DEVELOPMENT OF NATURAL VEGETABLE SANITIZER FROM THAI LOCAL HERBS

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ABSTRACT

Nowadays, food safety becomes public concern, especially in fresh produce; fresh vegetables and fruits. The CDC has been reported at least 7 outbreaks from fresh produce in the past 3 years; for example, *Escherichia coli* O121 in raw clover sprouts (2014), *E. coli* O157:H7 in ready-to-eat salads (2013), *Salmonella* Saintpaul in cucumbers (2013), *Salmonella* Typhimurium and Newport in cantaloupe (2012), and *E. coli* O157:H7 in spinach and spring mix salad (2012). Washing, before consuming, is the most important step in order to prevent foodborne outbreak in fresh produces. Therefore, this research was aimed to develop vegetable sanitizer from crude extracts of *Acacia concinna* (Willd.) D.C (Som-poi), *Capsicum annuum* (Chili), *Cymbopogon citratus* (Lemongrass), *Citrus hystrix* (Kaffir lime) and *Chrysanthemum indicum* L. (Chrysanthemum) in order to reduce foodborne bacteria in fresh vegetable. These herbs have been reported for their antibacterial activity (Pitinidhipat and Yasurin, 2012; Utami *et al*., 2012; Saenghiruna and Yasurin, Lazuardi *et al*., 2013; 2013; Piya-isarakul and Yasurin, 2013; Saenghiruna *et al*., 2014; Dung *et al*., 2014). The 18 treatments with 3 different crude extracts percentage (0.5, 1 and 1.5%), 3 different volumes (3, 5, and 10 ml.) and 2 different soaking times (10 and 20 min) were investigated for their antibacterial synergistic activity using *Lactuca sataiva* (Lettuce) as vegetable model. The MPN, total place count on PCA, BHI agar for *Listeria monocytogenes* count, and SS agar for *Salmonella* sp. count were used to evaluate the antibacterial efficiency of vegetable sanitizer. The results showed that treatment 18 (using 10 ml of 1.5% crude extracts with 20 min soaking time) gave the highest bacterial efficiency statistically by vegetable sanitizer comparing with the commercial brand. The MPN number, the total plate count, and the *L. monocytogenes* count were 1.34 ±0.19 MPN/ml, 1.62 ± 1.02 log CFU/ml, and 1.31 ± 0.40 log CFU/ml, respectively. The *Salmonella* sp. was not found. The t-test has been done by using SAS on log CFU/ml with $P \leq 0.05$. It was found that crude extract percentage, used volume, and soaking time affected the antibacterial efficiency of this vegetable sanitizer.

Keywords: *Vegetable sanitizer, Thai Local Herb, Crude Extract, Foodborne bacteria*

1.INTRODUCTION

Nowadays, food safety becomes public concern, especially in fresh produce; fresh vegetables and fruits. The Centers for Disease Control and Prevention (CDC) has been reported at least 7 outbreaks from fresh produce in the past 3 years; for example, *Escherichia coli* O121 in raw clover sprouts (2014) [1], *E. coli* O157:H7 in ready-to-eat salads (2013) [2], *Salmonella* Saintpaul in cucumbers(2013) [3], *Salmonella* Typhimurium and Newport in cantaloupe (2012) [4], and *E. coli* O157:H7 in spinach and spring mix salad (2012) [5]. Washing, before consuming, is the most important step in order to prevent foodborne outbreak in fresh produces. Interestingly, Thai herbs has been used for a large range of purposes including medicine, nutrition, flavorings, beverages, dyeing, repellents fragrances, cosmetics, charms, smoking, and industrial uses. Therefore, this research was aimed to develop vegetable sanitizer from crude extracts of Thai herbs.

Acacia concinna (Willd.) D.C (Som-poi), *Capsicum annuum* (Chili), *Cymbopogon citratus* (Lemongrass), *Citrus hystrix* (Kaffir lime), and *Chrysanthemum indicum* L. (Chrysanthemum) have been reported for their antibacterial activity under different extraction conditions. [6] [7] [8][9][10] In addition, these herbs have been reported for their antibacterial activity and also contain innumerable constituents and are valuable sources of new and biological activity molecules possessing antimicrobial properties [11].

