

PRELIMINARY INVESTIGATIONS OF ANTIMICROBIAL SCREENING OF CRUDE EXTRACTS OF SPONGES AND GORGONIANS SPECIES FROM SAUDI RED SEA COAST

ZEBA PERVEEN¹, SULTAN AL-LIHAIBI², ABDULMOHSIN AL-SOFYANI³,
G.R. NIAZ² AND JEAN-MICHEL KORNPBST⁴

¹HEJ, Institute of Chemistry, University of Karachi, Pakistan

²Marine Chemistry Department, Faculty of Marine Science,
King Abdulaziz University, Jeddah, Saudi Arabia

³Marine Biology Department, Faculty of Marine Science,
King Abdulaziz University, Jeddah, Saudi Arabia

⁴Institute of Substances and Organisms from the Sea (ISOMer)
University of Nantes, France

ABSTRACT

Some of the marine organisms have been found to possess chemotherapeutic properties. The active ingredients can be used as antibacterial, antifungal, antitumor and antiviral agents. These compounds are sometimes difficult to be synthesized in laboratory. Since very little work has been done in the past on the organisms found in the Red Sea, therefore some sponges and gorgonians were collected from the sea near the coast of Jeddah. Our results indicated that *Sh. boydii*, *E. coli*, and *S. aureus*, exhibited susceptibility to some crude extracts of sponges and gorgonians. Ampicillin, Amoxicillin and Cefuroxime were used as standard drugs. During the screening of antifungal activity, it was noted that *Elisella sp.* and *Axinellida sp.* showed significant activity against *C. lunata*, *T. longifusus* and *M. canis*. Myconazole, and Ketoconazole were used as standard drugs for comparison.

INTRODUCTION

The history of drugs is woven with plants, animals and minerals from earlier times. They are mostly of terrestrial origin with smaller number coming from the sea. Despite the wide availability of medicinally useful compounds a continuing search for new antimicrobial agents is still the primary need of the time. These compounds are generally smaller molecules ideally suited to serve either themselves or through chemical modifications as potential new pharmaceutical agents for the treatment of variety of ailments. The scientific community is focusing it's efforts on the isolation and characterization of biologically active compounds derived from marine organisms. It has been demonstrated that marine

organisms are excellent sources of new drugs, (Faulkner, 1995; Fenical, 1996). One may find exotic compounds arranged in completely different ways from those found on land plants and animals. Some of the antibiotics may have drawbacks in the sense that they have either limited antimicrobial spectrum or are associated with some side effects or contraindications. The combination of the genetic versatility of the microbes and widespread over use of antibiotics has also led to increasing clinical resistance of previously sensitive microorganisms and the emergency of previously uncommon infections. It has therefore become highly desirable to explore new molecules exhibiting prominent activity against pathogenic organisms with less undesirable effects (Vlietinck, 1987). In the early 1950's, the first

Table 1b:
Antibacterial activity of Crude Extracts of Gorgonians

Bacterial cultures	Zone of Inhibition in mm			Standard Drugs (200 µg/100µl)		
	<i>Rumphella aggregata</i>	<i>Elisella sp.</i>	<i>Acarboria erythracea</i>	Ampicillin	Amoxicillin	Cefuroxime
<i>S. aureus</i>	-	7	-	22	21	21
<i>S. pyogenes</i>	-	-	-	20	20	20
<i>A. hydrophilla</i>	-	-	-	19	19	19
<i>C. diphtheriae</i>	6	-	6	16	-	15
<i>B. subtilis</i>	-	-	-	18	19	19
<i>E. coli</i>	6	6	6	-	-	19
<i>S. typhi</i>	-	-	-	21	20	18
<i>Sh. boydii</i>	6	-	6	21	21	18
<i>K. pneumoniae</i>	-	-	-	9	-	19
<i>P. mirabilis</i>	-	-	-	20	20	20
<i>Ps. aeruginosa</i>	-	-	-	12	-	-

Key: mm: Zone of Inhibition, - No Antibacterial Activity, Concentration of test sample: 200 µg/100µl)

Table 2
MIC Values of Crude Extracts of the Sponges against Pathogenic Bacteria

Bacterial Culture	MIC Value in µg/ml				
	<i>Ircinia sp.</i>	<i>Phyllospangia sp.</i>	<i>Subercamollis</i>	<i>Axinellida sp.</i>	<i>Simularia sp.</i>
<i>Staphylococcus aureus</i>	90	-	-	90	95
<i>Cornebacterium diphtheriae</i>	85	-	-	-	-
<i>Bacillus subtilis</i>	85	80	-	-	-
<i>Salmonella typhi</i>	75	85	90	-	-

