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Potential of Edible Plants as Remedies of Systemic Bacterial Disease Infection in Cultured Fish

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Abstract: Extracts of 16 edible plant species that are available in wet market of Terengganu, Malaysia were screened for antibacterial activity against ten isolates of pathogenic fish bacteria including *Aeromonas hydrophila*, *Citrobacter freundii*, *Edwardsiella tarda*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalatiae*, *Streptococcus aginosus*, *Vibrio alginolyticus*, *V. parahaemolyticus* and *V. vulnificus*. All the isolates in the present study were sensitive to extract of *Allium sativum* (L.) (Alliaceae), *Citrus microcarpa* (Bunge.) (Rutaceae) and *Citrus aurantifolia* (Christm.) Swingle (Rutaceae). Minimum inhibitory concentration values of plant extracts against tested bacteria were ranged from 62.5 mg/ml to 7.81 mg/ml. Thus, *A. sativum*, *C.* microcarpa and *C. aurantifolia* have great potential to replace commercial antibiotics that are available in the market to combat systemic bacterial disease infection in cultured fishes.

Key word: Antimicrobial · Aquaculture · Edible plant

INTRODUCTION

The decline of fish from natural aquatic resources and the increasing demands for fish, shrimp and other aquatic organisms from the market are two main factors expanding aquaculture nowadays. Antibiotics have played major role in aquaculture in terms of health management. Theirs advantages include being readily available, having multiple disease efficacies and their versatile application. However, their residues may accumulate in the flesh of fish; subsequently it has become a human health hazard [1]. Due to this reason, antibiotics can no longer be applied legally in aquaculture. Many countries including Singapore and the European Union have banned imported aquaculture products which are detected to contain antibiotic residues. In view of this new drugs should be developed to replace the antibiotics that are needed to prevent and control of diseases in aquaculture.

Currently, many researchers primarily focus and explore the antimicrobial properties that can be derived from plants. Many studies showed that the extract from plants have a great potential to be used as antimicrobial agents. For instance, the study of [2] showed that garlic and clove extracts are bactericidal against *Staphylococcus epidermis* and *Salmonella typhi* that are human pathogenic bacteria. A study by [3] revealed that *Citrus* fruit juices are effective in preventing infection by *Vibrio* species. Thus, the potential of plant extract as chemotherapy agent cannot be denied. However, the study on the antimicrobial properties of plants against fish pathogenic bacteria is still lacking. Therefore, this study was carried out to explore and find out the potential of edible plants to combat fish pathogenic bacteria.

MATERIALS AND METHODS

Microorganisms: Bacterial used in the present study were isolated from moribund fishes and shrimps and identified using commercial identification kit (BBL Crystal, USA). The bacterial isolates were *Aeromonas hydrophila*, *Citrobacter freundii*, *Streptococcus*

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agalatiae and *Streptococcus aginosus* isolated from red hybrid tilapia (*Tilapia* sp.), *Escherichia coli* and *Edwardsiella tarda* isolated from African Catfish (*Clarias gariepinus*), *Staphylococcus aureus* isolated from goldfish (*Carrassius aureus*), *Vibrio alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* isolated from tiger shrimp (*Penaeus monodon*). Overnight cultured bacteria in Brain Heart Infusion broth (Oxoid, England) were adjusted to a suspension of 10^6 CFU/ml by using bio photometer (Eppendoff, Germany).

Preparation of edible plants extracts: A total of 16 types of edible plants were bought from Kuala Terengganu's local market in Malaysia on December 2006. These edible plants are *Abelmoschus esculentus* (L.) (Malvaceae), *Allium ascalonicum* (L.) (Alliaceae), *A. cepa* (L.) (Alliaceae), *A. fistulosum* (L.) (Alliaceae), *A. sativum* (L.), *Centella asiatica* (L.) (Apiaceae), *Citrus microcarpa* (Bunge.), *C. aurantifolia* (Christm.) Swingle, *Curcuma longa* (L.) (Zingiberaceae), *Cymbopogon citrate* (DC.) Stapf. A (Gramineae), *Mentha arvensis* Jacq. (Lamiaceae), *Parkia speciosa* Hassk. (Fabaceae), *Polygonum minus* Huds. (Polygonaceae), *Psophocarpus tetragonolobus* (L.) (Fabaceae), *Solanum lycopersicum* (L.) (Solanaceae) and *Zingiber officinale* Rosc. (Zingiberaceae). All the plants were identified by Dr Chuah Tse Seng from Department of Agrotechnology, Faculty of Agrotechnology and Food Science, Universiti of Malaysia Terengganu (UMT), Malaysia. All the voucher specimens of the plants were kept in Laboratory of Fish Disease in UMT. In the present study, three different methods were applied; namely method A, method B and method C.

