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Antimicrobial activity of cinnamon oil against bacteria that cause skin infections

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Abstract: Skin and soft-tissue infections are among the most common infections which may lead to serious local and systemic complications. New antibiotics with activity against resistant gram-positive and gram-negative pathogens are urgently needed (Brook, 2002). Although antimicrobial drugs have greatly reduced the incidence of certain infection, in some parts of the world, mortality rates from infectious diseases are as high as before the arrival of antimicrobial drugs (Talaro et al., 1999). Therefore, there has been a constant increase in the search of alternative and efficient compounds aimed at partial or total replacement of antimicrobial chemical drug (Gupta et al., 2008). Identification of the antimicrobial activity in *Cinnamomum zeylanicum (C. zeylanicum)* bark against bacterial skin infection were studied for the potential uses as alternative remedies in the treatment of skin infections. The cinnamon oil, obtained from distillation process was measured against *Staphylococcus aureus (S. aureus)* (ATCC 25923), *Sterptococcus pyogenes (S. pyogenes)* (ATCC 19615), *Staphylococcus epidermidis* (*S. epidermidis)* (ATCC 12228) and *Pseudomonas aeruginosa (P. aeruginosa)* (ATCC 10145) at three different concentrations (25%, 50% and 100%) using disk diffusion technique. Out of four bacteria tested, three bacteria were found to be sensitive towards cinnamon oil including *S. aureus, S. pyogenes* and *S. epidermidis*. On the other hand, *P. aeruginosa* exhibited high resistance to cinnamon oil. Cinnamon oil showed promising inhibitory activity even in low concentration against *S. pyogenes* with minimal inhibitory concentration (MIC) value of 1.6%. Cinnamon oil showed significant antimicrobial effect against *S. aureus* and *S. epidermidis* with MIC value of 6.3% and 12.5% against both bacteria. Cinnamon oil showed antimicrobial activity against majority of the tested bacteria ($p<0.05$) and it can be a good source of antimicrobial agents against bacteria that causes skin infections.

Key words: Antimicrobial activity; Cinnamomum zeylanicum; Bacteria that causes skin infections

1. Introduction

Skin is a large, complex organ that covers the external surface of the body. The important functions of the skin include control of body temperature, prevention of loss of fluid from the body tissues and the synthesis of vitamin D (Nester et al., 2001). As the skin is the major part of the body that is exposed to the environment, it is frequently subjected to cuts, punctures, burns or chemical injury, as well as hypersensitivity reactions. These injuries provide a way for pathogens to enter and infect the skin and underlying tissues (Nester et al., 2001; Shimeld and Rodgers, 1999). The other line of attack that can cause microbial disease of the skin is microbial toxin-mediated skin damage at the target site body (Mims et al., 2004).

S. aureus and group A β-haemolytic streptococci (*Sterptococcus pyogenes*) are the most common cause of skin diseases and superficial wound infections (Brook, 2002). *S. aureus* may transiently colonize the skin of newborn infants, the skin in 20% to 40% of healthy individuals, and the skin of atopic patients (Chiller et al., 2001). *S. aureus* usually invades the skin through wounds, follicles or skin glands. It causes minor skin infections such as boils or abscesses as well as more serious postoperative wound infections (Mims et al., 2004).

S. pyogenes is acquired through contact with other people with infected skin lesions and may first colonize on normal skin before invasion through in the epithelium (Mims et al., 2004; Shimeld and Rodgers, 1999). Pyogenic infections appearing after local invasion of the skin are called pyoderma or erysipelas. It is caused by *S. pyogenes*. Pyoderma, or streptococcal impetigo, is marked by burning, itching papules that break and form a highly contagious yellow crust (Talaro et al., 1999).

S. epidermidis has little virulence and are normal flora of the skin. However, it is capable of causing serious disease if the host defenses are impaired (Shimeld and Rodgers, 1999). *S. epidermidis* is the common cause of small abscess of little consequence around stitches (Nester et al., 2001). Infection of prosthetic heart valves is often caused by *S. epidermidis*. Infections of other types of prosthetic devices, shunts, dialysis catheters, implants, or grafts are common with *S. epidermidis* (Shimeld and Rodgers, 1999). Therefore, any time an indwelling device or foreign object is introduced, as with

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implants, shunts, or intravascular lines, patients are at high risk for *S. epidermidis* infections (Shimeld and Rodgers, 1999).

