

Inhibition of *Edwardsiella tarda* and Other Fish Pathogens by *Allium sativum* L. (Alliaceae) Extract

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Abstract: This study was conducted to investigate the potential of garlic (*Allium sativum*) as an antimicrobial agent to alternate commercial antibiotics for aquaculture use through evaluation of susceptibility of tested pathogenic bacteria and determination of minimum inhibitory concentration (MIC) value. The susceptibility of 18 isolates of *Edwardsiella tarda* including two Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae*) and another four Gram negative bacteria (*Citrobacter freundii*, *Escherichia coli*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*) against ampicillin, kanamycin and tetracycline were determined by Kirby-Bauer disc diffusion method whereas the sensitivity of tested bacteria against aqueous extract of *A. sativum* were evaluated through hole plate diffusion method. The MIC of antibiotics and aqueous extract of *A. sativum* were determined by two-fold serial dilution with concentration ranging from 50 mg mL⁻¹ to 0.024 mg mL⁻¹ and 500 mg mL⁻¹ to 0.24 mg mL⁻¹, respectively. The MIC values of ampicillin and kanamycin were ranged from 12.5 mg mL⁻¹ to < 0.024 mg mL⁻¹ and 0.391 mg mL⁻¹ to 0.049 mg mL⁻¹, respectively whereas the MIC values for tetracycline were ranged from 0.391 mg mL⁻¹ to < 0.024 mg mL⁻¹. On the other hand, the MIC values for *A. sativum* were ranged from 62.5 mg mL⁻¹ to 7.81 mg mL⁻¹. The aqueous extract of *A. sativum* showed huge potential as antimicrobial agent to alternate commercial antibiotic for aquaculture use as all the tested fish systemic pathogenic bacteria were found to be sensitive to the extract.

Key words: *Edwardsiella tarda* % Fish pathogenic bacteria % *Allium sativum* % MIC

INTRODUCTION

Allium sativum has cultivated for many centuries ago due to their long storage and portable. *A. sativum* usually used for cooking because of its pungent flavour [1]. It can be as seasoning as well as condiment. *A. sativum* has been reported possess medicinal values [2]. It reported can improve cholesterol profile, enhance immune system, preventing and fighting common cold, anticancer property and regulate sugar level in blood [3]. Since 1858, antimicrobial property of *A. sativum* has been reported and it was used as antiseptic to prevent gangrene during World War I and II [3]. Many studies were reported antimicrobial properties of *A. sativum* especially for human and food pathogens. For instance, [4] reported that garlic extract can inhibit the growth of 17 species of *Mycobacterium* spp. [5] found that active compound of garlic, allicin, has effect on various enzymes which can affect metabolism of virulence bacteria. Furthermore, [6]

reported that garlic extract can inhibit the growth of those bacteria that were resistance to certain antibiotics. Thus, in this study *A. sativum* extract has been studied for its antimicrobial property against fish pathogenic bacteria.

MATERIALS AND METHODS

Plant materials and antibiotic: *Allium sativum* or garlic bulb was bought from local market in Kuala Terengganu. The sample was crush into small pieces and exposed overnight to UV light in the laminar flow. The sample was then blended with sterile water at concentration of 1g/ml. The blended sample was filtered through muslin cloth and the extract was kept at 4°C until used.

Antibiotic disks that applied in this study were tetracycline 30µg/disk, kanamycin 30 µg/disk and ampicillin 10 µg/disk (Oxoid, England) for screening test. Whereas antibiotics (Sigma, USA) in powder form were

used to determine (minimum inhibitory concentration) MIC values against the present isolates.

Microorganisms: The bacteria strains that applied in the present study were local clinical isolates from diseased fish and shrimp. They were 18 isolates of *Edwardsiella tarda*, two Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae*) and another four Gram negative bacteria (*Citrobacter freundii*, *Escherichia coli*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*). All the bacteria in the present study were identified using commercial identification kit (BBL Crystal, USA). The bacterial was cultured in tryptic soy broth (TSB) (Merck, Germany) for 24 h and its concentration was adjusted into 10^6 CFU/ml for hole diffusion assay and minimum inhibitory concentration (MIC) determination using biophotometer (Eppendoff, Germany).

