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Full Length Research Paper

Antimicrobial properties of tropical plants against 12 pathogenic bacteria isolated from aquatic organisms

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Disk diffusion technique was used to determine the antibacterial activity of aqueous and methanolic extracts of edible tropical plant against 12 clinical and pathogenic bacterial strains isolated from aquatic animals. They were *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Vibrio vulnificus*, *Vibrio cholerae*, *Escherichia coli*, *Citrobacter freundii*, *Edwardsiella tarda*, *Aeromonas hydrophilla*, *Salmonella* sp., *Schewanella putrifaciens* and *Streptococcus* sp. The zone of inhibition varies depending on bacterial species and type of extract. The average diameter of inhibition zones ranges from without inhibition zone to 12 mm and without inhibition zone to 11 mm for water and methanolic extract, respectively. All the 9 plants tested showed antimicrobial activity against one or more species of bacteria. The most active antimicrobial plants were *Colocasia esculenta*, *Citrus microcarpa*, *Centella asiatica* and *Morinda citrifolia.*

Key words: Antimicrobial activity, edible plants, crude extract.

INTRODUCTION

Aquaculture is a growing industry in Malaysia. For instance, the total value of aquaculture production was recorded RM 1.275 billion in Malaysia. Due to intensive fish farming system, medications are needed to maintain animal health and manage fish populations. Commercial antibiotic such as oxolinic acid and tetracycline were applied in fish feed against a variety of bacterial pathogens of fish. Incorporating antibiotic into fish feed exposed antibiotic into environment, subsequently the emergence of antibiotic resistance among pathogenic bacterial was observed. For example, the first isolation of *Aeromonas salmoncida* resistant to a specific antibiotic has often been reported shortly after the introduction of the antibiotic into aquaculture. Thus it was clearly shown that the use of commercial antibiotic in aquaculture increases antibiotic resistance among bacteria in the exposed ecosystems. This resistance can disseminate through the environment and can be transmitted to a variety of bacterial species, including bacteria that can infect humans. Due to increase of antibiotic resistant among pathogenic bacteria, there has been the urgency for scientist to find new drugs against these pathogenic bacteria.

One of the solutions to solve antibiotic resistant incident problem among pathogenic bacteria is to develop new drug from natural sources such as plant. The biological activity of many plants has been known through scientific research and any literature search via the internet or elsewhere, would reveal that numerous new publications are added to the scientific literature review every day. Many efforts have been made to discover new antimicrobial or drug from various sources such as microorganism, animals and plants. Systematic screening of them may result new findings, drug.

In the present study, 9 types of edible plants were screened for their antimicrobial activities against 12 strains of pathogenic bacteria isolated from aquatic animals.

MATERIALS AND METHODS

Plant materials

Aloe vera, *Colocasia esculenta*, *Citrus microcarpa*, *Centella asiatica, Ipomoea reptans*, *Morinda citrifolia, Murraya koenigii*, *Pandanus odoratissimus* and *Passiflora foetida* were obtained from

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Table 1. Antibacterial activities (diameter of inhibition zone, mm) of methanol crude extracts of edible tropical plants.

Plant	Part tested	AH	CF	EC	ET	Sal	Str	SP	VA	VC	VH	VP	VV
Aloe vera	Whole plant	-	-	-	-	-	-	-	-	-	-	-	7
Centella asiatica	Whole plant	-	-	-	-	-	7	-	7	-	-	-	7
Citrus microcarpa	Leaf	-	7	-	-	-	7	-	11	-	-	8.5	9
Colocasia esculenta	Leaf	-	-	-	-	-	-	-	-	-	-	-	-
Ipomoea reptans	Leaf	-	7	-	-	-	-	-	8	-	-	7	8
Ipomoea reptans	Stem	-	7	-	-	-	-	-	9.5	-	7	-	-
Murraya koenigii	Leaf	-	-	-	-	-	-	-	-	-	-	-	-
Morinda citrifolia	Fruit	-	-	-	7	-	7	-	9.5	-	8	10.5	8
Pandanus odoratissimus	Leaf	-	-	7	-	-	-	-	-	9	-	-	-
Passiflora foetida	Leaf	-	-	-	-	-	-	-	-	-	-	-	-
Passiflora foetida	Stem	-	7	-	-	-	-	-	7	-	-	-	-

AH: *Aeromonas hydrophilla*, CF: *Citrobacter freundii*, EC: *Escherichia coli*, ET: *Edwardsiella tarda*, , Sal: *Salmonella* sp, SP: *Schewanella putrifaciens*, Str: *Streptococcus* sp., VA: *Vibrio alginolyticus*, VP: *V. parahaemolyticus*, VH: *V. harveyi*, VV: *V. vulnificus*, VC: *V. cholerae.*

Mengabang Telipot; a rural area in Terengganu, Malaysia. The plants were identified based on their physical characteristics.