Method A: The first batch of edible plants was prepared according to the method of [4] with some modification. Each edible plant was air-dried and finely blended. Sterile distilled water was added to make a concentration of 10% (dry weight of matter/volume of water. The suspensions were kept for 24 h at room temperature for hydration and extraction. Then, they were stored until at –20°C until antibacterial assay was conducted.

Method B: The second batch of edible plants was prepared according to the method of [5]. The edible plants were air dried and finely blended. Samples of edible plants (20 g) were soaked for 5 days in 100 ml of 70% methanol. For the aqueous extracts, edible plants were soaked for 2 days in 100 ml of water. All extracts were filtered through No. 1 Whatman paper and evaporated to dryness in current air at 30°C. Then, the dry crude extracts were irradiated with ultraviolet light for 24 h for sterilization. Finally, the extracts were concentrated to 500 mg/ml in 70% methanol or sterile water and stored at 4°C.

Method C: The third batch of edible plants was prepared immediately after bought from local market. The edible plants were subjected to ultraviolet for 30 min. Then, they were cut and finely blended. Finally, the samples were concentrated to 1 g/ml in sterile water and stored at 4°C until antibacterial assay was conducted.

Antibacterial assay: The antibacterial activity of the edible plants extracts was performed by the hole-plate diffusion method whereas the sensitivity of the tested bacteria against antibiotic disks that applied in the present study such as sulphamethoxazole (25 µg/disk), nalidixic acid (30 µg/disk) and ampicillin (10 µg/disk) was determined by the disk diffusion method. The bacterial suspension was swab on the Mueller Hinton (Oxoid, England) and left for 10 min. Holes were aseptically bored into agar with a hollow punch and 60 µl of the extracts were placed into wells with a sterile pipette. For the antibiotic disks were placed on the inoculated agar plate. The plates were incubated at 37°C for 24 h. All the tests were run in duplicate. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the holes. Antibacterial activity was assessed qualitatively as: (-), no inhibition; (+), zone of inhibition 10 – 19 mm; (++), zone of inhibition 19 – 29 mm; (+++), zone of inhibition 29 – 39 mm; (++++), > 39 mm.

Minimum Inhibitory Concentration (MIC) value determination: Minimum inhibitory concentration (MIC) test was carried out for those plant extracts or antibiotics that showed inhibition zone against the tested bacteria. MIC of plant extracts and antibiotics against the tested bacteria was determined using two fold dilution method in microtiter plate. The concentration of the plant extracts and antibiotics were ranged from 500 to 0.24 mg/ml and 50 to 0.024 mg/ml, respectively. Each assay was run in triplicates. The inoculated plates were incubated for 37°C for 24 h. After incubation period, the MIC values were determined by observing the turbidity of the wells in the microtiter plate. Microtiter plate that showed no turbidity was interpreted as negative (no growth) of the tested bacteria and presence of turbidity was interpreted as positive. The MIC was defined as the lowest concentration of plant extracts or antibiotics that can inhibit the growth of the tested bacterial.

RESULTS AND DISCUSSION

This is the first report that has revealed the antimicrobial properties of edible plants available local market of Terengganu, Malaysia. The objective of this study was to find out the potential edible plants extracts to replace commercial antibiotic as chemotherapy agent to combat fish systemic bacterial diseases. The results of the present study have been shown in Table 1-6. Although Method A, B and C generated different results of antimicrobial property of each edible plant, it clearly showed that *A. sativum* and other 2 types of lime extracts prepared by using Method A and C have great potential to be applied in aquaculture health management

Table 1: Antimicrobial actvities of 16 edible plants extracts prepared using method A

