



Effective of Reducing Bacteria in Chicken for Using Mango Peel Extracts with Acetic acid

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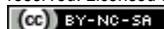
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Abstract: Objective of this research is to select mango peel extract of Chok-Anan, Mahachanok, Rat, Keawsavey and Namdokmai, extracted by 90% ethanol and water, which had highest polyphenol content and antibacterial ability. It was found that ethanol extraction had significant higher ($p \leq 0.05$) yield than water extraction. The ethanol extraction of Chok-Anan Peel had the maximum yield of 12.21 ± 0.39 %. It had the highest total phenolic compound 315.25 ± 23.32 mg gallic acid equivalents/g dry of mango Peel extract and highest total flavonoid compound 195.63 ± 13.36 mg catechin equivalents/g dry of mango Peel extract. The antibacterial activity against 5 species of Chicken associated food poisoning bacteria, the Chok-Anan extract with ethanol shows the best activity against *Bacillus cereus* and *Pseudomonas aeruginosa* with MIC of 0.78 and 6.26 mg/ml and MBC of 12.5 and 12.5 mg/ml, respectively. When pieces of chicken were dip in the mixture of mango peel extract solution (12.5 mg/ml) and Acetic acid solution (minimum inhibitory concentration of 5 species microorganism, 5 % of acetic acid) for half minute, then washed sterile water immediately, it could reduce total bacteria to 3.25 ± 0.28 log CFU/g from total bacteria started 7.83 ± 0.90 log CFU/g.

1. INTRODUCTION

Microbial contamination in chicken is a major problem that deteriorates chicken quality and may not be safe from some foodborne pathogens. The major cause is the lack of proper hygiene management, especially in most slaughterhouses in Thailand that are not up to standard and the meat is not handled properly until reaching the consumer (Guamán-Balcázar, 2017; K, et al., 2019; Nadar, & Rathod, 2019). To solve these problems, the research was conducted by using using natural extracts to reduce microbial contamination. There are many plants that have antibacterial properties such as turmeric, galangal, lemongrass, garlic, etc., as well as mango peels, which are waste materials (Oliveira, et al., 2016; Oliver-Simancas, et al., 2021). Leftovers, which provide extracts that can fight many types of bacteria. Mango peel is an unused agricultural waste material. The properties of the mango peel are composed of high polyphenols and carbohydrates. When the mango peel is used to extract substances to reduce bacteria in the food, it is one way to reuse the waste from mangoes and ethanol extract was able to inhibit gram-positive bacteria better than gram-negative bacteria (Thitilertdecha, et al., 2008; Sanchez-Sanchez, et al., 2017; Rofeal, et al., 2022). Reducing bacteria in fresh chicken products organic acids are often used

as lactic and acetic acids (Yang & Li, 2013). Therefore, this research was interested in the combination of mango peel extract and acetic acid to reduce bacterial count in chickens.

2. MATERIALS AND METHODS

2.1 Extraction from mango peels of different species

Commercial collection of some mango peels such as Chok-Anan, Mahachanok, Rat, Keawsavey and Namdokmai from Amphoe Mueang Yala Province by choosing raw green mango peels dried at 60 °C for 24 hours and then packed in a vacuum-sealed plastic bag. The extraction was then carried out by pulverizing the freeze-dried samples sift to the same size with a sieve. Extracted by weighing 10 g of samples, adding 90 % ethanol, shaking in the dark at 150 rpm for 48 h, filtering with a thin white cloth and centrifuging at 3,500 rpm for 15 min was evaporated with a rotary evaporator at a temperature of 60 °C. The water extraction was done the same as the above method by using water instead of ethanol. Then evaporated in a rotary evaporator at 80 °C and stored in a brown bottle at 4 °C (Kaur, et al., 2010; De-Sousa Guedes, et al., 2016; Kim, et al., 2017; Fernández-Ponce, et al., 2018).

