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Antimicrobial Activity of Some Seaweed Collected from South East Coast of Jeddah, Saudi Arabia

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ABSTRACT

Three marine seaweeds were collected from south east coast of Jeddah, Saudi Arabia. They were identified as species belonging to genera Hypnea, Turbinaria and Padina. They were extracted using hot water and five organic solvents. Their antimicrobial activities were determined against some clinical isolates of bacteria using disc diffusion assay. All the tested Padina and Hypnea extracts demonstrated activity against one or more of the tested bacteria. The methanolic extracts of Padina and Hypnea were the most active extracts compared to hot water and other organic extracts. They exhibited a broad spectrum of antibacterial activity against all the tested bacteria except Mycobacterium with MICs ranged from 125-400 µg/ml. Contrary to this, Turbinaria sp. did not show any detectable bactericidal activities. The toxicity for methanolic algal extracts was determined at the cell level by Biochemical Induction Assay and the Bioassay using Artemia salina as the test organism. No toxicity was detected against A. salina for the three tested algal extracts up to 300 µg/ml while at 400 µg/ml, the extracts of both Turbinaria sp. and Hapnea sp. showed some toxicity. By contrast, no toxicity for the methanolic algal extracts was detected by Biochemical Induction assay at 400 µg/ml. Only the extract of Turbinaria sp. had antitumor activity against Erlish cell line. The extracts showing good antimicrobial activities are undergoing further analysis to identify the active constituents which can be used as pharmaceutical compounds.

Key words: *Seaweed, Jeddah Coast, Marine Algae, Antimicrobial Activity, Mic, Antitumor.*

INTRODUCTION

Seaweeds are renewable living resources used by common people as low calorie food, food additives, animal feed, and fertilizers in many parts of the world [1]. They have good nutritional importance because they are rich in carbohydrates, protein, vitamins, minerals and dietary fibers [2]. Because of increasing resistance of microorganisms to existing antibiotics, there is an increasing need for new antibiotics. Seaweeds or macro algae are considered as a source of secondary metabolites and bioactive compounds with antibacterial, antifungal, antiviral, antineoplastic, antifouling and anti-inflammatory activities. Green, brown and red algae offer particularly a rich source of natural and bioactive molecules [3-6]. They are providing valuable sources for the development of new drugs against cancer, microbial infections and inflammations [7-9].

Special attention has been reported for antiviral, antibacterial and/or antifungal activities related to marine algae against several pathogens [10]. As an efficient strategy of investigation, organic solvents have been used to extract the possible lipid-soluble active principles from macroalgae with activities against different human pathogens [11, 12]. Espechel et al. (1984) [13] indicated the antimicrobial activities of eight different marine algae collected in Southern Argentina. The extracts Sargassum, Padina and Gracilaria collected from different coastal regions of India suppressed the growth of some pathogenic bacteria and fungi [14]. Similarly, the methanolic extracts from thirty two

species of macroalgae from the Atlantic and Mediterranean coast of Morocco, belonging to Chlorophyta and Phaeophyta showed a significant capacity of antibacterial activities [15]. Since seaweeds offer particularly a rich source of bioactive molecules, they were screened for antimicrobial activities after extraction with organic solvents (Chloroform, ethanol, methanol and hexane).

Many tested algal extracts have many biological activities. Therefore, this study is made to screen three common algae, Padina, Hypnea and Turbinaria for their antimicrobial activities, toxicity, and antitumor productions. The most active isolated algal can be used in production of pharmaceutical compounds used in medicine.

MATERIALS AND METHODS

Seaweed sample collection

Algal samples were collected from Red Sea coast of Saudi Arabia at Shuaba, about 82 km south of Jeddah (longitude 35° 91\\ - 39° 24\\ East and latitude 15° 56\\ - 20° 52\\ North), during summer season, 2015 at depths ranging between 0.3 m and 1. 5 m. They were collected in polyethylene bags by divers along the coast of the previous localities. Thereafter, they were transported wet to the lab in ice pack at 4°C. A complete sample of each collected algae was botanically identified at Biology Department, KAU, Saudi Arabia.

Preparation of crude extracts

Each dried algal material (l00 g) was powdered in a mortar and extracted two successive times at room temperature by continuous stirring for 16 h with 100 ml of different organic solvents including : acetone, chloroform, hexane, ethanol and methanol in addition to desterilized water. The organic extracts were collected and concentrated until dryness using rotary evaporator at 40°C but the water extract was lyophilized. The dried organic and water algal crude extracts were dissolved in 5 ml DMSO and screened for their biological activates.