	AH	CF	EC	ET	SA	StA	StAs	VA	VP	VV
Abelmoschus esculentus L. (Malvaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Allium ascalonicum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium cepa L. (Alliaceae) (Rhizome)	+	-	-	+	-	-	-	-	+	-
Allium fistulosum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium sativum L. (Alliaceae) (Rhizome)	+	+	+	++	++	++	+	++	+	++
Centella asiatica L. (Apiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Citrus microcarpa L. (Rutaceae) (Fruit)	+	+	+	++	++	+	+	+	+	+
Citrus aurantifolia L. (Rutaceae) (Fruit)	+	+	+	++	++	+	+	++	++	++
Curcuma longa L. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Cymbopogon citratus (DC.) Stapf. A (Gramineae) (Stem)	-	-	-	-	-	-	-	-	-	-
Mentha arvensis Jacq. (Lamiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Parkia speciosa Hassk. (Fabaceae) (Fruit)	++	-	-	-	+	+	+	-	+	-
Polygonum minus Huds. (Polygonaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Psophocarpus tetragonolobus L. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Solanum lycopersicum L. (Solanaceae) (Fruit)	-	-	-	-	-	-	-	+	-	-
Zingiber officinale Rosc. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-

Aeromonas hydrophila, (AH); *Citrobacter freundii*, (CF); *Edwardsiella tarda*, (ET); *Escherichia coli*, (EC); *Staphylococcus aureus*, (SA); *Streptococcus agalatiae*, (StA); *Streptococcus aginosus*, (StAs); *Vibrio alginolyticus*, (VA); *Vibrio parahaemolyticus* (VP) and *Vibrio vulfinificus* (VV), (-), no inhibition; (+), zone of inhibition 10 – 19 mm; (++), zone of inhibition 19 – 29 mm; (+++), zone of inhibition 29 – 39 mm; (++++), > 39 mm

Table 2: Antimicrobial activities of 16 edible plants extracts prepared using method B

	AH		CF		EC		ET		SA		StA		StAs		VA		VP		VV	
Plant extracts (Family) (Part)	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
Abelmoschus esculentus L. (Malvaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allium ascalonicum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	+
Allium cepa L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	++	-	+	-	-	-	-	-	+	-	+	-	+
Allium fistulosum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	+
Allium sativum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Centella asiatica L. (Apiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrus microcarpa L. (Rutaceae) (Fruit)	+	+	+	++	++	+	+	+	+	+	+	+	+	++	++	+	+	+	+	+
Citrus aurantifolia L. (Rutaceae) (Fruit)	+	+	+	++	++	+	+	++	++	++	+	+	+	++	++	+	+	++	++	++
Curcuma longa Linn. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Cymbopogon citratus (DC.) Stapf. A (Gramineae) (Stem)	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Mentha arvensis Jacq. (Lamiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parkia speciosa Hassk. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polygonum minus Huds. (Polygonaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Psophocarpus tetragonolobus L. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Solanum lycopersicum L. (Solanaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zingiber officinale Rosc. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

Aeromonas hydrophila, (AH); *Citrobacter freundii*, (CF); *Edwardsiella tarda*, (ET); *Escherichia coli*, (EC); *Staphylococcus aureus*, (SA); *Streptococcus agalatiae*, (StA); *Streptococcus aginosus*, (StAs); *Vibrio alginolyticus*, (VA); *Vibrio parahaemolyticus* (VP) and *Vibrio vulfinificus* (VV), (-), no inhibition; (+), zone of inhibition 10 – 19 mm; (++), zone of inhibition 19 – 29 mm; (+++), zone of inhibition 29 – 39 mm; (++++), > 39 mm

Table 3: Antimicrobial activities of 16 edible plants extracts prepared using method C

Plant species (Part)	AH	CF	EC	ET	SA	StA	StAs	VA	VP	VV
Abelmoschus esculentus L. (Malvaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Allium ascalonicum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium cepa L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium fistulosum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium sativum L. (Alliaceae) (Rhizome)	++++	++	++	+++	+++	++	++	++	++	++
Centella asiatica L. (Apiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Citrus microcarpa L. (Rutaceae) (Fruit)	+	+	+	++	++	+	+	+	+	+
Citrus aurantifolia L. (Rutaceae) (Fruit)	+	+	+	++	++	+	+	++	++	++
Curcuma longa Linn. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Cymbopogon citratus (DC.) Stapf. A (Gramineae) (Stem)	-	-	-	-	-	-	-	-	-	-
Mentha arvensis Jacq. (Lamiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Parkia speciosa Hassk. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Polygonum minus Huds. (Polygonaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Psophocarpus tetragonolobus L. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Solanum lycopersicum L. (Solanaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Zingiber officinale Rosc. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-