Therefore the objective of this research is to develop vegetable sanitizer from Thai local herbs; *A. concinna* (Willd.) D.C (Som poi), *C. annuum* (Chili), *C. citratus* (Lemongrass), *C. hystrix* (Kaffir lime), and *C. indicum* L. (Chrysanthemum).

2.MATERIALS AND METHODS

2.1.Preparation of crude extract

Plant samples: *A. concinna* (Willd.) D.C (Som -poi), *C. annuum* (Chili), *C. citratus* (Lemongrass), *C. hystrix* (Kaffir lime), and *C. indicum* L. (Chrysanthemum) were bought from local fresh market on Bangkapi, Bangkok, Thailand. The herbs were cut into small pieces and dried in air oven (Memmert, UM500) at 45°C for 20 hours. Then, dried herbs were blended by using blender to reduce the size of herbs. The herb powder was stored in refrigerator at 4°C until use.

2.2.Preparation of crude extract

The 20 g of herbs powder was weight on Top-loaded balance (1 decimal) (ZEPPER model ES-300) Then, 100 ml 95 % Ethanol was added and soaked for 24 hours at room temperature and shake at 120 rpm by shaking incubator (LSI-3016R model). After that, the liquid part was filtered out. Then, supernatant was evaporated paper by rotary evaporator at 120 rpm, 45°C for 15 minutes until supernatant become slurry crude extract. The crude extract was diluted to 0.2g/ml by using 95% ethanol. It was kept in freezer at -20°C until use.

2.3.Preparation of Som-poi water

The 100g *A. concinna* (Willd.) D.C (Som poi) power was boiled with 500 ml water for 30 minutes, stirred every 10 minutes. The temperature was in range between 95-98°C. Then Som-poi water was cool down to room temperature before using as vegetable sanitizer base.

2.4.Preparation of vegetable sanitizer

For one vegetable sanitize using the 10 ml Polysorbate Tween20 was weighed, mixed with 90ml Som-poi water and stirred for 15 minutes. Then, 0.2g Disodium-ethylenediaminetetraacetate (2NaEDTA) was added and stirred for 15 minutes. Then 4 types of 95% crude crude extracts; *C.*

annuum (Chili), *C. citratus* (Lemongrass), *C. hystrix* (Kaffir lime), and *C. indicum* L. (Chrysanthemum) were added with 3 different percentage (0.5, 1 and 1.5%). The formulas were in the table1.

Table1 The formula of vegetable sanitizer

95% Crude Ethanolic Extract 0.2 g/ ml	Percentage		
	Formula 1	Formula 2	Formula 3
Lemongrass *(C. hystrix)*	0.5	1.0	1.5
Chili (*C. annuum*)	0.5	1.0	1.5
Kaffir lime *(C. citrates)*	0.5	1.0	1.5
Chrysanthemum *(C. indicum* L.)	0.5	1.0	1.5

2.5.Antibacterial Assay

Lactuca sataiva (Lettuce) was used as a vegetable model. The lettuce was soaked. The swap method was used to evaluate antibacterial efficiency. The plate count agar (PCA), Brain Heart Infusion (BHI) agar, and Salmonella-Shigella (SS) agar were used for total plant count, *Listeria monocytogenes* count, and *Salmonella* sp. respectively. The most probable number (MPN) method was used for coliform bacteria count by using Lauryl sulfate tryptose (LST) broth. The 3×3×2 factorial design in the randomized complete block design (RCBD) was applied to this study. There were 3 formulas (formula 1, 2, and3), 3 used volume (3, 5, and 10ml), and 2 soaking time (10 and 20 min), total of 18 treatments. Each treatment was done duplicate 3 times independently. The results were compared with 2 commercial brands; Veggie and Jidrada.