P. aeruginosa is an aerobic gram-negative rod that is found in moist environments (Chiller et al., 2001). Due to its resistance to soaps, dyes, quaternary ammonium disinfectants, drugs, drying, and temperature extremes, it is a frequent contaminant of humidifiers, ventilators, intravenous solutions, and anesthesia and resuscitation equipment (Talaro et al., 1999). *P. aeruginosa* commonly causes infections in weakened hosts, especially burn victims and cyctic fibrosis patients (Ingraham et al., 2004). Although infections in immunocompromised hosts are significantly more common and more serious, *P. aeruginosa* can occasionally cause disease in healthy individuals (Mims et al., 2004). Ecthyma gangrenosum, a few painful maculopapular skin lesions, can occur primarily in immunosuppressed patients in the setting of *Pseudomonas* sepsis (Chiller et al., 2001).

For many years, antibiotics were regarded as the miracle cure of all infectious diseases. However, antibiotic drugs by its nature involve contact with foreign chemicals that can harm human tissues (Talaro et al., 1999). One of the problems that exist in the treatment of infectious diseases with the antibiotics is that many of the offending microorganisms are similar in their biology to human. As a result, most substances that are toxic to microorganisms are also toxic to patients (Shimeld and Rodgers, 1999). The major side effects of drugs fall into one of three categories which include direct damage to tissue through toxicity, allergic reactions and disruption in balance of normal microbial flora (Talaro et al., 1999). The other problem in treatment of infectious diseases is the increasing number of bacterial resistance to antimicrobial agents (Shimeld and Rodgers, 1999).

Therefore, there has been a constant increase in the search of alternative and efficient compounds aimed at partial or total replacement of antimicrobial drugs (Gupta et al., 2008). One of the areas which are of considerable interest is plant extract (Smith-Palmer et al., 2004). *C. zeylanicum* is one of the best known spices for thousands of years (Hoffmann and Manning, 2002). *C. zeylanicum* has been used for both as a vapor and in drinks and foods as preservative and for preventing infection (Lis-Balchin, 2006). It is also known as Ceylon cinnamon, true cinnamon, kulit kayu manis, Ceylon-zimtbaum and cannelle de Ceylan (Blumenthal et al., 2000). It belongs to the family Lauraceae and is cultivated in warmer climates worldwide, most notably in the West Indies and East Asia (Hoffmann and Manning, 2002). Moreira et al., 2007 findings suggest that essential oil from *C. zeylanicum* could arise as a promising alternative antimicrobial compounds to be inserted in pharmaceutical formulations that are used to treat mycoses of different clinical severities caused by diatomaceous molds.

In the present study, the antimicrobial activities of cinnamon oil are investigated against bacteria that cause skin infections. This activity is determined by using disk diffusion assay. Panels of bacteria potentially capable of causing skin infections were tested including *S. aureus, S. epidermidis, S. pyogenes and P. aeruginosa*. The sensitivity of the bacteria to the cinnamon oil was determined by zones of inhibition around the disks.

2. Methodology

2.1. Extraction and steam distillation

The extraction of cinnamon oil was performed according to the method developed by Gupta et al. (2008). The cinnamon bark was grounded in a grinding machine (Moulinex) in order to obtain a fine dry powder. 150g of the cinnamon powder was weighed using an analytical balance (Denver M120). 750mL of 50% ethanol (Univar) was soaked in 150g cinnamon powder in a 2L beaker for 48 hours at room temperature. The mixture was then filtered (Whatman No. 54) and the filtrate materials were distillated in a simple distillation apparatus (Toshniwal). Heat was applied to the distillation flask for 4 hours and maintained at temperature 80^{o}C. The recovered material was allowed to settle and the oil was withdrawn by using 50-200µL micropipette.

2.2. Preparation of different concentration of cinnamon extract

Three different concentrations of cinnamon oil, 25%, 50% and 100% were prepared by mixing the cinnamon oil in dimethylsulfoxide (DMSO) (Univar) using vortex mixture. Once the cinnamon oil was dissolved in pure DMSO, this was also sterilized, and thus, a very costly and time-consuming step of membrane filtration sterilization was omitted (Gupta et al., 2008).