Screening for antibacterial activity: Hole diffusion method was used as described by [7] for screening antimicrobial property of *A. sativum* extract whereas disc diffusion was carried for antibiotic sensitivity test according to [8]. For those antibiotics that showed inhibition zone against tested bacteria were further studies for MIC test.

Determination of MIC value: Minimum Inhibitory Concentration (MIC) of *A. sativum* extract and antibiotics against the tested bacteria was determined using two fold dilution method in microtiter plate. The concentration of the *A. sativum* extract and antibiotics were ranged from 500 mg mL⁻¹ to 0.24 mg mL⁻¹ and 50 mg mL⁻¹ to 0.024 mg mL⁻¹, respectively. Each assay was run in triplicates. The inoculated plates were incubated for 37 °C for 24 hours. After incubation period, the MIC values were determined by observed the turbidity of the wells in the microtiter plate. Well of the microtiter plate that showed no turbidity was interpreted as no growth of the tested bacteria. The MIC was defined as the lowest concentration of *A. sativum* extract or antibiotics that can inhibit the growth of the tested bacterial.

RESULTS AND DISCUSSION

Table 1-4 were showed Minimum Inhibitory Concentration (MIC) of *A. sativum*, kanamycin, ampicillin and tetracycline, respectively, against tested bacteria in the present study. The MIC values of ampicillin and kanamycin were ranged from 12.5 mg mL⁻¹ to <0.024 mg mL⁻¹ and 0.391 mg mL⁻¹ to 0.049 mg mL⁻¹, respectively whereas the MIC value for tetracycline was ranged from

Table 1: Minimum inhibitory concentration of *Allium sativum* extract against the tested bacteria

Concentration (mg mL ⁻¹)	500	250	125	62.5	31.25	15.63	7.81	3.91	1.95	0.98	0.49	0.24
ET 1	-	-	-	-	-	+	+	+	+	+	+	+
ET 2	-	-	-	-	-	+	+	+	+	+	+	+
ET 3	-	-	-	-	-	+	+	+	+	+	+	+
ET 4	-	-	-	-	-	+	+	+	+	+	+	+
ET 5	-	-	-	-	-	+	+	+	+	+	+	+
ET 6	-	-	-	-	-	+	+	+	+	+	+	+
ET 7	-	-	-	-	-	+	+	+	+	+	+	+
ET 8	-	-	-	-	-	+	+	+	+	+	+	+
ET 9	-	-	-	-	-	-	+	+	+	+	+	+
ET 10	-	-	-	-	-	+	+	+	+	+	+	+
ET 11	-	-	-	-	-	+	+	+	+	+	+	+
ET 12	-	-	-	-	-	+	+	+	+	+	+	+
ET 13	-	-	-	-	-	+	+	+	+	+	+	+
ET 14	-	-	-	-	-	+	+	+	+	+	+	+
ET 15	-	-	-	-	-	+	+	+	+	+	+	+
ET 16	-	-	-	-	-	+	+	+	+	+	+	+
ET 17	-	-	-	-	-	-	+	+	+	+	+	+
ET 18	-	-	-	-	-	-	-	+	+	+	+	+
CF	-	-	-	-	+	+	+	+	+	+	+	+
EC	-	-	-	-	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	+	+	+	+	+
VV	-	-	-	-	-	-	-	+	+	+	+	+
SA	-	-	-	-	+	+	+	+	+	+	+	+
SAG	-	-	-	-	+	+	+	+	+	+	+	+

- Absence of growth, + presence of growth, CF = *Citrobacter freundii*, EC = *Escherichia coli*, ET = *Edwardsiella tarda*, VP = *Vibrio parahaemolyticus*, VV = *Vibrio vulnificus*, SA = *Staphylococcus aureus* and SAG = *Streptococcus agalactiae*

