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Research Article

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Screening of antimicrobial activity of some marine invertebrate extracts collected from Tabuk region, Kingdom of Saudi Arabia

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ABSTRACT

The recent appearance of growing number of bacteria resistant to conventional antibiotics has stimulated the search for novel antimicrobial compounds from variety sources including marine sources. Four genera of marine invertebrates were collected from Tabuk region, Kingdom of Saudi Arabia. Bioactive metabolites were extracted using methanol and tested for its activity against sets of Gram +ve bacteria, Gram-ve bacteria, yeast and fungi using the agar disk diffusion method. The results showed different patterns of antibacterial activity. About 75% of the marine invertebrate extracts presented significant activity against yeast. No activity shown with fungi. These findings suggest that the marine invertebrates are a potential source of novel antimicrobial compounds. These results in relation with antibacterial and antiyeast activities in vitro open the way for complementary investigation in order to purify and identify active molecules.

Key words: Antimicrobial activity, Marine invertebrates, Methanolic extract, Pathogenic microorganisms.

INTRODUCTION

The effective use of conventional drugs available in markets has been severely affected due to the increasing incidence of development of multiple resistance mechanism in microbes [1]. The emergence of new diseases is also on the rise. Hence, there is always a constant need for search to find new drug leads with novel mechanisms of action to combat these problems. It is no wonder that the nature has formed the basic and wonderful source for invention of new and innovative drugs as the secondary metabolites from plants, animals and microorganisms exhibit striking structural diversity [2]. The marine life constitutes almost 80% of the world biota [3]. Microorganisms in sea water and sediments may be high as 10^6 per milliliter respectively [4]. During the last decade, there has been an increase in research on marine crustaceans, mollusks and echinoderms with particular interest on their secondary metabolites with desirable antimicrobial properties [5]. More or less 10,000 pharmacologically bioactive compounds have been derived from marine invertebrates. From the natural products isolated from marine organisms, only less than 1% has been examined so far for pharmacological activities [6]. The marine compounds posses many biological activities like antihelminthic, antibacterial, anticoagulant, antifungal, antimalarial, antiplatelet, antiprotozoal, antituberculosis and antiviral properties [7].

EXPERIMENTAL SECTION

-Specimen Collection

Four marine invertebrate organisms (*Penaeus* sp., *Portunus* sp., *Astacus* sp. and *Sepia* sp.) were collected from Tabuk region at the Gulf of Aqaba. The organisms were cleaned and stored at -20^o C until used for extraction.

- Preparation of Extracts

Each marine invertebrate sample (400g wet weight) was homogenized in a blender. The macerate was subjected to an extraction with methanol by soaking at ambient temperature. The combined methanolic extract was filtered through 0.2 μm Millipore filter and concentrated under vacuum on a rotary evapotator at low temperature. The filtrate was screened for the antimicrobial activities using the agar disk diffusion method. The invertebrate shells were cleaned, rinsed with water and dried. The shell extract was prepared based on the procedure of Chen *et al.* [8].

- Test microorganisms and culture media

Test microorganisms such as *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive bacteria), *Escherichia coli* and *Pseudomonas aeruginosae* (Gram-negative bacteria), *Candida albicans* (yeast) and *Aspergillus niger* (fungi) used in this study were kindly provided by Prof. Dr. H.M. Rifaat, Microbial Chemistry Department, National Research Center, Cairo, Egypt. All the isolated bacteria were grown on nutrient broth at 37º C, while yeast and fungi in saboroud broth using standard procedure of WHO [9].

- Antibacterial Assay

The antibacterial activity of invertebrate extracts was performed using the agar disk diffusion assay [10]. 20 ml of sterile nutrient agar was poured in petridishes allowed to set at 37º C and then inoculate uniformly with 0.1 ml of test microorganisms. Disk of Whatman no. 1 filter paper were cut out using an office punch and autoclaved at 121º C for 15 min. Each sterile disk was dipped in 100 μl of the various extracts and placed on the inoculated agar plates and incubated at 37º C for 18-24 h. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the disks.