Microorganisms used

The antimicrobial activities of the different algal extracts were tested against different pathogenic and non pathogenic bacteria. They were obtained from culture collection of Laboratoire de Biochimie Microbienne, Fac. De Pharmacie, Nancy, France.

The antimicrobial activities

The media used was nutrient agar for bacteria, Sabouraud dextrose agar for yeasts and fungi. The antimicrobial activity for each algal extract was detected against different test organisms. The used bacteria were Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella sp., Shigella sp., Corynebacterium michiganese and Mycobacterium. The technique used was agar well diffusion method described by Attaie et al., (1987) [16]. Petri dishes with 10 ml of nutrient agar were prepared, previously inoculated with 0.1 ml of a 24 h broth culture of tested bacteria. Four wells (6 mm) were made and each well was filled with 25µl of the algal extract in DMSO. The inoculated plates were incubated for 24 h at 37°C. After incubation, the diameter of the inhibition zone was measured. Dimethyl sulfoxide (DMSO) was used as a negative control and Ampicillin as the standard antibacterial agent. The antimicrobial activity was taken on the basis of diameter of the zone of inhibition, and the mean of three readings was represented.

The minimal inhibitory concentration

The minimal inhibitory concentration (MIC) was determined by the method suggested by Chand et al. (1994) [17] and modified by Aly and Gumgumjee (2011) [18] using 96 well ELISA trays. Each well was filled with 175µl bacterial suspension, 4x106CFU ml^{-1}, followed by 20 µl of the tested extracts in DMSO which was used as the control. The ELISA trays were incubated for 40 minutes, followed by addition of 5 µl of a 0.2% (w/v) Fluorescein diacetate in acetone and then the incubation was continued for 90 minutes more. The obtained green color was measured at 490 nm using MR7000 automatic ELISA tray reader.

Antitumor activity of the algal extracts

Under a humidified atmosphere air with 5% CO2, Ehrlich carcinoma cell line was grown at 37°C for 48 h in RPMI 1640 medium (Sigma, USA) with 10 % fetal calf serum [18]. The collected cells were incubated with 400 µm/ml of the tested algal extract for 24 hrs., centrifuged for 2 min at 5000 rpm and counted using hemacytometer and trypan blue (Sigma, USA) in normal saline. LD50 (lethal dose for 50 %) was determined and compared to the anticancer agent (Bleomycin).

Toxicity of the algal extract

The toxicity of algal extracts was determined at the cell level using Biochemical Induction Assay, E. coli BR513 as test organism [19] and using Bioassay, Artemia salina as the test organism [20].

Statistical analysis

Each experiment was carried out in three replicates, the mean of variable ± standard deviation was calculated and significant differences between control and samples were calculated using t-test.

RESULTS

Many investigations were carried out to discover algal products that inhibit pathogenic microorganisms including bacteria and fungi. The antimicrobial activities of three algal samples collected from Red Sea coast of Saudi Arabia at Shuaba, about 82 km south of Jeddah were investigated against some Gram positive and negative bacteria including Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella sp., Shigella sp., Corynebacterium michiganese and Mycobacterium using agar well diffusion method. The tested algae were extracted using different solvents. The antimicrobial activity was measured by the zone of inhibition for all the different test organisms. The crude extracts of Padina and Hypnea revealed a wide range of antimicrobial activity against the tested pathogens while all the extracts of Tepinaria showed no antibacterial activities. The results were tabulated in the tables 1, 2 for the crude extracts of Padina and Hypnea, respectively.

The different extracts of Padina showed antibacterial activities against Staphylococcus aureus and Escherichia coli. The methanolic extract of Padina sp. Was the most active with antibacterial index of 80 (Table 1). The maximum inhibition was noticed against E. coli (15 mm) followed by Pseudomonas aeuroginosa (13mm). Moderate antimicrobial activities were exhibited by ethanol, acetone, chloroform and hexane extracts. The lowest activities were obtained by water extract where the inhibition zone was ranged from 8-10mm and with antibacterial index of 50. None of the Padina extracts showed activities against Mycobacterium sp.

The methanolic extract of Hypnea showed promising antibacterial activities against all the tested bacteria especially Escherichia coli, Pseudomonas sp., Shigella sp., Klebsiella sp., Bacillus subtilis and Staphylococcus aureus. While chloroform and hexane extracts of Hepnea showed moderate antibacterial activities with inhibition zone ranged from 10-13mm. Weak antibacterial activity was obtained by water extract of Hypnea (diameter of the inhibition zone ranged from 8-9 mm). All Hypnea extracts showed no activity for Mycobacterium.

