

# Antimicrobial Activity and Synergic Effect of *Cinnamomum burmannii*'s Essential Oil & its isolated compound (Cinnamaldehyde)

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**Abstract**—*Cinnamomum burmannii* is one of the species in *Cinnamomum* genus and belongs to the Lauraceae family. It has a common name called Indonesian Cassia. The aims of the present study were to investigate the antimicrobial activity of *C. burmannii*'s essential oil and its major compound, cinnamaldehyde against selected microorganisms. This study also aimed to study the synergic effect of *C. burmannii*'s essential oil as well as cinnamaldehyde upon combining with the current antibiotics. Steam distillation was used for extraction of the essential oil. The isolation of active compound from the essential oil was conducted using column chromatography and the characterization of the compound was analyzed using spectroscopic techniques. The antimicrobial assay was done using micro-dilution methods against three microorganisms in triplicate. The present study was able to clarify the crucial role of cinnamaldehyde as a potent antimicrobial compound of the *C. burmannii*'s essential oil.

**Keywords**—Antimicrobial, Cinnamaldehyde, Essential oil, Synergic effect.

## I. INTRODUCTION

MULTIPLE drug resistance has occurred due to the indiscriminate use of commercial antimicrobial drugs in treatment of infectious disease [1]. In addition to this issue, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [2]. The use of antimicrobial agents in combination are currently captured the research interest in order to overcome the widespread of infectious diseases caused by the multi-drug resistant organisms (MDROs).

It is well known that medicinal and aromatic plants containing active compounds are able to inhibit microbial growth and act as antimicrobial agents. The bioactivities of plant oils have demonstrated great influences in the field of

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pharmaceuticals as an alternative medicine and natural therapies [3]. *C. burmannii* was primarily distributed in Asia and Australia. Traditionally, it was used to treat flatulence, nausea [4], colic, dyspepsia, diarrhea, as well as colds, influenza, fevers, arthritis, and rheumatism [5]. Cinnamons are rich in essential oils which mainly consist of cinnamaldehyde as its bioactive compounds. A numbers of studies on the cinnamons antimicrobial activity had been documented previously but very little reported on their interaction effects upon combination with antibiotics.

Hence, the present study was designed to extract the essential oil of *C. burmannii*'s bark and isolated its major compound, cinnamaldehyde. Both of the essential oil and cinnamaldehyde were subjected to the antimicrobial assay as well as interaction effect upon combining each of them with the current antibiotics.

## II. METHODOLOGY

### A. Sample Preparation

*C. burmannii*'s barks were collected from Bukit tinggi, West Sumatra province, Indonesia on March 2012. The bark was then cut into pieces and ground to produce coarse powder using a grinder. Ground samples had been kept in closed container and stored at room temperature until further used.

### B. Steam Distillation

Essential oil from the ground sample of *C. burmannii*'s barks was extracted using steam distillation technique. Liquid-liquid extraction was done for the collected distillate to separate the essential oil from water by adding dichloromethane to the mixture of water and essential oils in a separating funnel with a ratio of 1:3. The essential oil was collected and dried over anhydrous sodium sulfate to remove any traces of water. The extracted essential oil was stored at 4 °C until further use [6].

### C. Column Chromatography

Column chromatography was used to isolate cinnamaldehyde from the essential oil. Silica gel with 230-400 mesh (Merck, Germany) had been used as a stationary phase. A solvent system of 10% ethyl acetate in *n*-hexane was

used to fractionate the sample efficiently.

#### D. Compound identification

Isolated compound was identified by Fourier Transform Infrared Spectroscopy (FTIR) analysis. Nuclear Magnetic Resonance Spectroscopy ( $^1\text{H}$  NMR &  $^{13}\text{C}$  NMR) was also done using Bruker 500 MHz NMR spectroscopy, in  $\text{CD}_3\text{OD}$ .

#### E. Media Preparation

Muller-Hinton Agar (MHA) and 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 prepared. All of the prepared media were sterilized at  $121\text{ }^\circ\text{C}$  for 15 minutes, using an autoclave. After finish, the freshly prepared and cooled agar medium was poured into flat-bottomed petri dishes at approximately 4 mm and allowed to solidify at room temperature. Finally, both of the agar plates and broth were stored at  $4\text{ }^\circ\text{C}$ .

#### F. Preparation of Antibiotic Stock

Antibiotics were purchased in a powders form. The powders were accurately weighed and dissolved in methanol to yield the required concentration. Antibiotics stock solutions were prepared using the formula:  $(1000/P) \times V \times C = W$ , where P = potency of the antibiotic, V = volume in mL required, C = final concentration of solution and W = weight of the antimicrobial to be dissolved in V [7].

#### G. Microbial Strains

Three reference strains of human pathogens were used in this study which included one Gram positive (*Staphylococcus aureus* ATCC 25923), one Gram negative (*Escherichia coli* ATCC 8739) and one fungal strains (*Candida albicans* IMRC 533/11A).

