Antimicrobial Activity of Selected Philippine Marine Sponges

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Abstract

The extracts of the eleven species of fresh and dried marine sponges belonging to eight families under Class Demospongia were tested for their antimicrobial activity. The test microorganisms used were: ***Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida utilis*** *and* ***Aspergillus niger****. Ten species (****Adocia*** *sp.,* ***Pellina*** *sp.,* ***Cribrochalina*** *sp.,* ***Xestospongia*** *sp.,* ***Trachyopsis*** *sp.,* ***Halichondria*** *sp.,* ***Phyllospongia foliascens****, both juvenile and mature* ***Biemna fortis*** *and* ***Plakortis*** *sp.) demonstrated varied antimicrobial activity depending on the state of the material and on the solvent used. Extracts from fresh sponge samples gave greater inhibition compared with extracts from dried ones. Alcohol solvent showed greater efficiency in extracting active components from fresh sponges while methanol toluene solvent was efficient in dried sponges. For all ten species, inhibition was found to be greater against Gram-positive bacteria but* ***Adocia*** *sp.,* ***Pellina*** *sp.,* ***Cribcrochalina*** *sp.,* ***Trachyopsis*** *sp.,* ***Suberites*** *sp., and* ***Phyllospongia foliascens*** *inhibited fungi. The* ***Mycale*** *sp. did not show any antimicrobial activity.*

Introduction

Sponges were established to contain a great number of novel compounds of diverse chemical patterns and spectra that have antimicrobial properties. This activity is related to their adaptation to live as filter feeders and strainers of myriads of bacteria and microplankton.

Starting in 1952, Nigrelli observed the antibiotic effects of some tropical sponges by placing fresh sponge fragments on seeded petri dishes. He discovered that fluids extracted from freshly collected sponges showed a relatively high degree of antibacterial activity. Material extracted from dried or dead sponges demonstrated little or no such activity, and the

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number of bacteria were seen to increase considerably within relatively short time. In subsequent studies it was found that extracts of large number of sponges from either frozen or dried material made with water, ethanol, methanol, or acetone were inhibitory against a number of microorganisms. Antagonistic property of sponges against a pink yearst. *Ianthella* sp., was reported by Buck and Meyers (1962). Stempien (1966) discovered an antibiotic substance from a sponge of the genus *Agelas* and had observed that organic and aqueous extracts of the sponge have broad spectrum antibiotic effects. Later, a new antimicrobial substance "ectyonen" from a red beard sponge, *Microciona prolifera*, was isolated.

Sharma and Burkholder (1967) conducted a study on sterols, pigments and introgenous bases which were extracted from sponges and revealed the presence of antibiotic substance in the extracts. One of the compounds extracted from *Verongia* sp. was shown to be a derivative of dibromocyclohexadienone and considered among the most active. Sharma *et al* (1968) were able to show similar activity in extracts of *Haliclona viridis, Clionata celata* and *Tedania ignis*. In some cases extracts which were antifungal were also antibacterial.

Burkholder and Ruetzieler (1969) showed that out of 31 sponge species they collected, 18 species showed antimicrobial activity. There were several antibacterial compounds from *Dysidea herbacia* that yielded two active antibacterial substances. One was pentabromo derivative (2-hydroxydiphenyl ether) and another a dibromo derivative of 2-hydroxydiphenyl ether. In 1974, the U.S. National Science Foundation sponsored a scientific expedition aboard the R/V Alpha Helix Vessel. This expedition examined 831 species along West Coast of Baja California and in the Gulf of California. Seventy-one (71) species of poriferans gave high incidence of antimicrobial activity. Of these species, 32% were against *Bacillus subtilis*, 18% against *Escherichia coli*, and 17% against *Penicillium atrovenetum* and 13% against *Saccharomyces cerevisiae*. The second expedition was held in 1978. Out of the 187 species of poriferans examined, 82% had high antimicrobial and antiviral activities. Of this 41% was against *Bacillus subtilis*, 19% against *Saccharomyces cerevisiae*, 14% against *Escherichia coli*, and 11% against *Penicillium atrovenetum*, while the rest were active only against viral test organisms. This expedition also noted that at least three sponges had similar spectra and apparently the same antimicrobial compounds. One of these, the rope-like orange sponge *Ptilocaulis* aff.*P. spiculifer* (Lamarck)yielded extracts that appeared to be the most active against Gram-positive and Gram-negative bacteria, yeast and filamentous fungi. The active substances were identified as ptilocaulin and isoptilocaulin.

Phillipson and Rhinehart (1983) isolated two peroxy acids from an unidentified genus of the Family Plakinidae which they found to be very

active against fungal species. Matsunaga *et al.* (1985) identified an antimicrobial peptide *discodermin A* from a marine sponge *Discodermia kiiensis*. Methanolic extracts of this sponge exhibited significant activities against fungi, Gram-negative and Gram-positive bacteria, and also inhibited the development of starfish embryos.

