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Screening for Antimicrobial Activity of Various Extracts of *Acanthophora spicifera* (Rhodomelaceae, Ceramiales) from Malaysian Waters

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Abstract: The antimicrobial activities of eight crude extracts of local *Acanthophora spicifera* (Vahl) Borgesen acquired from two different extraction; soxhlet extraction and solvent partitioning were studied. These extracts were tested *in vitro* against 18 bacteria, 3 yeasts and 6 fungi strains by Disc diffusion method. The results revealed that methanol and ethyl acetate extract from solvent partitioning exhibited broader spectrum activity against tested bacterial strains. This two extracts showed inhibition zones against strains of *Bacillus cereus* ATCC 10876, *Bacillus licheniformis* ATCC 12759, Menthicilin Resistance *Staphyloccocus aureus* (MRSA), *Pseudomonas aeruginosa* ATCC 27853, *Yersinia* sp. and *Citrobacter freundii*. While methanol extracts from Soxhlet extraction and butanol from solvent partitioning had no effect against *P. aeruginosa* ATCC 27853, the other six extracts exhibited antibacterial activity against this opportunistic strain. Extracts showed a moderate average zone of inhibition ranged from 9.00-14.00 mm. *Yersinia* sp. showed the higher inhibition zones of 12.00 mm. However, no antifungal activity observed against tested strains of yeasts and fungi. The MIC and MBC values of selected bacterial strains tested ranged from 31.25-1000 μg mL^{-1} and 500-2000 μg mL^{-1}.

Key words: *Acanthophora spicifera*, antimicrobial activity, Disc diffusion method, minimum bactericidal concentration, minimum inhibitory concentration, Malaysia

INTRODUCTION

Decreasing efficiency and resistant of pathogens to antimicrobial drugs made the search of a new antimicrobial agent an important strategy for the establishment of alternative therapies in difficult handling infections. For example, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* that causes diseases like diarrhea, mastitis, abortion and upper respiratory complications (Levine, 1987; Jawetz *et al*., 1995). In recent years, multiple resistances in human pathogenic microorganism have developed due to the indiscriminate used of antibiotic drugs commonly employed in the treatment of infectious diseases. The undesirable side effects of certain antibiotics and the emergence of previously uncommon infections have forced scientists into looking for new antibiotic substances from various sources like marine macroalgae.

Marine macroalgae are the renewable living sources which are used as food, feed and fertilizer in many parts of the world. They are low calories foods but rich in vitamins and minerals. They are also potentially good source of proteins, polysaccharide and fibers. Besides that it has been reported that macroalgae serve as an important source of bioactive natural substances (Smit, 2004). Many metabolites isolates isolated from macroalgae have been shown to possess bioactive effects (Faulkner, 2002). Previous studies have proved that macroalgae possess broad range of biological activities such as antibiotics, antiviral, anti-inflamatory, cytotoxic and antimitotis (Navqi *et al*., 1980; Cacamese *et al*., 1981; Fenical and Paul, 1984; Hodgson, 1984; Ballesteros *et al*., 1992; Bhosale *et al*., 2002). Harder (1917) was the first to observe antimicrobial substances by algae. Since then, the demand for screening of natural bioactive compounds has widened the interest of researchers.

In this study, *A. spicifera* (Vahl) Borgesen *(Ceramiales: Rhodophyta)* or commonly known as spiny seaweed is chosen as subject. This alga is a rhodophycean alga with a wide distribution throughout the tropics and subtrophics (Kilar and McLachlan, 1986). It is also one of the most abundant red algal species found on reef flats (Joikel and Morrissey, 1986). *A. spicifera* is an erect plant with solid cylindrical branches (2-3 mm wide) which is branched either sparingly too repeatedly. Main branches have short, determinate branches, irregular shapes and spinose with spines numerous and radially arranged. Thus, it is also called spiny seaweed. The morphology vary as a result of environment in which it grows. Red, purple, yellow,

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orange and brown morphophytes are all in common natural habitat. To date, research on biologically active substances of this species is rather limited (Aihara and Yamato, 1968; Prakash *et al*., 1989; Wahidulla and Kamat, 1991; Wahidulla *et al*., 1986, 1991, 1998; Wang *et al*., 1998) especially in Malaysia.