- Antifungal Assay

This activity was tested against fungal pathogens using the disk diffusion assay method as described for antibacterial assay. The plates were incubated at 28º C for 48 h. The antifungal activity was evaluated by measuring the zone of growth inhibition surrounding the disks.

RESULTS AND DISCUSSION

A screening for antimicrobial activity of extracts from different marine invertebrates was conducted. Many workers described the antibacterial activity of marine invertebrate extracts [11 & 12]. The antimicrobial activity from four invertebrate specimens namely *Penaeus* sp., *Portunus* sp., *Astacus* sp. and *Sepia* sp. has been evaluated against four pathogenic bacteria (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeuroginosa* and *Escherichia coli*) and two pathogenic fungi (*Candida albicans* and *Aspergillus niger*). Methanol extraction was done on both muscles and shell of *Penaeus* sp., *Portunus* sp. and *Astacus* sp., while in *Sepia* sp. The antibacterial activity of *Penaeus* muscles extract gave the same inhibition zone (10.0 mm) against *Staphylococcus aureus* and *Escherichia coli*, while the zones of inhibition were 8.0 mm and 9.0 mm on *Bacillus subtilis* and *Pseudomonas aeuroginosa* respectively. The highest zone of inhibition caused by *Penaeus* shell extract was 9.0 mm on *Staphylococcus aureus*, while no activity indicated on *Escherichia coli*. The growth inhibition zones were 6.0 mm and 7.0 mm on *Pseudomonas aeuroginosa* and *Bacillus subtilis* respectively (Table 1 & Fig. 1). The obtained results revealed that *Penaeus* muscles extract showed antibacterial activity on all tested bacteria in comparison with its shell which had no activity on *Escherichia coli*. Previous studies [13 & 14] reported a broad spectrum antimicrobial activity in the exoskeletons of several crustacean species including *Penaeus*, *Astacus* and *Portunus*.

Astacus muscles extract gave the same growth inhibition zones on *Bacillus subtilis*, *Pseudomonas aeuroginosa* and *Escherichia coli* (11.0 mm) and 8.0 mm on *Staphylococcus aureus*. The growth inhibition zone caused by *Homarus* shell extract on *Bacillus subtilis* and *Staphylococcus aureus* was 10.0 mm. The zones of inhibition on *Pseudomonas aeuroginosa* and *Escherichia coli* were 6.0 mm and 9.0 mm respectively (Table 1 & Fig. 1). An important activity against the bacterial strains was also observed with the extract of *Astacus* muscles and shells. Many workers came to the same conclusion [15, 16 & 17]. The characteristics of this activity may be due to the presence of antimicrobial peptides [18]. Also, the chitin binding capabilities of the crustacean exoskeleton had a wide spread internal defense factor [17 & 19].

An important activity against the bacterial strains was also observed with the extract of *Astacus* muscles and shells. Many workers came to the same conclusion [15, 16 & 17]. The characteristics of this activity may be due to the presence of antimicrobial peptides [18]. Also, the chitin binding capabilities of the crustacean exoskeleton had a wide spread internal defense factor [17 & 19].

The highest antibacterial activity of *Portunus* muscles extract (12.0 mm) was indicated on both *Bacillus subtilis* and *Staphylococcus aureus*, followed by (8.0 mm) inhibition zone on *Pseudomonas aeuroginosa*. No activity was

recorded on *Escherichia coli*. The highest inhibition zone produced by *Portunus* shell extract was recorded on *Bacillus subtilis* and *Staphylococcus aureus* (12.0 mm). *Portunus* shell extract activity, against *Pseudomonas aeuroginosa* and *Escherichia coli* was 6.0 mm and 9.0 mm respectively (Table 1 & Fig. 1). *Portunus* shell extract showed antibacterial activity on Gram +ve and Gram –ve bacteria, while crab muscles extract had no activity on *Escherichia coli*. The obtained results are in accordance with those obtained by many workers [20, 21 & 22].