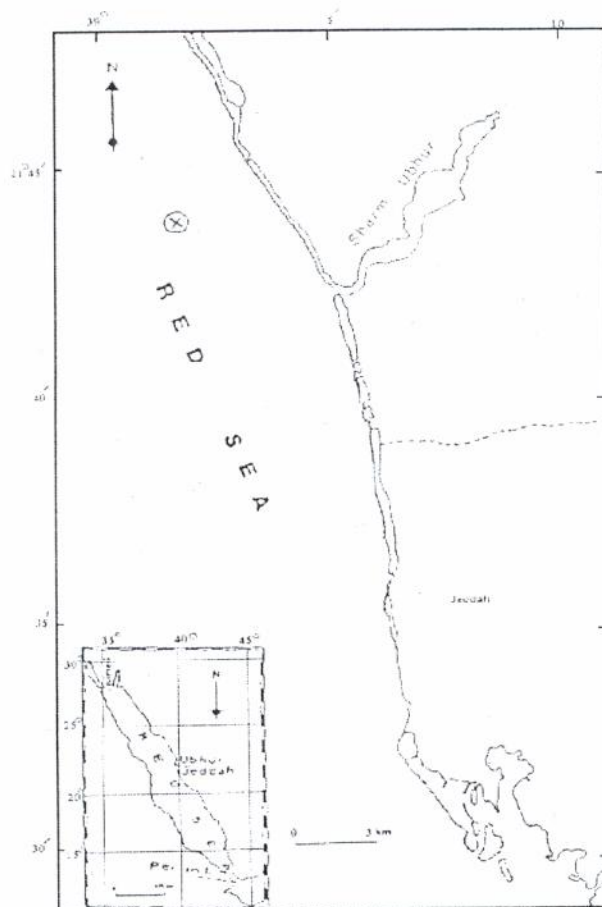
compounds exhibit a diversity of chemical structure (Scheuer, 1991 and 1995). Some of the sponges and related organisms have been found to be associated with antibacterial, antifungal, antiviral and antitumor activity (Higa, 1994, Avasti and

Bhakumi, 1993). The present work describes the collection and identification of various species of sponges and gorgonians and effects of their crude extract on some gram positive and gram-negative bacteria and some of the fungi.

Table 4
MIC Values of crude extracts of the sponges and Gorgonians against pathogenic Fungi

Fungal culture	MIC Values in $\mu\text{g/ml}$						
	<i>Acanthella carteri</i>	<i>Hyrtios erecta</i>	<i>Suberera mollis</i>	<i>Siphonchalina sp.</i>	<i>Simularia sp.</i>	<i>Rumphella aggregata</i>	<i>Axinellida sp.</i>
<i>Microsporium canis</i>	-	-	-	275	-	200	200
<i>Trichophyton longifusus</i>	-	250	-	-	325	250	225
<i>Curvularia lunata</i>	350	225	250	300	300	275	250

Unit = $\mu\text{g/ml}$.



test organisms with solutions containing different concentrations of the test compound.

The antifungal activity was determined by Agar tube dilution method (Paxton, 1991).

Test tubes having sterile Sabourand dextrose agar were inoculated with test compounds in different concentrations and kept in slanting position at room temperature for solidification. Test fungal cultures were inoculated

diversity of substances whose structure when isolated may not have terrestrial counterpart. The pattern of the antimicrobial activity varied with species. However, it can be concluded that *Ircinia sp.*, *Simularia sp.* and *Phyllospongia sp.* exhibited more promise for future studies. Some of these extracts have shown definite activity as antifungal agents and may prove to be potentially important and may be developed into value added products by preparing their derivatives.

An overall picture of these tables unveils the fact that these crude extracts were more active as antifungal agent rather than antibacterial agent. Moreover the extract may contain more than a dozen compounds in all. If the active principle is isolated in a pure form then one might get better results; it may also act as a starting material for semi-synthetic drugs, and it may prove to efficacious in medicinal chemistry.

ACKNOWLEDGEMENTS

The authors are grateful to Dr .J. Vacelet, Oceanological Centre, Marseille, France, for identification of all sponge species.

