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Effects of extraction conditions on yield, total phenolic contents and antibacterial activity of methanolic *Cinnamomum zeylanicum* Blume leaves extract

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Abstract

Increase of foodborne diseases has promulgated the development of new natural food additive with high extraction yield to eliminate food pathogenic organisms. One such possibility is the use of plant product as antibacterial agents with non-conventional method to enhance the yield. In this study, cinnamon leaves (*Cinnamomum zeylanicum* Blume) were subjected to ultrasonic assisted extraction (UAE) using response surface methodology (RSM) to optimise extraction yield and total phenolic contents. The effect of two independent factors, extraction temperature (x1: 25-40°C) and extraction time (x2: 15-45 minutes) were investigated. Optimum extraction yield and total phenolic contents of cinnamon leaves were 27.49 ± 1.59% and 3987 ± 79.10 mg GAE/g which were closely as predicted using RSM (28.34%, 4048 mg GAE/g), respectively. The optimum condition of extraction yield (40°C and 45 minutes) showed the maximum zone of inhibition against all tested foodborne pathogens (7.33 ± 0.50 to 13.22 ± 0.44 mm), whereas optimum condition of total phenolic contents (33°C and 31 minutes) showed the lowest zone inhibition (6.78 ± 0.67 mm to 11.67 ± 1.41 mm). The minimal inhibitory concentration (MIC) values range from 97.65 to 6250.00 µg/mL and minimal bactericidal concentration (MBC) values from 6.25 to 50.00 mg/mL. These results indicated that UAE method is excellent in producing significantly the highest of extraction yield, total phenolic contents and act as a potential natural antibacterial agent even using low extraction temperature and short time.

Introduction

Cinnamomum zeylanicum Blume (Lauracae) is a tropical tree which often grown as bush and has thick bark or branches (Thomas and Kuruvilla, 2012). C. zeylanicum is also known as *Cinnamomum verum* (Swetha *et al*., 2014) normally grows in Sri Lanka, Madagascar, India and Indochina (Simitzis *et al*., 2014). The *C. zeylanicum* extract has shown the presence of various precious chemical constituent such as flavonoid, alkaloids, steroids, tannins, saponins and phenol compounds which beneficial as flavouring, antimicrobial and antioxidant agents. Previous studies showed that bark and leaves of *C. verum* contained high phenolic content (2708.7 µg GAE/g) and has ability to inhibit the growth microorganisms such as *Escherichia coli, Staphylococcus albus, Staphylococcus aureus, Listeria monocytogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Salmonella* Typhimurium and Saccharomyces cerevisiae (Maidment *et al*., 2006; Prasad *et al.*, 2009; Unlu *et al*., 2010; Mith *et al.*, 2014; Saleem *et al*., 2015).

Traditional extraction methods such as maceration and Soxhlet are very time-consuming and require large quantities of solvents. Nevertheless, various extraction methods have been developed recently to enhance the extraction processes. Ultrasonic assisted extraction (UAE) is one of the non-conventional extraction methods which use cavitation phenomena and mass transfer enhancement to disturb the cell wall. Therefore, this approach has increased the extraction efficiency, reducing production cost and give high purity of the plant extract (Chemat *et al.*, 2011). Moreover, UAE can enhance the extraction processes such as dried clove bud (Alexandru *et al*., 2013), anthocyanin from blackberry (Ivanovic *et al*., 2014) and phenolic contents and yield from *Pistacia atlantica* subsp. (Rezaie *et al*., 2015). In fact, it is

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useful for developing, improving and optimising the mathematical modelling such as response surface methodology (RSM) during extraction process in all part of plant extracts to increase the extraction efficiency. RSM is a mathematical modelling which has advantage over the classical methods to obtain more information from a small number of experiments and possibility to evaluate the interaction effect between different variables on the response (Cui *et al.*, 2011). Thus, RSM was employed to optimise the extraction conditions including extraction time and extraction temperature on the extraction yield and total phenolic content. The leaves were further extracted using optimum extraction conditions from UAE. The antibacterial activity; diameter of inhibition zones, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the optimum extraction conditions were also determined against selected foodborne pathogens. The extraction yield, total phenolic contents and antibacterial activities were compared with Soxhlet methods.

