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A Comparative Study on Characteristic and Antimicrobial Activity of Cinnamon (*C. Zeylanicum*) Oil Small-Sized Emulsion By Phase Inversion Composition and Phase Inversion Temperature Methods

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Abstract: Cinnamomum zeylanicum (C. zeylanicum) is species of cinnamon, which a native plant to Sri Lanka, India and Madagascar that consist of a variety of biologically active compounds that makes C. zeylanicum able to act as an antimicrobial agent against both Gram-negative and Gram-positive bacteria. The ability of C. zeylanicum to act as antimicrobial agent could make it as a natural preservative in food products. However, the cinnamon oil that extracted from C. zeylanicum is in a lipophilic compound which are insoluble in water and hard to be applied directly to the food products as preservatives. These problems may be overcome by formulating essential oils into small-sized emulsions to increase solubility and stability of droplets. This study will help to produce small-sized emulsions from cinnamon oil as natural preservative in food products by using phase inversion composition (PIC) and phase inversion temperature (PIT) methods. Different formulation ratios between cinnamon essential oil to grape seed carrier oil were used. The antibacterial activity of small-sized emulsions was evaluated using minimum inhibitory concentration (MIC) and minimum inhibitory zone (MIZ) assays against Bacillus cereus (B. cereus), Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus). Small-sized emulsions produced by PIT are smaller than small-sized emulsions by PIC with 2.84 μm average droplet size. All sample mixtures of cinnamon oil with grape seed oil were found to be more acidic in the range of 4.23 to 4.78. Turbidity of small-sized emulsions produced by PIT method have a lower absorbance value than small-sized emulsions produced by PIC which could promote a better stability of emulsions. The antibacterial study found that all sample mixture small-sized emulsions of cinnamon oil able to inhibit B. cereus, E. coli and S. aureus at minimum concentration 12.50 %, 6.25 % and 12.50 % respectively. Development of inhibition zone against B. cereus, E. coli and S. aureus were found with 0.70 cm,

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0.95 cm and 0.80 cm respectively. Therefore, application of high concentration of surfactant and high temperature does give impact to the size and uniformity of emulsions and its ability in inhibit bacteria. The small-sized emulsions produced by PIC and PIT methods of cinnamon oil with combination grape seed oil is proven to be an alternative treatment with antibacterial activity and also can act as an antibacterial agent in preservation of foodsk.

Keywords: Antibacterial activity, Cinnamon zeylanicum, Minimum Inhibitory Concentration,

1. Introduction

Cinnamomum zeylanicum (C. zeylanicum) is species of cinnamon, which a native plant to Sri Lanka, India and Madagascar. Cinnamon is a plant with multiple uses, including as food spices, flavouring, and medicinal sources due to its aromatic fragrance, sweet, and flavourful properties. Phytochemical studies proved that *C. zeylanicum* consist of a variety of biologically active chemicals [1]. The extraction of leaves and barks contain major compound of trans-cinnamaldehyde, eugenol, camphor, trans-cinnamyl acetate, and β-caryophyllene [2]. However, studies also show the presence of coumarin in *Cinnamomum* species which found to be hazardous as it leads to liver [3]. Nonetheless, among the species, *C. zeylanicum* has the lowest level of coumarin compared to the others [4]. In addition, one of the biologically active chemicals, trans-cinnamaldehyde is a compound that makes *C. zeylanicum* able to act as an antimicrobial agent against both Gram-negative and Gram-positive bacteria [2].

In recent years, demands for natural compounds as food preservatives increase to fight against food-borne pathogens and extend shelf life of product [5]. Natural food preservatives can be obtained from plants such as cinnamon oil from *Cinnamomum*. Cinnamon oil is a lipophilic compound which is insoluble in most food products to act as natural preservatives [6]. However, emulsification methods could be applied by mixing the cinnamon oil with aqueous, surfactant and co-surfactant to create high solubility small-sized emulsions in food products [5]. Before evolution of small-sized emulsions, coarse emulsions of natural preservatives have been applied in food industry as preservatives to inhibit growth of pathogens in food products [7]. Coarse emulsions known as thermodynamically stable dispersion system, but not kinetically stable colloidal dispersion system over time which could contribute to droplet instability and further physical instability occurrence such as creaming, sedimentation, globule flocculation and Ostwald ripening [8]. These problems may be overcome by formulating essential oils into smaller size emulsions. Smaller size emulsions have high solubility and stability of droplets than microemulsions due to the smaller size of droplets which causes Brownian movement controls gravitational forces avoiding droplets from deforming and become physically instable [6,8].

Objectives for this study are to extract cinnamon *(C. zeylanicum)* oil by Soxhlet extraction using ethanol and generate small-sized emulsions with 1:1, 1:2, and 1:3 oil to surfactant ratios by using phase inversion composition (PIC) and phase inversion temperature (PIT) methods. Determination on size of droplets of emulsions was measured and the physicochemical characteristic of small-sized emulsions examined. Furthermore, the antimicrobial activity of cinnamon oil small-sized emulsions also evaluated by using minimum inhibitory concentration (MIC) and minimum inhibitory zone (MIZ) on *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli.*

2. Literature Review

2.1 Cinnamon and Benefits

Cinnamon is one of aromatic herb plants locally grown around Asia countries including Southeast Asia, China and Australia. Cinnamon or the origin name called *Cinnamomum* has diverse species based

on their botanical characteristics and there are several main species of *Cinnamomum.* Cinnamon is a herb species that has the specialty for many applications because it consists of bioactive compounds such as cinnamaldehyde, eugenol, cinnamic acid and cinnamate [9]. Different parts of cinnamon such as barks, leaves, root bark, fruit, buds and flowers contain different types of compounds [10]. The presence of those compounds make cinnamon used as agents in antimicrobial, antioxidant, anti-inflammatory, and antidiabetic [2]. Numerous studies of *C. zeylanicum* have been done for *in-vitro* and *in-vivo* research such as cinnamaldehyde for antimicrobial purposes where *C. zeylanicum* act as antimicrobial agent [11]. The inhibition of bacterial growth is done with action of cinnamaldehyde compounds by disturbing the cell membrane of bacteria to promote cell death [10].