2.6.Statistical Analysis

The data were analyzed using SAS (Statistical Analysis System for 158 Windows, Varsion 9.2, 2006, SAS Institute Inc., Cary, NC).

3.RESULTS AND DISCUSSION:

The vegetable sanitizer antibacterial efficiency is showed in table 2 – 5.

Table2 The MPN/ml

Note: Different superscript show significant different (P≤0.05)

Formula	Used Volume (ml)	Soaking Time (minutes	
		10	20
Formula 1	3	2.95 ± 2.57 b	2.38 ± 0 d
	5	2.50 ± 2.10 d	2.18 ± 0 f
	10	2.18 ± 0 f	1.88 ± 1.46 i
Formula 2	3	2.47 ± 2.16 d	2.380 ± 0 d
	5	2.26 ± 1.72 f	2.05 ± 1.19 f
	10	1.97 ± 0 i	1.75 ± 1.16 i
Formula 3	3	2.34 ± 1.24 d	2.05 ± 1.19 d
	5	2.18± 0 f	2.05 ± 1.19 f
	10	1.85 ± 0.80 i	1.34 ±0.19 j
Un-wash	-	3.04 ± 0.00 a	-
Wash	-	1.66 ± 0.00 c	-
Soak with tap water	-	2.36 ± 1.24 d	2.32 ± 0.00 d
Veggi	6	2.32 ± 0.00 d	1.42 ± 0.46 j
Jidrada	3	2.66 ± 0.00 c	0.92 ± 1.22 j

Table3 The log CFU/ml of total plate count on PCA

Formula	Used Volume (ml)	Soaking Time (minutes)	
		10	20
Formula 1	3	2.39 ± 0.70 b	2.31 ± 1.18 d
	5	2.29 ± 1.26 d	2.05 ± 0.76 f
	10	2.27 ± 0.76 f	2.21 ± 0.81 i
Formula 2	3	2.25 ± 1.40 d	2.15 ± 1 d
	5	2.21 ± 1.02 f	2.18 ± 1.01 f
	10	2.18 ± 1 i	2.11 ± 1.42 i
Formula 3	3	2.04 ± 1 d	2.03 ± 1.06 d
	5	2.24 ± 0.70 f	1.92 ± 1.18 f
	10	2.15 ± 1 i	1.62 ± 1.02 j
Un-wash	-	2.46 ± 1 a	-
Wash	-	2.30 ± 1.30 c	-
Soak with tap water	-	2.39 ± 1.51 d	2.26 ± 1.42 d
Veggi	6	2.04 ± 1.42 d	1.40 ± 0.64 j
Jidrada	3	2.32 ± 1.18 c	1.99 ± 1.32 j

Note: Different superscript show significant different (P≤0.05)

Table4 The Log CFU/ml of *Listeria monocytogenes* count on BHI agar

Formula	Used Volume (ml)	Soaking Time (minutes	
		10	20
Formula 1	3	1.84 ± 0.88 b	1.73 ± 1.02 d
	5	1.87 ± 0.72 d	1.94 ± 1.18 f
	10	1.92 ± 0.76 f	1.60 ± 1 i
Formula 2	3	1.81 ±1.15 d	1.74 ± 1.12 d
	5	1.73 ± 0.46 f	1.61 ± 0.74 f
	10	1.86 ± 1.06 i	1.65 ± 0.67 i
Formula 3	3	1.90 ± 1 d	2.13 ± 0.76 d
	5	1.740 ± 1 f	1.92 ± 0.76 f
	10	1.75 ± 0.76 i	1.31 ± 0.40 j
Un-wash	-	1.78 ± 1.30 a	-
Wash	-	1.73 ± 1.30 c	-
Soak with tap water	-	1.70 ± 11 d	1.71 ± 1.17 d
Veggi	6	1.67 ±0.76 d	1.15 ± 0 j
Jidrada	3	1.57 ± 0.40 c	1.73 ± 1.02 j

Note: Different superscript show significant different (P≤0.05)

