

The effect of Allyl isothiocyanate-derived radish on antimicrobial activity in seafood.

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Abstract

Most Thai people like to eat seafood. It had reported that one Thai person consumes 33.73 kilograms of seafood per year and the rate increase every year. In seafood, there are pathogenic microorganisms such as *vibrio parahemolyticus* that cause food poisoning and *Escherichia coli* that cause diarrhea. The current treatment is treated by giving antibiotics to the patient, but if used too much, it may cause residue and result in reduced antibiotics. Therefore, research has been conducted to inhibit bacteria with herbs. Japanese study reported that allyl isothiocyanate that was found in wasabi can be effective in eliminating microbes *V. parahemolyticus* and *E. coli*, Moreover AITC can be found in many radishes. For example, radish, which is a plant that is easy to find in Thailand and can yield year-round. Therefore, this study aims to studied AITC extracts from radish at concentrations of 25%, 50%, 75% w/v obtained from soaking in 95% ethanol and fermented for 7 days to test the antimicrobial efficacy from shrimp, fish, oyster, squid and crabs by disk diffusion method. The three concentrations of AITC were compared with pure AITC 94%, distilled water was negative control and doxycycline was the positive control.

Keyword: radish, antimicrobial activity, *Vibrio parahaemolyticus*, *Escherichia coli*

1. Introduction

Most Thai people love eating seafood. The Food and Agricultural Organization showed that one Thai person consumes approximately 33.73 kilograms of seafood per year and the rate increases every year. Although seafood is delicious, it can be related to foodborne illness because seafood has pathogenic bacteria such as *V. parahaemolyticus* which is the cause of food poisoning, and *E. coli* which is the cause of Diarrhea. (Maliha Afreen and Ilknur Ucak et al., 2021) These microbes would be destroyed at high temperatures but some pathogenic bacteria remain. (Joe , David and robert et al.,1973) Antibiotics are used to treat this illness but antibiotic residues can induce and accelerate antibiotic resistance development. (RUDN University 2022) A Japanese study reported that wasabi has allyl isothiocyanate which is the main component related to antimicrobial activity against foodborne pathogens. (Hasegawa et al., 1999). For this reason, the study aims to test the efficiency of radish which is known as Thai plants

that have allyl isothiocyanate similar to wasabi killing *V. parahaemolyticus* and *E. coli* in seafood.

2. Method and Experiment Detail

2.1 Bacterial culture and identification

For *V. parahaemolyticus* culture. Added PBS dilution water which was an enrichment medium to prepare diluted samples and streak them on TCBS agar. The light microscope was used to identify the appearance of bacteria. *V. parahaemolyticus* appear as round, opaque, green, or bluish colonies on TCBS agar because they do not ferment sucrose.

2.2 DNA extraction

Test cultures were grown in 3.0 ml Luria-Bertani broth at 37°C overnight. The cell suspension was transferred to a 1.5 ml microfuge tube. Centrifuged it at 10,000 rpm, 25°C for 2 min. The supernatant was discarded. Added 1 ml of sterile distilled water and vortex to mix. The cell suspension was centrifuged at 10,000 rpm, 25°C for 5 min. The supernatant was discarded and

again 1 ml of sterile distilled water was added. After the vortex, incubated the cell suspension at 60°C for 10 min in waterbath. Immediately the tube was immersed in ice and kept for 10 min. Centrifuged it at 10,000 rpm for 15 sec. the supernatant was transferred to a new tube. DNA is ready to be used as a template for *toxR* genes detection in *V. parahaemolyticus*.

PCR was carried out using 0.5 ml microfuge tubes. The total volume reaction mixtures were 10.0 μl which contained 2.7 μl sterile distilled water, 5.0 μl 2X Phusion HSII High-

Fidelity PCR (Thermo Scientific, Lithuania), 0.5 μl of each primer and 1.0 μl DNA template. The cycling conditions were as follows; initial denaturation at 94°C for 3.3 min, denaturation at 94°C for 1 min, annealing at 59.6°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 4 min at the end of 35 cycles. Checked the results with Gel doc. *V. parahaemolyticus* appeared as 368 bp.

2.3 Radish extraction and Preparation

Blended radish using a blender. Radish was soaked in ethanol at 95% for 7 days. Filtered it with cheesecloth and no 1. Whatman filter paper. Evaporated the solvent with Rotary vacuum evaporation. Freeze-dried crude extract to get radish powder. Diluted it with sterile distilled water 100 μl at 3 concentrations. At 25% prepared 2.5 mg, 50% prepared 5 mg and 75% prepared 7.5 mg.

2.4 Antimicrobial activity test

Test the antimicrobial activity of radish extract by disk diffusion method. *V. parahaemolyticus* was cultured in Luria-Bertani broth at 35-37°C overnight. Transferred it to 0.85% NaCl to make the bacterial turbidity equal to the McFarland standard No.0.5. Swabbed it on the Mueller Hinton Agar (supplemented with 2% w/v NaCl) using sterile Cotton Tipped Applicators. 6 mm. diameter of filter paper disks impregnated with 15 μl of antimicrobial agent placed on the

surface. Incubated at 37°C for 18-24 h. Observed and recorded the inhibition zone of radish extract at various concentrations compared to 94% pure AITC (Thermo Scientific, China). In this experiment, Doxycycline was used as a positive control, and sterile distilled water was used as a negative control.