Tayo (1986) and Raymundo *et al.* (1981) conducted a study on the antibacterial activity of some Philippine marine sponges. Tayo found that the aqueous and methanolic extracts of *Dysidea herbacia* showed high antibacterial activity was observed in the methanolic extracts of *Spirastrella vagabunda* and *Sigmadocia symbiotica* which inhibited *Bacillus subtilis*.

Materials and Methods

Collection and Identification of Samples

Sponges were harvested from Kauswagan, Lanao del Norte in Mindanao on September 9, 1986. Each distinct species or kind was separately placed in plastic bags, properly labeled and placed in buckets with ice. Assays using fresh materials were done upon arrival in the laboratory. Parts of the samples were stored in the freezer for the later experiments using dried materials. Procedures for morphological studies on the sponges were undertaken according to microtechniques suggested by Mr. Rodolfo Caberoy, curator of sponges at the National Museum. Verification of the identity of the specimens was made against type specimens available at the National Museum and descriptions in literature (Gray, 1867; Gray, 1872; Wilson, 1925; de Laubenfels,1935; Clemente, 1941; Arnt, 1943; Levi, 1959; Ruelo, 1964; Esmero, 1978; Lendenfeld, 1888; 1889; Weerdt and van Soest, 1986; Hummelinck and van der Steen, 1980; Dendy, 1905; Bergquist, 1970; 1978; Vacelet, Vasseur, and Levi, 1976).

Preparation of Extracts

Fresh and dried samples were extracted separately with respective solvents. Sponge samples were thoroughly rinsed with tap water to wash out salt water. Contaminants were removed from the samples and were cut into pieces for the extraction process. Two grams of the samples were then pounded in a mortar and pestle until thoroughly minced. Ten milliliter (10 ml) of either 95% ethanol, a mixture of methanol-toluene (3:1) and distilled water were added and the mixture stirred slightly to allow the dissolution of active substances in the sample. The mixture was then centrifuged at 3000 RPM and transferred to sterile vials. Dried sponge samples were also

extracted in the same manner as that of fresh samples.

Antimicrobial Assay: Filter Paper Disc Method

Nutrient Agar (NA) was used for growing the bacteria and Malt Extract Agar (MEA) for the fungal test organisms. Test organisms used were *Staphylococcus aureus* Rosenbach (UPCC 143), *Bacillus subtilis* (Ehrenberg) Cohn (UPCC 6633), *Pseudomonas aeruginosa* (Schroeter) Migula (UPCC, 196/ATCC 27833), *Escherichia coli* (Migula) (UPCC 196/ATCC 25922),one mold *Aspergillus niger* (UPCC 3450) and one yeast *Candida utilis* (UPCC 2001/NRRLY-900). These organisms were obtained from the Culture Collection of the University of the Philippines-Natural Science Research Institute.

The method used by Raymundo *et al.* (1981) was followed. The activities of each sponge extract per solvent used were compared with the effects of a known broad spectrum antibiotic (Streptomycin) and the respective solvents used in the experimental runs. The sterile filter paper discs were also immersed into the particular solvents and dried in the same manner as that of the discs impregnated with test extracts.

Results

Identification of the Different Sponges

The sponges used in this study were identified as follows: *Adocia* sp. Gray and *Pellina* sp. (Family Adociidae); *Cribrochalina* sp. Schidmt, *Xestospongia* sp. Dendy (Family Nephiospongiidae; *Halichondria* sp. (Pallas) (Family Halichondriidae); Suberites sp. Bergquist (Family Clavulidae); *Phyllospongia foliascens* (Burton) Pallas (Family Spongiidae); *Biemna fortis* Gray (Family Descacidonidae); *Plakortis* sp. Schulze (Family Plakinidae); and *Myçale* sp.(Carter) (Family Mycalidae).

Antimicrobial Activity of Different Sponges

Adocia sp. Gray

Of all the sponge species used, *Adocia* sp., had the widest spectrum of antimicrobial activity in so far as the ethanolic extracts were concerned. Table 1 shows that its fresh extracts inhibited all the microorganisms except *E. coli*. Its dry extracts showed activity against *E. coli*, S. *aureus*, and *B. subtilis*. Ethanolic and methanol-toluene extracts from fresh samples also inhibited *Aspergillus niger*. Methanol-toluene extracts of this species showed

results similar to that of ethanol except that *E. coli* was inhibited by both fresh and dry extracts. The aqueous extracts of Adocia sp., gave a different result, only Gram-positive bacteria were inhibited by both extracts from fresh and dried materials.