Materials and Methods

Plant materials

Cinnamomum zeylanicum Blume (Voucher number: SK 2517/14) leaves were obtained from Laman Herba Maeps Mardi, Serdang Selangor. The leaves were identified by plant botanist and deposited at Phytomedicine Herbarium of Institute of Bioscience, Universiti Putra Malaysia. Then, the leaves were dried for 24 hours in an oven at 40°C and ground into fine powdered (400 µm) form with a grinder (Wenqiang *et al*., 2007; Saleem *et al*., 2015). The leaves powders were sealed in an opaque plastic packaging prior to analysis.

Bacterial strains

Foodborne bacteria such as *E. coli* ATCC 11229, *P. aeruginosa* ATCC 15692, *K. pneumonia* ATCC 13773, *S.* Typhimurium ATCC 13311, *L. monocytogenes* ATCC 19111, *S. aureus* ATCC 29213, *B. cereus* ATCC 10876 and *B. subtilis* ATCC 11774 were obtained from American Type Culture Collection (Rockville, Maryland, United States) at Lab of Natural Products (Microbiology Laboratory), Institute of Bioscience, Universiti Putra Malaysia. These foodborne bacteria were stored at 4°C on nutrient agar slant, sub-cultured every two weeks and used for further microbial analyses.

Preparation of C. zeylanicum *leaves extract by ultrasonic assisted extraction (UAE)*

The 25 g of cinnamon powder was weighed precisely and extracted with 250 mL of methanol using ultrasonic bath system. Methanol was used as a solvent and purchased from System (Malaysia). The ultrasound extraction parameters such as extraction temperature (25 to 40°C) and time (15 minutes to 45 minutes) were optimised using RSM. The extracts were filtered with Whatman No. 1 (Whatman International Ltd., Middlesex, England). Then, concentrated under vacuum using rotary evaporator (Buchi, Flawil, Switzerland) at 50°C and increased to 85°C for 30 seconds two times with 150 rpm to yield methanol-free extract (Zainin *et al.*, 2013). The extracts were sealed in the opaque glass bottle prior to analysis. The UAE method was compared Soxhlet in terms of extraction yield, total phenolic contents and bacteria inhibition.

The extraction yields were determined as below (Tahmouzi, 2014):

$$\text{Extraction yield (\%)} = \frac{\text{The weight of extract (g)}}{\text{The weight of cinnamon leaves powder (g)}}$$

Preparation of C. zeylanicum *leaves extract by Soxhlet extraction*

Soxhlet extraction is a conventional extraction method and it was applied in this study to compare with UAE methods. 5 g of cinnamon leaves powder with particle size of 400 µm was extracted with 200 mL of methanol for 8 hours at 64.7°C. Later, the extracts were removed by rotary vacuum evaporator and kept at 4°C (Zhao and Zhang, 2014). The extracts were sealed in the opaque glass bottle prior to analysis.

Determination of total phenolic contents

The total phenolic contents of crude extracts from cinnamon leaves were determined by Folin-Ciocalteu methods. 0.2 mL of 1 mg/mL crude extracts were mixed with 0.5 M Folin-Ciocalteu. The mixtures were shaken for 6 minutes. Next, 75 g/L sodium carbonate anhydrous were added and shaken for 30 seconds. After 2 hours, the absorbances were measured at 760 nm (Yang and Chuang, 2012). Gallic acid (0.05 to 0.25 mg/mL) was used for calibration of a standard curve. The calibration curve showed the linear regression at $R^2 > 0.9736$. The equation for the gallic acid calibration curve was $Y = 0.1204X + 0.1572$, where Y was absorbance reading, and X was gallic acid equivalents (GAE) in milligrams of GAE per gram of dried extract. The results were expressed as gallic acid equivalents (GAE) in milligrams of GAE per gram of dried extract. In this study, the effect of extraction condition on total phenolic contents and bacteria inhibition were studied.

