruption to the bacterial cytoplasmic membrane (Utchariyakiat et al., 2016). The mechanism occurs when the bioactive compound cinnamaldehyde binds to the FtsZ protein and forms Z rings at the site of cell division (Domadia et al., 2007).

CbBO has antibacterial qualities against the standard isolate *K. pneumoniae* ATCC 700603, with MIC and MBC values of 0.125 mg/mL and 0.25 mg/mL, respectively. The concentration required to kill the standard isolate *K. pneumoniae* ATCC 700603 was twice that required to halt its growth. Research on the antibacterial potential of cinnamon oil from different cinnamon species and bacterial isolates has been carried out. The antibacterial potential of *Cinnamomum cassia* against the standard isolate *Escherichia coli* O157:H7 ATCC 35150 discovered MIC and MBC values of 0.5 lL/mL and 1 lL/mL. In contrast, *Cinnamomum verum* against *Escherichia coli* O157:H7 ATCC 35150 acquired the same MIC and MBC values of 0.5 lL/mL (Santos et al., 2017). When comparing the MIC and MBC values of *Cinnamomum cassia* and *Cinnamomum verum* to the standard isolate *Escherichia coli* O157:H7 ATCC 35150, they were less than those of CbBO against the standard isolate *K. pneumonia* ATCC 700603. This variation was caused by the many cinnamon species utilized, which naturally varied. Different bacterial isolates studied will exhibit varying features in terms of chemical composition, particularly resistance to antimicrobial agents.

The antibacterial activities of herbal *Monarda didyma* essential oils (MDEO) against *K. pneumoniae* were investigated by Chen et al. (2023). Using a broth microdilution experiment, the MIC and MBC of MDEO against the strains of CRKP were determined. MDEO has antibacterial properties against carbapenem-resistant *K. pneumoniae* (CRKP), with both MIC and MBC values of 1.25 mg/mL. The same MIC and MBC results have demonstrated the effectiveness of MDEO as a bactericidal agent (Chen et al., 2023). The potential differences between the isolate CRKP and the standard isolate *K. pneumoniae* ATCC 700603 resulted in a greater dose of CbBO to kill *K. pneumoniae* ATCC 700603 compared to the herbal MDEO. The standard isolate *K. pneumoniae* ATCC 700603 has been known as a strong biofilm

producer (Shadkam et al., 2021). Without using any human genetic material, Chen's research applied 20 CRKP subcultures obtained from the laboratory of the First Affiliated Hospital of Jiamusi University. The CRKP test generated similar MIC and MBC values for MDEO (MIC = MBC = 1.25 mg/mL).

Research on the antibacterial properties of various herbal components and other chemicals against *K. pneumoniae* has been conducted, one of which was related to the antibacterial activity of *C. verum* leaves against *K. pneumoniae*. The essential oil isolated from *C. verum* leaves has demonstrated strong antibacterial and antibiofilm properties against *K. pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, with an MIC of 0.5 mg/mL and an MBC of 1.0 mg/mL. Its high eugenol concentration further contributed to these qualities. The lowest values of biofilm inhibition and eradication (MBIC50 and MBEC) were 1.0 mg/mL and 4.0 mg/mL. SEM revealed deteriorated cell walls, a decrease in biofilm density, and shrinking cells (Wijesinghe et al., 2021). Eugenol antibacterial test results reported that the MIC of eugenol against the four tested CRKP was 0.2 mg/mL (Qian weidong, 2020). The fact that less CbBO concentration is required to inhibit *K. pneumoniae* ATCC 700603 than both *C. verum* and eugenol essential oils indicates that CbBO has more potential than the other two.

Biofilms have been a major contributor to pathogenesis; it is an aggregation of microbial organisms enclosed in an extracellular polymeric matrix. The biofilm matrix gives the bacteria physical protection, encourages the transfer of antibiotic-resistant genes, and can promote microbial antibiotic resistance, bacterial durability, and bacterial proliferation (Jamal et al., 2018; Qian weidong, 2020 ; Chen et al., 2023). A prior retrospective investigation disclosed a substantial correlation between increased mortality in infected patients and CRKP, which had a high propensity to generate biofilms. High doses of antimicrobial medicines are required to eliminate biofilms. However, because of their associated drug toxicity, it is frequently not achievable (Di Tella et al., 2019). Many studies have highlighted the potential of EOs as a natural material to inhibit and eradicate biofilms.

