Revista Internacional de BOTANICA EXPERIMENTAL ΦYTON International Journal of EXPERIMENTAL BOTANY

Fundada en 1951 por Founded 1951 by
Miguel Raggio & Nora Moro-Raggio
Editor: Dr. Miguel Raggio
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina

53° ANIVERSARIO 2004: 165-170 53rd ANNIVERSARY

Active fractions from four species of marine algae

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Abstract. A bioassay-directed is utilized to detect substances with biological activity from *Gracilaria tikvahiae, Ulva lactuca, Ulva fasciata* and *Sargassum fluitans.* In a preliminary assessment, polar and non polar extracts of four species of marine protoctist form were screened for antibacterial and antifungal properties against seven microorganisms by the diffusion method, non polar extracts of *Sargassum fluitans*,and polar extracts of *Gracilaria tikvahiae* inhibited the growth of more than four microorganisms. Extracts were separated using chromatography column and fractions were tested against *Stapylococcus aureus* and *Candida albicans.* The eighty fraction of petroleum ether of S. fluitans exhibited high activity against *C. albicans*, MIC 0.16 µg/mL.

In recent years several marine bacterial and protoctist forms have been confirmed as important source of new compounds potentially useful for the development of chemotherapeutic agents. Previous investigations of the production of antibiotic substances by aquatic organisms point to these forms as a rich and varied source of antibacterial and antifungal agents.

Antimicrobial substances produced by benthic tropical marine forms were studied by Allen & Dawson (1) and were screened by them against *E. coli* and *S. aureus*. Mesmar & Abussaud (2) reported that most effective antibacterial agents were species from the marine protoctist phylum Pr 15. Chlorophyta (green), followed by Pr 12. Phaeophyta (brown) and Pr 13. Rhodophyta (red). König & Wrigth (3) provide an overview of the literature related to the three forms that contain naturally active products. Species of the genera studied have been widely reported for their antimicrobial activities. Acrylic acid, the first compound with antibiotic

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We wish to thank CONACYT
Received 19.IV.2003: accepted 28.V.2003

activity of any of these marine forms, was obtained from the genus Gracilaria (4). Antiviral activity of the alcohol extract of *Ulva fasciata* was reported by Sharma & Bhakuni (1992). Antibiotic substances of *Sargassum natans* were isolated by Martínez & Casillas (7), from Sargassum, which they subsequently characterized (6). Glombitza & Sukopp (4) report polihidroxphenyl ether with antimicrobial activity from the brown alga *Sargassum spinuligerum*.

The nonpolar extracts obtained by successive extractions using benzene, chloroform and methanol exhibited high antimicrobial activity according to Sastry & Rad (9).

This study reports the screening for antimicrobial activity of the chlorophyte (*Ulva fasciata, Ulva lactuca*), rhodophyte (*Gracilaria tikvahiae*) and phaeophyte (*Sargassum fluitans*), all of which are marine forms.

MATERIALS & METHODS

Plant material. The marine material used in this study was collected from the coast of the Gulf of Mexico at N 20° 28' 28", W. 97° 51' 05" during August 1994. Samples of these forms were authenticated by Dra. Leticia Villarreal Rivera of our Department of Botany and have been deposited in the herbarium of the Faculty of Biology at Universidad Autónoma de Nuevo León.

Chemical extraction and fractionation. The extracts were obtained by macerating 30 g of the dried plant in cold petroleum ether for 48 h, with the resultant extract being filtered and then concentrated to dryness in a rotary evaporator under reduced pressure. Chloroform, acetone, ethanol, methanol or water extracts were obtained similarly. The extracts were diluted in different solvents for approximately 2.5 mg/ml, then sterilized by passage through 0.45 µm filter.

The active extract was analyzed chromatographically on silica gel 60 (Baker) eluted with benzene acetone gradient into several fractions giving 42 fractions tested against *S. aureus* and *C. albicans*.

Microorganisms. The strains used in this study (*E.coli, S.aureus, S, enteriditis, S. epidermidis, P. aeruginosa, S, faecalis* and *C. albicans*), were provided by Manuel Rodriguez Q. (Department of Microbiology Fac. Medicina, Universidad Autónoma de Nuevo León), the bacterial strains were grown and maintained on nutrient agar slants. Yeast was cultured on Sabouraud dextrose 45 agar slants. The inoculated agar was incubated a 37°C.

