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Biological Activity of Extracts from Some Mediterranean Macrophytes

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Abstract

Seventy one species of marine macrophytes from the Central Mediterranean have been screened for the production of antibacterial, antifungal, antiviral, cytotoxic and antimitotic compounds. Sixty five of the species displayed some kind of activity and most of them were active on more than one organism or cell tested. Antifungal activity was the most widespread (70% of the plants), whilst the incidence of antibacterial activity was extraordinarily low (6% of the plants). Of the plants tested 21% showed antiviral activity, 35% were cytotoxic and nearly 50% had antimitotic properties. The maximum level of activity was found among the Chlorophyta; some members of the Bryopsidales (*Flabellia petiolata, Caulerpa prolifera, Halimeda tuna*) were the most active species. Most of the dominant species in Mediterranean phytobenthic communities (*Corallina elongata, Lithophyllum lichenoides, Phyllophora crispa, Cystoseira* spp., *Halopteris* spp., *Codium* spp., *Halimeda tuna, Valonia utricularis, Posidonia oceanica, Zostera noltii* and *Cymodocea nodosa*) exhibited strong antifungal properties.

Introduction

Marine plants have been repeatedly recognized as producers of biologically active substances. Specific studies on seaweeds, carried out in the Atlantic, Pacific and Indian oceans, have demonstrated antibacterial, antifungal and antiviral (e. g. Burkholder *et al.* 1960, Hornsey and Hide 1974, Naqvi *et al.* 1990, Rinehart *et al.* 1981, Reichelt and Borowitzka 1983, Hodgson 1984) activities, as well as ichthyotoxicity (e. g. Paul and Fenical 1986), cytotoxicity (e. g. Paul and Fenical 1991) and antimitotic properties (e. g. Munro *et al.* 1991). Antibacterial, antifungal and antiviral activities have also been investigated in some Mediterranean algae (Berti *et al.* 1963, Khaleafa *et al.* 1975, Porzi and Minelli 1975, Caccamese and Azzolina 1979, Barbagallo *et al.* 1979 a, b, Serarols *et al.* 1982, Pesando and Caram 1984, Caccamese *et al.* 1980, 1981, 1985) and the seagrass *Posidonia oceanica* (Bernard and Clement 1983, Bernard and Pesando 1989).

The goal of this study was to increase the knowledge of the chemically-mediated bioactivity in the marine Mediterranean flora by examining its most representative macrophytes, including species not previously tested, and performing antimitotic and cytotoxic tests.

Material and Methods

Samples were taken by SCUBA diving in October 1988 during a survey aboard the oceanographic vessel 'García del Cid'. The zones investigated included 27 sampling stations off the Balearic and Columbretes Archipelagos (Western Mediterranean). Each sampling station was sampled exhaustively on deep to shallow transects 4 m wide. The extensive sampling provided 71 species coming from the main phytobenthic communities present in the Western Mediterranean.

Species were identified on board, just after collection. Voucher specimens were preserved in 5% formaldehyde. Tests for biological activities were started within three hours of collection of the samples. A mixture of from two to four specimens of each species was used in the bioassays in order to reduce intraspecific variability due to uncontrolled exogenous causes.

Small pieces (2 g wet weight) of various specimens of each species were drained for 1 min, weighed, homogenized together in the proportion of 1 g of alga per 10 ml of 3 : 1 methanol/toluene, and centrifuged (Rinehart *et al.* 1983). Sterile paper discs, 6 mm in diameter, were soaked with 10 μl of the supernatant, air-dried and used in the activity assays.

Antibacterial and antifungal activities of the crude organic extracts were determined by the diffusion method (Bergquist and Bedford 1978, Thompson *et al.* 1985) on cultures of two strains of bacteria, *Escherichia coli* and *Bacillus subtilis*, and two of fungi, *Candida albicans* and *Aspergillus niger*. The nutrient-agar plates were incubated overnight at 37 °C and zones of inhibition around the discs were measured. The mean value of the inhibition zones on the two microorganisms used to test each type of antimicrobial activity was scored: (0) no growth inhibition, (1, 2 and 3) inhibition zone less than 2 mm, between 2 and 4 mm, and more than 4 mm wide, respectively.