Methanol extract of *Sepia* muscles did not show any antibacterial activity against all tested bacterial pathogens. The highest growth inhibition zone of *Sepia* ink extract was recorded on *Bacillus subtilis* (14.0 mm) followed by *Staphylococcus aureus* (12.0 mm), while no activity was shown on *Pseudomonas aeuroginosa* and *Escherichia coli* (Table 1 & Fig. 1). Anand and Edward [23] reported moderate antibacterial activity from the extracts of various cephalopods which not in accordance with the obtained results. The highest activity against Gram +ve bacteria and yeast was indicated by *Sepia* ink extract which is in accordance with many workers [24 & 25]. The *Sepia* ink has proved to play various primary roles in the world of alternative medicine and has widest range of the therapeutic applications [26].

The antifungal activity tests showed that methanol extract of the marine invertebrate specimens produced by *Penaeus* muscles, *Penaeus* shell, *Astacus* muscles, *Astacus* shell, *Portunus* muscles, *Portunus* shell and *Sepia* ink on *Candida albicans* were 8.0, 10.0, 8.0, 9.0, 6.0 and 12.0 mm respectively, while no activity produced by *Penaeus* shell and *Sepia* muscles (Table 2 & Fig. 2). None of the marine invertebrate extracts specimens show any activity against *Aspergillus niger* (Table 2 & Fig. 2). All the marine invertebrate extracts showed considerable activity on *Candida albicans* except *Sepia* muscles. The highest activity against yeast was indicated by the extractions of *Sepia* ink, which confirm the results obtained by Bharthi *et al.* [25] who reported anticandidal activities of *Sepia aculeate* ink extract. No activity was recorded against *Aspergillus niger*. Ramasamy *et al.* [24] mentioned that the methanolic extracts from selected species of marine invertebrate showed no activity against different fungal strains.

Table (1) Antibacterial activity of marine invertebrates methanol extract

Marine sample	Code No.	Growth inhibition diameter (mm)			
		Bacillus subtilis	Staphylococcus aureus	Pseudomonas aeuroginosa	Escherichia coli
Penaeus muscles	1	10	9	10	8
Penaeus shell	2	0	6	9	7
Astacus muscles	3	11	11	8	11
Astacus shell	4	9	6	10	10
Portunus shell	5	9	6	12	12
Portunus muscles	6	0	8	12	12
Sepia muscles	7	0	0	0	0
Sepia ink	8	14	12	0	0

Table (2) Antifungal activity of marine invertebrates methanol extract

Marine organism	Code No.	Growth inhibition diameter (mm)	
		Candida albicans	Aspergillus niger
Penaeus muscles	1	8	0
Penaeus shell	2	0	0
Astacus muscles	3	10	0
Astacus shell	4	8	0
Portunus shell	5	6	0
Portunus muscles	6	9	0
Sepia muscles	7	0	0
Sepia ink	8	12	0