Minimal inhibitory concentration for the methanol extracts of the three tested algae were calculated for different test organisms (Table 3). The basic quantitative measures of the in vitro activity of the active substance are the minimum inhibitory concentration. The MIC is the lowest concentration of the active substance that results in inhibition of the visible growth under standard conditions. MIC of Ampicillin, calculated for bacteria, was ranged from 5-10 µg/ml.

Toxicity and antitumor activity

Methanol extracts of both Turbinaria and Hypnea showed a potent cytotoxic effect using standardised short-term toxicity test where Artemia salina was used as test organism. They showed mortality percentage >50 % (Table 4). The detected cell toxicity of A. salina was ranged from 9-29 % at 100-300 µg/ml of the crude extracts. The obtained results showed that there was an increase in the toxicity of Turbinaria and Hypnea extracts with their elevated concentrations. The maximum toxicity of 65 and 55 % were observed at 400 µg/ml of crude extract of Turbinaria and Hypnea, respectively. Cell toxicity was confirmed using Biochemical Induction Assay (BIA) and E. coli as test organisms. Both of the extracts could be a promising source of cytotoxic components. No toxicity was observed for the different concentration of methanol extract of Padina up to 400 µg/ml.

No antitumor activity was recorded with the application of the crude extract of Padina and Hapnea species. The antitumor activity was observed for the methanolic extract of *Turbinaria* against Erlish ascites carcinoma cell line.

DISCUSSION

Among marine organisms, algae are a large and diverse group of organisms from which a wide range of secondary metabolites have been isolated. A number of these compounds possess biological activity. Nagi et al., (2009) [21], Chiheb et al., (2009) [15] and Anbu-Jeba-Sunilson et al., (2009) [22] reported that marine algae are rich in active constituents showing antimicrobial activity. Furthermore, the ethanolic extract of Sargassum ilicifolium, Padina tetrastromatica and the methanolic extract of Gracilaria corticata showed maximum bacterial inhibition and the lowest antibacterial activities were noticed with chloroform extracts [14]. From Persian Gulf, the extracts of the collected red algae Gracilaria salicornia and Hypnea flagelliformis have strong antimicrobial activity due to the presence of acrylic acid, terpenes, heterocyclic compounds containing sulfur, chlorellin derivatives and halogenated aliphatic compounds [23]. Sastry et al. (1994) [24] noticed that algae have antibacterial activity against Gram-

positive and Gram-negative pathogenic strains after successive extraction with benzene, chloroform and methanol. Likewise, Mahasneh et al. (1995) [25] have shown antibiotic activity in organic extracts of six species of marine algae against multi-antibiotic resistant bacteria. Thus, it is evident from the results that these algae possess antimicrobial activity. Further studies are aimed at isolation and purification of phyto-constituents responsible for antimicrobial activity. Antimicrobial activity depends on algal type, the season of collection, and the efficiency of extraction of their active materials including used organic solvents and the method of extraction and assay.

Özdemir et al. (2001) [26] found that the Spirulina extracts, obtained from various solvents exhibited antimicrobial activity on both Gram-positive and Gram-negative organisms which may be indicative of the presence of broad spectrum antibiotic compounds. Numerous pharmacological active constituents like alkaloids, saponins, glycosides, tannins, chlorellin derivatives, acrylicacid and halogenated substances were identified as antimicrobial agents from algae. The results from the present study showed that Gram negative bacteria are more susceptible to algal extracts than Gram positive bacteria due to the differences in their cell wall structures and compositions, and this result was also supported from earlier works with different species of seaweeds [27]. Further in Gram-negative bacteria, the lipids of the outer membrane act as a target to many environmental substances including antibiotics, and the presence of a thick murine layer in the cell wall of Gram positive also prevents the entry of the inhibitors [28]. The overall antimicrobial activity assessed from the above results indicates the presence of active constituents in the extractions of seaweeds, thus they can be considered as potential sources of bioactive compounds acting as antibiotic or cytotoxic compounds.

In 21st century, cancer is a dangerous disease, it has increasing occurrence and it has caused about 25% of all the deaths in humans. Algal activity against cancer cell lines was documented, and many algae have shown cytotoxic and antitumor activities [29]. The methanolic extract of Turbinaria sp. acts as potential antitumor agents, while the extract of Hypnea and padina has shown promising cytotoxic activity. Similarly, Barchi et al. (1984) [30] found out that liphophilic extracts of Oscillatoria accutissima were anti-neoplastic and showed toxic activities. Codium tomentosum, Jania rubens and Padina pavonia collected from Alexandria coast displayed 20% inhibition of tumor initiation [31]. The secondary products of algae, bromophenols, carotene and steroids showed antitumor activity [32]. Moreover, sulfated metabolites like fucoidans from the brown algae showed excellent activity against different tumor cell lines [33].