Antiviral tests were performed with herpes simplex virus, type I (HSV) cultured in kidney cells of monkey (CV-1), and with vesicular stomatitis virus (VSV) in kidney cells of hamster (BAK) as previously described (Schröder *et al.* 1981). Mean values of the zones of virus inhibition on the two viruses were scored as in the case of cytotoxic activity.

Antimitotic tests were performed on leukemic cells of mice (L1210) by determining the percentage of inhibition of cell growth. The assays were scored: (0) no inhibition, (1) 1–25% inhibition, (2) 26–50% inhibition, and (3) 51–100% inhibition.

Cytotoxic tests were performed on kidney cells of monkey (CV-1) incubated for 3 days at 37 °C. Cytotoxicity was calculated by determining zones of cell inhibition (Rinehart *et al.* 1983) and graded: (0) no growth inhibition, (1) inhibition zone to 1 mm, (2) inhibition zone from 1 to 2 mm, and (3) inhibition zone from 2 to 4 mm.

Control tests with the solvent were performed for every assay.

Results and Discussion

Table I summarizes the degree of bioactivity of each species. Among the 71 species tested, only 6 (*Spirulina* sp., *Amphiroa beauvoisii*, *Peyssonnelia rubra*, *Polysiphonia subulifera*, *Phyllariopsis brevipes* and *Zonaria tournefortii*) did not show any kind of activity, and only one (*Flabellia petiolata*) was active in all tests.

Antibacterial activity was extremely rare in the plants screened (6%). Only the red alga *Falkenbergia rufolanosa* (tetrasporophyte of *Asparagopsis armata* Harvey) and the green algae *Caulerpa prolifera*, *Flabellia petiolata* and *Palmophyllum crassum* displayed antibacterial activity. Antibacterial activity found by other authors in Mediterranean seaweeds was considerably larger; it ranged between 13% (Pesando and Caram 1984) and nearly 50% (Barbagallo *et al.* 1979, Caccamese *et al.* 1981, 1985) of the species tested. No previous record of antibacterial activity for *Palmophyllum crassum* has been found in the literature, while *Falkenbergia rufolanosa* (Serarols *et al.* 1982, Pesando and Caram 1984), *Flabellia petiolata* (Paul and Fenical 1991) and *Caulerpa prolifera* (Caccamese and Azzolina 1979) were already known to exhibit strong antibacterial activity. Different species shown to be without antibacterial activity in our screening tests have been found to be active by other authors also working with Mediterranean specimens. Seasonal (Burkholder *et al.* 1960; Hornsey and Hide 1976) as well as geographical (Conover and Sieburth 1963, Caccamese *et al.* 1989) differences in activity could explain these dissimilarities.

Antifungal activity was widespread in all the groups and it amounted to 70% of the tested macrophytes. This extensive and high activity found in our samples had never been observed in other Mediterranean surveys (Caccamese and Azzolina 1979, Caccamese *et al.* 1980, 1985, Pesando and Caram 1984, Bernard and Pesando 1989). The most dominant plants in Mediterranean phytobentic communities such as the seagrasses, *Cystoseira* spp., *Halopteris* spp., *Codium* spp., *Halimeda tuna*, *Corallina elongata* and *Mesophyllum lichenoides* strongly inhibited the growth of fungi. Seasonal and geographical changes in activity must also be taken into account to explain differences between our results and those found by other authors. Nevertheless, no reasons seem to explain this high level of antifungal activity, coupled with a low level of antibacterial activity, exhibited by our samples.

Only 21% of the plants showed antiviral activity, and this was mainly concentrated in members of the Chlorophyta. Nevertheless, strong inhibition of viral growth was only found in the blue green alga *Aphanocapsa littoralis*, the encrusting coralline *Lithophyllum expansum* and the brown alga *Cystoseira balearica*. Antiviral activity in Mediterranean seaweeds has only been reported by Caccamese *et al.* (1980, 1981), who used tobacco mosaic virus (TMV) inoculated on tobacco plants. Although no conclusions can yet be drawn, it seems possible that there is a higher activity of Mediterranean macrophyte extracts against plant viruses than against animal viruses.