2.2 Formation of Small-Sized Emulsions

Emulsions is small-sized particle which consist a mixture of two immiscible liquid which classified as oil-in-water (O/W) and water-in-oil (W/O). Small-sized emulsions are unstable from thermodynamic aspect, but it is stable system in term of kinetic aspects. The stability of an emulsion is mainly depending on the size of droplets and the uniformity of particle sizes. The particle size of small-sized emulsions ranging from 100 μm to 10 μm [12]. Small-sized emulsions can be produced in two ways, using high energy methods and low energy methods [13]. In previous study, small-sized emulsions of cinnamon oil prepared by high-pressure homogenization have been used to preserve vacuumed ground beef. It is proven that the addition of cinnamon oil small-sized emulsions can inhibit the growth of bacteria, lipid oxidation and decreasing the pH of fresh ground beef during storage compared to the control sample [14].

Phase inversion composition (PIC) method is also known as emulsion inversion point. This method involves constant temperature but altering the composition of water in the emulsion system. Water is added to dilute the mixture to form intended structures and introduce a kinetically stable system in small-sized emulsions. Water is added until system reach critical water concentration and inversion of phase occurs from water-in-oil (W/O) to oil-in-water (O/W). While for phase inversion temperature (PIT) method requires the elevation of temperature above critical temperature of a system. The rises of temperature in emulsification cause the non-ionic surfactants to be more hydrophilic and having changes on their chemical configuration, where the curvature of surfactant turns into different structure resulting a small-sized emulsion. Small-sized emulsification are done with uses of surfactant in its procedure because surfactant plays an important role in the formation of small-sized emulsions [12]. Among the small molecule surfactant, Tween-80 are mostly used in emulsifications because of its excellent solubility for essential oils and water due to having both hydrophilic and hydrophobic characteristic. Besides, a study also proved the small-sized emulsification of essential oil affected by type and concentration of the surfactant used using the phase inversion temperature method (PIT). The results of good delivery system are based on the solubility of non-ionic surfactant over temperature [15].

2.3 Assessment on Antimicrobial Activity

Antimicrobial activity is an activity that stops or inhibits the growth of microorganism at a certain place. Minimum inhibitory concentration (MIC) and minimum inhibitory zone (MIZ) tests are employed in this study to determine the antimicrobial activity of small-sized emulsions of cinnamon oil. MIC assay is a method used to determine the lowest possible concentration of an antimicrobial agent in inhibition of bacteria. This method is carried out by letting the antimicrobial agent incubated overnight with bacteria. The diluted cinnamon oil and bacterial suspension were added into 96 well plates and incubated at 37 °C for 18-24 hours [11]. Indicator such resazurin blue dye solution is added to indicate the growth of bacteria after incubation. MIZ assay or agar disc diffusion assay is another assay that determines antimicrobial activity by the diameter of inhibition of an antimicrobial agent. It is done by placing discs infused with potential antibacterial agent on top of agar and swabbed bacterial suspension [11]. The performance of cinnamon oil in their studies was compared with a positive control

in inhibiting bacteria. Three bacteria are involved in this study. Gram-positive bacteria and gram-negative bacteria of food-borne pathogens used. *Bacillus cereus* and *Staphylococcus aureus* are a gram-positive bacterium, while *Escherichia coli* is a gram-negative bacterium. All these three bacteria responsible for a minority of foodborne illnesses causing severe nausea, vomiting, diarrhoea and can be found in dairy and food products.

3. Methodology

3.1 Material and Equipment

Cinnamon plants kindly provided by Nasuha Herbs Plantation, 95% ethanol (HmBG, United Kingdom), Grape seed oil (Biobasic, Canada), Tween 80 (Biobasic, Canada), Nutrient agar (Merck, Germany), Nutrient broth (Merck, Germany), Escherichia coli (ATCC 43888, US), Staphylococcus aureus (ATCC 25923, US), and Bacillus cereus (ATCC 10876, US). Micropipette (Eppendorf, Germany), Microbiological Incubator (Fisher Scientific, US), Microplate Reader (Fisher Scientific, US), Soxhlet Extractor (Buchi, UK), Rotary Evaporator (Buchi, UK), Vacuum Drying Oven (Daihan Labtech Co. Ltd, Korea), Analytical Grinder (Polymix, Malaysia), Inverted Microscope (Olympus, Japan), 96-Well Plates (Eppendorf, Germany), DR6000 UV-Vis Spectrophotometer (Labomed,Inc., Germany), Turbidity Meter (Cole-Parmer, India), pH Meter (Hanna Instruments H13220-02, England), Laboratory Magnetic Stirrer Hot Plate (Fisher Scientific, US), Conductivity Meter (Hanna Instruments, US).