Preparation of plant extract

Dried plant (15 g) was macerated with 80% methanol or water (450 ml) for five days and then filtered. The filtered was evaporated to a thick residue at 40°C.

Preparation of extract solution

The extracts were reconstituted according to the solvent that was used to extract them with the concentration of 250 mg/ml. Then the samples were kept in a water bath for 20 min at 100°C to dissolve plant extract into solvent.

Microorganisms

Vibrio alginolyticus, *Vibrio parahaemolyticus* and *Vibrio harveyi* were isolated from tiger shrimp (*Penaeus monodon*). *Vibrio vulnificus*, *Vibrio cholerae* and *Escherichia coli* were isolated from oyster (*Crassostrea iredalei*). *Citrobacter freundii*, *Edwardsiella tarda*, *Aeromonas hydrophilla* and *Salmonella* sp. were isolated from red tilapia (*Tilapia* sp). *Schwanella putrifacien* and *Streptococcus* sp were isolated from marine fish. The isolated bacteria were identified using BBL commercial identification kit with additional 15 conventional biochemical tests and confirmed by reference to Bergey Manual. All the bacteria were grown overnight in Tryptic Soy Broth (TSB) (Merck, Germany). The inoculum size of each test strain was standardized by adjusting the optical density (O. D) of the bacterial suspension to turbidity 1.0 O. D.

Screening for antibacterial activity

The disc diffusion method was used to evaluate the antibacterial activity. Mueller Hinton agar (Oxoid, England) was prepared in plates as the media for test bacteria. Sterilized filter paper discs (Whatman No. 1) with the diameter 6 mm were impregnated with 20 ul of each of the extracts (250 mg/ml) to give a final concentration of 5 mg/disc and left to dry under laminar flow cabinet. Disks injected with 20 ul of methanol and sterilized water served as negative controls. The bacterial inoculum was spread evenly onto surface of the Mueller Hinton agar plates using a sterilized cotton bud before the extract discs were positioned on the inoculated agar surface. Each extract was assayed in triplicate. Sterile distilled water and methanol served as negative control. All the plates were incubated for 24 h. The antibacterial activity was interpreted from the size of the diameter of zone inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the discs.

RESULTS

The *in vitro* antibacterial activities of the methanol and aqueous crude extracts of different parts of *A. vera*, *C. esculenta*, *C. microcarpa*, *C. asiatica, I. reptans*, *M. citrifolia, M. koenigii P. odoratissimus* and *P. foetida* against 12 pathogenic bacteria isolated from aquatic animals were examined in the present study and their potency was assessed by the presence or absence of inhibition zones and zone diameters (Table 1 and Table 2). The methanol and aqueous extracts of all plants screened showed various inhibitory effects (7 – 11 mm) and (7 – 12 mm), respectively. No effects were detected for *Aeromonas hydrophila*, *Salmonella* sp and *Shewanella. putrefaciens* against methanol extracts while *Escherichia coli* and *Salmonella* sp were resistant to all aqueous extract. *Streptococcus* sp., *E. coli*, *A. hydrophila* and *Edwardsiella tarda* were more resistant to most extracts used in the present study. The largest inhibition zone was observed from methanol and aqueous extracts against *V. alginolyticus* (11 mm) and *V. harveyi* (12 mm), respectively. *A. vera* whole plant aqueous extract showed inactivity against the listed bacteria, however, *A. vera* whole plant methanol extract showed only effect to *V. vulnificus* with diameter of the inhibition zone 7 mm. Methanol extract of *C. asiatica* whole plant showed 7 mm inhibition zone against *V. alginolyticus*, *V. vulnificus* and *Streptococcus* sp while inhibiton zone was found in *C. freundii* and all *Vibrio* sp. except *V. vulnificus* against

Table 2. Antibacterial activities (diameter of inhibition zone, mm) of aqueous crude extracts of edible tropical plants.