Table 1. Antimicrobial activity of *Adocia* sp. Gray

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	-	+	-	++	+	-
P. aeruginosa	++	+	-	-	-	-
S. aureus	++	++	+	+	++	++
B. subtilis	++++	++	+	++	+	++
C. utilis	+	-	-	-	-	-
A. niger	++	+	-	-	-	-

Legend: meand zones of inhibitions (mm)

no inhibition		=	-
1 -	10	=	+
11 -	20	=	++
21 -	30	=	+++
31 -	40	=	++++
41 -	50	=	+++++

Pellina sp. de Laubenfels

The effect of this sponge.extract is rather variable in regard to the different solvents. Ethanol extracts from dried materials inhibited *E. coli*, while methanol-toluene extracts of the same dried material inhibited *P. aeruginosa*. The methanol-toluene extracts from fresh materials inhibited A. niger only. None of the microorganisms used was inhibited by either of the fresh or dried sponge aqueous extracts (Table 2).

Table 2. Antimicrobial activity of *Pellina* sp. de Laubenfels

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	-	-	-	+	-	-
P. aeruginosa	-	-	-	-	+	-
S. aureus	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-
C. utilis	-	-	-	-	-	-
A. niger	-	+	-	-	-	-

Legend: meand zones of inhibitions (mm)

no inhibition		=	-
1	- 10	=	+
11	- 20	=	++
21	- 30	=	+++
31	- 40	=	++++
41	- 50	=	+++++

Cribrochalina sp. Schmidt

Extracts of both ethanol and methanol-toluene using fresh materials inhibited consistently all the test bacteria (*E. coli, P. aeruginosa, S. aureus, and B. subtilis*), while aqueous extracts from fresh materials inhibited only the Gram-positive bacteria (*S. aureus* and *B. subtilis*). Extracts from dried materials with both methanol-toluene and distilled water inhibited *P. aeruginosa* including the two Gram-positive bacterial while its ethanolic extracts inhibited all the bacteria except *P. aeruginosa* (Table 3).

Xestospongia sp. (Ducchassiang)

The effect of fresh ethanol extracts and dried methanol-toluene extracts were practically the same having inhibited all bacterial except that the latter also inhibited a yeast, *Candida utilis*. The aqueous extracts of fresh and dried materials showed consistent results in inhibiting the two Gram-positive bacteria but with apparently higher activity in the dried samples (Table 4).

Trachyopsis sp. Dendy

Ethanolic extracts of fresh materials inhibited all the bacteria while extracts from the dried sample inhibited only the Gram-positive bacteria.

Table 3. Antimicrobial activity of *Cribrochalina* sp. Schidmt

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	+	+	-	+	-	-
P. aeruginosa	++	+	-	-	+	+
S. aureus	++	++	+	++	++	+
B. subtilis	++	++	+	++	++	++
C. utilis	-	-	-	-	-	-
A. niger	-	+	-	-	-	-

Legend: meand zones of inhibitions (mm)

no inhibition		=	-
1 -	10	=	+
11 -	20	=	++
21 -	30	=	+++
31 -	40	=	++++
41 -	50	=	+++++

Table 4. Antimicrobial activity of *Xestospongiae* sp. Ducchassing

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	++	++	-	+	++	-
P. aeruginosa	+++	-	-	-	+	-
S. aureus	++	++	+	-	++	+++
B. subtilis	++	++	++	++	++	++
C. utilis	-	-	-	-	++	-
A. niger	-	-	-	-	-	-

Legend: meand zones of inhibitions (mm)

no inhibition		=	-
1 -	10	=	+
11 -	20	=	++
21 -	30	=	+++
31 -	40	=	++++
41 -	50	=	+++++

On the other hand, extracts of both fresh and dried materials with methanol-toluene as solvents inhibited all the microorganisms except the mold, *Aspergillus niger*. The aqueous extracts from fresh and dried samples inhibited the two Gram-positive bacteria. The latter also demonstrated activity against *E. coli* (Table 5).

Table 5. Antimicrobial activity of *Trachyopsis* sp. Dendy

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	+	+	-	-	+	+
P. aeruginosa	++	++	-	-	+	-
S. aureus	++	++	++	++	++	++
B. subtilis	++	++	++	+	++	++
C. utilis	-	+	-	-	+	-
A. niger	-	-	-	-	-	-

Legend: meand zones of inhibitions (mm)

no inhibition		=	-
1 -	10	=	+
11 -	20	=	++
21 -	30	=	+++
31 -	40	=	++++
41 -	50	=	+++++

Halichondria sp. (Pallas)

Methanol-toluene extracts from dried sponge samples yielded a positive result. It inhibited the two Gram-negative bacteria, *E. coli* and *P. aeruginosa* although, its inhibition was very minimal (Table 6).

Suberites sp. Bergquist

Ethanolic extracts of fresh and dried materials inhibited *A. niger*. Fresh ethanolic extracts also inhibited *P. aeruginosa* while dried ethanolic extracts inhibited *E. coli*. On the other hand, fresh methanol-toluene extracts inhibited *A. niger* while dried methanol-toluene extracts inhibited both Gram-negative bacteria. Neither fresh nor dried aqueous sponge extracts showed any antimicrobial activity (Table 7).