#### H. 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, respectively [7]. The bacteria was incubated at  $37\text{ }^\circ\text{C}$  for 24 hours while for fungus strain, incubation was done at room temperature ( $25\text{--}27\text{ }^\circ\text{C}$ ) for 48 hours. The optical densities (OD) of the incubated bacterial and fungal inoculums were measured using spectrophotometer. The bacteria and fungus turbidity were measured at 600 and 494 nm, respectively [8].

#### I. Minimum Inhibitory Concentration (MIC) Assay

The MIC of essential oil as well cinnamaldehyde was determined by the broth micro-dilution method [9]. 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  $10^7$  and  $10^4$  CFU/mL of bacteria and fungi,

respectively. After that, 30  $\mu\text{L}$  of the 10 mg/mL stock of essential oil and cinnamaldehyde were transferred into the inoculated well (270  $\mu\text{L}$ ). Three-fold serial dilution was performed by transferring 100  $\mu\text{L}$  from the highest concentration of treated culture into the next well so that the final volume of each well was 200  $\mu\text{L}$ . Finally, all of the tested plates were incubated. The standard antibiotics were tested in the same manner as the stated steps. The concentration that gave zero optical growth was considered as MIC. All of the tests were done in triplicates.

#### J. Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) assay

Treated cultures containing concentrations equal to and higher than the MIC value were transferred (100  $\mu\text{L}$ ) and 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.

#### K. Fractional Inhibitory Concentration (FIC) assay

Interactions upon the combination of natural antimicrobials agents (drug A) with antibiotics (drug B) against bacteria or fungus strain were evaluated using broth micro-dilution method [10]. The essential oil and cinnamaldehyde stock were prepared and tested in combination with each of the antibiotics [11]. The antibiotics used in this test were ampicillin, chloramphenicol and streptomycin for each of the bacteria strain or nystatin and amphotericin B for the fungus strain. The combination of treated strain was serially diluted by three-fold and tested in triplicates. After the incubation period, the MIC value of agent in combination was determined and compared with the MIC value of agent alone.

The distinction between additive, synergy, antagonism and indifference effect were evaluated by Fractional Inhibitory Concentrations (FIC) and sum of the FIC index: Synergy (FIC index  $\leq 0.5$ ), additive ( $0.5 < \text{FIC index} \leq 1$ ), indifference ( $1 < \text{FIC index} \leq 2$ ) and antagonism (FIC index  $> 2$ ) (Mackay et al., 2000). The calculations [10], [12] were as followed:

FIC of drug A = MIC of drug A in combination/MIC of drug A alone;

FIC of drug B = MIC of drug B in combination/MIC of drug B alone;

FIC index = FIC of drug A + FIC of drug B

### III. RESULTS

*Trans*-cinnamaldehyde was identified based on the following evidences: IR (neat) max  $\text{cm}^{-1}$ : 688.75, 748.48, 973.21, 1626.06, 1676.46, 2742.74, 2815.18;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz): 7.41-7.43 (m, 2H), 7.68-7.71 (m, 2H), 7.43 (dd, 1H), 7.47 (d, 1H, J=16), 6.78 (dd, 1H, J=4.3 and 16 Hz), 9.67 (d, 1H, J=4.3);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz):

134.4, 130.0, 128.6, 131.0, 153.7, 128.8, 194.7.

Table I showed the analysis result of MIC values obtained for essential oil and cinnamaldehyde against *E. coli*, *S. aureus* and *C. albicans*. The MIC values of antibiotics used for selected strains such as ampicillin, chloramphenicol, streptomycin, amphotericin B and nystatin were also recorded. Both of the essential oil and cinnamaldehyde possessed the same MIC value against all of the tested strains at 333µg/mL.

MBC values were obtained upon the observation of no growth on the incubated plates. Essential oil as well as cinnamaldehyde had the same MBC value which was 1000 µg/mL against both of *E. coli* and *S. aureus*. However, lower concentration of MBC value (333µg/mL) was obtained when cinnamaldehyde was tested against *C. albicans*. The result of MBC values was shown in the Table II.

Essential oil and cinnamaldehyde were found to have indifference effect upon combination with all the tested antibiotics (ampicillin, chloramphenicol, streptomycin) against *E. coli* and *S. aureus* with the FIC index between 1 and 2. Their combination with nystatin as well amphotericin B against *C. albicans* was also found to give indifference effect with the FIC index of 1.55 and 1.35, respectively. Table III, Table IV, Table V and Table VI summarized the MIC values of each agent upon combination and the calculated FIC index.

TABLE I  
MINIMUM INHIBITORY CONCENTRATION RESULT

Microorganisms	Minimum Inhibitory Concentration (MIC) value (µg/mL)						
	Oil	Cin	A	C	S	AB	N
<i>E. coli</i>	333	333	18.5	0.7	0.7	-	-
<i>S. aureus</i>	333	333	18.5	0.7	0.7	-	-
<i>C. albicans</i>	333	333	-	-	-	0.2	6.2

Cin = Cinnamaldehyde, A = Ampicillin, C = Chloramphenicol, S = Streptomycin, AB = Amphotericin B, N = Nystatin, - = not determined.