REFERENCES

- Akaniro, J.C. Vidaurre, C.E., Stuttman, H.R., and Marks, M.I., (1990). Antimicrobial agents and chemotherapy; American Society of Microbiology, Washington, U.S.A., **34**: 1880-1884.
- Amade, P., Psedano, D., and Chevolot, L., (1982), Antimicrobial activity of marine Sponges, from French Polynesia and Brittany. *Mar. Biol.* **70**: 223-228.
- Avasthi, K. and Bhakuni, D.S., (1993). Marine Nucleosides. *Indian J. Of Het. Chem.* **2**: 203-218.
- Bergmann, W., and Burke, D.C. (1955). The nucleoside of sponges, spongothy-midine and spongouridine. *J. Org. Chem.* **20**: 1501-1507.
- Faulkner, D.J., Thompson, J.E. and Walker, R.P., (1985), Screening and bioassay for biological active substances from forty marine sponges from San Diego, California. *Mar. Biol.* **88**: 11-12.
- Faulkner, D.J., (1995), Chemical Riches from the Oceans. *Chem. In Britain*, **31**: 680-684.
- Fenicle, W. (1996), Status of new drugs from marine organisms. *Oceanography* **9**(1): 23-27.
- Higa, T., Tanaka, T.I., Kitamura, A., Kyoma, T., Takashi, M., and Uchida, T., (1993). Bioactive compounds from marine sponges. *Pure and App. Chem.* **66**: 2227-2230.
- Newbold, R.W., Jensen, P.R., Fenical, W. and Pawlik, J.R. (1999), Antimicrobial activity of Caribbean Sponge extracts. *Aq. Microb. Ecol.* **19**: 279-284.
- Numata, A. and Iritani, M. (1997). Novel antitumor metabolites produced from a fungal strain from a sea hare. *Tetrahedron. Lett.* **88**(47): 8215-8218.
- Paxton, I.D., (1991). Methods in Plant Biochemistry, Assays for antifungal activities, Academic Press, London, 3rd. Ed., **6**: pp. 33-45.
- Scheuer, P.J. (1991). Drugs from the Sea. *Chem. and Ind.* **5**: 276-279.
- Scheuer, P.J. (1995). Marine Natural Products Research; A Look into the Dive Bag. *J. of Nat. Prod.* **58**: 335-343.
- Tringali, C., (1997). Bioactive metabolites from marine algae, recent results, Benthan Science Publishers, London, Vol. I, pp. 375-394.
- Viletinck, A.J., (1987), Topics in Pharmaceutical Sciences, Elsevier Science Publication, Amsterdam, pp. 249-262.
- Washington, J.A., and Sutter, V.L., (1980). Agar and Microbroth dilution procedures, American Society of Microbiology, Washington, 3rd. Ed., pp. 453-462.

PRELIMINARY INVESTIGATIONS OF ANTIMICROBIAL SCREENING OF CRUDE EXTRACTS OF SPONGES AND GORGONIANS SPECIES FROM SAUDI RED SEA COAST

ZEBA PERVEEN¹, SULTAN AL-LIHAIBI², ABDULMOHSIN AL-SOFYANI³,
G.R. NIAZ² AND JEAN-MICHEL KORNPBST⁴

¹HEJ, Institute of Chemistry, University of Karachi, Pakistan

²Marine Chemistry Department, Faculty of Marine Science,
King Abdulaziz University, Jeddah, Saudi Arabia

³Marine Biology Department, Faculty of Marine Science,
King Abdulaziz University, Jeddah, Saudi Arabia

⁴Institute of Substances and Organisms from the Sea (ISOMer)
University of Nantes, France

ABSTRACT

Some of the marine organisms have been found to possess chemotherapeutic properties. The active ingredients can be used as antibacterial, antifungal, antitumor and antiviral agents. These compounds are sometimes difficult to be synthesized in laboratory. Since very little work has been done in the past on the organisms found in the Red Sea, therefore some sponges and gorgonians were collected from the sea near the coast of Jeddah. Our results indicated that *Sh. boydii*, *E. coli*, and *S. aureus*, exhibited susceptibility to some crude extracts of sponges and gorgonians. Ampicillin, Amoxicillin and Cefuroxime were used as standard drugs. During the screening of antifungal activity, it was noted that *Elisella sp.* and *Axinellida sp.* showed significant activity against *C. lunata*, *T. longifusus* and *M. canis*. Myconazole, and Ketoconazole were used as standard drugs for comparison.