3. Result and Discussion

3.1 Isolation of *V. parahaemolyticus* from seafood

Examples of all 5 types of seafood were shrimp, oyster, squid, fish, and crab. They were diluted with PBS by tenfold dilution. The substance will be obtained at the 4th level of dilution which were 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} . Repeated the experiment 3 times. Each type of seafood selected 4 tubes with the most opaque. *V. parahaemolyticus*-like organisms were found growing on TCBS agar on approximately 8 plates of total 20 plates. 2 isolates of *V. parahaemolyticus* were detected from shrimp. (Table 1)

Type of seafood	Number of sample plates	<i>V. parahaemolyticus</i> -like organisms		<i>V. parahaemolyticus</i> isolated from seafood (Number of plates)
		Number of plates	Percentage	
Shrimp	4	2	50	2
Oyster	4	3	75	0
Squid	4	2	50	0
Fish	4	1	25	0
Crab	4	0	0	0
Total	20	8	40	2

Table 1. Isolation of *V. parahaemolyticus* from seafood

3.2 PCR of *Vibrio* isolates from seafood samples.

Detection of *toxR* gene of *V. parahaemolyticus* isolated from seafood by polymerase chain reaction (PCR) electrophoresed on 1.5 % (w/v) agarose gel. Bacteria strains were positive result to have *toxR*-gene producing amplicon of approximately 300 bp molecular size similarly to 368 bp which is specific identification of *V. parahaemolyticus* size. (Fig. 1)

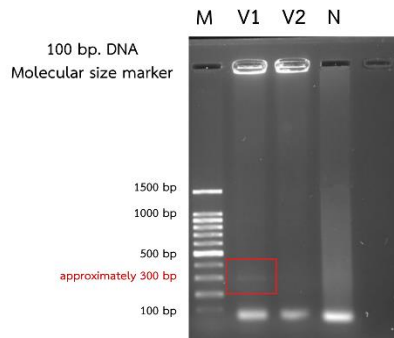


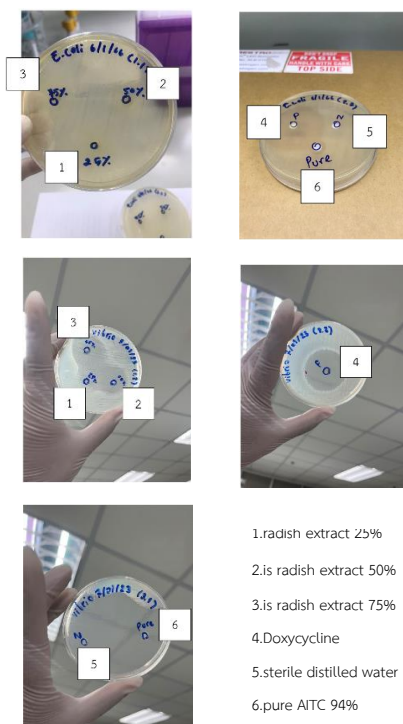
Fig. 1 Gel electrophoresis of the *toxR* gene (368bp) detection of *V. parahaemolyticus* from seafood

3.2 Antimicrobial efficacy of radish extract at various concentrations.

Antimicrobial agent	Inhibition zone (mm)	
	<i>V. parahaemolyticus</i>	<i>E. coli</i>
Radish 25%	4.3	0
Radish 50%	7.15	0
Radish 75%	11.15	0
Pure AITC 94%	N/A	3.33
Doxycycline (P)	34.3	19.33
Sterile distilled water (N)	0	0

Table 2. Inhibition zone measurement of radish extract against *V. parahaemolyticus* and *E. coli* at various concentrations.

From the table, the inhibition zone of Pure AITC is not available because it can inhibit a lot of *V. parahaemolyticus* Seeing as a clear and wide band that cannot measure the diameter.



1. radish extract 25%
2. is radish extract 50%
3. is radish extract 75%
4. Doxycycline
5. sterile distilled water
6. pure AITC 94%

From the experiment, it was found that all concentrations of radish extract were able to inhibit *V. parahaemolyticus* following the clear zones but the ability to inhibit bacteria is less than Pure AITC 94% and Doxycycline. At higher concentrations, clear zones will occur larger than at less concentration.

On the other hand, radish extract did not cause an inhibition zone of *E. coli*. In addition, Pure AITC 94% and Doxycycline can inhibit *E. coli* but the ability to inhibit is less than in *V. parahaemolyticus*.

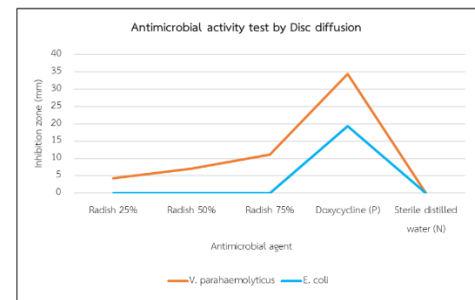


Fig. 2 Antimicrobial activity test of radish extract against *V. parahaemolyticus* and *E. coli* at various concentrations.

4. Conclusion

The results showed that *Vibrio* is a common foodborne pathogen found in undercooked seafood, especially shrimp and shellfish. The inhibition zone of *V. parahaemolyticus* was directly proportional to the concentration of radish extract but radish extract had no effect on *E. coli* inhibition. This is expected to be because the concentration is too low. The finding in this study may be useful in studying the antimicrobial activity of radish and could be further developed into a product. We hope this research helps people pay more attention to food hygiene and eat more cooked seafood.

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