2.3. Disk preparations

Under aseptic conditions, sterilized filter paper disks (6mm in diameter) were impregnated with 20µL of 25%, 50% and 100% concentration of cinnamon oil. The impregnated disks were dried in laminar flow for 15 minutes at room temperature. The dried disks can be used afterward for the disk diffusion assay of cinnamon oil against the panels of bacteria.

2.4. Antimicrobial susceptibility test (AST)

Bacteria strain used in this study include *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *S. pyogenes* (ATCC 19615) and *P. aeruginosa* (ATCC 10145). One loop of the stock culture from ATCC was streaked on sheep blood agar (Columbia) and incubated at 37^{o}C for 18 hours. One colony from the

cultured sheep blood agar was taken to perform conformation test. The confirmation tests include catalase, coagulase, oxidase and gram stain. One more colony from the sheep blood agar was inoculated in 5mL brain heart infusion BHI broth (Oxoid) and incubated at 37°C with gently shaking at 60 strokes per minute for 18 hours.

The AST was performed according to the method used by Prabuseenivasan et al., 2008, disk diffusion assay. 0.1 mL of the bacterial inoculum was spread over the plates containing Mueller-Hinton (MH) agar (Oxoid) using a sterile cotton swab to obtain uniform microbial growth on the media. The disks impregnated with different concentrations of extract were placed on the agar surface. Standard disk was used as reference control. These included vancomycin (30µg) for *S. aureus* and *S. epidermidis*, bacitracin (85µg) for *S. pyogenes* and ticarcillin (10µg) for *P. aeruginosa*. DMSO was used as a negative control disc. The plates were left for 30 minutes at room temperature to allow the diffusion of cinnamon oil and incubated at 37°C for 18 hours. After the incubation period, the diameter of zone of inhibition (mm) was measured. Studies were performed in triplicate and the mean value was calculated.

2.5. Minimal inhibitory concentration (MIC)

Of the 4 bacteria tested, only those that showed sensitivity against cinnamon oil were selected for further tests for MIC determination by macrodilution method. The macrodilution broth susceptibility test was performed as described by the National Committee for Clinical Laboratory Standards. 100% concentration of cinnamon oil was serially diluted two fold in 1 mL BHI broth to produce a series of tube containing decreasing concentrations of the extract (100%, 50%, 25%, 12.5%, 6.3%, 3.1%, 1.6% and 0.8%). 1 mL of the inoculum was added into all tubes except the control tubes. Each tube was mixed well and incubated at 37°C for 18 hours. Inhibition of bacterial growth in each tube containing test extract was judged by comparison with growth in control tube containing 100% cinnamon oil, fresh BHI broth and inoculated BHI broth. The MIC was determined as the lowest concentration of extract inhibiting visible growth of each organism on the agar plate.

3. Results

3.1. Disk diffusion assay

The antimicrobial activities of cinnamon oil and control drugs against *S. aureus, S. pyogenes, S. epidermidis* and *P. aeruginosa* are as shown in Table 1. Three different concentrations of the cinnamon oil (25%, 50% and 100%) were tested against panel of bacteria that causes skin infections. *S. aureus, S. pyogenes and S. epidermidis* showed sensitive toward the cinnamon oil whereas *P. aeruginosa* showed resistance towards all concentrations of cinnamon oil tested. The positive control using specific antibiotic showed a clear zone around the antibiotic whereas there was no inhibition zone observed around the negative control (Table 1).

Table 1: Antimicrobial activities of cinnamon oil; Inhibition zones (mm) of cinnamon oil (25%, 50% and 100%) against *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 19615), *S. epidermidis* (ATCC 12228) and *P. aeruginosa* (ATCC 10145) on Mueller-Hinton agar medium and corresponding minimum inhibitory concentration (MIC) values; Each value is the average of three independent replicates [NZ, no zone; ND, not done].

Bacteria	Cinnamon oil			Control drug	
	Inhibition zone (mm)			Drug	Inhibition zone (mm)
	25%	50%	100%		
S. aureus	22.3	23.3	25.3	Vancomycin	19
S. pyogenes	25.3	30.0	36.0	Bacitracin	34
S. epidermidis	16.7	26.7	31.3	Vancomycin	17
P. aeruginosa	NZ	NZ	NZ	Ticarcillin	25

The cinnamon oil extract at 25%, 50% and 100% concentrations were found to inhibit *S. aureus* with clear zone diameters of 22.3mm, 23.3mm and 25.3mm respectively whereas the inhibition zone of vancomycin (positive control) was 19mm. Based on Fig. 1, the result of T test analysis shown that cinnamon oil was significantly inhibited the growth of *S. aureus* at concentration 50% and 100% if compared with positive control with p-value less than 0.05.