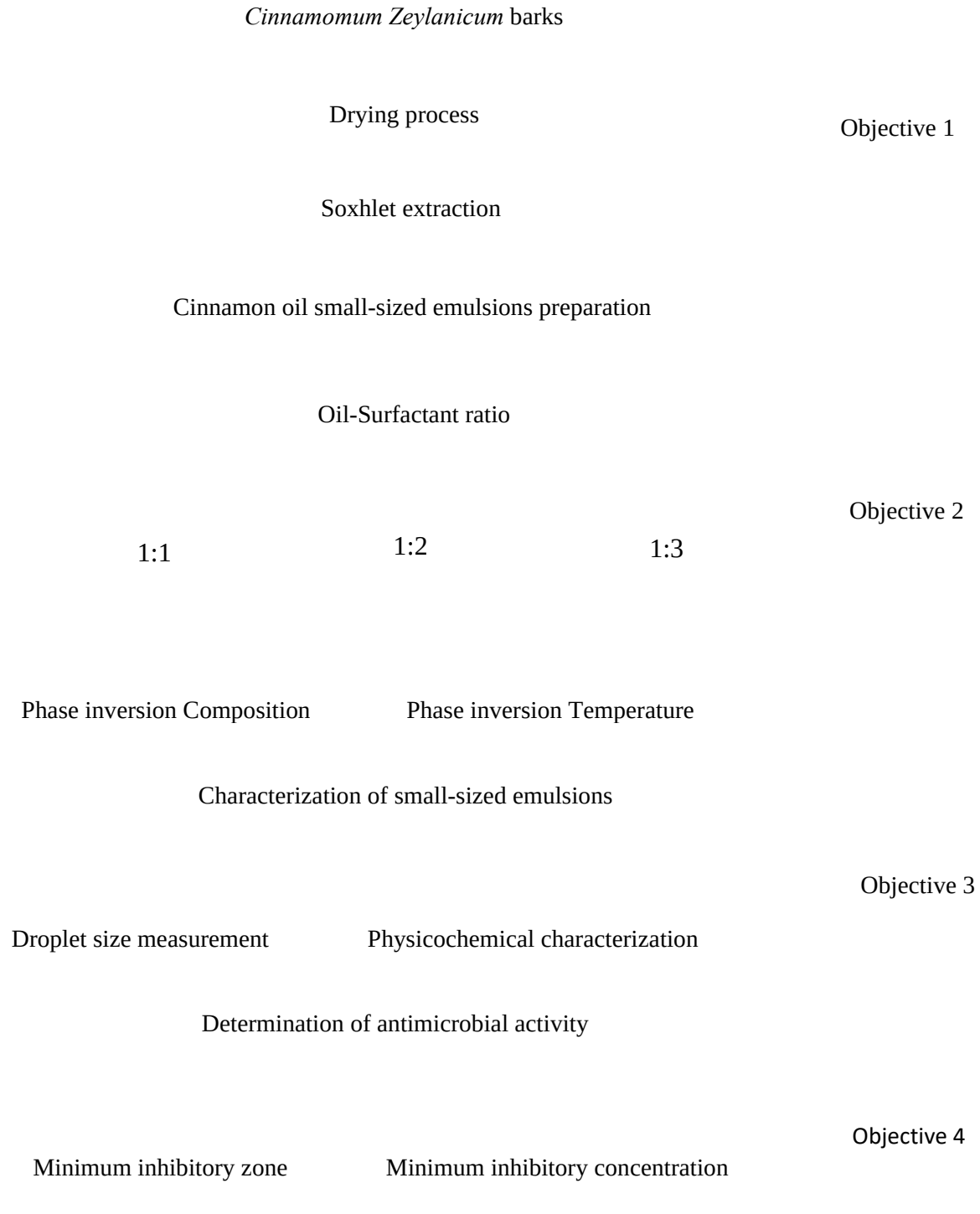

Figure 1: Flowchart of experimental design

3.2 Soxhlet extraction

About 20 g of cinnamon barks and leaves powder were weighed and placed into the extraction thimble-holder and 200 ml of fresh solvent of 95.0 % ethanol was filled gradually into the round bottom flask. The cinnamon leaves and barks powder were extracted at 78 °C and left for about 6 hours until siphon tube appeared colourless [16]. After the extraction, the products were collected and purified using vacuum filter pump and rotary evaporator at temperature of 34 °C. After that, the samples were left under fume hood for one hour to make sure all the ethanol left in the oil crude was completely vaporized to the environment.

3.3 Small-sized emulsions preparation

3.3.1 Phase inversion composition (PIC) method

The samples were prepared by using different formulation ratios of cinnamon essential oil to grape seed carrier oil that was shown in Table 3.1 for phase inversion composition method. These ratios were selected based on study conducted by [17].

Table 1: Composition of Different Cinnamon Oil small-sized emulsions For Phase Inversion Composition

Formulation Code	Oil: Surfactant Ratio (V/V)	Percent Composition of Different Component in Formulations			
		Oil		Surfactant	Water
		Cinnamon	Grape Seed		
PIC1	1:1	4	6	10	130
PIC2	1:2	4	6	20	120
PIC2	1:3	4	6	30	110

PIC method involves titrating the aqueous phase into the organic phase at constant temperature. Organic phase consists of oil phase of cinnamon oil and grape seed oil as carrier oil and Tween 80 as surfactant. Oil phase and surfactant were mixed with different surfactant-oil-ratio (SOR). The SOR were 1:1, 1:2, and 1:3 by increasing the surfactant volume. The organic phase was prepared by mixing the cinnamon oil with carrier oil and surfactant for 30 minutes at 750 rpm agitation speed. Then, the aqueous phase was added by titration using burette into the organic phase to produce small-sized emulsions with flow rate of 4 mL/min while continuous stirring at 750 rpm for 60 minutes under room temperature.