Some of the previous studies on this species were reported from India (Navqi *et al*., 1980), Coast of Urla, Turkey (Tuney *et al*., 2006), Southern Brazil (Lima-Filho *et al*., 2002) and Hawaiian (Perez *et al*., 1990). Therefore, the aim of the present study was to evaluate the antibacterial and antifungal activities of a marine alga *A. spicifera* which is abundantly available in Malaysian waters. These pre-screening results reported herein could serve as a basis to isolate and identify the antibacterial compounds from seaweeds extracts as a source of natural antibacterial agents for application in pharmaceutical industries.

MATERIALS AND METHODS

Sample collection and preparation: A fresh *A. spicifera* was collected by hand picking method from the flouting buoy used for floating cage aquaculture in pulau gedung, penang. Seaweed was collected during January, August and September 2008 and was identified by referring to a book entitle Rumpai Laut Malaysia (Ismail, 1995) and finally was authenticated by Associate Professor Shaida Fariza Sulaiman from School of Biological Science, University Sains Malaysia.

Microorganisms: About 18 bacterial strains which were divided into two groups, American Type Culture Collection (ATCC) (Rockville, MD and U.S.A) and clinical isolates groups were used in this study. The ATCC group consisted of *Bacillus cereus* ATCC 10876, *Bacillus licheniformis* ATCC 12759, *Bacillus spizizenii* ATCC 6633, *Staphylococcus aureus* ATCC 12600, *Staphylococcus epidermidis* ATCC 12228, *Shigella boydyii* ATCC 9207, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas stutzeri* ATCC 17588.

The clinical isolates used were *Acinitobacter anitratus*, *Bacillus subtilis*, *Citrobacter freundii*, *Escherichia coli*, *Erwinia* sp., *Klebsiella pneumoniae*, Methicillin resistance-*Staphylococcus aureus* (MRSA) and *Yersinia* sp. Three yeasts strains which were *Candida utilis*, *Candida albicans* and *Saccahromyces cerevisiae* and six fungal strains which were *Aspergillus niger*, *Microsporum gypseum*, *Penicillium* sp., *Rhizopus* sp., *Trichoderma viridae* and *Trichophyton rubrum* were also studied. All of the bacterial, yeast and fungal strains were obtained from the Industrial Biotechnology Research Laboratory and Plant Phytochemistry laboratory, School of Biological Science, University Sains Malaysia. The bacterial isolates were maintained on Nutrient Agar (NA) slants incubated at 37°C for 24 h while Potato Dextrose Agar (PDA) slants were used to maintain the yeast (at 30°C for 24 h) and fungal cultures at 30°C for 24-72 h (until sporulation). All the cultures were kept at 4°C until further used.

Extract preparation: After collected from sampling site, the fresh algal sample was rinsed several times with sea water to removed debris and epiphytes prior transported to the laboratory. At the laboratory, the algal sample was further washed with tap water and gently brushed with a soft brush to remove epiphytes and other marine organisms. The algal was then dried in a dryer at 60°C for 24-72 h. Then, the dried algal sample was cut into small pieces and powdered using an electrical blender.

Extraction procedure: The algal powdered sample was extracted using two methods; Soxhlet extraction and solvent partitioning.

Soxhlet extraction method: About 20 g of powdered algal sample was placed into the soxhlet apparatus and the extraction was performed based on solvents polarity. The solvents used were hexane followed by ethyl acetate, chloroform and methanol (v/v), respectively. The extraction process took about 24-48 h to complete which was indicated by the change of solvent colours to colourless in the soxhlet extraction chamber. The extracts were evaporated using a rotary evaporator under reduced pressure until an oily paste formed. The paste extracts were then left air dried in the fume cupboard. The pastes were kept at 4°C for further used.

Solvent partitioning method: Approximately, 50 g of powdered sample was added into 500 mL methanol at the ratio 1:10 (w/v) and soaked for 3 days at temperature (28±2°C). Removal of the algal residue from the solvents was done by filtration through a double layered cheese cloth. The filtrate was concentrated using a rotary evaporator under reduced pressure. The methanol extract obtained was further partitioned in diethyl ether (3×100 mL). Consequently, the aqueous layer formed was further partitioned in ethyl acetate (3×100 mL) and butanol (3×100 mL) (Duan *et al*., 2006). Fractions collected were evaporated to dryness under reduced pressure using a rotary evaporator and left air dried in fume cupboard into paste form. Then the dried paste extracts were stored at 4°C for further used.