Disc-diffusion assay

Antibacterial activities of C. zeylenicum leaves were determined by disc-diffusion method according to Clinical and Laboratory Standard Institute (CLSI, 2012) standard. A sterile paper disc (6 mm) was impregnated with 10 µl of 100 mg/mL extract; UAE extract (optimum conditions from extraction yield/ total phenolic contents) and Soxhlet extract. The extracts were dissolved in 10% dimethyl sulfoxide (DMSO). 10% DMSO did not kill bacteria that being tested in this study. Discs impregnated with 0.1% of chlorhexidine were used as positive control and DMSO was used as negative control. The plates were incubated at 37°C for 24 hours and observed any clear zone surrounding. The test was performed in triplicate to verify the results and antibacterial activity was expressed as the mean inhibition diameter (mm) produced. This assay was done for three times with three replicates (n = 3 x 3).

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

The MIC and MBC were studied in order to determine the effect of extraction conditions (temperature and time) on antibacterial activities. The extracts from optimum UAE were compared with Soxhlet method in term of MICs and MBCs. The determination of MIC and MBC were done according to methods recommended by CLSI (2012). The MICs and MBCs of C. zeylanicum conducted against eight foodborne pathogens in 96-well microtiter plate using two fold standard broth microdilution method with an inoculum of approximately 10^7 - 10^8 CFU/ mL which was compared with 0.5 Mc Farland. A 100µL of 100 mg/mL *C. zeylanicum* extracts stock solution were mixed and diluted two-folds with test organisms in Mueller-Hinton broth (MHB). Column 12 of the microtiter plates contained the highest concentration of the extract, while column 3 contained the lowest concentration. Column 2 served as positive growth control for all samples (only MHB and inoculum). Meanwhile, column 1 acted as a negative control (only 200 µL MHB, no inoculum and antibacterial agent). The microtiter plates were incubated aerobically at 37°C for 24 hours. The MIC was defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of microorganisms after overnight incubation while the MBC as the lowest concentration of antimicrobial that will prevent the growth of an organisms after subculture onto antibiotic free media (Andrews, 2001). The MBC was determined for each foodborne pathogen from MIC by removing the media from each well which showing to visible growth. The plates were incubated at 37°C for 24 hours. MIC and MBC were tested for all bacteria strains for three times with three replications each (n = 3 x 3).

Experimental design and statistical analysis

Optimisation studies have been done with 14 designed experiments (runs), 6 centre point in central composite designs and two independent variables (extraction time and temperature). The least square multiple regression methodology was used to reveal the relationship between the independent (extraction temperature, extraction time) and dependent (extraction yield, total phenolic contents) variables. The multiple regression equation was used to fit the second order polynomial equation based on experimental data as in Eq. (1):

$$Y = C + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2 \quad (1)$$

where Y represents the predicted response, C is the constant, β_1 and β_2 are linear coefficients. Then, β_3 and β_4 are quadratic coefficients. Lastly, β_5 is interaction coefficients. X_1X_2 are the independent similar as X_1 and X_2. The models are determined by the coefficient of determination (R^2). The coefficient of determination (R^2) is defined as the regression of sum of the squares proportion to the total sum of square that illustrates the adequacy of the model. R^2 ranges from 0 to 1. The model is more accurate when R^2 values closer to 1 (Yolmeh *et al.*, 2014). All results reported as mean ± standard deviation of triplicate measurements. Significant difference (p-value < 0.05) within means was analysed by analysis of variance (ANOVA) and Tukey's honestly significantly difference (HSD) test. All the data were assessed by MINITAB 16.