Plant	Part tested	AH	CF	EC	ET	Sal	Str	SP	VA	VC	VH	VP	VV
Aloe vera	Whole plant	-	-	-	-	-	-	-	-	-	-	-	-
Centella asiatica	Whole plant	-	11	-	-	-	-	-	8	9	8	8	-
Citrus microcarpa	Leaf	-	7	-	8	-	7	-	7	-	7	7	7
Colocasia esculenta	Leaf	-	9	-	-	-	-	-	9	11	12	9	8
Ipomoea reptans	Leaf	-	-	-	-	-	-	-	-	-	-	-	-
Ipomoea reptans	Stem	-	-	-	-	-	-	-	-	7	-	-	-
Murraya koenigii	Leaf	11	8	-	-	-	-	7	8	-	7	-	7
Morinda citrifolia	Fruit	-	7.5	-	7	-	-	8.5	9.5	7	9.5	7	7
Pandanus odoratissimus	Leaf	-	-	-	-	-	-	-	-	-	-	-	-
Passiflora foetida	Leaf	-	-	-	-	-	-	-	-	8	-	-	-
Passiflora foetida	Stem	-	9	-	-	-	-	-	7	11	-	9	8

AH: *Aeromonas hydrophilla*, CF: *Citrobacter freundii*, EC: *Escherichia coli*, ET: *Edwardsiella tarda*, Sal: *Salmonella* sp, SP: *Schewanella putrifaciens*, Str: *Streptococcus* sp., VA: *Vibrio alginolyticus*, VP: *V. parahaemolyticus*, VH: *V. harveyi*, VV: *V. vulnificus*, VC: *V. cholerae.*

aqueous extracted *C. asiatica* whole plant. *C. microcarpa* leaf methanol and aqueous extracts showed effect against *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *C. freundii* and *Streptococcus* sp. *C. esculenta* leaf methanol extract showed not active against listed bacteria; however, its aqueous extract showed effect against all tested *Vibrio* sp and *C. freundii*. *M. citrifolia* fruit aqueous extract showed more effect against the tested bacteria compared to the methanol extract. Methanol extract of *Murraya koenigii* showed no effect to the tested bacteria; however, aqueous extract of these plant possess antibacterial activities against *V. alginolyticus*, *V. harveyi*, *V. vulnificus*, *A. hydrophila*, *C. freundii* and *S. putrefaciens*. 66% of the tested bacteria showed inhibition zone against aqueous extract of *M. citrifolia* fruit, while aqueous extract of *M. citrifolia* showed effect against 50% of the tested bacteria. *Pandanus odoratissimus* leaf extracted with methanol showed inhibition to all tested bacteria except for *Vibrio cholerae* with the inhibition zone 7 mm. On the other hand, *P. odoratissimus* leaf aqueous extract showed effect to *E. coli* and *V. cholerae*. *P. foetida* leaf was found no effect to the tested bacteria except its aqueous extract that showed inhibition zone against *V. cholerae*. However *P. foetida* stem was found inhibition zone aganist *C. freundii* and *V. alginolyticus*. While, its aqueous extract has inhibition effect on the growth of *C. freundii, V. alginolyticus, V. cholerae, V. parahaemolyticus* and *V. vulnificus*.

DISCUSSION

Generally, plants extracts are usually more active against gram positive bacteria than gram negative bacteria. (Basri and Fan, 2005). According to Abu-Shanab et al. (2004) gram negative bacteria are more resistant to plants extract compared to gram positive bacteria. This may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism. This is in support of the present finding which showed that only 1 out of 22 plants was effective on the growth of *E. coli* while *Salmonella* sp. was resistant to all tested plant extracts. However, in the present study it was found that only 4 out of 22 plants extracts inhibited *Streptococcus* sp, a gram positive bacteria. On the other hand, 12 out of 22 plants extracts showed affect on the growth of *V. cholerae*, *V. alginolyticus* and *C. freundii*, gram negative bacteria. The incidence of bacteria resistant to plant methanol extracts was recorded as 106 cases while 96 cases were recorded as resistant to plant aqueous extracts. Many studies revealed that plants methanol extracts inhibited the growth of tested bacteria more than plants aqueous extracts. For instance, the study of Karaman et al. (2003) showed that *Juniperus oxycedrus* aqueous extract showed no effect to 56 species of bacteria; however methanol extract of *J. oxycedrus* inhibited the growth of 24 out of 56 bacteria species. Another study of Abu Shanab et al. (2005) showed that methanol extracts of the dried ripe berries of *Rhus coriaria* species inhibited all tested bacteria; however, its aqueous extract showed effect on 2 out of 5 tested bacteria. However, in the study of Nasar-Abbas and Halkman (2004), *Rhus coriaria* was extracted using water. The aqueous extract inhibited all tested 12 types of food borne bacteria including pathogens. Furthermore, the finding of Nair et al. (2005) reported that *Hibicus rosa-sinensis* aqueous extract performed more effectively than methanol extract against tested bacteria.