Table 6. Antimicrobial activity of *Halichondria* sp. (Pallas)

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	-	-	-	-	+	-
P. aeruginosa	-	-	-	-	+	-
S. aureus	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-
C. utilis	-	-	-	-	-	-
A. niger	-	-	-	-	-	-

Legend: meand zones of inhibitions (mm)

no inhibition		=	-
1 -	10	=	+
11 -	20	=	++
21 -	30	=	+++
31 -	40	=	++++
41 -	50	=	+++++

Table 7. Antimicrobial activity of *Suberites* sp. Bergquist

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	-	-	-	+	+	-
P. aeruginosa	+	-	-	-	+	-
S. aureus	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-
C. utilis	-	-	-	-	-	-
A. niger	++	++	-	++	-	-

Legend: meand zones of inhibitions (mm)

no inhibition		=	-
1 -	10	=	+
11 -	20	=	++
21 -	30	=	+++
31 -	40	=	++++
41 -	50	=	+++++

Phyllospongia foliascens (Burton) Pallas

Extracts from fresh materials using all the three solvents gave positive inhibitions against the two Gram-positive bacteria.

The ethanol and methanol-toluene extracts of the same material also inhibited C. *utilis*. The extracts from dried materials using methanol-toluene and distilled water solvents inhibited only *B. subtilis* (Table 8).

Table 8. Antimicrobial activity of *Phyllospongia foliascens* Burton (Pallas)

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-
S. aureus	++	++	+	-	-	-
B. subtilis	+++	+++	++	-	++	+
C. utilis	+	+	-	-	+	-
A. niger	-	-	-	-	-	-

Legend:

meand zones of inhibitions (mm)		
no inhibition	=	-
1 - 10	=	+
11 - 20	=	++
21 - 30	=	+++
31 - 40	=	++++
41 - 50	=	+++++

Biemna fortis Gray (juvenile)

Ethanolic extracts of fresh materials produced inhibitions against the two Gram-positive bacteria including one Gram-negative bacterium (*E. coli*). Extracts from dried materials in the same solvent did not show any inhibition to all the microorganisms used in this study. Methanol-toluene extracts from fresh materials inhibited *E. coli* while dried materials inhibited the two Gram-negative bacteria. Aqueous extracts from either fresh or dried sponge samples did not demonstrate any antimicrobial activity (Table 9).

Biemna fortis Gray (mature)

Ethanolic extracts of fresh materials inhibited all four bacteria while extracts of dried materials did not show any activity. Methanol-toluene extracts of fresh material inhibited *E. coli* and *S. aureus*, Gram-negative and Gram-positive bacteria, respectively. Dried material extracts in methanol-toluene solvent inhibited *E. coli* only. The aqueous sponge extracts from neither fresh nor dried materials effected any inhibition (Table 10).

Table 9. Antimicrobial activity of *Biemna fortis* Gray (juvenile)

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	+	+	-	-	+	-
P. aeruginosa	-	-	-	-	+	-
S. aureus	++	-	-	-	-	-
B. subtilis	++	-	-	-	-	-
C. utilis	-	-	-	-	-	-
A. niger	-	-	-	-	-	-

Legend:

meand zones of inhibitions (mm)		
no inhibition	=	-
1 - 10	=	+
11 - 20	=	++
21 - 30	=	+++
31 - 40	=	++++
41 - 50	=	+++++

Plakortis sp. Schulze

Extracts from dried materials of this species using ethanol and methanol-toluene as solvents gave positive inhibition against Gram-negative bacteria except that the latter extract showed activity against *S. aureus*. However, ethanolic extracts of fresh materials of this sponge species also inhibited *P. aeruginosa*. The aqueous extracts for either fresh or dried materials did not demonstrate antimicrobial activity (Table 11).

Table 10. Antimicrobial activity of *Biemna fortis* Gray (mature)

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	+	+	-	-	+	-
P. aeruginosa	+	-	-	-	-	-
S. aureus	+	+	-	-	-	-
B. subtilis	++	-	-	-	-	-
C. utilis	-	-	-	-	-	-
A. niger	-	-	-	-	-	-

Legend: meand zones of inhibitions (mm)

no inhibition		=	-
1	- 10	=	+
11	- 20	=	++
21	- 30	=	+++
31	- 40	=	++++
41	- 50	=	+++++

Table 11. Antimicrobial activity of *Plakortis* sp. Schulze

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	-	-	-	+	+	-
P. aeruginosa	+	-	-	+	+	-
S. aureus	-	-	-	++	-	-
B. subtilis	-	-	-	-	-	-
C. utilis	-	-	-	-	-	-
A. niger	-	-	-	-	-	-

Legend: meand zones of inhibitions (mm)

no inhibition		=	-
1	- 10	=	+
11	- 20	=	++
21	- 30	=	+++
31	- 40	=	++++
41	- 50	=	+++++

Mycale sp. (Carter)

Of the eleven sponge species used, this is the only species which consistently gave negative results in all of the three solvents all throughout the experiment (Table 12).