Antimicrobial activity test. Test was performed by the diffusion method (Rios et al) using 1 ml. of inoculum contains 10 microorganisms per plate. Extract-impregnated discs (10 µg per disc) were placed on the

agar Muller Hinton and incubated at 37°C overnight. Sterile solvents were used as negative control and various dilutions of ampiciline and penprocilin were used as positive control. Experiments were carried out in quadruplicate. Antimicrobial activities of crude extracts were indicated by clear zones of growth inhibition The MIC value was taken as the lowest concentration of compound which inhibited the growth of the test microorganisms after 24 h of incubation at 37°C

RESULTS & DISCUSSION

The comparative preliminary analysis of the macerated and soxhlet extracts (Table 1) showed that macerated extracts are the more active. A total the 42 crude extracts corresponding to species were tested (Table 2); antimicrobial activity was found in ether extracts of *Sargassum fluitans* against *E. coli, S. aureus, Candida albicans* and *S. epidermidis;* these pharmacological activities have been reported in lipophilic extract of phaeophytas by Tringali et al. acetone extracts of *Gracilaria foliifera exhibited* activity against microorganisms mentioned, and *P. aeruginosa,* antimicrobial activity of extract genus *Gracilaria* was already reported (Hoppe et al, Nagal et al). The results of genus *Ulva* showed moderate activity against *C. albicans* and *S. aureus* and was not more considered in the present study.

Active extracts the *S. fluitans* and *G. tikvahiae* were separated using chromatography column, and fractions were tested against these microorganisms (Table 3), eigth fractions of petroleum ether of S. fluitans exhibited high activity against C. albicans, MIC 0.16 µg/mL

Table 1.– Comparison of extraction soxhlet Vs dipping

Solvent	Extraction	*E. coli*	*S. aureus*	*C. albicans*	*Ps. aeruginosa*	*S. enteriditis*	*S. epidermidis*	*S. faecalis*
Pet.Ether	Soxhlet	Neg	++	-	Neg	Neg	Neg	Neg
	Dipping	+++	++	+++	+	+	+++	Neg
Chloroform	Soxhlet	++	-	+	Neg	Neg	Neg	-
	Dipping	+	+	++	Neg	+	+++	Neg
Acetone	Soxhlet	+	+	-	+	Neg	Neg	Neg
	Dipping	+++	-	Neg	+	+	+	Neg
Ethanol	Soxhlet	+++	-	+	+	Neg.	Neg	
	Dipping	++	Neg	Neg	++	Neg	+	Neg
Methanol	Soxhlet	-	+	+	-	+	+	+
	Dipping	+	-	-	+++	+	+	+++
Water	Soxhlet	Neg	Neg	+	Neg	Neg	Neg	Neg
	Dipping	Neg	Neg	-	Neg	+	Neg	Neg

Agar diffusion method: the antimicrobial activity is expressed as the ratio of the inhibition zone of the extract (± 2.5 mg/ml), to the inhibition zone of the reference ampiciline (2.5 mg/ml), (+++) more than 15 mm, (++) 12 to 14 mm, (+) 8 to 11 mm, Neg no inhibition, (-) no effected.

Table 2 .– Results of the antimicrobial screening of algae

Sargassum fluitans Extrait	Solving	*E. coli*	*S. aureus*	*C. albicans*	*Ps. aeruginosa*	*S. enteriditis*	*S.epidermidis*	*S.faecalis*
Pet.Ether	Ethanol	+++	++	+++	+	+	+++	Neg
Pet.Ether	Acetone	-	+++	-	-	Neg	-	-
Pet.Ether	Chloroform	-	+++	-	-	-	-	-
Chloroform	Ethanol	+	Neg	+++	Neg	+	+++	Neg
Chloroform	Acetone	-	Neg	-	++	-	-	-
Chloroform	Chloroform	-	-	-	+	-	-	-
Acetone	Ethanol	+++	+++	+++	Neg	+	++	Neg
Acetone	Ethanol	++	+	+	+	Neg	++	Neg
Ethanol	Dichloromethane	-	-	-	Neg	++	-	+++
Methanol	Ethanol	+	Neg	+++	Neg	Neg	+	Neg
Gracilaria tikvahiae								
Extract	Solving							
Chloroform	Ethanol	-	+++	+++	+	+	+++	Neg
Chloroform	Chloroform	++	+	-	-	Neg	-	-
Acetone	Ethanol	+++	-	-	-	-	-	-
Ethanol	Ethanol	+++	+++	+++	Neg	+	+++	Neg
Ethanol	Et /Acetone	+++	+++	-	++	-	-	-
Methanol	Ethanol	++	-	-	+	-	-	-
Methanol	Chlorofom	Neg	+++	+++	Neg	+	++	Neg
Water	Et/acetone	Neg	-	+	+	Neg	++	Neg
Water	Acetone	+++	Neg	-	Neg	++	-	+++
Ulva lactuca								
Extract	Solving							
Ether Petr.	Chloroform	-	+	++	-	+++	+++	Neg
Cloroform	Ethanol	++	+	+	++	+++	+++	Neg
Cloroform	Chloroform	+++	-	-	+	-	-	-
Acetone	Ethanol	+++	+	+	+++	+++	++	Neg
Ethanol	Ethanol	+++	++	+	++	+++	+	Neg
Ethanol	Et/acetone	+++	-	Neg	Neg	++	Neg	Neg
Methanol	Ethanol	++	++	++	++	Neg	++	-
Ulva fasciata								
Extrait	Solving							
Pet. Ether	Ethanol	+++	+	+	++	++	+++	+
Pet. Ether	Chloroform	-	-	-	++	-	-	—
Chloroform	Ethanol	+++	+	Neg	++	Neg	Neg	Neg
Chloroform	Chloroform	+++	-	++	Neg	Neg	Neg	Neg
Acetone	Ethanol	++	Neg	+	+	-Neg	+	Neg
Acetone	Chloroform	-	Neg	-	+	Neg	-	-
Ethanol	Ethanol	+++	-	Neg	Neg	++	++	Neg
Ethanol	Water	-	-	-	Neg	-	-	-
Methanol	Ethanol	+++	+	++	++	++	+	++
Methanol	Water	Neg	Neg	-	Neg	-	Neg	Neg
Methanol	Ethanol	+++	Neg	-	Neg	-	-	-
Water	Ethanol	-	-	Neg	Neg	++	+++	Neg
Water	Acetone	+++	+	+	+	-	-	-
Water	Water	Neg	Neg	Neg	Neg	-	Neg	-