Antimicrobial activity test: Antimicrobial activity was evaluated by a disc diffusion method (Darah *et al*., 2006). About 100 mg of crude extracts was dissolved in 1 mL of methanol. Extracts were vortex to ensure thorough mixing. Each assay in this experiment was performed in triplicate. Chloramphenicol (for bacteria) and ketoconazole (for yeasts and fungi) was used as the standard antimicrobial agent.

The test microbes were removed aseptically with an inoculating loop from slants and suspended into universal bottles containing 10 mL of sterile distilled water. The turbidity of the bacterial and yeasts suspension was compared with 0.5 Mc Farland standard and adjusted to 1.0×10^5 and 1.0×10^6 cells mL^{-1}, respectively. While the density of spore suspension for fungi was determine using haemocytometer and adjusted to 4.0×10^5 spore mL^{-1}. The suspension (1 mL) was added into 20 mL of molten Nutrient Agar (NA) and gently shakes before poured it into a sterile petri dish.

The Potato Dextrose Agar (PDA) was used for yeasts and fungi. The mixtures were left to solidify for 15 min. Sterile paper discs (6 mm diameter) prepared from Whatman no. 1 filter paper were impregnated with 20 μL of 100 mg mL^{-1} extracts (2 mg $disc^{-1}$) or chloramphenicol (30 μg mL^{-1}) or ketoconazole (30 μg mL^{-1}) and air dried. After drying, discs were aseptically placed on the nutrient agar (for bacteria) and potato dextrose agar (for fungi and yeasts). Chloramphenicol (for bacteria) and ketoconazole (for yeasts and fungi) were used as positive controls. Whereas, diluting solvent (methanol) was used as a negative control. The inoculated plates were incubated at 37°C for 24 h for bacteria, 30°C for 24 h for yeasts and 30°C for 24-72 h for fungi. Results were recorded by measuring the zones of growth inhibition surrounding the disc (mm).

Determination of Minimum Inhibitory Concentrations (MIC): The determination of MIC was conducted using tube dilution method (Treagan and Pulliam, 1982) with a slight modification. Briefly, extracts were subjected to a series of serial dilution to give final concentrations between 3.9-2000 μg mL^{-1}. Extracts (1000 μL) with different concentration was added aseptically into different labeled test tubes containing 1500 μL sterile Nutrient Broth (NB). Then, 500 μL of bacterial suspension was inoculated into respective test tubes.

The test tubes were vortex and incubated at 37°C for 21-24 h. The MIC value was measured by comparing the turbidity of the whole series of the test tubes with a negative control (nutrient broth inoculated with bacteria) and two positive controls (nutrient broth with extract and nutrient broth only). MIC value was stated as the highest concentration that showed no turbidity which indicates no growth of bacteria. Each test was performed in triplicate. In this study, only selected bacteria with the inhibition zone of >8.0 mm was tested.

Determination of Minimum Bactericidal Concentration (MBC): The minimal bactericidal concentration (MBC) was performed according to Doughari (2006). A loop full of broth from the test tube that show no visible growth which regarded as the MIC value was streaked onto the sterile Nutrient Agar (NA) plate and incubated at 37°C for 24 h. The Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration of extract that completely prevented microbial growth and was determined by visible inspection of the NA plates. MBC assays were carried out in triplicates.

Determination of mechanisms of antibiosis (bacteriostatic or bactericidal): The mechanism of antibiosis of the extracts was calculated using the ratio of MBC/MIC as described by Shanmughapriya *et al*. (2008). When the ratio of MBC/MIC was ≤2.0, the extract was considered bacteriostatic and otherwise bactericidal. If the ratio is ≥16.0 the extract was considered ineffective.

RESULTS

The antimicrobial activities of *Acanthophora spicifera* extracts against microorganisms were examined in this study and their potency were assessed by the diameter of presence inhibition zones and the MIC values. The antimicrobial activity of the various extract of *A. spicifera* from partitioning method is shown in Table 1. Among the extracts, crude Methanol Extract (ME) showed antibacterial activity against 2 g negative bacteria tested, *P. aeruginosa* ATCC 27853 and *Yersinia* sp. with inhibition zones of 10.00-14.00 mm diameter. The extract also showed some antibacterial activity against gram positive bacteria, *Bacillus cereus* ATCC 10876, *B. licheniformis* ATCC 12759, MRSA and *S. aureus* ATCC 12600 with about 9.00 mm diameter of inhibition zones. The rest of the bacteria seem to be resistance to the extract.