CONCLUSION

This study reported the antimicrobial activity of different organic and water extracts of the 3 selected algae collected from Jeddah against some Gram positive and negative bacteria. Generally, the recorded antibacterial activity in the algal extracts was due to the active compounds that can be extracted, purified and used as pharmaceutical drugs. The algal extracts in different solvents exhibited different antimicrobial activities, and no solvent recorded effectively antibacterial activity against all the test bacteria.

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Table 1. Antimicrobial activity of Padina extracts obtained with different organic solvents against different tested bacteria

Tested microorganisms	Inhibition zones (mm) of the different algal extracts**					
	Methanol	Ethanol	Acetone	chloroform	Hexane	H_2O (control)
Escherichia coli	19±0.1	10±0.1	11±0.2	10±0.1	10±0.0	8±0.6
Pseudomonas aeroginose	15±0.2	13±0.3	10±0.1	10±0.1	11±0.2	8±0.2
Shigella sp.	13±0.3	12±0.2	10±0.1	15±0.2	11±0.1	8±0.1
Klebsiella sp.	11±0.2	12±0.1	10±0.2	10±0.1	11±0.1	8±0.1
Bacillus subtilis	11±0.1	11±0.1	11±0.1	10±0.3	10±0.0	8±0.0
Staphylococcus aureus	11±0.1	10±0.2	13±0.2	10±0.2	15±0.2	10±0.2
Mycobacterium	-	-	-	-	-	-
Antibacterial index***	80*	68*	65*	65*	68*	50

*: significant results compared to control, ** Inhibition zone ,7–10-mm =weak activity ; 1 0–15-mm = good activity; (15–20 mm very good activity and (-) =No activity. *** Activity index was calculated as the sum of the net values of inhibition zone diameters (mm) against the tested bacterial strains

Table 2. Antimicrobial activity of different Hypnea extracts obtained with different organic solvents against different tested bacteria

Microorganisms	Inhibition zones (mm,) against investigated extracts**					
	Methanol	Ethanol	Acetone	chloroform	Hexane	H_2O (control)
Escherichia coli	12±0.0	11±0.1	11±0.1	10±0.3	10±0.0	8±0.1
Pseudomonas aeroginose	12±0.2	09±0.1	11±0.1	10±0.1	11±0.2	8±0.3
Shigella sp.	12±0.1	10±0.1	12±0.2	12±0.2	11±0.1	9±0.2
Klebsiella sp.	12±0.1	09±0.1	07±0.1	13±0.3	11±0.1	8±0.1
Bacillus subtilis	12±0.0	10±0.1	10±0.1	13±0.3	10±0.0	8±0.1
Staphylococcus aureus	12±0.1	10±0.1	10±0.0	10±0.3	15±0.2	8±0.2
Mycobacterium sp.	-	-	-	-	-	-
Antibacterial index***	74*	59*	61*	68*	68*	49

*: significant results compared to control, ** Inhibition zone ,7–10-mm =weak activity ; 1 0–15-mm = good activity ; (15–20 mm very good activity and (-) =No activity. *** Activity index was calculated as the sum of the net values of inhibition zone diameters (mm) against the tested bacterial strains

Table 3. The minimal inhibitory concentrations of the methanolic algal extracts in addition to Ampicillin (control) for different tested bacteria

Minimal inhibitory concentration (MIC, µg/ml)				
Microorganisms	Control antibiotic (Ampicillin)	Padina sp.	Turbinaria sp.	Hypnea sp.
Escherichia coli	10	100	>400	250
Pseudomonas aeroginose	5	125	>400	250
Shigella sp.	5	125	>400	250
Klebsiella sp.	5	150	>400	250
Bacillus subtilis	2	250	>400	200
Staphylococcus aureus	2	250	>400	250
Mycobacterium	>400	>400	>400	>400

Table 4. The toxicity and antitumor activity of the three methanolic algal extracts

Conc. (µg/ml) / Algal extract	Toxicity (LD_{50}, µg/ml)	Toxicity (Biochemical Induction Assay)	Antitumor Activity against Erlish ascites carcinoma (400 µg/ml)
Control (Bleomycin)	>400	-	+
Padina sp.	400>	-	-
Turbinaria sp.	375*	+	+
Hypnea sp.	350*	-	-