2.2 Determination of phenolic content and total flavonoid content

The total phenolic content was determined by the method Folin-Ciocalteu Colorimetry (AOAC, 1990). Gallic acid was used as standard solution and report the results as weights mg gallic acid equivalents/g dry of mango peel extract and the total flavonoid content was determined by colorimetric assay (AOAC, 1990), using catechin as the standard solution and reporting the results in weight (mg) catechin equivalents/g dry of mango peel extract (Jahurul, et al., 2015; Chi, et al., 2019; Cheng, et al., 2021)

2.3 Antibacterial ability test by Filter paper disk agar diffusion technique (Kirby-Bauer Test)

Five strains of bacteria that cause food poisoning, namely *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15422 and *Salmonella Enteridis* DMST 17368. It was taken from the field of renewable energy technology, Faculty of Science, Technology and Agriculture Yala Rajabhat University culture in Nutrient broth (NB) incubated at 37 °C for 8-12 h, adjust cell concentration to 10^6 - 10^7 CFU/ml, spread plate on Mueller-Hinton agar (MHA), then drop extract on top 8 µl of the prepared medium was placed on a 6 mm filter paper disk, incubated for 5 min, then placed on the medium in a culture dish, stored at 4 °C for 30 min, and then incubated at 37 °C for 18 h. The size of the resulting clear zone was measured using standardized tetracycline as a positive control and 50 % (V/V) DMSO as a negative control (López-Cobo, et al., 2017; Klangmuang, & Sothornvit, 2018; Nandhavathy, et al., 2021)

2.4 Determination of Minimal inhibition concentration (MIC) and Minimal bactericidal concentration (MBC)

The test was performed by using macro broth dilution technique. Twelve sterile test tubes were taken and numbered on the tubes. Mueller Hinton broth was then aspirated into tubes 2-12, 1 mL each, and the extract was extracted into tubes 1 and 2, 1 mL each, shake well and pipet the sample in tube 2. In tube 3, this was done up to tube 11, and 1 ml of solution was aspirated in tube 11. Tube 12 contained only the culture medium, which was a positive control, in order from tubes 1 to 11. The initial concentration was 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20 and 0.10 mg/ml, respectively. For acetic acid, the initial concentration was 24, 12, 6, 3, 1.5, 0.75, 0.38, 0.19, 0.09, 0.05 and 0.02, respectively. Then, 1 ml of the desired bacteria prepared above was added to each tube. All tubes were incubated at 37 °C for 18 h except *B. cereus*. ATCC 1729 were incubated at 30 °C mg/ml. The antibacterial activity was tested by determining MBC by streaking the lowest concentration of MIC tubes onto NA medium and then incubating them at 37 °C for 24 h (Yenjit, et al., 2010; Zhang, et al., 2020; Ribeiro, et al., 2021).

2.5 The combination of mango peel extract and acetic acid reduced bacteria in chicken.

The chicken sample was prepared from the market in Yala, Yala Province, Thailand. The weighing 200g in average. The experiment was divided into 4 treatments, which were exposed to sterile distilled water. Experience the mango seed extract solution. Exposure to acetic acid and exposure to a solution of mango peel extract together with acetic acid with the lowest concentration of antimicrobial of the five tested microorganisms were immersed in the solution for 0, 1, 3, and 5 min, then rinse with sterile water. Dry on a wire rack to drain. After each treatment, microorganisms were counted by spreading plates on NA

medium and incubating them at 37 °C for 24 h (Torres-León, et al., 2016; Naeem, et al., 2018; Jiang, et al., 2021)

3. RESULTS AND DISCUSSION

3.1 Mango peel extract content with 90% ethanol and water

When 90% ethanol and water were used as solvents for the extraction of mango seeds, it was found that amount of substance extracted with ethanol was significantly higher than that of water ($p \leq 0.05$). CN, MH, RT, KW and ND extracted with ethanol had 12.21 ± 0.39 , 11.27 ± 0.61 , 9.21 ± 0.85 , 9.21 ± 0.85 , 10.55 ± 0.17 and 11.26 ± 0.93 %, respectively. The aqueous extract had $8.13 \pm 0.51b$, 4.03 ± 0.18 , 4.23 ± 0.24 , 7.94 ± 0.32 and 7.54 ± 0.47 %, as shown in Figure 1.