The Diethyl Ether Extract (DEE) only showed antibacterial activity against Gram negative bacteria *P. aeruginosa* ATCC 27853 and *Yersinia* sp. with about 9.00 mm diameter of the inhibition zones. However, the Ethyl Acetate Extract (EAE) showed a slightly better antibacterial activity against gram positive bacteria (*B. cereus* ATCC 10876, *B. licheniformis* ATCC 12759,

B. subtilis and *S. epidermidis* ATCC 12228) and gram negative bacteria (*A. anitratus* and *P. aeruginosa* ATCC 27853).

The butanol extract (BUT) showed antibacterial activity against gram positive (*B. licheniformis* ATCC 12759 and *B. spizizenii* ATCC 6633) and gram negative (*C. freundii*). On the other hand, only extract from ME, DEE and BUT showed activity against *C. albicans*. Unfortunately, there was no extract that can inhibit the fungal species.

Table 2 shows the antibacterial activity from various extract of *A. spicifera* obtained from Sohxlet extraction method. It seems that only the Hexane (HXN) extract showed some antibacterial activity against gram positive (MRSA) and gram negative bacteria (*P. aeruginosa* ATCC 27853).

Furthermore, the Chloroform (CM) and Ethyl Acetate (EAE) Extracts only showed activity against *P. aeruginosa* ATCC 27853 whereas Methanol Extract (ME) did not show any effect on the test microorganisms. All the extracts obtained from sohxlet method did not show any activity against yeasts and fungal species. In this study, chloramphenicol was used as a positive control for bacteria and ketoconazole was used as a positive control for yeast and fungi. Based on the results, gram positive bacteria were found more susceptible to the various crude extracts used.

Overall, 5 gram positive bacteria and 3 gram negative bacteria were inhibited by the extracts. The results also revealed that all the various solvent used had different efficiency in the capability in extracting compounds. Table 3 shows the MIC values of the extract from the two extraction methods against five selected bacteria, *P. aeruginosa* ATCC 27853, MRSA, *B. licheniformis* ATCC 12759, *C. freundii* and *Yersinia* sp.

The MIC values against the test bacteria were within the range of 31.25-1000 μg mL^{-1} while the MBC values were between 500-2000 μg mL^{-1}. As for the determination of antibiosis, only methanol extract from partitioning and ethyl acetate extract from soxhlet extraction exhibited bactericidal effect against *P. aeruginosa* ATCC 27853 at the ratio of MBC/MIC of 2.0.

Table 1: Antimicrobial activity of various partitioning extracts[P] of *Acanthophora spicifera*

Microorganisms	ME[P]	DEE[P]	EAE[P]	BUT[P]	C	K
Bacteria						
Gram positive						
Bacillus cereus ATTC 10876	+	-	+	-	++	ND
Bacillus licheniformis ATTC 12759	+	-	+	+	-	ND
Bacillus spizizenii ATTC 6633	-	-	-	+	+++	ND
Bacillus subtilis	-	-	-	-	+++	ND
MRSA	+	-	++	-	+++	ND
Staphylococcus aureus	+	-	-	-	+++	ND
Staphylococcus aureus ATTC 12600	-	-	-	-	+++	ND
Staphylococcus epidermidis ATTC 12228	-	-	+	-	+++	ND
Gram negative						
Acinobacter anitratus	-	-	-	-	++	ND
Citrotobacter freundii	-	-	+	++	++	ND
Erwinia sp.	-	-	-	-	+++	ND
Escherichia coli	-	-	-	-	++	ND
Klebsiella pneumoniae	-	-	-	-	+++	ND
Klebsiella pneumoniae ATTC 13883	-	-	-	-	++	ND
Pseudomonas aeruginosa ATTC 27853	++	+	+	-	-	ND
Pseudomonas stutzeri ATTC 17588	-	-	-	-	+	ND
Shigella bodyii	-	-	-	-	+++	ND
Yersinia sp.	++	+	-	-	+++	ND
Yeasts						
Candida albicans	+	+	-	+	ND	++
Candida utilis	-	-	-	-	ND	-
Saccharomyces cerevisiae	-	-	-	-	ND	+
Fungi						
Aspergillus niger	-	-	-	-	ND	+++
Microsporum gypseum	-	-	-	-	ND	-
Penicillum sp.	-	-	-	-	ND	+
Rhizopus sp.	-	-	-	-	ND	++
Trychophyton rubrum	-	-	-	-	ND	+
Trichoderma viridae	-	-	-	-	ND	+