TABLE II  
MINIMUM BACTERICIDAL CONCENTRATION RESULT

Microorganisms	Minimum Bactericidal Concentration (MBC) value (µg/mL)						
	Oil	Cin	A	C	S	AB	N
<i>E. coli</i>	1000	1000	>500	>500	500	-	-
<i>S. aureus</i>	1000	1000	>500	>500	500	-	-
<i>C. albicans</i>	1000	333	-	-	-	6.2	18.5

Cin = Cinnamaldehyde, A = Ampicillin, C = Chloramphenicol, S = Streptomycin, AB = Amphotericin B, N = Nystatin, - = not determined.

TABLE III  
FIC INDEX OF ESSENTIAL OIL AGAINST BACTERIA

Combined agents (µg/mL)	<i>E. coli</i>			<i>S. aureus</i>			Evaluation
	MIC	MIC	FIC	MIC	MIC	FIC	
	A	B		A	B		
EO + A	55.5	27.8	1.67	55.5	27.8	1.67	Indifference
EO + C	2	1.05	1.51	2	1.05	1.51	Indifference
EO + S	2	1.05	1.51	2	1.05	1.51	Indifference

EO = Essential Oil, A = Ampicillin, C = Chloramphenicol, S = Streptomycin, MIC A = MIC of sample, MIC B = MIC of antibiotic.

TABLE IV  
FIC INDEX OF ESSENTIAL OIL AGAINST FUNGUS

Combined agents (µg/mL)	<i>C. albicans</i>			Evaluation
	MIC	MIC	FIC	
	A	B		
EO + AB	0.37	0.27	1.35	Indifference
EO + N	18.5	9.25	1.55	Indifference

EO = Essential Oil, AB = Amphotericin B, N = Nystatin, MIC A = MIC of sample, MIC B = MIC of antibiotic.

TABLE V  
FIC INDEX OF CINNAMALDEHYDE AGAINST BACTERIA

Combined agents (µg/mL)	<i>E. coli</i>			<i>S. aureus</i>			Evaluation
	MIC	MIC	FIC	MIC	MIC	FIC	
	A	B		A	B		
Cin + A	55.5	27.8	1.67	55.5	27.8	1.67	Indifference
Cin + C	2	1.05	1.51	2	1.05	1.51	Indifference
Cin + S	2	1.05	1.51	2	1.05	1.51	Indifference

Cin = Cinnamaldehyde, A = Ampicillin, C = Chloramphenicol, S = Streptomycin, MIC A = MIC of sample, MIC B = MIC of antibiotic.

TABLE VI  
FIC INDEX OF CINNAMALDEHYDE AGAINST FUNGUS

Combined agents (µg/mL)	<i>C. albicans</i>			Evaluation
	MIC	MIC	FIC	
	A	B		
Cin + AB	0.53	0.27	1.35	Indifference
Cin + N	18.5	9.25	1.55	Indifference

Cin = Cinnamaldehyde, AB = Amphotericin B, N = Nystatin, MIC A = MIC of sample, MIC B = MIC of antibiotic.

#### IV. DISCUSSION

Based on the result obtained, the MIC values of essential oil and cinnamaldehyde against the bacteria and fungus strain were similar at 333 µg/ mL which meant that at this concentration, the optical growth of microorganism were inhibited or lack of visual turbidity was observed as compared to the control.

The MBC test had establish a value of 1000 µg/ mL as the MBC value for the essential oil against *E. coli*, *S. aureus* and *C. albicans*. However, for cinnamaldehyde, lower concentration was seen against *C. albicans* at 333 µg/ mL as compared to *E. coli* and *S. aureus* at 1000 µg/ mL. The MBC value of cinnamaldehyde against *C. albicans* was found to be similar with its MIC value. This observation suggested that cinnamaldehyde might act as a fungicidal agent to this microorganism.

The combination therapy of the *C. burmannii*'s essential oil as well as cinnamaldehyde was observed to give indifference effect in combination with the ampicillin, chloramphenicol, streptomycin, amphotericin B and nystatin against all of the tested microorganisms. This finding might imply that the use of cinnamaldehyde alone was already effective in inhibiting the growth of microorganisms tested which might be an alternative way of combating the emergence of antibiotics resistance [13]. Purified compounds of essential oils had been reported to inhibit a great variety of microorganisms [14]. It was clear that the antimicrobial

activity of the crude essential oil was attributed to the cinnamaldehyde as its sole potent compound.

#### V.CONCLUSION

The potential of cinnamaldehyde to be an effective antibiotic was discovered. The problem of antibiotics resistance and their harmful side-effect can be resolved by the use of bioactive compound in the essential oil. Future study may be needed in order to postulate the mechanism of cinnamaldehyde against the microorganisms.

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