Table I. Activity levels of the species tested. The meaning of the activity indices are explained in the text. Activities: BAC, antibacterial; FUN, antifungal; AV, antiviral; CT, cytotoxic; AM, antimitotic.

Species	BAC	FUN	AV	CT	AM
Cyanophyta					
Aphanocapsa littoralis Hansgirg	0	1	3	1	1
Rivularia mesenterica Thuret	0	2	1	1	0
Spirulina sp.	0	0	0	0	0
Rhodophyta					
Acrodiscus vidovichii (Meneghini) Zanardini	0	0	0	1	1
Amphiroa beauvoisii Lamouroux	0	0	0	0	0
Amphiroa cryptarthrodia Zanardini	0	1	0	0	1
Amphiroa rigida Lamouroux	0	1	0	0	2
Botryocladia botryoides (Wulfen) J. Feldmann	0	1	0	0	0
Corallina elongata Ellis *et* Solander	0	2	1	0	0
Corallina granifera Ellis *et* Solander	0	1	0	0	1
Falkenbergia rufolanosa stadio (Harvey) Schmitz	3	1	0	0	1
Gelidiella ramellosa (Kützing) J. Feldmann *et* Hamel	0	0	0	1	1
Gelidium pusillum (Stackhouse) Le Jolis	0	1	0	0	0
Griffithsia schousboei Montagne	0	1	0	0	1
Jania adhaerens Lamouroux	0	1	0	0	1
Jania rubens (Linné) Lamouroux	0	1	0	0	0
Kallymenia requienii J. Agardh	0	0	1	0	1
Laurencia microcladia Kützing	0	2	0	0	1
Laurencia pelagosae (Schiffner) Ercegovic	0	1	0	0	0
Lithophyllum expansum Philippi *sensu* Lemoine	0	0	3	0	1
Lithophyllum lichenoides Philippi	0	2	–	–	–
Lithothamnion corallioides Crouan *et* Crouan	0	1	0	0	0
Lithothamnion valens Foslie	0	0	0	1	0
Lophosiphonia subadunca (Kützing) Falkenberg	0	1	0	0	1
Mesophyllum lichenoides (Ellis) Lemoine	0	1	0	1	1
Peyssonnelia bornetii Boudouresque *et* Denizot	0	0	0	1	3
Peyssonnelia rosa-marina Boudouresque *et* Denizot	0	0	0	2	1
Peyssonnelia rubra (Greville) J. Agardh	0	0	0	0	0
Peyssonnelia squamaria (Gmelin) Decaisne	0	0	0	1	1
Phyllophora crispa (Hudson) Dixon	0	2	0	0	0
Polysiphonia subulifera (C. Agardh) Harvey	0	0	0	0	0
Sphaerococcus coronopifolius Stackhouse	0	1	0	0	1
Spongites hauckii (Rothpletz) Ballesteros	0	1	–	–	–
Spongites ramulosa (Philippi) Kützing	0	1	–	–	–
Tricleocarpa oblongata (Ellis *et* Sol.) Huisman *et* Borowitzka	0	0	0	1	0
Vidalia volubilis (Linné) J. Agardh	0	0	0	0	0
Phaeophyta					
Aglaozonia chilosa stadio Falkenberg	0	1	0	0	0
Cladostephus hirsutus (Linné) Prud'homme van Reine	0	1	0	0	0
Cystoseira balearica Sauvageau	0	2	2	1	1
Cystoseira compressa (Esper) Gerloff *et* Nizamuddin	–	2	0	0	0
Cystoseira ercegovicii Giaccone	0	2	0	0	0
Cystoseira mediterranea Sauvageau	0	2	0	0	1
Cystoseira spinosa Sauvageau	0	2	–	–	–
Cystoseira zosteroides (Turner) C. Agardh	0	2	0	0	1
Dictyopteris membranacea (Stackhouse) Batters	0	0	0	2	0
Dictyota dichotoma (Hudson) Lamouroux	0	1	0	1	1
Dictyota dichotoma v. *intricata* (C. Agardh) Greville	0	1	1	0	0
Halopteris filicina (Grateloup) Kützing	0	2	0	0	1
Halopteris scoparia (Linné) Sauvageau	0	2	0	0	1
Lobophora variegata (Lamouroux) Womersley	0	1	0	0	0
Padina pavonica (Linné) Thivy	0	0	0	1	1
Phyllariopsis brevipes (C. Agardh) Henry *et* South	0	0	0	0	0
Sargassum hornschuchii C. Agardh	0	1	0	0	0
Zanardinia prototypus (Nardo) Nardo	0	1	1	1	1
Zonaria tournefortii (Lamouroux) Montagne	0	0	0	0	0
Chlorophyta					
Anadyomene stellata (Wulfen) C. Agardh	0	1	0	0	0
Caulerpa prolifera (Forskal) Lamouroux	1	0	1	1	3
Chaetomorpha capillaris (Kützing) Boergesen	0	1	0	0	0
Cladophora coelothrix Kützing	0	1	0	0	0
Cladophoropsis monodensis (Kützing) Boergesen	0	1	0	1	1