Results and Discussion

The extraction yield (%) and total phenolic contents (mg GAE/g) of cinnamon leaves were optimised through RSM. All of the 14 designed experiments with central composite designs were conducted for optimising two parameters (temperature and time) in central composite (Table 1). Then, the antibacterial activity of extract from optimised conditions were studied against *E. Coli, P. aeruginosa, S. Typhimurium, K. pneumonia, L. monocytogenes, S. aureus, B. cereus* and *B. subtilis*. Effectiveness of UAE methods in producing higher extraction yield, total phenolic content and broader bacterial inhibitions were compared with Soxhlet

Table 1. Extraction yield and total phenolic contents in methanolic extract using RSM

Run	Temperature (x_1)	Time (x_2)	Extraction Yield % (Y_1)	Total phenolic contents, mg GAE. g^{-1} (Y_2)
1	27	41	19.60	2939
2*	33	30	20.68	4025
3	38	19	20.80	3641
4	27	19	19.56	2367
5	38	41	25.16	2907
6*	33	30	20.80	4060
7*	33	30	20.76	4079
8*	33	30	19.72	4041
9*	33	30	20.84	4012
10	25	30	19.64	2227
11	33	45	20.18	3394
12	33	15	19.84	2861
13*	33	30	20.40	4039
14	40	30	25.84	1972

Note; *, centre point

methods.

Effect of extraction condition on extraction yield and total phenolic content of crude extract

Preliminary studies using single factor experiments have been conducted before RSM trials in order to select the type of solvent, the ratio of water: solvent, range for extraction temperature and time. The different types of solvents such as chloroform, hexane, ethanol and methanol have been tested. Methanol showed the highest extraction yield (27.49 ± 1.59%) among all tested solvents. Rezaie *et al*. (2015) stated that the efficiency of the different solvent extraction methods strongly depends on the composition of plant materials. According to them, methanol was efficient in extraction as methanol and ethanol are the polar protic solvent which can be attributed to the fact that alcohols participate in the chemical reactions related to the polar carbon–oxygen and oxygen–hydrogen bonds. As a consequence, the unshared electron pair of the oxygen atom in alcohols are more available than that of water molecules. Therefore, methanol and ethanol have ability to extract glycosidic and non-glycosidic phenolic compounds from the plant cells better than non polar solvent.

Therefore, methanol have been selected for preliminary studies such as the ratio of water: methanol (100:00 to 00:100), extraction time (15 to 60 minutes) and extraction temperature (25 to 60°C) (Figure1). From the preliminary study, the extraction yields were 9.6% ± 0.4 to 27.49% ± 1.59 when the leaves extracted by ratio of water: methanol (100:00 to 00:100). The ratio of water: methanol (00:100) contributed the highest extraction yield (27.49% ± 1.59). In extracting the plant product, the solvent viscosity is one of the physical property that affects the extractability. In facts, low viscosity solvents have high diffusivity which easily allow the diffusion of solvents into the pores of the plant matrices in order to leach out the bioactive compounds. Furthermore, in less viscous solvents, the cavitational bubbles were produced easily because the ultrasonic intensity applied can easily exceed the molecular forces of the solvent. Therefore, the ultrasonic behaviours of solvents were considerably affected by their viscosity (Rezaie *et al*., 2015). When the extraction processes for 15 to 60 minutes, the extract yielded from 19.91% ± 1.55 to 27.50% ± 1.42. When the leaves were exposed for 25 to 60°C, the extraction

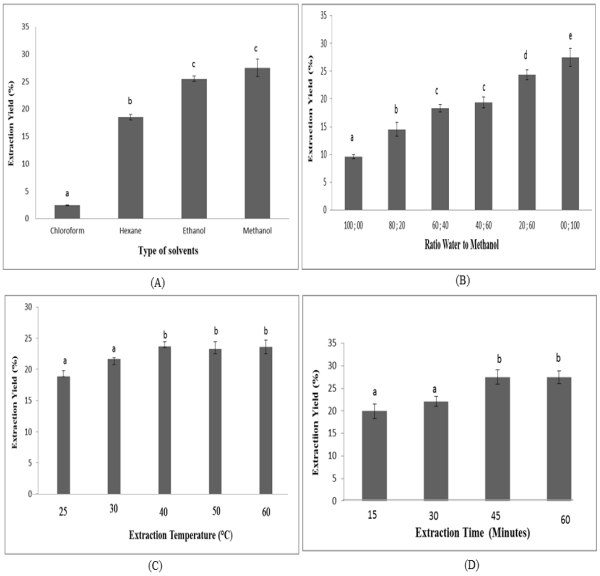

Figure 1. The preliminary studies to determine the range of RSM

yields increased from 18.86% ± 0.93 to 23.62 ± 1.05. However, there was no significant different ($p>0.05$) on extraction yield (%) when the extract exposed for 40, 50, 60°C and 45, 60 minutes. Therefore, the ranges of temperatures (25 to 40°C) and time (15 to 45 minutes) have selected for optimisation process.