INTRODUCTION

The history of drugs is woven with plants, animals and minerals from earlier times. They are mostly of terrestrial origin with smaller number coming from the sea. Despite the wide availability of medicinally useful compounds a continuing search for new antimicrobial agents is still the primary need of the time. These compounds are generally smaller molecules ideally suited to serve either themselves or through chemical modifications as potential new pharmaceutical agents for the treatment of variety of ailments. The scientific community is focusing it's efforts on the isolation and characterization of biologically active compounds derived from marine organisms. It has been demonstrated that marine

organisms are excellent sources of new drugs, (Faulkner, 1995; Fenical, 1996). One may find exotic compounds arranged in completely different ways from those found on land plants and animals. Some of the antibiotics may have drawbacks in the sense that they have either limited antimicrobial spectrum or are associated with some side effects or contraindications. The combination of the genetic versatility of the microbes and widespread over use of antibiotics has also led to increasing clinical resistance of previously sensitive microorganisms and the emergency of previously uncommon infections. It has therefore become highly desirable to explore new molecules exhibiting prominent activity against pathogenic organisms with less undesirable effects (Vlietinck, 1987). In the early 1950's, the first

Table 1b:
Antibacterial activity of Crude Extracts of Gorgonians

Bacterial cultures	Zone of Inhibition in mm			Standard Drugs (200 µg/100µl)		
	<i>Rumphella aggregata</i>	<i>Elisella sp.</i>	<i>Acarboria erythracea</i>	Ampicillin	Amoxicillin	Cefuroxime
<i>S. aureus</i>	-	7	-	22	21	21
<i>S. pyogenes</i>	-	-	-	20	20	20
<i>A. hydrophilla</i>	-	-	-	19	19	19
<i>C. diphtheriae</i>	6	-	6	16	-	15
<i>B. subtilis</i>	-	-	-	18	19	19
<i>E. coli</i>	6	6	6	-	-	19
<i>S. typhi</i>	-	-	-	21	20	18
<i>Sh. boydii</i>	6	-	6	21	21	18
<i>K. pneumoniae</i>	-	-	-	9	-	19
<i>P. mirabilis</i>	-	-	-	20	20	20
<i>Ps. aeruginosa</i>	-	-	-	12	-	-

Key: mm: Zone of Inhibition, - No Antibacterial Activity, Concentration of test sample: 200 µg/100µl)

Table 2
MIC Values of Crude Extracts of the Sponges against Pathogenic Bacteria

Bacterial Culture	MIC Value in µg/ml				
	<i>Ircinia sp.</i>	<i>Phyllospangia sp.</i>	<i>Suberemollis</i>	<i>Axinellida sp.</i>	<i>Sinularia sp.</i>
<i>Staphylococcus aureus</i>	90	-	-	90	95
<i>Corneybacterium diphtheriae</i>	85	-	-	-	-
<i>Bacillus subtilis</i>	85	80	-	-	-
<i>Salmonella typhi</i>	75	85	90	-	-

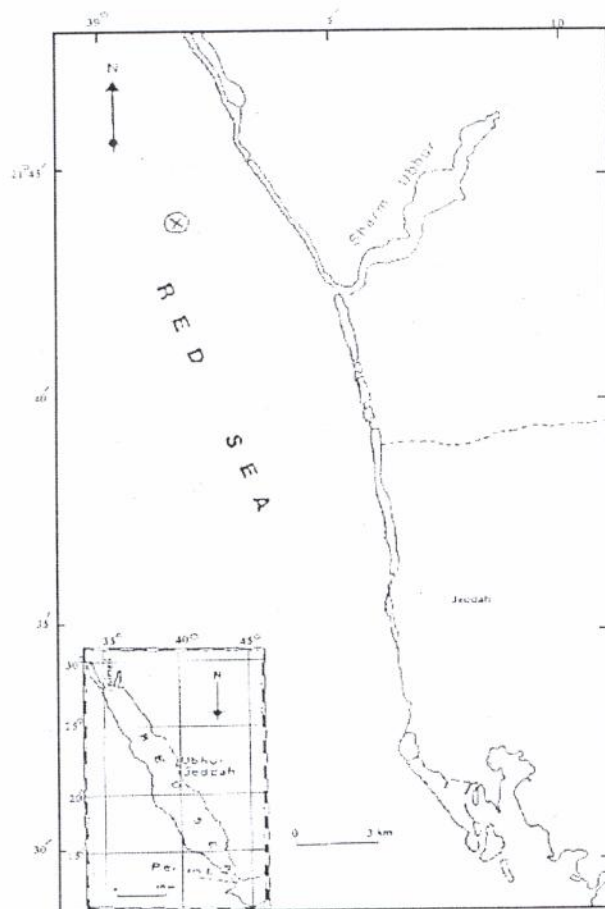
compounds exhibit a diversity of chemical structure (Scheuer, 1991 and 1995). Some of the sponges and related organisms have been found to be associated with antibacterial, antifungal, antiviral and antitumor activity (Higa, 1994, Avasti and

Bhakumi, 1993). The present work describes the collection and identification of various species of sponges and gorgonians and effects of their crude extract on some gram positive and gram-negative bacteria and some of the fungi.