Table 12. Antimicrobial activity of *Mycale* sp. (Carter)

Microorganism	Fesh Samples			Dried Samples		
	ETOH	MeOH Toluence	DW	EtOH	MeOH Toluence	DW
Escherichia coli	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-
S. aureus	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-
C. utilis	-	-	-	-	-	-
A. niger	-	-	-	-	-	-

Legend: meand zones of inhibitions (mm)

no inhibition	=	-
1 - 10	=	+
11 - 20	=	++
21 - 30	=	+++
31 - 40	=	++++
41 - 50	=	+++++

The positive control (Streptomycin) showed its wide spectrum of activity against bacteria but not with fungi. Apparently, some sponge extracts gave similar diameter measurement of inhibition. The negative controls which is comprised by the solvents used in this study gave no inhibitions.

Based on the results presented in Table 13 (summary table), sponge extracts which have broad spectrum activity are the following: *Adocia* sp., *Xestospongia* sp., *Biemna fortis* (juvenile and mature samples), *Trachyopsis* sp., *Cribrochalina* sp., and *Plakortis* sp. The species which inhibited Gram-negative bacteria are: *Pellina* sp., *Halichondria* sp., and *Suberites* sp., while *Phyllospongia foliascens* inhibited Gram-positive bacteria only.

Of all sponge extracts used, only extracts from *Adocia* sp., inhibited both mold and the yeast. *Xestospongia* sp., *Trachyopsis* sp., and *Phyllospongia*

Table 13. Summary of Antimicrobial Activity of Eleven Marine Sponge Species collected from Lanao del Norte, Philippines

Bateria / Sponge Species		Fungi Gram +	Gram -	CU	AN
Adocia sp.	(bs)	+	+	+	+
Pellina sp		-	+	-	+
Cribrochalina sp.	(bs)	+	+	-	+
Xestospongia sp.	(bs)	+	+	+	-
Trachyopsis sp.	(bs)	+	+	+	-
Halichondria sp.		-	+	-	-
Suberites sp.		-	+	-	+
P. foliascens		+	-	+	-
Biemna fortis (j)	(bs)	+	+	-	-
Biemna fortis (m)	(bs)	+	+	-	-
Plakortis sp.	(bs)	+	+	-	-
Mycale sp.		-	-	-	-

Legend :

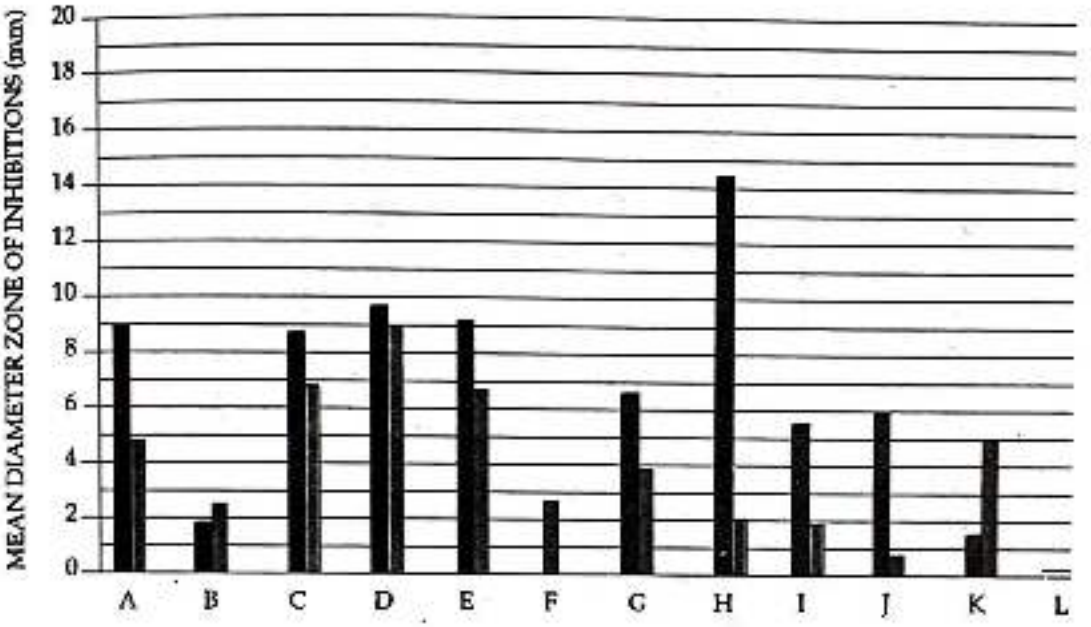
+	=	there is growth inhibition
-	=	no inhibition
j	=	juvenile form
m	=	mature form
CU	=	*Candida utilis*
AN	=	*Aspergillus niger*
bs	=	broad spectrum

foliascens inhibited *Candida utilis* while *Pellina* sp., *Suberites* sp., and *Cribrochalina* sp. inhibited *Aspergillus niger.*