For *S. pyogenes*, the inhibition zone diameters were 25.3mm, 30.0mm and 36.0mm respectively whereas the diameter of inhibition zone of bacitracin (positive control) was 34mm. According to Figure 2, cinnamon oil has significant value of antimicrobial effect at concentration 25%, 50% and 100% if compared to each other, as well as the positive control against *S. pyogenes* because the *p*-value were less than 0.05.

At the same concentrations of cinnamon oil that were tested on *S. epidermidis* showed inhibitory zones of 16.7mm, 26.7mm and 31.3mm whereas vancomycin (positive control) showed diameter of inhibition zone of 25mm. According to Figure 3, cinnamon oil at concentration 25%, 50% and 100% were considered significant if compared with each other with *p*-value were less than 0.05. As cinnamon oil at those concentrations had shown significance value, the result of *p*-value was also less than 0.05 if compared with positive control.

The cinnamon oil showed inhibitory effect against the panel of bacteria except *P. aeruginosa* which did not show inhibitory effect to 25%, 50% and 100% concentrations of cinnamon oil.

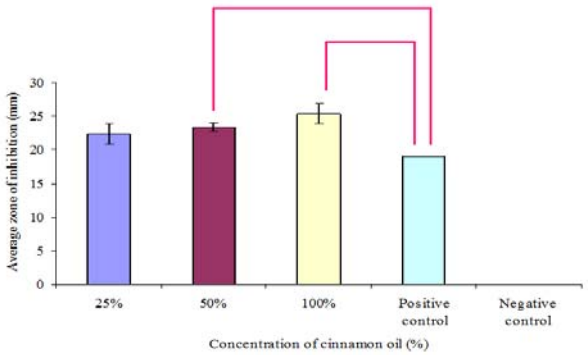

Fig. 1: Bar chart for average zones of inhibition (mm) of cinnamon oil (25%, 50% and 100%) and the controls against *S. aureus* (ATCC 25923) on Muller-Hinton agar medium after 18 hours incubation at 37°C.

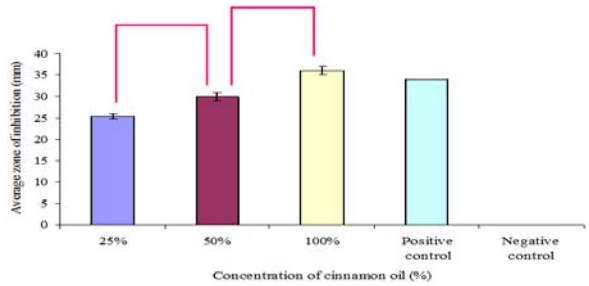

Fig. 2: Bar chart for average zone of inhibitions (mm) of cinnamon oil (25%, 50% and 100%) and the controls against *S. pyogenes* (ATCC 19615) on Muller-Hinton agar medium after 18 hours incubation at 37°C.

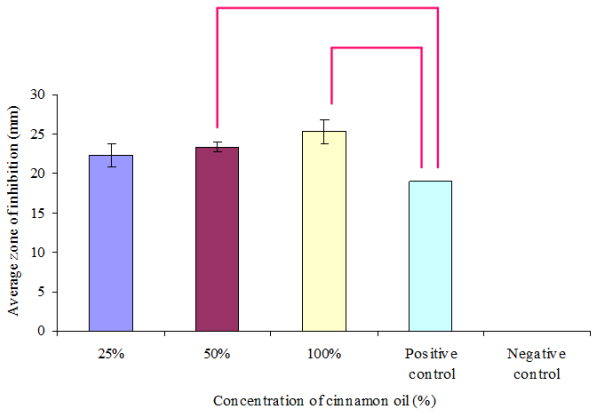

Fig. 3: Bar chart for average zones of inhibition (mm) of cinnamon oil (25%, 50% and 100%) and the controls against *S. epidermidis* (ATCC 12228) on Muller-Hinton agar medium after 18 hours incubation at 37°C.