Table5 The Log CFU/ml of *Salmonella* sp. count on SS agar

Formula	Used Volume (ml)	Soaking Time (minutes)	
		10	20
Formula 1	3	2.39 ± 0.70 b	2.31 ± 1.18 d
	5	2.29 ± 1.26 d	2.05 ± 0.76 f
	10	2.27 ± 0.76 f	2.21 ± 0.81 i
Formula 2	3	2.25 ± 1.40 d	2.15 ± 1 d
	5	2.21 ± 1.02 f	2.18 ± 1.01 f
	10	2.18 ± 1 i	2.11 ± 1.42 i
Formula 3	3	2.04 ± 1 d	2.03 ± 1.06 d
	5	2.24 ± 0.70 f	1.92 ± 1.18 f
	10	2.15 ± 1 i	1.62 ± 1.02 j
Un-wash	-	2.46 ± 1 a	-
Wash	-	2.30 ± 1.30 c	-
Soak with tap water	-	2.39 ± 1.51 d	2.26 ± 1.42 d
Veggi	6	2.04 ± 1.42 d	1.40 ± 0.64 j
Jidrada	3	2.32 ± 1.18 c	1.99 ± 1.32 j

Note: ND = Not Detectable

From table 2 to 4, un-wash and wash treatment gave the lowest bacterial efficiency statistically. The MPN number, the total plate count, and the *L. monocytogenes* count were 3.04 ± 0.00 a, 1.66 ± 0.00 c MPN/ml, 2.46 ± 1 a, 2.30 ± 1.30 c log CFU/ml, and 1.78 ± 1.30 a, 1.73 ± 1.30 c log CFU/ml, respectively. But use 0.5% crude extract the result show that The MPN number, the total plate count, and the *L. monocytogenes* count it is not different with soak with tap water treatment. When increasing the percentage of crude extract (at 1%) the result different from the 0.5% crude extract.

According to table 2-4, showed that highest crude extracts percentage, volumes, and soaking times gave better results in bacterial efficiency statistically.

This result indicates that crude extract concentration, used volume, and soaking affect on antibacterial efficacy. The result showed that using treatment 18; 10 ml of formula 3 with 20 min soaking time, gave the highest bacterial efficiency statistically. The

MPN number, the total plate count, and the *L. monocytogenes* count were 1.34 ±0.19 MPN/ml, 1.62 ± 1.02 log CFU/ml, and 1.31 ± 0.40 log CFU/ml, respectively with the 1.5% crude extracts, 10 ml. and 20 minutes for soaking times. While, the 0.5 and 1 % crude extracts, 3 and 5 ml, and 10 minutes for soaking times are not significant different.

For the commercial brand used 6 ml and 20 minutes for soaking times of Veggi brand. It gave the highest bacterial efficiency statistically. The MPN number, the total plate count, and the *L. monocytogenes* count were 1.42 ± 0.46 MPN/ml, 1.40 ± 0.64 log CFU/ml, and 1.15 ± 0 log CFU/ml, respectively.

When compare between Veggi brand; 20 min soaking time, and treatment 18; 10 ml of formula 3 with 20 min soaking time. As the result shows that The MPN number, the total plate count, and the *L. monocytogenes* count were 1.42 ± 0.46 j, 1.34 ±0.19 j MPN/ml, 1.40 ± 0.64 j, 1.62 ± 1.02 j log CFU/ml, and 1.15 ± 0 j, 1.31 ± 0.40 j log CFU/ml, respectively. The bacterial efficiency statistically are not significant different between Veggi brand and treatment 18. In general gram positive bacteria demonstrated more sensitivity to the spice. [12] Therefore, in treatment 18 use the formula contain chilli (*C. annuum*) and Chrysanthemum *(C. indicum L.*), it produce promising antibacterial substances against *B. cereus* and *L. monocytogenes* too. [19] So the *L. monocytogenes* (gram positive) are sensitive in this treatment 18.

In this experiment, the *Salmonella* sp. from table 5 was not found in the lettuce (*L. sataiva)*. So, we found that the crude extracts of herbs have effect in bacterial efficiency in this experiment.