Table 2: Minimum inhibitory concentration of kanamycin against the tested bacteria

Concentration (mg mL ⁻¹)	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.098	0.049	0.024
ET 1	-	-	-	-	-	-	-	-	-	-	-	+
ET 2	-	-	-	-	-	-	-	-	-	-	-	+
ET 3	-	-	-	-	-	-	-	-	-	-	-	+
ET 4	-	-	-	-	-	-	-	-	-	-	+	+
ET 5	-	-	-	-	-	-	-	-	-	-	-	+
ET 6	-	-	-	-	-	-	-	-	-	-	-	+
ET 7	-	-	-	-	-	-	-	-	+	+	+	+
ET 8	-	-	-	-	-	-	-	-	+	+	+	+
ET 9	-	-	-	-	-	-	-	-	-	+	+	+
ET 10	-	-	-	-	-	-	-	-	-	-	-	+
ET 11	-	-	-	-	-	-	-	-	-	-	-	+
ET 12	-	-	-	-	-	-	-	-	-	-	-	+
ET 13	-	-	-	-	-	-	-	-	-	-	-	+
ET 14	-	-	-	-	-	-	-	-	-	-	-	+
ET 15	-	-	-	-	-	-	-	-	-	-	-	+
ET 16	-	-	-	-	-	-	-	-	-	-	-	+
ET 17	-	-	-	-	-	-	-	-	-	-	-	+
ET 18	-	-	-	-	-	-	-	-	-	-	-	+
CF	-	-	-	-	-	-	-	-	+	+	+	+
EC	-	-	-	-	-	-	-	-	-	+	+	+
VP	-	-	-	-	-	-	-	-	+	+	+	+
VV	-	-	-	-	-	-	-	-	-	-	-	+
SA	-	-	-	-	-	-	-	-	-	+	+	+
SAG	-	-	-	-	-	-	-	-	-	+	+	+

- Absence of growth, + presence of growth, CF = *Citrobacter freundii*, EC = *Escherichia coli*, ET = *Edwardsiella tarda*, VP = *Vibrio parahaemolyticus*, VV = *Vibrio vulnificus*, SA = *Staphylococcus aureus* and SAG = *Streptococcus agalactiae*

Table 3: Minimum inhibitory concentration of ampicillin against the tested bacteria

Concentration (mg mL ⁻¹)	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.098	0.049	0.024
ET 1	-	-	-	-	-	-	-	-	-	-	-	-
ET 2	-	-	-	-	-	-	-	-	-	-	-	+
ET 3	-	-	-	-	-	-	-	-	+	+	+	+
ET 4	-	-	-	-	-	-	-	-	-	-	-	+
ET 5	-	-	-	-	-	-	-	-	+	+	+	+
ET 6	-	-	-	-	-	-	-	-	-	-	-	+
ET 7	-	-	-	-	-	-	-	-	+	+	+	+
ET 8	-	-	-	-	-	-	-	-	+	+	+	+
ET 9	-	-	-	-	-	-	-	-	+	+	+	+
ET 10	-	-	-	-	-	-	-	-	-	-	-	-
ET 11	-	-	-	-	-	-	-	-	-	-	-	-
ET 12	-	-	-	-	-	-	-	-	-	-	-	-
ET 13	-	-	-	-	-	-	-	-	-	-	-	-
ET 14	-	-	-	-	-	-	-	-	-	-	-	-
ET 15	-	-	-	-	-	-	-	-	-	-	-	-
ET 16	-	-	-	-	-	-	-	-	-	-	-	-
ET 17	-	-	-	-	-	-	-	-	-	-	-	-
ET 18	-	-	-	-	-	-	-	-	-	-	-	-
CF	-	-	-	-	-	-	-	-	+	+	+	+
EC	-	-	-	-	-	-	+	+	+	+	+	+
VP	-	-	-	+	+	+	+	+	+	+	+	+
VV	-	-	-	-	-	-	+	+	+	+	+	+
SA	-	-	-	-	-	-	-	-	-	+	+	+
SAG	-	-	-	-	-	-	-	-	-	+	+	+