3.3.2 Phase inversion temperature (PIT) method

The samples were prepared by using different formulation ratios of cinnamon essential oil to grape seed carrier oil that was shown in Table 1 for phase inversion temperature method. These ratios were selected based on study conducted by [18].

Table 2: Composition of Different Cinnamon Oil Small-sized Emulsions for Phase Inversion Temperature

Formulation Code	Oil: Surfactant Ratio (V/V)	Percent Composition of Different Component in Formulations			
		Oil		Surfactant	Water
		Cinnamon	Grape Seed		
PIT1	1:1	4	6	10	80
PIT2	1:2	4	6	20	70
PIT2	1:3	4	6	30	60

Using this method, the phase inversion temperature of a system was be obtained before forming small-sized emulsions by measuring the electrical conductivity of the system while being heated. From the conductivity measurements, a graph of temperature versus conductivity is plotted. Once the PIT is known, the system will be heated up to the PIT value to form the small-sized emulsions. The organic phase was mixed with different oil-to-surfactant ratio shown in Table 2 for 3 minutes, then aqueous phase was added. Then, the mixture was mixed for 30 minutes with magnetic stirrer. After mixing step, the mixture was heated up to PIT value. Once it reaches the PIT value, the mixture is rapidly cooled from PIT value to room temperature to form the small-sized emulsions by partially immersed the beaker into ice with continuous stirring [18].

3.4 Characterization of emulsions

The characteristics are based on physical and chemical characteristic of the small-sized emulsions. A few tests were run to characterize the small-sized emulsions in terms of droplet size measurement, pH and turbidity.

3.4.1 Droplet size measurement

The droplet size of cinnamon oil emulsions was determined using Inverted Microscope at 20X magnification. The small-sized emulsions were placed on the glass slide and observed through ImageJ software by selecting 20 random droplets. (n=2)

3.4.2 pH measurement

The pH cinnamon oil small-sized emulsions were tested by using a pH meter. The readings were taken in duplicate for confirmation.

3.4.3 Turbidity measurement

The cinnamon oil small-sized emulsions sample were placed in quart cuvette and placed in UV-visible spectrophotometer at 600 nm wavelength. The turbidity was obtained by the optical density readings read by the UV-visible spectrophotometer and distilled water is used as reading reference [13].

3.5 Antibacterial assay

The tests used in this study are minimum inhibitory concentration and minimum inhibitory zone. These tests methods are further explained in section 3.5.1-3.5.3.

3.5.1 Preparation of bacterial culture and agar preparation

Three strains of bacteria *Bacillus cereus (B. cereus*), *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli (E. coli)* were kindly provided from Microbiology laboratory, Faculty of Applied Science and Technology (FAST), UTHM. The strains were cultured in Nutrient broth and incubated for 24 hours at 37 ºC for determination of antibacterial activity. 1.6 g of Nutrient broth powder was suspended in 200 mL distilled water and 6.9 g of Nutrient agar powder was mixed with 300 mL of distilled water. Both Nutrient broth and agar autoclaved at 121 ºC for 15 minutes for the sterilization purpose.

3.5.2 Assessment of Minimum inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the samples against three selected bacteria was determined with the microdilution method. 100 µL of samples diluted in two-fold dilution were added to each well that contains 100 µL of bacteria inoculums were added into each well except for column 12 which is the broth sterility control well. The 96-well plates were then incubated at 35 ˚C for 18 hours. After incubation, resazurin blue dye was added and incubated for another 2 hours. The observation of colour changes was made, and absorbance was recorded using microplate reader at 390 nm wavelength [19].

3.5.3 Assessment of Minimum Inhibitory Zone (MIZ)

400 µL of cultures of bacteria were poured and spread evenly on the Nutrient agar plate using glass spreader. In aseptic condition, sterilized filter paper with diameter of 6 mm was prepared and impregnated with 50 µL of samples and antibiotic were placed on the agar surface. Procedure above must be completed in 15 minutes time to prevent contamination occurs. Agar plates were incubated for 24 hours at 37 ºC. Inhibitory zones of the control strain were examined by measuring the diameters (mm) with a calliper [19].

3.6 Statistical Analysis

Data were analyzed using repeated measures one-way or two-way ANOVA using Microsoft Excel. Data are expressed as mean ± S.E.M and a P value < 0.05 was considered statistically significant.

4. Results and Discussions

4.1 Determination of droplet size

All samples were observed under Inverted microscope with 20X magnification by measurement of 20 random droplets. The sizes of droplets were compared between emulsions produced by PIC and PIT methods with different surfactant to oil ratio.

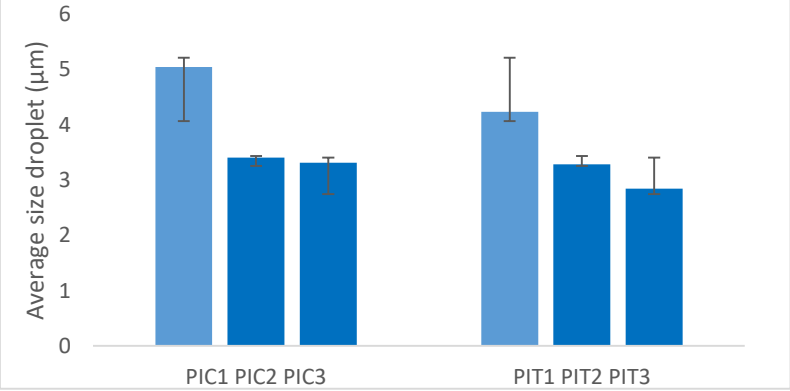

Figure 2: Effect of three different SOR which are 1:1, 1:2 and 1:3, for phase inversion composition method and stated as PIC1, PIC2 and PIC3, respectively. The difference in SOR are 1:1, 1:2 and 1:3 for phase inversion temperature method and stated as PIT1, PIT2 and PIT3, respectively. Values are mean ± S.E.M (n=2)