However the using treatment 18; 10 ml of formula 3 with 20 min soaking time, gave the highest bacterial than the commercial brand because the activity of four herbs crude extracted (Lemongrass, chilli, kaffir lime, and Chrysanthemum) against the growth of MPN number, the total plate count, the *L. monocytogenes* count and the *Salmonella* sp. count. And also volume of sanitizer has effect in bacterial efficiency because the results show that when we use 10ml of sanitizer it better result compare with commercial brand. As the result, soaking time is the most important for this experiment. The better soaking is about 20 minutes has effect in bacterial efficiency.

In The Centers for Disease Control and Prevention (CDC) has been reported at least 7 outbreaks from fresh produce in the past 3 years; for example, the coliform bacteria as *Escherichia coli* O121 H7 in ready-to-eat salads, *Salmonella* Saintpaul in cucumbers, *Salmonella* Typhimurium in cantaloupe and *Listeria monocytogenes* in farm. In order to prevent foodborne outbreak in fresh produces so we need to reduce the microorganism in fresh vegetables and fruits from the natural herbs.

For the first herbs was use in this experiment, lemongrass (*C. citratus*), it was among the oils that exhibited antibacterial activity against all the *Listeria* strains tested and correlate to in research and found out that not only the oil form of lemongrass that showed activity against *L. monocytogenes* but also the ethanolic extraction form.[13]

Next, chilli (*C. annuum*) contains capsaicin which is reported as antimicrobial agent. [14] And also adding 1% w/v of dried chili in BHI can slightly inhibited the growth of *L. monocytogenes*. So increasing the amount of chilli might increase the inhibition activity. [15] Spices also rich in phenolic compounds and besides exerting antimicrobial effect they

may preserve the foods by reducing lipid oxidation as they are reported to have significant antioxidant activity. [16]

And, Kaffir lime (*C. hystrix*) peels also contain antimicrobial compounds. Kaffir lime (*C. hystrix*) peel crude ethanolic extract has antibacterial activity against of *Salmonella* sp. and other enterobacteria. Thus, not only kaffir lime peel has activity against *Sallmonella* sp. but also against *L. monocytogenes*.[17] The study of Kaffir lime show that the highest activity measured by using agar disc diffusion are 95% ethanolic extraction of kaffir lime peel. The inhibition effects of the extractions were 10.17 ± 1.96. Kaffir lime *C. hystrix*) peel crude ethanolic extract showed antibacterial activity against 20 serotypes of *Salmonella* sp. and 5 species of other enterobacteria. [18]

Chrysanthemum *(C. indicum L.*) it's always used in traditional drug formula for the treatment of several infectious disease such as pneumonia, colitis stomatitis, cancer, fever and sore and used to treat vertigo, pertussis and hypertensive symptom.[19] In Chrysanthemum *(C. indicum L.*) has antibacterial activity 1.17 ± 0.85 mm in vitro antibacterial screening results of crude 95% ethanolic extracted under normal stress so it contain antimicrobial compounds.[20]

Finally, Som poi (*A. concinna* (Willd.) D.C) is an important medicinal plant in Thailand and throughout Asian countries. This investigation was performed in order to demonstrate the antimicrobial potential against the fungal causative agents of ringworm and opportunistic infections. The study showed that the crude extract of *A. concinna* pod consisted of alkaloids, flavonoids, saponin and tannin but none of antraquinone and cyanotic glycosides. [21]

4.CONCLUSION

As the results showed that treatment 18 (using 10 ml of 1.5% crude extracts with 20 min soaking time) gave the highest bacterial efficiency statistically by vegetable sanitizer comparing with the commercial brand. The MPN number, the total plate count, and the *L. monocytogenes* count were 1.34 ±0.19 MPN/ml, 1.62 ± 1.02 log CFU/ml, and 1.31 ± 0.40 log CFU/ml, respectively, (P≤0.05). The *Salmonella* sp. was not found. And also, it was found that the difference in the activity was found when using different volume of vegetable sanitizer and soaking time.

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