Strong inhibitory effects on CRKP biofilm development were demonstrated by eugenol (Qian weidong, 2020), paeoniflorin (Weidong, 2020), and ursolic acid (Qian et al., 2020), as exhibited by FESEM and CLSM images, as well as the crystal violet staining assay (Chen et al., 2023). Paeoniflorin's minimum inhibitory concentrations (MICs) for *K. pneumoniae* ATCC 43816 strain were 600 μg mL-1, while CRKP-1, CRKP-5, CRKP-12, and CRKP-15 had an MIC of 1200 μg mL-1. Thus, CRKP-12 was adopted in other studies (Weidong, 2020). Ursolic acid (UA) has useful qualities, including its antibacterial, antifungal, and anti-inflammatory capabilities. Apple pomace produces the bioactive chemical compound ursolic acid, which has antibacterial, antifungal, and anti-inflammatory properties. One of the bioactive chemicals obtained from apple pomace has drawn increased attention from researchers (Cargnin & Gnoatto, 2017). The MICs of ursolic acid against *K. pneumoniae* ATCC 43816 strain were 400 μg mL−1 and 800 μg mL−1 for CRKP-1, CRKP-2, CRKP-8, and CRKP-10, respectively (Qian et al., 2020). Certain traditional plants also contain pentacyclic triterpene UA, which has been depicted to have antibacterial, antiprotozoal, anti-inflammatory, and anticancer properties (Cargnin & Gnoatto, 2017).

K. pneumoniae ATCC 700603 has become a highly intense biofilm producer in this investigation. Following this study's results, the inhibitory potency of MDEO against CRKP was equal to the potential of CbBO in inhibiting the formation of biofilm of the standard isolate *K. pneumoniae* ATCC 700603 (MBIC value of 0.25 mg/mL). However, the concentration of CbBO required to eradicate the biofilm formed was twice as high (MBEC value of 0.5 mg/mL). The findings disclosed a concentration-dependent inhibition of CRKP biofilm development by MDEO. Following treatment with MDEO at MIC and 2MIC, the CRKP biofilm absorbance values at 570 nm were lowered by 51% and 60%, respectively, in comparison to the control (Chen et al., 2023). Compared to other compounds, such as eugenol, paeoniflorin, and ursolic acid, CbBO's MBIC and MBEC values against *K. pneumoniae* ATCC 700603 were comparatively lower, indicating a stronger potential for antibacterial and antibiofilm properties. In this analysis, several compounds, including cinnamaldehyde, were discovered in CbBO. The primary component of cinnamon oil, cinnamaldehyde, has been demonstrated to have antibacterial properties and to act on the plasma membrane by blocking the same target as chlorhexidine (Nuryastuti et al., 2009).

The cell envelope of gram-negative bacteria is composed of an outer, inner, and cytoplasmic membrane, with a thin peptidoglycan cell wall sandwiched in between. The outer membrane protects the germs from damage during infection while allowing adequate access to the environment (Chen et al., 2023). The outward secretion of specific compounds is made possible by porins and secretion systems. Numerous cellular processes, including solute transport, metabolic control,

mitochondria-coupled energy transduction mechanisms, and energy state preservation, depend on the permeability barrier provided by the cell membrane. The compounds in EO target the hydrophobic portion of the outer membrane (Chen et al., 2023). The production of exopolysaccharide (cellulose), flagellar development and function, and certain genes are all suppressed by trans-cinnamaldehyde, preventing the formation of biofilms (Amalaradjou et al., 2014). Additionally, trans-cinnamaldehyde has worked well to prevent and inactivate uropathogenic *E. coli* biofilms on polystyrene and latex urinary catheter surfaces, posing a significant threat to the medical community. The potential of cinnamaldehyde and its derivatives to eliminate persisters in *E. coli* was examined by Shen et al. (2017).

Persisters are common in bacteria, fungi, and parasites, causing recurrent infections and treatment-associated relapses (Shen et al., 2017). α-bromocinnamaldehyde had the most activity of all the chemicals tested; at concentrations up to 400N μg/mL, it exhibited a 100% death rate. Conversely, cinnamaldehyde disclosed a comparable 99.8% death rate across the same dosage range. When α-bromocinnamaldehyde was administered at a dose of 100 μg/mL, less than 1% of cells survived. However, all *E.coli* cells were eliminated by α-bromocinnamaldehyde at a dosage of 400 μg/mL, regardless of whether the cells were in the stationary phase or the exponential phase (Doyle & Stephens, 2019). CbBO, containing the highest molecule of cinnamaldehyde with an MBIV value of 0.25 mg/mL and an MBEC value of 0.5 mg/mL, inhibited and eradicated the biofilm of the standard isolate *K. pneumoniae* ATCC 700603.

CONCLUSION

The antibacterial and antibiofilm assay unveiled that CbBO had potential against the standard isolate *K. pneumoniae* ATCC 700603. The antibacterial potential was demonstrated by an MIC of 0.125 mg/mL and MBC of 0.25 mg/mL, while the antibiofilm was exhibited by an MBIC value of 0.25 mg/mL and an MBEC value of 0.5 mg/mL. The antibacterial and antibiofilm activities of CbBO could eradicate the standard isolate *K. pneumoniae* ATCC 700603 if the concentration takes two times their MIC and MBC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this research.

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