Aeromonas hydrophila, (AH); *Citrobacter freundii*, (CF); *Edwardsiella tarda*, (ET); *Escherichia coli*, (EC); *Staphylococcus aureus*, (SA); *Streptococcus agalatiae*, (StA); *Streptococcus aginosus*, (StAs); *Vibrio alginolyticus*, (VA); *Vibrio parahaemolyticus* (VP) and *Vibrio vulfinificus* (VV), (-), no inhibition; (+), zone of inhibition 10 – 19 mm; (++), zone of inhibition 19 – 29 mm; (+++), zone of inhibition 29 – 39 mm; (++++), > 39 mm

Table 4: Minimum Inhibitory Concentration (MIC) (mg/ml) value of 16 edible plants extracts prepared using method A

Antibiotic	AH	CF	EC	ET	SA	StA	StAs	VA	VP	VV
Ampicillin	-	-	-	-	≤0.024	-	≤0.024	-	-	-
Sulphamethoxazole	-	-	-	-	0.049	-	-	-	-	-
Nalidixic acid	≤0.024	0.049	≤0.024	-	-	≤0.024	-	≤0.024	≤0.024	≤0.024
Plant species (Part)										
Abelmoschus esculentus L. (Malvaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Allium ascalonicum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium cepa L. (Alliaceae) (Rhizome)	62.5	-	-	62.5	-	-	-	-	62.5	-
Allium fistulosum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium sativum L. (Alliaceae) (Rhizome)	62.5	62.5	62.5	31.5	31.5	31.5	62.5	31.5	62.5	31.5
Centella asiatica L. (Apiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Citrus microcarpa L. (Rutaceae) (Fruit)	62.5	62.5	62.5	31.5	31.5	62.5	62.5	62.5	62.5	62.5
Citrus aurantifolia L. (Rutaceae) (Fruit)	62.5	62.5	62.5	31.5	31.5	62.5	62.5	31.5	31.5	31.5
Curcuma longa Linn. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Cymbopogon citratus (DC.) Stapf. A (Gramineae) (Stem)	-	-	-	-	-	-	-	-	-	-
Mentha arvensis Jacq. (Lamiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Parkia speciosa Hassk. (Fabaceae) (Fruit)	31.5	-	-	-	62.5	62.5	62.5	-	62.5	-
Polygonum minus Huds. (Polygonaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Psophocarpus tetragonolobus L. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Solanum lycopersicum L. (Solanaceae) (Fruit)	-	-	-	-	-	-	-	62.5	-	-
Zingiber officinale Rosc. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-

Aeromonas hydrophila, (AH); *Citrobacter freundii*, (CF); *Edwardsiella tarda*, (ET); *Escherichia coli*, (EC); *Staphylococcus aureus*, (SA); *Streptococcus agalatiae*, (StA); *Streptococcus aginosus*, (StAs); *Vibrio alginolyticus*, (VA); *Vibrio parahaemolyticus* (VP); *Vibrio vulfinificus* (VV) and no test (-)

Table 5: Minimum Inhibitory Concentration (MIC) (mg/ml) value of 16 edible plants extracts prepared using method B

	AH		CF		EC		ET		SA		StA		StAs		VA		VP		VV	
Plant extracts (Family) (Part)	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
Abelmoschus esculentus L. (Malvaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allium ascalonicum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	62.5	-	62.5	-	-	-	-	-	62.5	-	62.5	-	62.5
Allium cepa L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	31.5	-	62.5	-	-	-	-	-	62.5	-	62.5	-	62.5
Allium fistulosum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	62.5	-	62.5	-	-	-	-	-	62.5	-	62.5	-	62.5
Allium sativum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	62.5	-	-	-	-	-
Centella asiatica L. (Apiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrus microcarpa L. (Rutaceae) (Fruit)	62.5	62.5	62.5	31.5	31.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	31.5	31.5	62.5	62.5	62.5	62.5	62.5
Citrus aurantifolia L. (Rutaceae) (Fruit)	62.5	62.5	62.5	31.5	31.5	62.5	62.5	31.5	31.5	31.5	62.5	62.5	62.5	31.5	31.5	62.5	62.5	31.5	31.5	31.5
Curcuma longa Linn. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	62.5	62.5	-	-	-	-	-	-	-	-	-	-	-
Cymbopogon citratus(DC.) Stapf. A(Gramineae)(Stem)	-	-	-	-	-	-	62.5	62.5	-	-	-	-	-	-	-	-	-	-	-	-
Mentha arvensis Jacq. (Lamiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parkia speciosa Hassk. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polygonum minus Huds. (Polygonaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Psophocarpus tetragonolobus L. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Solanum lycopersicum L. (Solanaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zingiber officinale Rosc. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	62.5	-	-	-	-	-	-	-	-	-	-	-	-