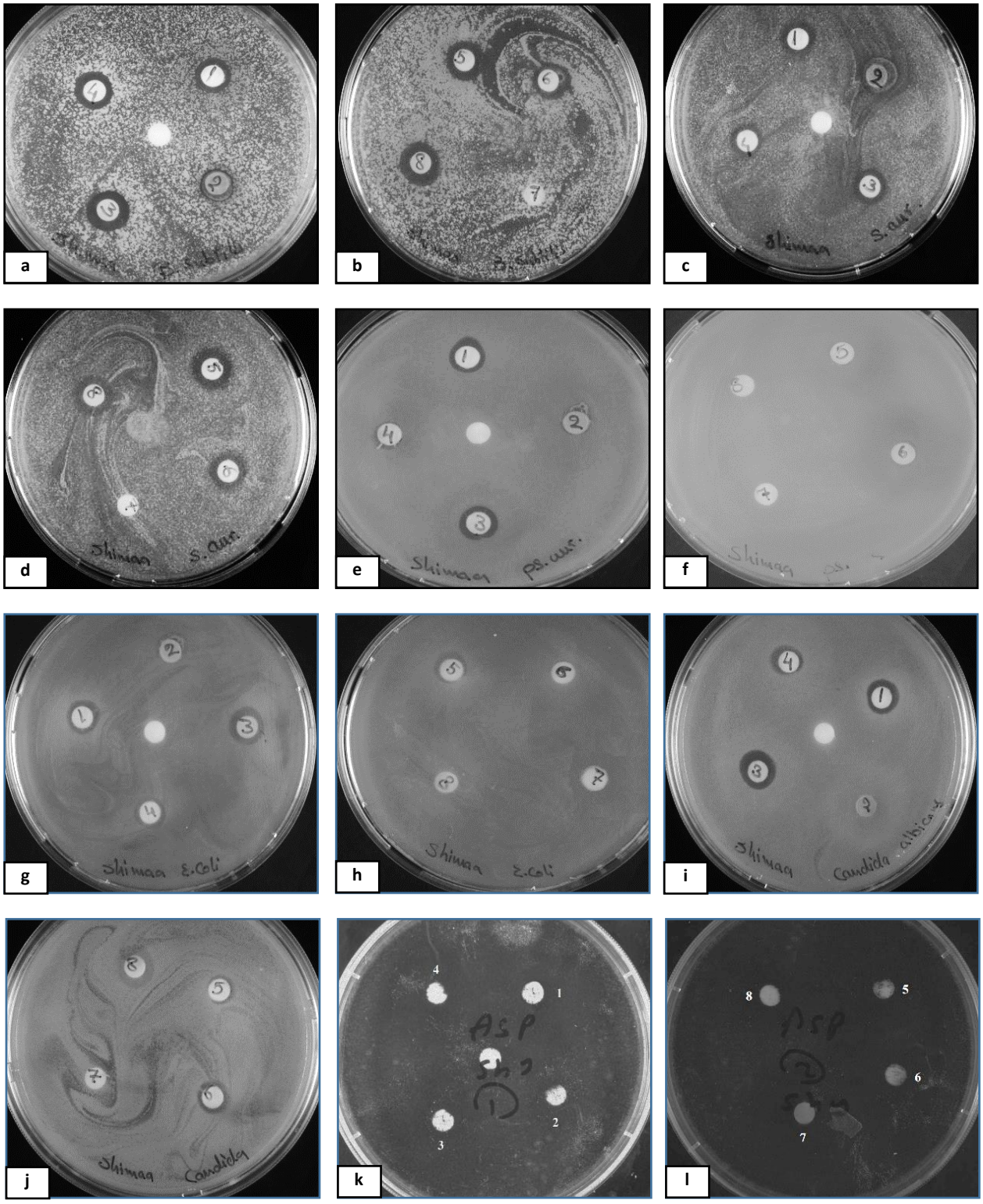

Fig. (1) Antimicrobial activity of marine invertebrate specimens: a&b *Bacillus subtilis*; c&d *Staphylococcus aureus*; e&f *Pseudomonas aeuroginosa*; g&h *Escherichia coli*; i&j *Candida albicans*; k&l *Aspergillus niger*

CONCLUSION

The present study indicated that marine invertebrates remain an interesting source for new antimicrobial metabolites. Four different genera of marine invertebrate were extracted and screened for activity against different set of bacteria, yeast and fungi. The results showed that all the investigated genera showing activities against bacteria and yeast. No antifungal activity was detected in all samples. Thus, Tabouk region are rich source of potent antibacterial and

antiyeast compounds. Among the investigated genera *Sepia* ink was the most promising invertebrate which providing high activity against *Bacillus subtilis* and *Candida albicans*. Isolation and purification of the active compounds is necessary in order to identify their chemical nature and to evaluate their potential as novel drugs. The problem of drug resistance by many microbes may now be dealt with considering marine organisms for antibiotic activity.

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