CONCLUSIONS

The present study confirms the traditional use the cold extract in marine plants. The four algae showed antimicrobial activity on several of the selected pathogenic microorganisms. The wider spectrum was presented for *Sargassum fluitans* and *Gracilaria follifera* showed grand activity, since it inhibited more 4 microorganisms. Most antimicrobial ac-

Table 3.– Results of the antimicrobial screening of fractions separated using chromatography column

Sargassum fluitans

Pethtoleum ether Extract			Cloroformic extract		
Fraction	*S. aureus*	*C. albicans*	Fraction	*S. aureus*	*C. albicans*
Sf 1.1	Neg	neg	Sf 2.1	+++	Neg
Sf 1.2	++	+	Sf 2.2	neg	neg
Sf 1.3	+	+	Sf 2-3	Neg	Neg
Sf 1.4	++	Neg	Sf 2.4	Neg	Neg
Sf 1.5	+	++	Sf 2.5	+++	Neg
Sf 1.6	+++	Neg	Sf 2.6	++	+++
Sf 1.7	+++	+++	Sf 2.7	+	++
Sf 1.8	Neg	++	Sf 2.8	+	Neg
Sf 1.9	++	++	Sf 2.9	Neg	neg
Sf 1.10	Neg	+++			
Sf 1.11	+++	+			
Sf 1.12	+	++++			
Sf 1.13	Neg	Neg			
Sf 1.14	neg	Neg			

G. Tikvahiae

Pethtoleum ether Extract			Chloroformic extract		
Fraction	*S.aureus*	*C.albicans*	Fraction	*S.aureus*	*C.albicans*
Gf 1.1	+	Neg	Gf 2.1	Neg	Neg
Gf 1.2	+	+	Gf 2.2	Neg	Neg
Gf 1.3	++	Neg	Gf 2-3	Neg	Neg
Gf 1.4	++	+++	Gf 2.4	Neg	Neg
Gf 1.5	+	Neg	Gf 2.5	+++	Neg
Gf 1.6	Neg	+++	Gf 2.6	Neg	Neg
Gf 1.7	+++	++	Gf 2.7	Neg	Neg
Gf 1.8	+++	++	Gf 2.8	+	++
Gf 1.9	+++	+	Gf 2.9	Neg	Neg
Gf 1.10	Neg	+++		+	+++
Gf 1.11	+++	+		Ethanolic	Extract
Gf 1.12	Neg	Neg			
Gf 1.13	Neg	Neg	Gf 4.1	++	Neg
Gf 1.14	Neg	+		Neg	Neg
Gf 1.15	++	+		+	+
Gf 1.16	Neg	Neg			

tivity was found in ether extracts of *Sargassum fluitans* against *E. coli, S. aureus, Candida albicans* and *S. epidermidis,* these pharmacological activities have been reported in lipophilic extracts of phaeophytas by Tringali et al; acetone extracts of *Gracilaria follifera* exhibited activity against microorganisms mentioned, and *P. aeruginosa,* antimicrobial activity of extract genus *Gracilaria* was already reported (Hoppe et al, Nagal

et al). The eight fraction of petroleum ether of *S. fluitans* exhibited high activity against *C. albicans* MIC 0.16 µg/mL The fact that the algae tested demonstrated antimicrobial activity, emphasizes the important role of traditional medicine in the search for antibiotic compounds from natural sources.

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