- Absence of growth, + presence of growth, CF = *Citrobacter freundii*, EC = *Escherichia coli*, ET = *Edwardsiella tarda*, VP = *Vibrio parahaemolyticus*, VV = *Vibrio vulnificus*, SA = *Staphylococcus aureus* and SAG = *Streptococcus agalactiae*

Table 4: Minimum inhibitory concentration of tetracycline against the tested bacteria

Concentration (mg mL ⁻¹)	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.098	0.049	0.024
ET 1	-	-	-	-	-	-	-	-	-	+	+	+
ET 2	-	-	-	-	-	-	-	-	-	+	+	+
ET 3	-	-	-	-	-	-	-	-	-	+	+	+
ET 4	-	-	-	-	-	-	-	-	-	+	+	+
ET 5	-	-	-	-	-	-	-	-	-	+	+	+
ET 6	-	-	-	-	-	-	-	-	-	+	+	+
ET 7	-	-	-	-	-	-	-	-	-	+	+	+
ET 8	-	-	-	-	-	-	-	-	+	+	+	+
ET 9	-	-	-	-	-	-	-	-	+	+	+	+
ET 10	-	-	-	-	-	-	-	-	-	-	+	+
ET 11	-	-	-	-	-	-	-	-	-	-	+	+
ET 12	-	-	-	-	-	-	-	-	-	+	+	+
ET 13	-	-	-	-	-	-	-	-	-	+	+	+
ET 14	-	-	-	-	-	-	-	-	-	+	+	+
ET 15	-	-	-	-	-	-	-	-	-	-	+	+
ET 16	-	-	-	-	-	-	-	-	-	-	+	+
ET 17	-	-	-	-	-	-	-	-	+	+	+	+
ET 18	-	-	-	-	-	-	-	-	+	+	+	+
CF	-	-	-	-	-	-	-	-	-	-	-	-
EC	-	-	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-	-	-
VV	-	-	-	-	-	-	-	-	-	-	-	-
SA	-	-	-	-	-	-	-	-	+	+	+	+
SAG	-	-	-	-	-	-	-	-	-	-	-	-

- Absence of growth, + presence of growth, CF = *Citrobacter freundii*, EC = *Escherichia coli*, ET = *Edwardsiella tarda*, VP = *Vibrio parahaemolyticus*, VV = *Vibrio vulnificus*, SA = *Staphylococcus aureus* and SAG = *Streptococcus agalactiae*

0.391 mg mL⁻¹ to < 0.024 mg mL⁻¹. On the other hand, the MIC value for *A. sativum* was ranged from 62.5 mg mL⁻¹ to 7.81 mg mL⁻¹. [9] claimed that generally plant extract was more effective in term of inhibit the growth of Gram positive bacteria than Gram negative bacteria. This was supported by the finding of [10] showed that extract of galls of *Quercus infectoria* can inhibit the growth of Gram positive bacteria better than Gram negative bacteria. However, garlic extract of the present study was found more effective to control Gram negative bacteria (except for *Citrobacter freundii* and *Escherichia coli*) than *Streptococcus agalactiae* and *Staphylococcus aureus*. The important finding of the present study showed *A. sativum* extract was found effective in controlling all the tested pathogenic bacteria. Thus, it is clearly showed that this plant has huge potential as antimicrobial agent for aquaculture use. However, [11] reported that *A. sativum* was not significantly can control *Mycobacterium marinum* infection in European sea bass culture. Although, kanamycin, ampicillin and tetracycline were found to be effective in control fish pathogenic bacteria at lower concentration than *A. sativum* extract but they were banned by many countries and Malaysian

government due to public health concern and environment.

CONCLUSION

A. sativum was found can control all the tested pathogenic fish bacteria however it is not as effective as antibiotics especially tetracycline. Tetracycline was the most effective among the tested antibiotics. Further study has to carry out as this study revealed the huge potential of *A. sativum* as antimicrobial agent for aquaculture use.

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