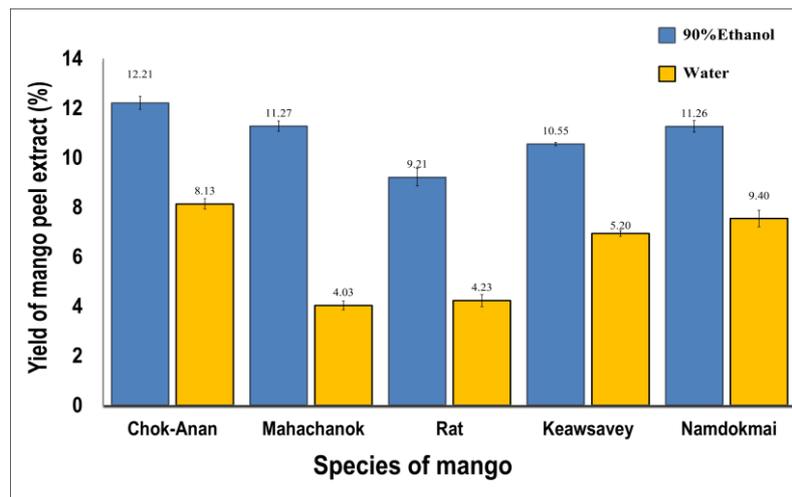


Figure 1: Mango peel extract content with 90% ethanol and water

3.2 Total Polyphenol Content in Mango Peel Extract

The phenolic and flavonoid contents of 5 mango peel extracts as Chok-Anan, Mahachanok, Rat, Keawsavey and Namdokmai were extracted with 90% ethanol and water. Total phenolic were 315.25, 254.32, 301.63, 198.36, and 305.02 mg gallic acid equivalents per gram dried mango peel extract weight, and 182.63, 145.32, 154.65, 125.69 and 152.32 mg catechin equivalents per weight. Extract 1 gram of dried mango peel, respectively. and the water extract had phenolic and total flavonoid contents of 195.63, 112.32, 136.32, 145.25 and 187.22 mg of catechin equivalents per gram of dried mango peel extract and 144.85, $115.22 \pm 15.43cd$, 138.84, 105.40 and 139.93 mg catechin equivalents per 1g dried mango peel extract weight, respectively.

3.3 Antibacterial ability from mango peel extract

The antibacterial activity against all 5 strains of food poisoning was tested using Tetracycline as a positive control and DMSO 50 % (V/V) of the solvent as a negative control. Ethanol gave better effect than water extract which Chok-Anan extracted with ethanol. Inhibitory against of *P. aeruginosa*, *B. cereus*, *S. aureus*, *S. Enteritidis*, *E. coli* were highest with clear zone sizes of 21.22 ± 0.42 , 17.23 ± 0.71 , 16.22 ± 0.21 , 15.24 ± 0.42 and 14.17 ± 0.27 mm., respectively. In the above research, which has a clear zone size similar to that of Namdokmai and Rat, Mahachanok and Keawsavey were not statistically different in activity against different strains of bacteria as shown in Table 1.

3.4 Minimum concentration of Chok-Anan extract and acetic acid solution that inhibits and kills food poisoning bacteria (Minimal inhibition concentration (MIC) and Minimal bactericidal concentration (MBC))

The MIC and MBC values of CN extract were determined by ethanol extraction. Chok-Anan showed that the inhibitory and bactericidal activity against *B. cereus* was 0.39 and 0.78 mg/ml, respectively, while *E. coli* and *S. Enteridis* were required. The peak inhibition and killing concentrations were 12.5 and 12.5 mg/ml, respectively, as shown in Table 2. The inhibitory effect was probably due to the polyphenols binding to the copper at the center of the active site when acetic acid was taken to determine the MIC and MBC values against the five-food poisoning bacterial strains. *P. aeruginosa* was found to have the lowest MIC of 6.26 %, indicating that *P. aeruginosa* was inhibited at 12.5 % concentration. The overall bactericidal activity was 3 % as shown in Table 2. Acetic acid can inhibit and kill bacteria by destroying the membrane layer, causing holes in that area, and acid destroying nitrogen bonds, resulting in protein degradation in microorganisms. come and prevent microorganisms from growing (Firouzabadi, et al.,2014; Guamán-Balcázar, et al., 2019; Engels, et al., 2019)

Table 1. Antibacterial activity of different strains of mango peel extract extracted with 90% ethanol when tetracycline 30 µg and DMSO were used as control.