Table 4
MIC Values of crude extracts of the sponges and Gorgonians against pathogenic Fungi

Fungal culture	MIC Values in $\mu\text{g/ml}$						
	<i>Acanthella carteri</i>	<i>Hyrtios erecta</i>	<i>Suberera mollis</i>	<i>Siphonchalina sp.</i>	<i>Simularia sp.</i>	<i>Rumphella aggregata</i>	<i>Axinellida sp.</i>
<i>Microsporium canis</i>	-	-	-	275	-	200	200
<i>Trichophyton longifusus</i>	-	250	-	-	325	250	225
<i>Curvularia lunata</i>	350	225	250	300	300	275	250

Unit = $\mu\text{g/ml}$.



test organisms with solutions containing different concentrations of the test compound.

The antifungal activity was determined by Agar tube dilution method (Paxton, 1991).

Test tubes having sterile Sabourand dextrose agar were inoculated with test compounds in different concentrations and kept in slanting position at room temperature for solidification. Test fungal cultures were inoculated

diversity of substances whose structure when isolated may not have terrestrial counterpart. The pattern of the antimicrobial activity varied with species. However, it can be concluded that *Ircinia sp.*, *Sinularia sp.* and *Phyllospongia sp.* exhibited more promise for future studies. Some of these extracts have shown definite activity as antifungal agents and may prove to be potentially important and may be developed into value added products by preparing their derivatives.

An overall picture of these tables unveils the fact that these crude extracts were more active as antifungal agent rather than antibacterial agent. Moreover the extract may contain more than a dozen compounds in all. If the active principle is isolated in a pure form then one might get better results; it may also act as a starting material for semi-synthetic drugs, and it may prove to be efficacious in medicinal chemistry.

ACKNOWLEDGEMENTS

The authors are grateful to Dr .J. Vacelet, Oceanological Centre, Marseille, France, for identification of all sponge species.

REFERENCES

- Akaniro, J.C. Vidaurre, C.E., Stuttman, H.R., and Marks, M.I., (1990). Antimicrobial agents and chemotherapy; American Society of Microbiology, Washington, U.S.A., **34**: 1880-1884.
- Amade, P., Psedano, D., and Chevolot, L., (1982). Antimicrobial activity of marine Sponges, from French Polynesia and Brittany. *Mar. Biol.* **70**: 223-228.
- Avasthi, K. and Bhakuni, D.S., (1993). Marine Nucleosides. *Indian J. Of Het. Chem.* **2**: 203-218.
- Bergmann, W., and Burke, D.C. (1955). The nucleoside of sponges, spongothy-midine and spongouridine. *J. Org. Chem.* **20**: 1501-1507.
- Faulkner, D.J., Thompson, J.E. and Walker, R.P., (1985), Screening and bioassay for biological active substances from forty marine sponges from San Diego, California. *Mar. Biol.* **88**: 11-12.
- Faulkner, D.J., (1995), Chemical Riches from the Oceans. *Chem. In Britain*, **31**: 680-684.
- Fenicle, W. (1996), Status of new drugs from marine organisms. *Oceanography* **9**(1): 23-27.
- Higa, T., Tanaka, T.I., Kitamura, A., Kyoma, T., Takashi, M., and Uchida, T., (1993). Bioactive compounds from marine sponges. *Pure and App. Chem.* **66**: 2227-2230.
- Newbold, R.W., Jensen, P.R., Fenical, W. and Pawlik, J.R. (1999), Antimicrobial activity of Caribbean Sponge extracts. *Aq. Microb. Ecol.* **19**: 279-284.
- Numata, A. and Iritani, M. (1997). Novel antitumor metabolites produced from a fungal strain from a sea hare. *Tetrahedron. Lett.* **88**(47): 8215-8218.
- Paxton, I.D., (1991). Methods in Plant Biochemistry, Assays for antifungal activities, Academic Press, London, 3rd. Ed., **6**: pp. 33-45.
- Scheuer, P.J. (1991). Drugs from the Sea. *Chem. and Ind.* **5**: 276-279.
- Scheuer, P.J. (1995). Marine Natural Products Research; A Look into the Dive Bag. *J. of Nat. Prod.* **58**: 335-343.
- Tringali, C., (1997). Bioactive metabolites from marine algae, recent results, Benthan Science Publishers, London, Vol. I, pp. 375-394.
- Viletinck, A.J., (1987), Topics in Pharmaceutical Sciences, Elsevier Science Publication, Amsterdam, pp. 249-262.
- Washington, J.A., and Sutter, V.L., (1980). Agar and Microbroth dilution procedures, American Society of Microbiology, Washington, 3rd. Ed., pp. 453-462.