3.2. Minimum Inhibitory Concentration (MIC)

Table 2 shows MIC values of cinnamon oil against *S. aureus*, *S. pyogenes* and *S. epidermidis*. MIC was performed on the bacteria that showed sensitivity towards the cinnamon oil extract in the disk diffusion assay. The MIC values of cinnamon oil against *S. aureus* and *S. pyogenes* were 6.3% and 1.6%. For *S. epidermidis*, a higher MIC value of 12.5% was observed. The inoculum of *S. aureus*, *S. pyogenes* and *S. epidermidis* that act as the control were turbid whereas the fresh broth and cinnamon oil were clear.

Table 2: The MIC of different concentrations of cinnamon oil against *S. aureus*, *S. pyogenes* and *S. epidermidis* obtained by the macrodilution method. Clear (C) indicates that the bacterial growth was inhibited and turbid (T) indicates that bacterial growth was not inhibited at the particular concentration

Bacteria	Concentration of cinnamon oil (%)							
	0.8	1. 6	3.1	6.3	12.5	25	50	100
S. aureus	T	T	T	C	C	C	C	C
S.pyogenes	T	C	C	C	C	C	C	C
S. epidermidis	T	T	T	T	C	C	C	C

4. Discussion

4.1. Antimicrobial Susceptibility Testing (AST)

There were a number of AST methods available to determine bacterial susceptibility to antimicrobials. Method used in the routine laboratory to test activity of antimicrobials includes agar dilution, macrodilution, microdilution and disk diffusion. In this study, disk diffusion assay and macrodilution assay were used to determined susceptibility of panel of bacteria to antimicrobial agent. The selections of methods were based on many factors such as practicality, ease of performance, availability of chemical reagents and materials, low cost, accuracy, and reliability.

4.2. Disk diffusion assay

Disk diffusion is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The results generated by bacterial in vitro AST were generally interpreted and reported as resistance, intermediate or susceptible to the action of a particular antimicrobial. An organism that is resistance is unlikely to be successfully treated with a given antibiotic (Bannister et al., 2006). In this disk diffusion assay, results were considered accurate and reliable as it follows the principle of standard methodology developed by NCCLS including 4mm thickness of the agar medium, pH range between 7.2 to 7.4 and the inoculum size approximately 1x108 cfu/ml (NCCLS, 2000).

Panel of bacteria tested for antimicrobial activity of cinnamon oil were including *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 19615), *S. epidermidis* (ATCC 12228) and *P. aeruginosa* (ATCC 10145). All tested bacteria shown susceptible to the cinnamon oil except for *P. aeruginosa*. These results were consistent with previous reports done by Abu-Shanab et al. (2004) on cinnamon extract regarding gram-positive and gram negative bacteria. The resistance of gram-negative bacteria (*P. aeruginosa*)

to plant extracts was not unexpected. This class of bacteria is more resistance than gram-positive bacteria (*S. aureus, S. pyogenes* and *S. epidermidis*). The others studies (Prabuseenivasan et al., 2006; Gupta et al., 2008; Hili et al., 1997) had shown that cinnamon extract and oil had strong activity against various food-borne microbes.

AST of 25%, 50% and 100% cinnamon oil against *S. aureus* showed larger zone of inhibition compared to zone of inhibition produced by positive control, vancomycin. Results also had shown that the higher the concentration of cinnamon oil, the larger the diameter of inhibition zone presented. At concentration 50% and 100% cinnamon oil, diameter of inhibition zone were considered significant if compared with positive control with *p*-value less than 0.05. These results suggest that cinnamon oil at concentration 50% and 100% has significance antimicrobial effect over positive control against *S. aureus*.

The diameter of inhibition zone of cinnamon oil at 100% concentration against *S. pyogenes* was larger than the positive control, bacitracin. However, cinnamon oil has significant value of antimicrobial effect at concentration 25%, 50% and 100% as well as the positive control against *S. pyogenes*. Therefore, it proved that cinnamon oil has significance value of antimicrobial effect against *S. pyogenes*.