Activity of Fresh and Dried Extracts of Sponges

In Figure 1, fresh sponge extracts of *Adocia* sp., *Cribrochalina* sp., *Trachyopsis* sp., *Phyllospongia foliascens*, and both juvenile and mature *Biemna fortis* have significantly greater antimicrobial activity than dried samples. For *Xestospongia* sp., and *Suberites* sp., although fresh extracts were apparently higher than extracts from dried samples, their difference is not statistically significant. In contrast, *Plakortis* sp. and *Halichondria* sp., showed significantly greater antimicrobial activity in dried than in fresh samples.

Figure 1. Antimicrobial activity of extracts from fresh and dried materials of eleven marine sponges.



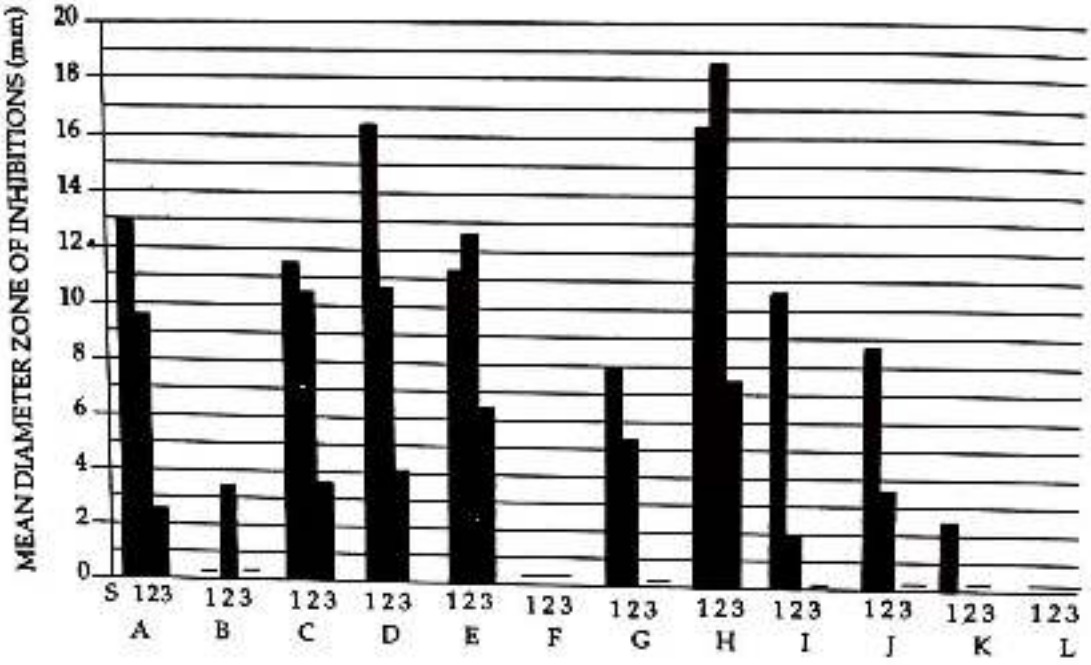
Both extracts from *Pellina* sp., did not show significant difference in its antimicrobial activity.

Effects of Solvents

Figure 2 shows the antimicrobial activity of extracts from fresh materials using three solvents. Diameter zones of inhibitions produced by ethanol extracts showed significantly higher in *Adocia* sp., *Xestospongia* sp., *Suberites* sp., both juvenile and mature *Bienma fortis* and *Plakortis* sp. than in other solvents. *Cribrochalina* sp., and *Trachyopsis* sp. showed no significant difference between methanol-toluene and ethanol solvents. In the case of *Pellina* sp. antimicrobial activity was detected only with methanol-toluene as solvent. In contrast, *Plakortis* sp. showed positive inhibitions agains microorganisms when ethanol solvent was used. Obviously, the aqueous extracts gave significantly lower inhibitory activity.

Figure 3 shows the antimicrobial activity of marine sponges using dried materials extracted with three different solvents. With the use of methanol-toluene as solvent the species *Xestospongia* sp., *Trachyopsis* sp., *Halichondria* sp. and *B. fortis* (both juvenile and mature) displayed signifi-

Figure 2. Antimicrobial activity of extracts form fresh materials of eleven sponge species using three different solvents.



cantly different activity than the rest. With the use of ethanol as solvent, the species *Adocia* sp., *Suberites* sp. and *Plakortis* sp. were those that displayed higher significant value than the rest of the solvents. The values obtained from the rest of the species were not significantly different from each other.