Aeromonas hydrophila, (AH); *Citrobacter freundii*, (CF); *Edwardsiella tarda*, (ET); *Escherichia coli*, (EC); *Staphylococcus aureus*, (SA); *Streptococcus agalatiae*, (StA); *Streptococcus aginosus*, (StAs); *Vibrio alginolyticus*, (VA); *Vibrio parahaemolyticus* (VP); *Vibrio vulfinificus* (VV) and no test (-)

Table 6: Minimum Inhibitory Concentration (MIC) (mg/ml) value of 16 edible plants extracts prepared using method C

Plant species (Part)	AH	CF	EC	ET	SA	StA	StAs	VA	VP	VV
Abelmoschus esculentus L. (Malvaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Allium ascalonicum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium cepa L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium fistulosum L. (Alliaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Allium sativum L. (Alliaceae) (Rhizome)	7.81	31.5	31.5	15.63	15.6	31.5	31.5	31.5	31.5	31.5
Centella asiatica L. (Apiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Citrus microcarpa L. (Rutaceae) (Fruit)	62.5	62.5	62.5	31.5	31.5	62.5	62.5	62.5	62.5	62.5
Citrus aurantifolia L. (Rutaceae) (Fruit)	62.5	62.5	62.5	31.5	31.5	62.5	62.5	31.5	31.5	31.5
Curcuma longa Linn. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-
Cymbopogon citratus (DC.) Stapf. A (Gramineae) (Stem)	-	-	-	-	-	-	-	-	-	-
Mentha arvensis Jacq. (Lamiaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Parkia speciosa Hassk. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Polygonum minus Huds. (Polygonaceae) (Leaf)	-	-	-	-	-	-	-	-	-	-
Psophocarpus tetragonolobus L. (Fabaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Solanum lycopersicum L. (Solanaceae) (Fruit)	-	-	-	-	-	-	-	-	-	-
Zingiber officinale Rosc. (Zingiberaceae) (Rhizome)	-	-	-	-	-	-	-	-	-	-

Aeromonas hydrophila, (AH); *Citrobacter freundii*, (CF); *Edwardsiella tarda*, (ET); *Escherichia coli*, (EC); *Staphylococcus aureus*, (SA); *Streptococcus agalatiae*, (StA); *Streptococcus aginosus*, (StAs); *Vibrio alginolyticus*, (VA); *Vibrio parahaemolyticus* (VP); *Vibrio vulfinificus* (VV) and no test (-)

since their extracts inhibited all the tested bacteria whereas their Minimum Inhibitory Concentration (MIC) value were ranged from 62.5 to 7.81 mg/ml. However, it is not as effective as all the tested antibiotics in terms of MIC value. By using Method C, *A. sativum, C.* microcarpa and *C. aurantifolia* extracts are easier to prepare, saving time and can be applied instantly as compared to Method A. Plants extracts that prepared using Method B are the most time consuming. Furthermore, the results obtai.ed by using Method B are not promising.

Allicin, ajoene, thiosulfinates and a wide range of other organosulphata compounds, are known to be the constituents linked to the garlic properties [6]. Among the *A. sativum*'s compound is well known is for its antimicrobial activity [7] however the majority of researches are on its usefulness against human pathogenic bacteria and food-borne bacteria. According to [3], the major compound of *Citrus* sp. is citric acid. Thus, the authors concluded that citric acid possess antimicrobial property against *Vibrio parahaemolyticus*, a food-borne pathogen. This conclusion is supported by the finding of the present study that *C. microcarpa* and *C. aurantifolia* can inhibit all the tested fish pathogen in the present study.

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