[a]Activity is classified according to the diameter of the inhibition zone (+++: ≥15 mm; ++: 10-14 mm; +: ≤9 mm; -: no activity; ND = Not Done), [P]extracts from partitioning extraction, ME = Methanol Extract, DEE = Diethylether Extract, EAE = Ethyl Acetate Extract, BUT = Butanol extract, C= Chloramphenicol, K = Ketoconazole

Table 2: Antimicrobial activity of various soxhlet extracts[S] of Acanthophora spicifera

Microorganisms	HXN[S]	CM[S]	EAE[S]	ME[S]	C	K
Bacteria						
Gram positive						
Bacillus cereus ATCC 10876	-	-	-	-	+++	ND
Bacillus licheniformis ATCC 12759	-	-	-	-	++	ND
Bacillus spizizenii ATCC 6633	-	-	-	-	+++	ND
Bacillus subtilis	-	-	-	-	+++	ND
MRSA	+	-	-	-	+++	ND
Staphylococcus aureus	-	-	-	-	+++	ND
Staphylococcus aureus ATCC 12600	-	-	-	-	+++	ND
Staphylococcus epidermidis ATCC 12228	-	-	-	-	+++	ND
Gram negative						
Acinobacter anitratus	-	-	-	-	+++	ND
Citrotobacter freundii	-	-	-	-	+++	ND
Erwinia sp.	-	-	-	-	+++	ND
Escherichia coli	-	-	-	-	+++	ND
Klebsiella pneumoniae	-	-	-	-	+++	ND
Klebsiella pneumoniae ATCC 13883	-	-	-	-	++	ND
Pseudomonas aeruginosa ATCC 27853	++	+	+	-	-	ND
Pseudomonas stutzeri ATCC 17588	-	-	-	-	++	ND
Shigella bodyii ATCC 9207	-	-	-	-	+++	ND
Yersinia sp.	-	-	-	-	+++	ND
Yeasts						
Candida albicans	-	-	-	-	ND	+++
Candida utilis	-	-	-	-	ND	+
Saccharomyces cerevisiae	-	-	-	-	ND	+
Fungi						
Aspergillus niger	-	-	-	-	ND	++
Microsporum gypseum	-	-	-	-	ND	-
Penicillum sp.	-	-	-	-	ND	+
Rhizopus sp.	-	-	-	-	ND	++
Trychophyton rubrum	-	-	-	-	ND	+
Trichoderma viridae	-	-	-	-	ND	-

[a]Activity is classified according to the diameter of the inhibition zone (+++: ≥15 mm; ++: 10-14 mm; +: ≤9 mm; -: no activity; ND = Not Done), [S]extracts from soxhlet extraction, HXN = Hexane Extract, CM = Chloroform extract, EAE = Ethyl Acetate Extract, ME = Methanol Extract, C = Chloramphenicol, K = Ketoconazole

Table 3: Minimum Inhibitory Concentration (MIC) values for selected microorganisms[c] studied on various Acanthophora spicifera extracts

Strains	Extracts	MIC (μg mL^{-1})	MBC (μg mL^{-1})	MBC/ MIC
Pseudomonas aeruginosa ATCC 27853	Methanol[P]	250.00	500	2.0
	Diethyl ether[P]	500.00	>2000	>2.0
	Ethyl acetate[P]	1000.00	>2000	>2.0
	Hexane[S]	125.00	2000	16.0
	Chloroform[S]	250.00	>2000	>2.0
	Ethyl acetate[S]	250.00	500	2.0
MRSA	Methanol[P]	125.00	>2000	>2.0
	Ethyl acetate[P]	500.00	>2000	>2.0
	Hexane[S]	500.00	>2000	>2.0
Bacillus licheniformis ATCC 12759	Ethyl acetate[P]	125.00	>2000	>2.0
Citrobacter freundii	Butanol[P]	125.00	>2000	>2.0
Yersinia sp.	Methanol[P]	31.25	>2000	>2.0
	Diethyl ether[P]	125.00	>2000	>2.0

[c]Only bacteria with inhibition zone = 8 mm was studied ([S]Represent Soxhlet extracts; [P]Represent partitioning extracts)

DISCUSSION

Macroalga are considered as sources of bioactive compounds and produce a great variety of secondary metabolites. Hornsey and Hide (1974) reported that 151 species of marine algal crude extracts exhibited activity against pathogenic bacteria but variation may exist in antibacterial activity of the algae. The differences may due to the efficiency of the extraction methods to recover the active metabolites, solvents used (Tuney *et al*., 2006), susceptibility of strains (Perez *et al*., 1990; Gonzalez del Val *et al*., 2001), assay methods and seasonal variation (Sasidharan *et al*., 2009).