In general, temperature was the most significant parameter which influenced extraction yield and total phenolic contents. The extraction yields increased as temperature and time increased. Tahmouzi (2014) stated, when the extraction temperature of Q. brantii leaf increased (30 to 90°C), the extraction yield would also increase.

The total phenolic contents also increased as temperature and time increased. However, total phenolic contents declined after the extracts were exposed at higher than 33°C. This might be due to improvement of mass transfer rate at high temperature (35 to 65°C) which caused higher solubility in the solvent such as ethanol, diethyl ether, acetone and ethyl acetate (Yolmeh *et al*., 2014). The high temperature (>90°C) promoted the degradation of some thermo-sensitive compounds such as flavonoid from Flos Populi (Sheng *et al.*, 2013). Another study by Chen *et al.* (2015) also stated that as temperature increased, the total phenolic contents increased. However, the total phenolic contents were decreased as the extraction temperature reached to 43°C.

The amounts of total phenolic contents were calculated as gallic acid equivalent. The range of total phenolic contents in methanolic cinnamon leaves were between 1972 to 4079 mg GAE/g. However, previous research by Yang *et al.* (2012) showed that the total phenolic contents of Cinnamomum cassia leave extracts immersed with ethanol were only 885 mg GAE/g.

Fitting the response surface models

The second order polynomial equations were applied to build mathematical models as n Eq. (2) and (3):

$$Y_1 = 63.8630 - 2.7041\ x_1 - 0.4143x_2 + 0.0384x_1^2 + 0.0192\ x_1\ x_2 \quad (2)$$

$$Y_2 = -38664 + 2238.1\ x_1 + 393\ x_2 - 31.4\ x_1^2 - 3.3\ x_2^2 - 5.8\ x_1\ x_2 \quad (3)$$

where Y_1 is the uncoded values of extraction yield (%),Y2 is total phenolic contents (mg GAE /g), x1 is extraction temperature (°C) and x2 is extraction time (minutes). The bigger p-value, the smaller the significance of the corresponding coefficient (Sheng *et al.*, 2013). In this study, the p-value was smaller than 0.05 indicated that the model was suitable for this experiment.

Table 2. Comparison between extraction methods on extraction yield and total phenolic contents

Extraction methods (optimum condition)	Extraction yield (%)	Total phenolic contents (mg GAE.g^{-1})
UAE (45min , 40 °C)	27.49 ± 1.59^a	-
UAE (33min , 31 °C)	-	3987 ± 79.10^a
Soxhlet (8 h, 64 °C)	20.01 ± 1.24^b	2837 ± 21.17^b

(-), not performed
Values are expressed as mean ± standard deviation (n=3). Means within a column followed by different letters (a, b) are significantly different at the level of $p<0.05$.

Optimisation of extraction condition of crude extract

In optimising t UAE methods, the numerical optimisation techniques were used. The extraction times (15 to 45 minutes), methanol as solvent and ratio of water: methanol (00:100) had selected to be analysed by RSM (Tables 1). The optimum condition for extraction yield was at 40°C and 45 minutes. The similar optimum temperature (40°C) gained by Ivanovic *et al.* (2014) in optimising blackberry using UAE. Then, extraction condition at 33°C for 31 minutes was found as optimum condition to extract total phenolic contents from *C. zeylanicum* leaves. In this study, the extraction yield and total phenolic contents were acquired as the predicted results whose desirability were equal to 1.000 (extraction yield) and 0.985 (total phenolic contents).