Sensitivity of Microorganisms to Sponge Extracts

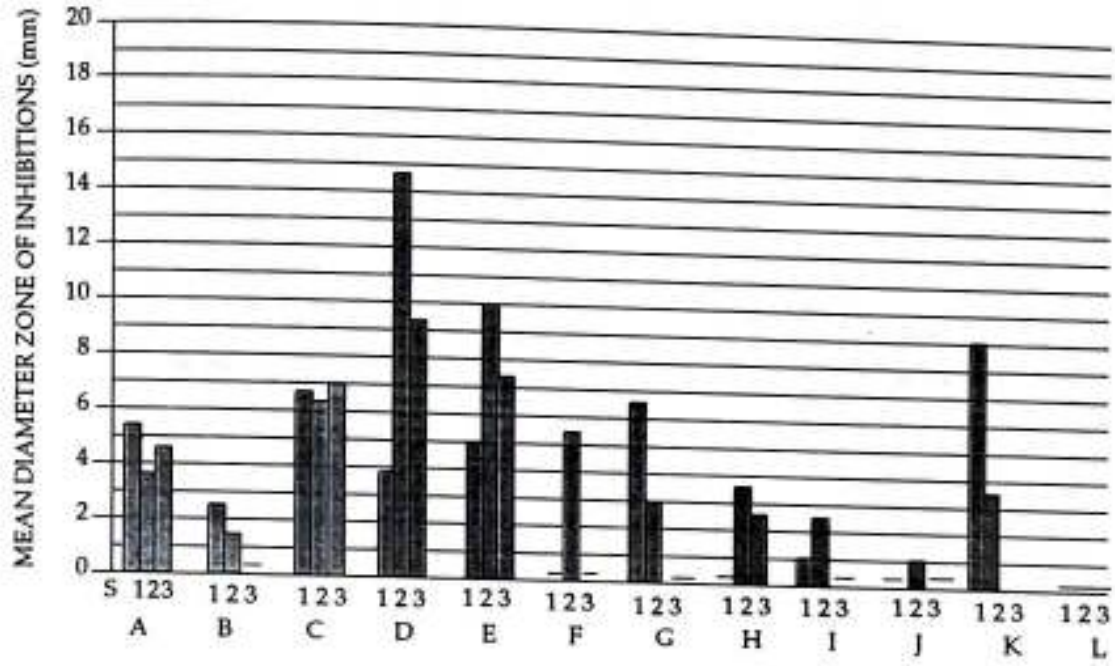
Of all the microorganisms used in the experiment, the two Gram-positive bacteria (*S. aureus* and *B. subtilis)* consistently demonstrated the highest susceptibility to sponge extracts whether from fresh or dried materials. This was followed by the Gram-negative bacteria namely *E. coli* and *P. aeruginosa*. The fungi were not inhibited by the aqueous sponge extracts and generally were less susceptible to the other sponge extracts.

Discussion

According to Rinehart (1981) and Hashimoto (1976), the presence of antimicrobial compounds in marine sponges is a general phenomenon. Both scientists also suggest that said compounds are utilized in the defensive strategy of these sedentary and filter-feeding animals.

This study confirms that sponges have metabolites capable of inhibiting microbial growth. Certain variations with respect to antimicrobial

Figure 3. Antimicrobial activity of extracts from dried materials of eleven sponge species using three different solvents.



A - Adocia sp.
B - Pellina sp.
C - Cribrochalina sp.
D - Xestospongia sp.
E - Trachyopsis sp.
F - Halichondria sp.
G - Suberites sp.
H - Phyllospongia sp.
I - Biemna fortis (juvenile)
J - Biemna fortis (mature)
K - Plakortis sp.
L - Mycale sp.
S1 - Ethanol (95%)
S2 - Methanol - toluene
S3 - Distilled water

LEGEND
Fresh
Dried

activity of each sponge species were observed. Some were shown to contain broad spectrum substances of metabolites affecting both Gram-positive and Gram-negative bacteria. Others had specific reactions to either of the two types of bacteria, while there were those which had inhibitory actions against fungi.

This noticeable difference in antimicrobial activity of sponge ext racts may be due to some reported evidence that the animal itself is capable of synthesizing the active compounds. Also, the possibility that such active metabolites may have been by-products of microorganisms or other symbionts associated with the sponge (Jakowska and Nigrelli, 1960).

Effects of Solvents

Apparently, the three solvents used in this study: 95% ethyl alcohol, mixture of methanol-toluene (3:1) and distilled water gave varied results. This only suggests that the active compounds have also varied chemical nature. Alcohol for instance is characteristically slightly polar with non-polar end (-R), methanol-toluene is a mixture of non-polar (toluene) and slightly polar (methanol), whereas water is definitely strong polar solvent. Based on these accounts, it is expected that the first two solvents are more effective in extracting active subtances from sponges for maximizing the dissolution of both polar and non-polar compounds.