Figure 2 shows that when surfactant concentration increased, the size of droplets decreased for both methods. The droplet diameter sequences produced by phase inversion composition (PIC) and phase inversion temperature (PIT) methods are PIC1> PIC2> PIC3 and PIT1> PIT2> PIT3 respectively. The size of droplets decreased from 5.05 μm to 3.31 μm for PIC method and 4.23 μm to 2.84 μm for PIT method when concentration of surfactant increases because the surfactant molecule that helps formation of emulsions between oil and water molecule by surrounding the emulsion droplet [12]. However, comparison between size of droplets from PIC and PIT method shows that the application of heat on the coarse emulsions did give impact to smaller size emulsions. The average droplet size shows that PIC produces larger droplet size compared to emulsions from PIT method. PIT applies temperature up to the inversion temperature of the coarse emulsions where the structure of emulsions tends to change from oil in water (O/W) to water in oil (W/O) emulsions [12]. The inversion of emulsion structure happened due to dehydration of surfactant molecule during the heating process because surfactant tends to be lipophilic when exposed to high temperature. The exposure of very low temperature after the heating process does make rapid inversion of emulsions from water in oil (W/O) back to oil in water (O/W) emulsions in smaller size droplet because the surfactant was hydrated rapidly due to rapid cooling process [20]. Therefore, it is suggested to use PIT method in producing small-sized emulsions.

4.2 Analysis of pH

The pH value of each small-sized emulsions produced by PIC and PIT methods were determined by using pH meter. The comparison between different concentration of surfactant used is shown in Figure 3 below.

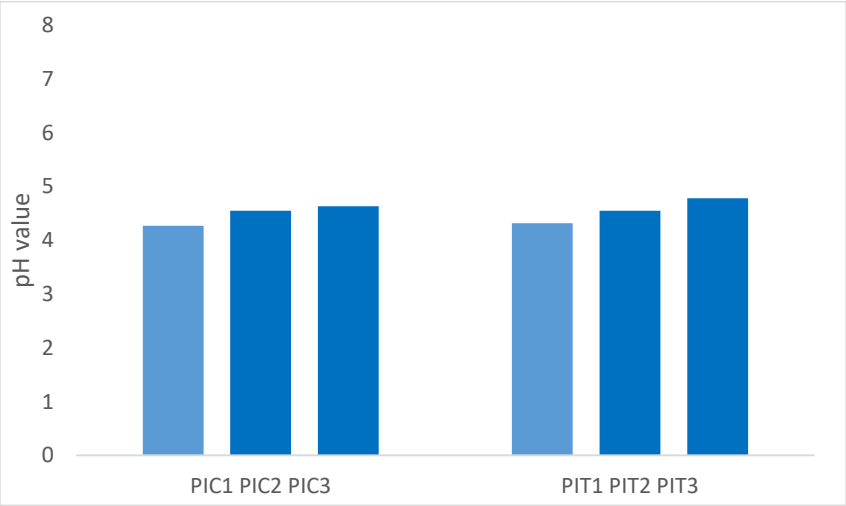

Figure 3: Comparison of pH between sample. Values are mean ± S.E.M. (n=2). Samples of three different SOR which are 1:1, 1:2 and 1:3, for phase inversion composition method and stated as PIC1, PIC2 and PIC3, respectively. The difference in SOR are 1:1, 1:2 and 1:3 for phase inversion temperature method and stated as PIT1, PIT2 and PIT3, respectively

There were no significant differences of pH of emulsions from both methods. The pH ranging from 4.23 – 4.78. The emulsions from PIC method consist of pH values from 4.23 to 4.63. While for small-sized emulsions from PIT shows pH, values range from 4.29 to 4.78. Both methods show acidic range of small-sized emulsions. The uses of different concentration of surfactant does give small effect on the pH of the emulsions. The trend of pH was increasing when the surfactant concentration was increased from 1:1 to 1:2 and 1:3. A previous study also shown that increasing in surfactant does increases the pH of emulsions [21]. This is because higher concentration of surfactant does helps to stabilize the electrostatic repulsion between particles surfactant molecules by making less adsorption of H^+ ions and increase the negative charge on the small-sized emulsions droplet surface for more stable small-sized emulsions [22].

4.3 Analysis of turbidity

Comparison on turbidity between samples with different surfactant ratios are shown in Figure 4 below.