Table I. Continued.

Species	BAC	FUN	AV	CT	AM
Codium bursa J. Agardh	0	2	0	0	0
Codium vermilara (Olivi) Delle Chiaje	0	2	0	0	0
Dasycladus vermicularis (Scopoli) Krasser	0	0	0	1	3
Flabellia petiolata (Turra) Nizamuddin	1	1	1	1	2
Halimeda tuna (Ellis *et* Solander) Lamouroux	0	2	1	1	3
Microdictyon tenuius (J. Agardh) Decaisne	0	1	0	0	0
Palmophyllum crassum (Naccari) Rabenhorst	1	0	1	1	1
Valonia utricularis (Roth) C. Agardh	0	2	1	3	1
Magnoliophyta					
Cymodocea nodosa (Ucria) Ascherson	0	2	1	1	0
Posidonia oceanica (Linné) Delile	0	2	0	0	0
Zostera noltii Hornemann	0	2	0	0	0

Cytotoxic activity was found in 35% of the total macrophytes screened and it was also outstanding (54%) in the green algae. The encrusting red alga *Peyssonnelia rosa-marina*, the brown alga *Dictyopteris membranacea* and the green alga *Valonia utricularis* were the species with the highest cytotoxic activity.

Antimitotic activity was common in all the algal divisions, but not activity was found in the seagrasses. Activity was found in between 45 and 55% of all tested species, depending on the algal group considered. The maximum of activity was found in the green algae *Dasycladus vermicularis*, *Halimeda tuna*, *Caulerpa prolifera* and *Flabellia petiolata*, and the red algae *Peyssonnelia bornetii* and *Amphiroa rigida*.

No previous records on antimitotic and cytotoxic properties of Mediterranean macrophytes have appeared in the literature, except those dealing with particular species present in other areas of the world. Different antimitotic and cytotoxic compounds have been isolated from *Halimeda tuna*, *Flabellia petiolata* and *Caulerpa prolifera* (Paul and Fenical 1991). These three plants exhibited strong antimitotic and cytotoxic activities in our screening tests. The brown alga *Dictyota dichotoma*, for which previously known cytotoxic metabolites have been reported (Enoki *et al.* 1980), was also cytotoxic in our tests. On the contrary, *Anadyomene stellata* did not show any sign of antimitotic activity in our tests, whilst specimens from Florida gave a strong antineoplastic response (Hodgson 1984). *Peyssonnelia rosa-marina*, *Dictyopteris membranacea* and *Valonia utricularis* were found to be highly cytotoxic, whilst *Amphiroa rigida*, *Peyssonnelia bornetii* and *Dasycladus vermicularis* had strong antimitotic activity. More research is needed on this subject before any conclusion can be drawn.

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