Validation of the model

To validate the equation, the experiments were conducted under optimised conditions such as extraction temperature (x1); 40°C, 33°C and extraction time (x_2); 45, 31 minutes for extraction yield and total phenolic contents, respectively. The validated extraction yield and total phenolic contents of cinnamon leaves were 27.49 ± 1.59% and 3987.00 ± 79.10 mg GAE /g which respectively similar as predicted by RSM which 28.34% and 4040.00mg GAE /g.

Comparison of UAE and Soxhlet in extraction yield, total phenolic contents and antibacterial activities

Response surface methodology was used to evaluate the optimum condition for UAE and compared with Soxhlet extraction method (Table 2). Soxhlet is a standard method and main references for evaluating the performances of solid-liquid extraction methods (Wang and Weller, 2006). In this study, the extraction yield and total phenolic contents of UAE methods (27.49 ± 1.59%, 3987 ± 79.10 mg

Table 3. Diameter of inhibition zone at optimum condition at different extraction methods

Extraction methods	Optimum conditions A) Yield B) TPC		Disc-diffusion (mm)							
	Temp (°C)	Time (Min)	*E. coli*	*P. aeruginosa*	*S.* Typhimurium	*K. pneumonia*	*L. monocytogenes*	*S. aureus*	*B. cereus*	*B. subtilis*
UAE	A) 40	45	Nd	7.33 ± 0.50^{aA}	8.11 ± 0.33^{aB}	12.78 ± 0.83^{aC}	9.33 ± 0.50 ^{aD}	11.33 ± 0.50^{aE}	12.78 ± 0.44^{aC}	13.22 ± 0.44^{aC}
	B) 33	31	Nd	6.78 ± 0.67^{aA}	7.22 ± 0.67^{bA}	11.56 ± 0.53^{bB}	9.22 ± 0.44^{aC}	11.67 ± 1.41^{aB}	11.33 ± 0.50^{bB}	11.33 ± 0.50^{bB}
Soxhlet	64.7	480	Nd	6.78 ± 0.97^{aA}	8.33 ± 0.87^{aB}	12.22 ± 0.44^{abC}	8.00 ± 0.50^{bB}	10.83 ± 0.44^{aD}	12.22 ± 0.44^{cC}	11.78 ± 1.48^{bCD}

Nd = Not detected, TPC= Total phenolic contents

Values are expressed as mean ± standard deviation (n=9). Means within a column followed by different small letters (a, b, c) are significantly different at the level of $p<0.05$. Means within a row followed by different capital letters (A, B, C) are significantly different at the level of $p<0.05$. Disc diameter 6.0 mm.

GAE/g) were significantly the highest ($p<0.05$) than Soxhlet methods (20.01 ± 1.24%, 2837.00 ± 21.17 mg GAE/g), respectively. The results show that UAE method was more efficient than Soxhlet method in extracting crude extract and total phenolic contents. This might be due to UAE method uses sound waves which create bubbles form and grow. Therefore, when the bubbles close to solid boundary, cavity collapse and produce high-speed jets of fluids and penetrate the samples which enhance extraction yield (Wang and Weller, 2006). Interaction between methanol and UAE ensure the extraction became efficient in cell walls degradation, which have unpolar character which cause compounds such as anthocyanins and other polyphenols can be released from cells (Lapornik *et al.*, 2005). Moreover, Yolmeh *et al.* (2014) also reached the same conclusion in extracting the natural pigment from annatto seed. According to Rezaie *et al.* (2015), the efficiency of the extraction methods (i.e: UAE) strongly depends on the matrix of plant materials as well as the type of extractable compound.