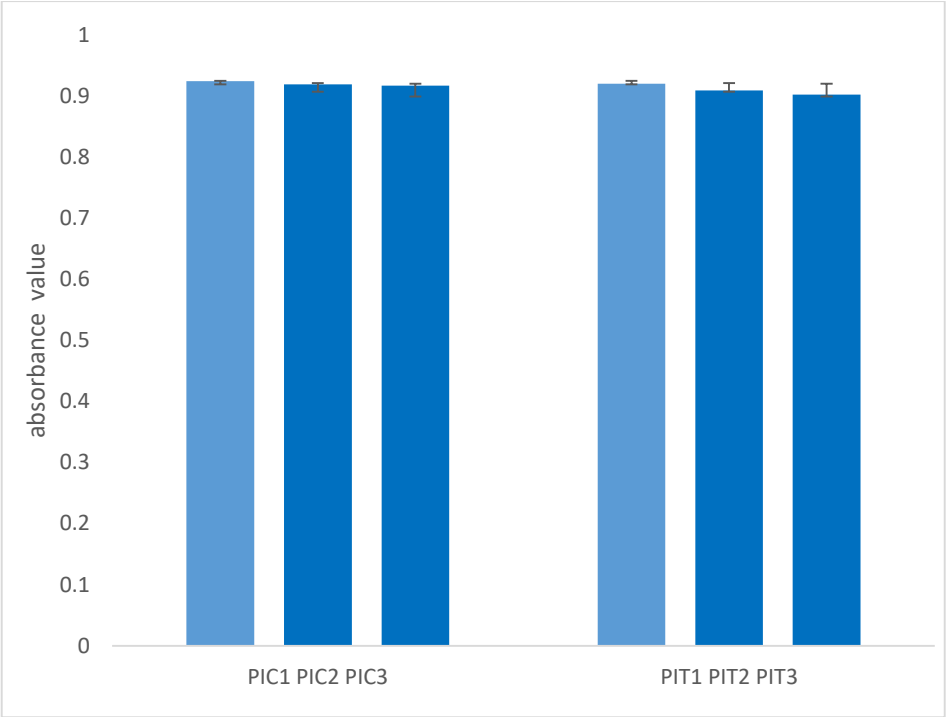

Figure 4: Comparison of turbidity between samples. Values are mean ± S.E.M. (n=2). Samples of three different SOR which are 1:1, 1:2 and 1:3, for phase inversion composition method and stated as PIC1, PIC2 and PIC3, respectively. The difference in SOR are 1:1, 1:2 and 1:3 for phase inversion temperature method and stated as PIT1, PIT2 and PIT3, respectively

Figure 4 shows no significant difference in absorbance value when surfactant concentration was increased in both methods. Hence, uses of different concentration of surfactant does not affect the turbidity of emulsions. However, comparison between PIC and PIT methods showed in Figure 4 prove that emulsions produced by PIT method have a slightly lower turbidity than samples subjected to PIC. Low absorbance value means the solution has a low turbidity. Based on the average size droplets, PIT method produces smaller size of emulsions than PIC method and emulsions should be transparent or translucent due to fine emulsions. Hence, small-sized emulsions of PIT method have lower turbidity value than emulsions of PIC due to higher amount formation of smaller particles of oil in water (O/W) inside the emulsions [23].

4.4 Antimicrobial activity

4.4.1 Minimum inhibitory concentration (MIC)

The absorbance of control sample was measured and used as reference for comparison of absorbance measurement of cinnamon oil small-sized emulsions. Positive control used was Tetracycline, a well-known antibiotic, water as negative control and nutrient broth was used as blank sample as shown in Table 3 The absorbance value for all samples against all three bacteria has been normalized by subtracting the value with the absorbance of water in order to obtain the actual value of diluted samples against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus.* The value of absorbance at 100 % emulsion concentration was set as reference to compare with other serial dilution in inhibiting bacterial growth.

Table 3: The absorbance value of Tetracycline, water and nutrient broth after incubation with *Escherichia coli, Staphylococcus aureus,* and *Bacillus cereus.* (n=2)

Sample	Absorbance value of *Bacillus cereus* + sample	Absorbance value of *Escherichia coli* + sample	Absorbance value of *Staphylococcus aureus* + sample
Tetracycline (Positive control)	3.565	3.475	3.662
Water (Negative control)	1.371	1.321	1.555
Nutrient broth (Blank sample)	0.074	0.066	0.065

a) *Bacillus cereus*

Figure 5: Percentage of absorbance measured by microplate reader at 390 nm. Samples are mean ± SEM (n= 2). PIC1, PIC2 and PIC3 represent different SOR used which are 1:1, 1:2 and 1:3, respectively by PIC method. PIT1, PIT2 and PIT3 represent different SOR used which are 1:1, 1:2 and 1:3, respectively by PIT method

From Figure 5 the minimum concentration of emulsions of cinnamon oil needed to inhibit *Bacillus cereus* is 12.5 % concentration. Maximum concentration of emulsions was used as reference with 100.0 % absorbance value to compare the value percentage of absorption for the dilution series. The absorbance of cinnamon oil small-sized emulsions was increased from 12.5 % to 100.0 % of concentrations where the colour of resazurin stay blue while pink colour was observed at 3.1 % and 6.2 % of concentration. This indicates that the inhibition of bacteria starts from 12.5 % of concentration. *Bacillus cereus* is a positive-gram bacterium which able to be unviable easily even at medium concentration of cinnamon oil due to lack of peptidoglycan layer on the outer part of cell [22]. There was no significant differences seen in the capability of all samples in inhibiting *B. cereus*.