In conjunction to this, various solvents and two extraction methods were implied in this study, aiming to extract maximum amount of bioactive compounds. Furthermore as stated by Yan *et al*. (1999) and Goli *et al*. (2004), a single solvent extraction compared to multiple extraction procedure is not sufficient to effectively extract respective compounds responsible for the activity.

Some studies concerning the effectiveness of extraction methods reported that methanol extraction yields higher antimicrobial activity than n-hexane and ethyl acetate (Rosell and Sirasvata, 1987; Moreau *et al*., 1988; Ahmad *et al*., 1998; Eloff, 1998; Sastry and Rao, 1994) whereas others reported that chloroform is better than methanol and benzene (Febles *et al*., 1995). Thus, it is clear that organic solvents provide a higher efficiency in extracting compounds for antimicrobial activities compared to water-based method (Masuda *et al*., 1997; Lima-Filho *et al*., 2002). Based on the results, partitioning

extracts showed broader spectrum activity against tested microorganisms as compared to soxhlet extracts. This implied, Soxhlet extraction method did not work well with *A. spicifera*.

Eloff (1998) stated that soxhlet extraction of dried materials using solvents with increasing polarity only work well for compounds that can withstand the temperature of the boiling solvents but cannot be used for thermolabile compounds as it able to changed the chemical structures of the compound and affected the biological activity. Findings obtained from this study, might suggest that antimicrobial compounds from this local *A. spicifera* were thermolabile compounds.

Results of the present study revealed that extracts were more susceptible to gram-positive compared to gram negative bacteria. In general, antibiotic substances appear to have more inhibitory effect towards gram positive than to gram negative group (Kumar *et al*., 2006). The major components in the cell wall of gram negative bacteria are the lipopolysaccharide layer along with proteins and phospholipids (Burn, 1988).

Thus, access of inhibitory compounds to the peptidoglycan layer of cell wall was blocked. Hence, resisting the gram negative strains to the lytic action of extracts exhibiting activity. There was a contrast between the current study conducted with previous studied done. Gupta *et al.* (1991) reported that methanolic extracts of *A. spicifera* had no inhibitory activity against *E. coli*, *P. aeruginosa*, *A. niger* and *C. albicans*. Tuney *et al.* (2006) also reported that diethyl ether crude extract of *Acanthophora nojadiformis* showed no inhibitory activity against *P. aeruginosa, Candida* sp., *Enterococcus faecalis* and *E. coli*.

It is also reported that methanolic extract did not show inhibitory activity against bacteria mentioned before. In contrast to the study, the results showed that methanol extract from partitioning inhibited the growth of most of the bacteria tested (*B. cereus* ATCC 10876, MRSA, *S. aureus* ATCC 12600 and *Yersinia* sp.) unexpectedly, *P. aeruginosa* ATCC 27853 and *C. albicans*.

On the other hand, *P. aeruginosa* ATCC 27853 was the most inhibited microorganism with six out of eight extracts from both extractions completely inhibiting its growth. The MIC values of these pathogenic bacteria sensitive to the extracts were in the range of 125-1000 μg mL^{-1}.

This alga species may thus be a source of natural and alternative drugs that can improve the treatment infections caused by this microorganism as the resistance of this strain towards commercialized antibiotic increases (Levison and Jawetz, 1992).

CONCLUSION

Antimicrobial properties of various extracts from seaweeds are now become a great interest among academia and industry worldwide, due to their possible uses as natural additive to compliment the synthetic ones. In this respect, the present study was conducted to evaluate the antimicrobial properties of local *A. spicifera* against various microorganisms. The results presented indicate that *A. spicifera* extracts exhibit a promising antimicrobial activity against pathogenic, *P. aeruginosa* ATCC 27853. As a result, it can be concluded that *A. spicifera* may be useful as sources of bioactive compounds and should be investigated for natural antibiotic. Further research should be made to identify and purify the active compounds.

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