Bacteria	Diameter of various substances					Tetracycline 30 µg
	Chok-Anan	Mahachanok	Rat	Keawsavey	Namdokmai	
<i>B. cereus</i>	17.23±0.71	10.57±0.41	14.50±0.23	10.17±0.41	16.50±0.84	16.21±0.20
<i>S. aureus</i>	16.22±0.21	9.17±0.41	12.58±0.41	7.83±0.41	13.53±0.41	18.16±0.41
<i>E. coli</i>	14.17±0.27	8.18±0.28	11.33±0.21	10.08±0.76	12.25±0.45	14.65±0.41
<i>P. aeruginosa</i>	21.22±0.42	14.75±0.61	17.55±0.41	9.77±0.41	16.24±0.52	22.43±0.41
<i>S. Enteridis</i>	16.22±0.21	14.67±0.82	15.17±0.41	8.92±0.20	14.25±0.42	17.84±1.02

* DMSO 50 % (V/V) had no antibacterial effect on test bacteria.

Table 2 MIC and MBC values of Chokanan Mango Peel Extract (CN) with ethanol and acetic acid solution.

Bacteria	Chokanan peel extract with ethanol (mg/ml)		Acetic acid (%)	
	MIC	MBC	MIC	MBC
<i>B. cereus</i>	0.39	0.78	0.75	0.75
<i>S. aureus</i>	1.56	6.25	0.75	0.75
<i>E. coli</i>	6.25	12.50	1.50	3.00
<i>P. aeruginosa</i>	6.25	12.50	0.75	3.00
<i>S. Enteridis</i>	3.13	6.25	0.75	1.50

3.5 The effect of using extract and acetic acid to reduce bacteria in chicken

The use of chicken meat was divided into 5 experimental groups. Then dipped in various solutions consisting of (1) water (2) Chokanan at a concentration of 12.5 mg/ml (3) 3% acetic acid (4) Chokanan at a concentration of 6.25 mg/ml with 1.5 % acetic acid and (5) Chokanan at a concentration of 1.5 % concentration of 12.5 mg/ml with 3% acetic acid at 0, 1, 3 and 5 min. The results showed that chickens had initial bacterial numbers of 7.83 ± 0.30 log CFU/g when immersed in 12.5 mg/ml of CN extract combined with 3% acetic acid at 30 s, which reduced the number of bacteria the most to 3.25 ± 0.28 log.

CFU/g if halved the concentration of Chokanan in combination with acetic acid the total number of bacteria was 6.73 ± 0.32 log CFU/g, which reduced the number of bacteria less than 1.33 log CFU/g when dipped in Chok-Anan at a concentration of 12.5 mg/ml. and 3% acetic acid. The total number of bacteria was 5.29 ± 0.24 log CFU/g, respectively. Chok-Anan solution reduced the total number of bacteria, possibly enzymatic inhibition with copper binding in the middle of the active site and acetic acid. The ability to reduce the number of bacteria may be due to the dissociation of acids and dissociated ions that can pass into the microbial cells. and cause the cells in the bacteria to not grow (Das, et al., 2019; Angamuthu, et al., 2021; Behzad, et al., 2021)

4. CONCLUSION

The 90% ethanol extraction had significant higher $p \leq 0.05$ yield than water extraction. The ethanol extraction of Chok-Anan Peel had the maximum yield of 12.21 ± 0.39 %. It had the highest total phenolic compound 315.25 ± 23.32 mg gallic acid equivalents/g dry of mango Peel extract and highest total flavonoid compound 195.63 ± 13.36 mg catechin equivalents/g dry of mango Peel extract. The best of antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa* with MIC of 0.78 and 6.26 mg/ml and MBC of 12.5 and 12.5 mg/ml, respectively. The pieces of chicken with deep in extract solution and acetic acid solution could reduce total bacteria to 3.25 ± 0.28 log CFU/g from total bacteria started 7.83 ± 0.90 log CFU/g.

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