Several previous studies have shown that there is a variety of compounds present in marine sponges. Majority of these are antibacterial and antifungal. Among the most active compounds are terpenes, polychlorinated metabolites from amino acids, polybrominated diphenyl ethers including pentabromodiphenyl and hexabromodiphenyl ether (Targett and Keenan, 1984). Also, according to Bergquist (1978), most halogenated terpenes and dibromotyrosine are strong antimicrobial agents more particlarly the furanosesterterpenes containing tetroic acid unit (e.g. *variabilin*). The halogens they contain are also strong oxidizing agents which account for the antimicrobial activity of these substances. The above compounds are readily soluble in non-polar solvents (Faulkner, 1986). This may explain the considerable greater inhibitory activity of sponge extracts made with 95% ethanol and methanol-toluene (3:1) solvents in the present study. For instance, ethanol extracts have the highest activity in seven species of the sponges compared with the methanol-toluene and distilled water. For aqueous extracts, five species out of eleven sponges demonstrated the antimicrobial activity, however, the extent of inhibition is very minimal compared with the other two solvents. This is very reasonable since water as strong as polar solvent dissolves only polar compounds, thus, only limited substances are found in the extract. From the above observations, it is apparent that active antibiotic substances are either polar or non-polar compounds. The data show that there are more non-polar compounds. The variety of probable antimicrobial substances from different species of sponges may explain the varying effects of the solvents used for extraction.

Effects of Fresh and Dried Materials

Fresh and dried sponge materials were separately extracted and the corresponding extracts were compared. Extracts from fresh samples showed greater inhibitory activities against microorganisms whereas extracts from dried samples showed lesser inhibition despite the fact that they were

concentrated. One implication regarding this is that most antibiotic substances should be retrieved while sponges are fresh. Another is that the active substances are heat labile and volatile. This is confirmed in the marked decrease in antimicrobial activity of extracts from dried materials as shown in this study. The probable reason for this is that there may be substances that have been modified during the drying process. This renders them less effective in inhibiting the microorganisms. The process of dehydration has destroyed other active substances and the heat stable compounds may have been less potent than the heat labile components compared may have been less potent than the heat labile components compared with those of fresh extracts.

Of the 11 sponge species, only *Halichondria* sp. showed inhibitory activity with the extracts from dried materials but not with its fresh sample extracts. The rest except *Mycale* showed antimicrobial activity for both extracts with considerable decreassed activity in dry extracts. The probable reason for this is the *Halichondria* sp. might have unique substances that have been activated when dried. Those species with antimicrobial activity even from extracts using dried samples may have heat stable active compounds.

Sensitivity of Microorganism to Sponge Extracts

Microorganisms vary in their response to different inhibitory substances. The factor that induces this difference may be the obvious difference in chemical composition of their cell walls. This stucture being the outermost organelle serves as barrier to the external millieu. The major chemical components in the cell walls of Gram-negative bacteria are peptidoglycans (Pelczar, 1997). Once the permeability of the cell wall is altered antibiotic substances may enter the protoplasm of the bacteria causing them to be destroyed or damaged. Such destruction is primarily the reason for its growth inhibition. Observations show that the Gram-positive bacteria consistently revealed high sensitivity to all sponge extracts. It simply implies that the active compounds present in the sponge are capable of destroying easily or at least interacting with peptidoglycans and teichoic acids lead to the inhibition of bacterial proliferation. Such activity is less pronounced in Gram-negative bacteria which may suggest reactivity otherwise. Other possible reaction is the ability of the sponge extracts to alter the permeability of the cell wall which may also allow penetration of active components into the cell. Such event may consequently block synthetic agents, possibilities which should be subjected to further investigation.

The results of this experiment reveal that only Gram-negative bacteria are inhibited by *Pellina* sp., a finding which differs form Stempien

(1966) who reported that this sponge contains active substances against a wide spectrum of microorganisms. Further, in this study, *Suberites* sp. was shown to be inhibitory to bacterial while that of Minale (1978) demonstrated antifungal activity. This present investigation also shows that methano-toluene extracts of *Halichondria* sp. exhibited antibacterial activity while the study of Kingston *et al.* (1982) revealed that the extracts of these species are cytotoxic and larvicidal. Obviously, there are differences of results which may only suggest possible geographical variation among sponge species.

Previous to this study, there are no reports on the presence of antimicrobial compounds in *Biemna fortis, Trachyopsis* sp., and *Suberites* sp. This investigation shows that ethanolic and methanol-toluene extracts of *B. fortis* contain antibacterial substances specifically against both Gram-positive and Gram-negative bacteria. It also confirms the antimicrobial activityof *Adocia* sp., (Kobayashi *et al.*, 1983), *Pellina* sp. (Stempien, 1966); *Phyllospongia foliascens* (Kobayashi *et al.*, 19982); *Plakortis* sp. (Minale, 1978); *Halichondria* sp. (Kingston *et al.*, 1982).

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