b) *Escherichia coli*

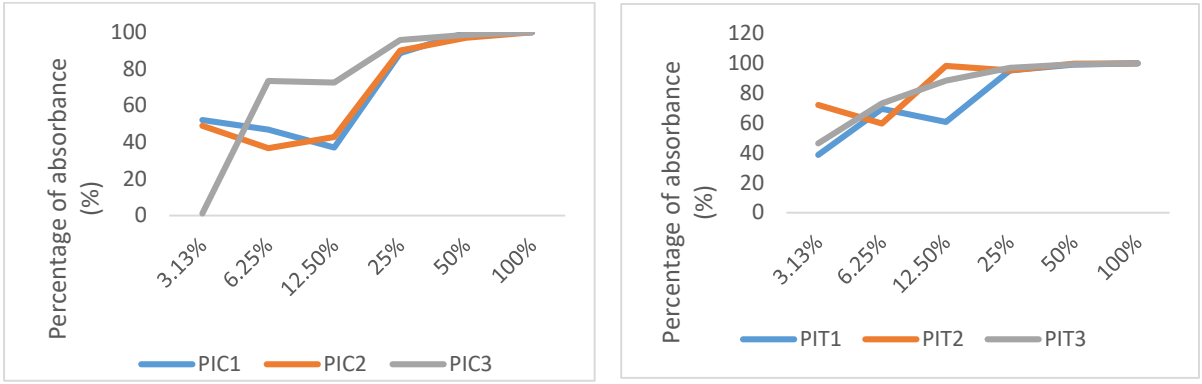

Figure 6: Percentage of absorbance measured by microplate reader at 390 nm. Samples are mean ± SEM (n= 2). PIC1, PIC2 and PIC3 represent different SOR used which are 1:1, 1:2 and 1:3, respectively by PIC method. PIT1, PIT2 and PIT3 represent different SOR used which are 1:1, 1:2 and 1:3, respectively by PIT method

Figure 6 shows the cinnamon oil small-sized emulsions produced by PIC and PIT method able to inhibit *Escherichia coli* with minimum concentration at 6.2 %. This is because there is no reduction of resazurin from blue to pink colour at 6.2 % concentration which indicate there is no presence of bacteria inside the well [25]. The pink colour was observed in well of 3.1 % concentration of cinnamon oil small-sized emulsions was due to failure inhibition of bacteria at very low concentration. Increased concentration of surfactant in small-sized emulsions of cinnamon oil should be able to inhibit the growth of bacteria better than low concentration of surfactant due to decreased size of emulsions.

c) *Staphylococcus aureus*

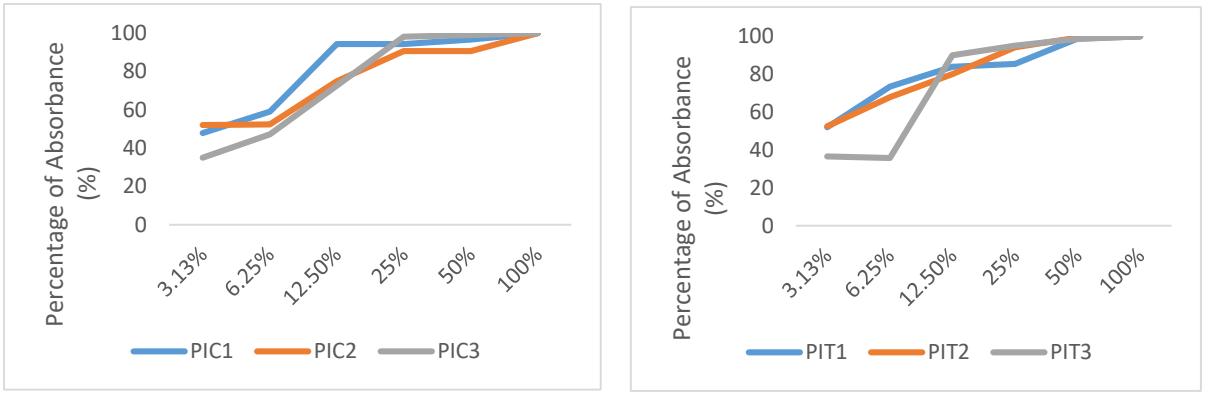

Figure 7: Percentage of absorbance measured by microplate reader at 390 nm. Samples are mean ± SEM (n= 2). PIC1, PIC2 and PIC3 represent different surfactant oil ratios used which are 1:1, 1:2 and 1:3, respectively by phase inversion composition method. PIT1, PIT2 and PIT3 represent different surfactant

From Figure 7, it is shown that absorbance value of small-sized emulsions of cinnamon oil increased when the concentration of cinnamon oil small-sized emulsions decreased. The lowest concentration of small-sized emulsions of cinnamon oil needed to inhibit growth of *Staphylococcus aureus* was 12.5 % for both PIC and PIT method. The inhibition of bacteria starts from 12.5 % to 100.0 % of concentration. At 6.2 % - 3.1 % concentration, the absorbance values were decreased due to pink colour product from reduction of resazurin blue dye. Cinnamon oil small-sized emulsions able to inhibit *Staphylococcus aureus* with lowest 12.5 % concentration due to the presence of cinnamaldehyde as antibacterial compound in cinnamon oil [22]. Tetracycline able to inhibit 100.0 % bacteria, thus it is used as reference.

4.2.2 Minimum inhibitory zone

The effects of the smaller size emulsions of cinnamon oil on inhibiting bacterial growth were tested. The bacteria chosen were *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*. All tested bacteria are susceptible to the cinnamon oil small-sized emulsions by showing inhibition zone.

a) *Bacillus cereus*

Tests were done on the positive control sample and negative control sample and being compared with all samples from PIC and PIT method.