Cinnamon oil was found to be effective against S. *epidermidis*. The cinnamon oil showed a higher and a stronger antimicrobial activity than vancomycin (positive control) at concentration 50% and 100%. Cinnamon oil at concentration 25%, 50% and 100% were considered significant if compared with each other with *p*-value were less than 0.05. As cinnamon oil at those concentrations had shown significance value, the result of *p*-value was also less than 0.05 if compared with positive control. This result suggested that cinnamon oil has significant antimicrobial effect against *S. epidermidis.*

Among the tested bacteria, *P. aeruginosa* proved to be most resistant to all different concentrations of cinnamon oil. This was due to no inhibition zone appeared around the disk impregnated with known volume and concentration of cinnamon oil. *P. aeruginosa* was an opportunistic pathogen which can grow in a variety of aqueous solutions, even in distilled water (Nester et al., 2001). The bacterium was widespread easily in the environment (Nester et al., 2001) and because of their hardy nature and low nutritional requirements, they was resistance not only to drugs, but also to soaps, dyes, quaternary ammonium disinfectants, drying, and temperature extremes (Shimeld and Rodgers, 1999; Talaro et al., 1999). Infections caused by *P. aeruginosa,* especially those with multi-drug resistance, are among the most difficult to treat with conventional antibiotics (Abu-Shanab et al., 2004). Shimeld and Rodgers (1999) stated that *P. aeruginosa* was never being treated with a single antimicrobial agent because the success rate was very low.

4.3. Minimum inhibitory concentration (MIC)

The diameter of zone of inhibition in disk diffusion assay around the antimicrobial disk is related to MIC for that particular bacterium and antimicrobial combination. Based on the result of disk diffusion assay and MIC obtained, the zone of inhibition correlates inversely with the MIC of the tested bacterium. Generally, the larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organisms.

In this study, due to the disk diffusion assay, *S. pyogenes* was found to be the most susceptible bacterium to the cinnamon oil with the diameter of zones of inhibition were 25.3mm, 30mm and 36mm at the concentration 25%, 50% and 100% cinnamon oil. The result of MIC showed that cinnamon oil was found to be most effective with the lowest MIC and MBC value of 1.6% against *S. pyogenes*.

The second most susceptible bacterium to the cinnamon oil if due to disk diffusion assay was *S. epidermidis* with the diameter of zones of inhibition were 16.7mm, 26.7mm and 31.3mm at concentration 25%, 50% and 100% cinnamon oil. The MIC value of cinnamon oil against *S. epidermidis* was 12.5% whereby the MIC value against *S. aureus* was 6.3%.

5. Conclusions

Based on the results obtained, cinnamon oil extract possesses an antibacterial activity against *S. aureus, S. pyogenes* and *S. epidermidis*. This study demonstrated that the essential oil from barks of *C. zeylanicum* has excellent antimicrobial activities against bacterial skin infection. It also showed that cinnamon oil was found to be a better antimicrobial agent, exhibiting broad range of antimicrobial activity against bacterial skin infection than the common chemical antibiotic used. Therefore, it has the potential to be used for medical purposes and to be utilized as antimicrobial of skin infections. However, the cinnamon extract did not possess antimicrobial effect against *P. aeruginosa*. Study of the other plant extracts as the antimicrobial agent for *P. aeruginosa* is required to inhibit the growth of this most common bacterial burn and wound infections on skin.

In this study, cinnamon oil had shown to possess antimicrobial effect against *S. aureus, S. pyogenes* and *S. epidermidis*. These *in vitro* studies of the antimicrobial activity of cinnamon oil extract need to be continued with the in vivo study such as level and rate of absorption, diffusion into the skin and tissue, metabolism, excretion and the possible toxicity and effect on the normal flora need to be performed before this natural plant extract can be commercially used as the alternative treatment regimen.

Some limitation had been found in this study. This study was lack of purification of the extraction compound, cinnamaldehyde. Purification of tested compound can be done through the application of high performance liquid chromatography (HPLC).

The other limitation in this study was the limitation of equipment used. Equipment used in the extraction of cinnamon oil was using the traditional equipment, simple distillation apparatus. More advanced equipment especially those that involve in the extraction process should be used. Besides that, because of no carbon dioxide incubator in the laboratory of this study, the AST of cinnamon oil against *Propionibacterium acnes*, bacteria that commonly causes acne cannot be done.

In conclusion, the objective of this study had been achieved as the antimicrobial activity of cinnamon oil extracted from the bark of *C. zeylanicum* against bacteria that causes skin infections had been identified and the results had been obtained. Cinnamon oil was proved to have antimicrobial effect for *S. aureus*, *S. pyogenes* and *S. epidermidis.*

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