C. zeylanicum leaves are well known in traditional medicine for their effectiveness as it contained phenolics and polyphenol such as eugenol, linalool, benzyl benzoate and (E)-cinnamaldehyde as major compounds (Subki *et al.*, 2013). The antibacterial activity of methanolic extract of *C. zeylanicum* was studied against *E. Coli, P. aeruginosa, S. Typhimurium, K. pneumonia, L. monocytogenes, S. aureus, B. cereus* and *B. subtilis* at different extraction methods (UAE with different extraction temperature/time and Soxhlet methods) by disc-diffusion assay. The present study found that the extracts obtained from optimum condition of UAE (40°C and 45 minutes) showed the broadest antibacterial activity by inhibiting the growth of all bacterial strains tested compared to Soxhlet methods (7.33 ± 0.50 to 12.78 ± 0.83 mm and 6.78 ± 0.97 to 12.22 ± 0.44 mm, respectively). There was significant different between UAE (extraction yield) and Soxhlet methods ($p<0.05$) against *K. pneumonia, S. Typhimurium, L. monocytogenes B. cereus* and *B. subtilis* Low inhibitory effect from Soxhlet extracts compared to UAE extracts indicated some of the active and heat sensitive ingredients were destroyed during high temperature exposure in boiling processes (Agnihotri *et al.,* 2014). Therefore, from the results, it can be concluded that UAE produced better extract to inhibit bacteria compared to extract by Soxhlet. Gram-positive bacteria were more susceptible (8.00 ± 0.50 to 13.22 ± 0.44 mm) to all extract than gram-negative bacteria (6.78 ± 0.67 to 8.33 ± 0.58 mm) except for *K. pneumonia* (11.56 ± 0.53 to 12.78 ± 0.83 mm). All of the extracts were not active against *E. coli* (Table 3).

Broth dilution assay was performed to determine the minimum inhibitory concentration (MIC). The inhibition of disc-diffusion assay against *E. coli* were not detected but the value of MIC could be determined. It is interesting to point out that the strains demonstrating the biggest inhibition zones by the diffusion method but contributed to the lowest MIC and MBC values similar to Mezni (2015). MIC and MBC values were shown in Table 4. Gram positive bacteria (*L. monocytogenes, S. aureus, B. cereus* and *B. subtilis*) were the most susceptible organisms to the selected extract with MIC values of 97.65 µg/mL. Among all of the extraction condition, UAE showed the lowest value of MBC against *L. monocytogenes* with value of 6.25 mg/mL. In this study, the MIC values were lower than MBC values. These suggest that all of the extracts were bactericidal at higher concentration and bacteriostatic at a lower concentration. Seanego and Ndip (2012) also suggest similar results in extract of Garcinia kola (Heckel) Seeds.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude extract against foodborne pathogens

Extraction methods	MIC (µg/ml)			MBC (mg/ml)		
	UAE (Extraction yield)	UAE (Total phenolic contents)	Soxhlet	UAE (Extraction yield)	UAE (Total phenolic contents)	Soxhlet
E. coli	1562.50	1562.50	6250.00	25.00	50.00	50.00
P. aeruginosa	1562.50	1562.50	1562.50	25.00	50.00	12.50
S. Typhimurium	781.25	781.25	195.31	12.50	25.00	12.50
K. pneumonia	390.25	390.25	390.25	25.00	25.00	12.50
S. aureus	97.65	97.65	97.65	25.00	12.50	12.50
L. monocytogenes	97.65	97.65	97.65	6.25	12.50	12.50
B. cereus	97.65	97.65	97.65	12.5	25.00	12.50
B. subtilis	97.65	97.65	97.65	12.5	25.00	12.50

Note: MIC = minimum inhibitory concentration (µg/ml), MBC = minimum bactericidal concentration (mg/ml)

Conclusion

RSM was successfully implemented for the optimisation of extraction yield (%) and total phenolic contents (mg GAE /g) of cinnamon leaves. The optimum extraction conditions were 40°C, 45 minutes for the highest extraction yield and 33°C and 31 minutes for the highest total phenolic contents. Extraction yield (%) and total phenolic contents (mg GAE /g) from UAE methods were similar as predicted by RSM's equation. The results suggested that the RSM approaches were effective to optimise the extraction of cinnamon leaves. The UAE method was suitable in producing the highest extraction yield and total phenolic contents compared to Soxhlet method. For antibacterial assay, UAE extract also showed the broadest antibacterial inhibition (mm) and lower MICs and MBCs compared to Soxhlet extraction methods against selected foodborne pathogens. Therefore, UAE method is suitable to be applied in spice extraction processes especially for the food industry.

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