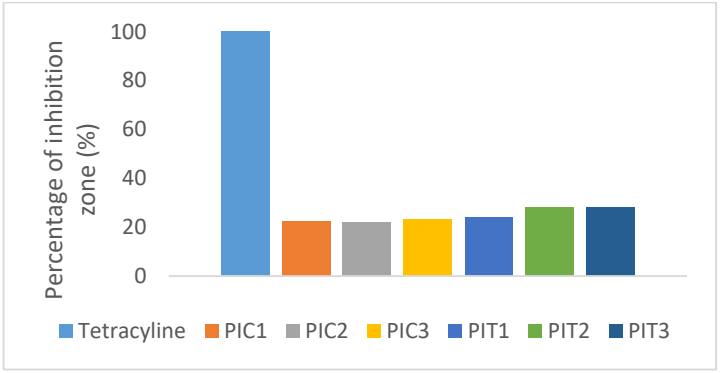

Figure 8: The comparison of percentage of inhibition zone between Tetracycline with all samples in inhibiting *B. cereus*. All percentage of inhibition zone values of each sample have been normalized by subtraction of negative control inhibition zone value. Value of samples are mean ± SEM. (n= 2)

Figure 8 shows the comparison of inhibition zone of *B. cereus* growth between Tetracycline and all samples produced by PIC and PIT method. It is shown that the highest inhibition zone between all samples is PIT2 and PIT3 as both share the same value. However, there is huge significant difference between ability of PIT2, PIT3 and Tetracycline in inhibiting *B. cereus*. Tetracycline shows 100.0 % inhibition zone while PIT2 and PIT3 needs four times volumes to have same effect on *B. cereus* growth inhibition. The inhibition zone by phase inversion composition (PIC) method shows increasing inhibition diameter when higher amount of surfactant concentration used in production of small size emulsions of cinnamon oil. Sample of PIC3 inhibit *Bacillus cereus* at average 0.58 cm which is higher than inhibition zone of PIC1 and PIC2. While for smaller size emulsions of cinnamon oil produced by PIT method shows highest inhibition with samples from PIT2 and PIT3. This shows that increasing the concentration of surfactant will give impact to the antimicrobial activity of small-sized emulsions [23].

b) *Escherichia coli*

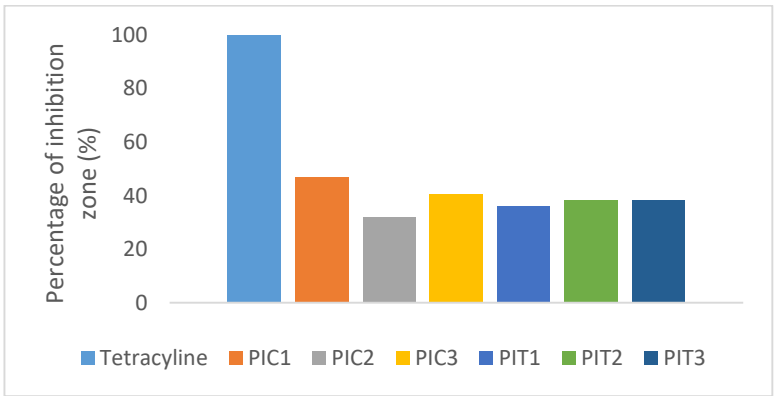

Figure 9: The comparison of percentage of inhibition zone between Tetracycline with all samples in inhibiting *E. coli*. All percentage of inhibition zone values of each sample have been normalized by subtraction of negative control inhibition zone value. Value of samples are mean ± SEM. (n= 2)

Figure 9 shows comparison of inhibition zone of *E. coli* growth between Tetracycline and all samples produced by PIC and PIT method. It is shown that the highest inhibition zone between all samples is PIC1. It is shows that using lowest concentration of surfactant able to inhibit with largest zone compared to other concentrations. Therefore, it is suggested to use lowest concentration (1:1 SOR) to inhibit growth of *E. coli* due to lower cost involved but with better effects in inhibit bacteria.

c) *Staphylococcus aureus*

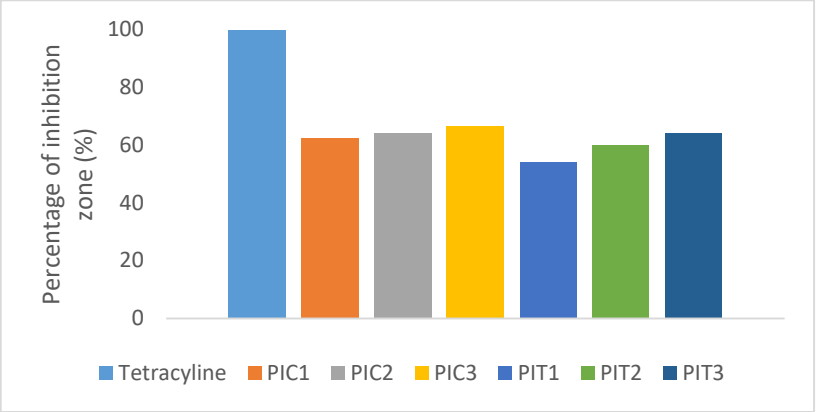

Figure 10: The comparison of percentage of inhibition zone between Tetracycline with all samples in inhibiting *S. aureus*. All percentage of inhibition zone values of each sample have been normalized by subtraction of negative control inhibition zone value. Value of samples are mean ± SEM. (n= 2)

Figure 10 shows comparison of effect in inhibit growth of *S. aureus* between Tetracycline and all samples produced by PIC and PIT method. It is shown that the highest inhibition zone between all samples is PIC3. However, there are huge significant difference between ability of PIC3 and Tetracycline in inhibiting *S. aureus*. Tetracycline shows 100.0 % inhibition zone while PIC3 needs double volumes to have same effect on *S. aureus* growth inhibition.

5. Conclusions

Low energy method could be used in producing small-sized emulsions of cinnamon oil. Application of high concentration of surfactant does give impact to the size and uniformity of emulsions The small sized emulsions of cinnamon oil with combination grape seed oil is proven to be an alternative treatment with antibacterial activity and also can act as an early intervention as natural preservative in food products. Antibiotics that are chemically synthesized can be replaced with high bioavailability as natural antibiotics with reduced risk of consumption.

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