A. vera is a stemless plant with one to several rosettes of thick, fleshy, non-thorny leaves and erect, yellow or red flower clusters. The origin of *A. vera* is North Africa and it is an ancient cultigen and forms the basis of a very large industry, mainly in Central America and the Southern USA. A resinous extract from dried leaves has been

administered internally as a strong laxative. The gel of *A. vera* is applied for wound treatment, skin care, tonic drink and stimulant laxative. Millions of litres of gel of *A. vera* are used annually for health drinks and dietary supplements. The active ingredient in *A. vera* is aloin (an anthrone C-glucoside) as main laxative compound, the gel contains complex polysaccharides, amino acid, minerals, glycoprotein, salicylic acid and enzymes and acetylated mannan as woung therapy. However, in the present study *A. vera* whole plant methanol extract showed only effect on the growth of *V. vulnificus*.

The main use of *C. asiatica* is for treating wounds, burns and ulcers, to accelerate healing and to prevent the formation of scar tissue following surgery. The active ingredients or the main compounds in *C. asiatica* are triterpenes asiatic acid and madecassic acid, together with triterpenoid ester glycoside, known as asiaticoside and brahminoside. Also present is a volatile oil (with *p*-cymol, β-caryophyllene and farnesene). In terms of wound healing, the triterpenoids are thought to stimulate the production of human collagen I, a protein associated with wound healing. Several trials are available that have shown efficacy in the treatment of wound, burns, ulcers and the prevention of scars. Animal experiments also show sedative, anti-inflammatory and antimicrobial activities. In the present study, *C. asiatica* whole plant methanol extract was found inhibited *Streptococcus* sp, *V. alginolyticus* and *V. vulnificus*, while its aqueous extract showed effect against *C. freundii*, *V. alginolyticus*, *V. cholerae*, *V. harveyi* and *V. parahaemolyticus*.

C. esculenta belongs to the family Araceae. It contains thiamine, riboflavin, niacin, oxalic acid, calcium oxalate and sapotoxin. Traditionally, it is used to settle the stomach, to prevent swelling and pain and to reduce fever. In the present study, *C. esculenta* leaf aqueous extract was found inhibiting the growth of all tested *Vibrio* sp and *C. freundii*. However, its methanol extract showed no effect to all tested bacteria. The study of Nair et al. (2005) found that *C. esculenta* leaf methanol extract was only effective to *Klebsiella pneumonia* NCIM 2719 but its aqueous extract showed no effect to all tested bacteria.

Morinda. citrifolia has been classified as a medicinal herb due to its therapeutic properties. Various parts of the plant, including the roots, stems, leaves and fruit have been consumed solely on the basis of the assumption that it possesses healing properties. Previous research reports on *M. citrifolia* state that the fruit juice of the plant has exhibited anti-tumor activity in mice and increased the life span of mice artificially implanted with Lewis lung carcinoma. In the present study, *M. citrifolia* antibacterial property has been screened and the result showed effect on the growth of 50 and 67% tested bacteria for methanol and aqueous extract, respectively. However, in the study of Sekander and Beck (2006) *M. citrifolia* fruit methanol extract showed no effect on the growth of *Staphylococcus aureus* and *Bacillus subtilis*.

M. koenigii is a small tree with dark grey bark distributed throughout Bangladesh, India, Nepal, Malaysia, Sri Langka ang Burma. Traditionally, the plant is used as a stimulant, stomachic, diarrhea and insect bites. The study of Rahman and Gray (2005) showed that petroleum ether extracted *M. koenigii* leaf inhibited the growth of *S. aureus*, *B. subtilis*, *E. coli* and *P. vulgaris*. However, the present study showed that only aqueous extract of *M. koenigii* leaf can be effective on the growth of *A. hydrophila, C. freundii, S. putrifacien, V. alginolyticus, V. harveyi* and *V. vulnificus*.

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