Habitually, control of biological fouling includes application of paints containing toxic substances that end up contaminating marine ecosystem. Many organisms prevent settlement of other species synthesizing secondary metabolites that could be used in the elaboration of environmentally friendly anti-fouling paints. This work evaluated the behavior of anti-fouling paints based on extracts from marine invertebrates in the Colombian Caribbean: *Agelas tubulata*, *Myrmekioderma gyroderma*, *Oceanapia peltata*, *Aplysina lacunosa*, *Neopetrosia próxima*, and *Holothuria glaberrima*. The painted panels were submerged in the port of Mar del Plata (Argentina); after 90 days in the sea significant differences were registered in the total coverage between the painted panels and the controls (p<0.05). The results obtained represent important progress toward using natural compounds in controlling encrustations.

**Abstract**

Habitually, the control of biological fouling includes application of paints containing toxic substances that end up contaminating marine ecosystem. Many organisms prevent settlement of other species synthesizing secondary metabolites that could be used in the elaboration of environmentally friendly anti-fouling paints. This work evaluated the behavior of anti-fouling paints based on extracts from marine invertebrates in the Colombian Caribbean: *Agelas tubulata*, *Myrmekioderma gyroderma*, *Oceanapia peltata*, *Aplysina lacunosa*, *Neopetrosia próxima*, and *Holothuria glaberrima*. The painted panels were submerged in the port of Mar del Plata (Argentina); after 90 days in the sea significant differences were registered in the total coverage between the painted panels and the controls (p<0.05). The results obtained represent important progress toward using natural compounds in controlling encrustations.

**Key words:** biofouling, sponges, sea cucumbers, anti-fouling paints.

**Resumen**

Habitualmente, el control de las incrustaciones biológicas incluye la aplicación de pinturas que contienen sustancias tóxicas que resultan contaminantes para el ecosistema marino. Muchos organismos previenen el asentamiento de otras especies sintetizando metabolitos secundarios que podrían utilizarse en la elaboración de pinturas antiincrustantes amigables con el medio ambiente. En este trabajo se evaluó el comportamiento de pinturas antifouling a base de extractos de invertebrados marinos del Caribe Colombiano: *Agelas tubulata*, *Myrmekioderma gyroderma*, *Oceanapia peltata*, *Aplysina lacunosa*, *Neopetrosia próxima* y *Holothuria glaberrima*. Los paneles pintados se sumergieron en el puerto de Mar del Plata (Argentina); luego de 90 días en el mar se registraron diferencias significativas en la cobertura total entre los paneles pintados y los controles (p<0.05). Los resultados obtenidos representan un avance importante hacia la utilización de compuestos naturales en el control de las incrustaciones.

**Palabras claves:** biofouling, esponjas, pepinos de mar, pinturas antifouling.

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Introduction

Biofouling is known as the attachment and growth of micro and/or macroorganisms on any natural or artificial submerged substrate. The formation of biofouling is a process that implies a series of stages that lead to the consolidation of a community of encrusting organisms subject to changes in function of the prevailing conditions in the system. One of the most widely accepted approaches considers this process formed by distinct phases that include biochemical conditioning of the surface followed by settlement and growth of pioneer bacteria, unicellular organisms, and late-stage prokaryotes (microfouling) and, finally, development of macroscopic organisms (macrofouling) (Wahl, 1989; Railkin, 2003; Chambers et al., 2006).

Marine biofouling seriously affects submerged structures (ships, platforms, buoys, culture farms, etc.) causing big economic losses of diverse nature. Particularly, attachment on hulls of vessels implies an increase between 40 and 50% in fuel consumption due to increased motion resistance (IMO- International Maritime Organization, 2002); in turn, settlement of organisms favors corrosion processes, generating high costs related to cleaning, removal, fairing, and repainting requiring millions of dollars yearly throughout the world. When the bottoms of vessels are not protected by antifouling systems they can accumulate up to 150 kg of biofouling per square meter in less than six months in the sea, which is why it is necessary to use effective methods for its control (Callow and Callow, 2003).

Use of anti-fouling paints is the most efficient method to control biofouling. The action mechanism of these paints is based on the controlled release of or pigments that contain generating a highly toxic interface that avoids attachment of organisms (Caprari and Lecot, 2001). Anti-fouling paints have very particular characteristics that, due to their mode of action, are totally differentiated from the other types known; the paint film modifies permanently its characteristics when it is submerged, with the fundamental objective being that of achieving adequate solubilization de toxic. The toxic loss has a critical minimum value that depends on the toxic used and on the paint formulation; below said value, the paint has no preventive action.

The initial solubilization of the biocide (initial leaching rate) is generally high, given that it results from excess toxics accumulated in the paint film. This value is important because the paint must start to act immediately after the immersion and the effectiveness will depend on the solubility of the toxic, on the characteristics of the vehicle, on the surface area of toxic particles, and on the conditions of temperature, salinity, and pH of the water. After several days of immersion, a constant leaching rate is reached.

During the last four decades, antifouling pigments have been used of excellent biocide power but highly contaminating and harmful to the biota. Among these, there are mercuric oxide (HgO), mercuric arsenate (AsO₃Hg₃), arsenic trioxide (As₂O₇), cuprous arsenite (AsO₃Cu₃), and organometallic substances; mainly tributyltin oxide (TBTO) and triphenyl tin oxide (TPTF). However, research conducted fundamentally in the northern hemisphere confirmed the impact of organoarsenic compounds added to paints on marine life; among these, deformation of shells and imposex phenomena in oyster populations (Bettin et al., 1996; Pereira et al., 1999; Wu et al., 2010), immune responses, neuro-toxic and genetic affections in fishes (IMO, 2002; Ferraro et al., 2004) and bio-accumulation in mammals (Yebra et al., 2004). As a consequence of these results, as of 1991, strict regulations were established for the use of this substance in many countries, later resulting in legislation that culminated in the total prohibition of the application of antifouling paints based on TBT since the start of 2003 and the prohibition of their manufacture as of 2008 (Chambers et al., 2006). By virtue of these restrictions, possible replacements have been developed like Irgarol 1051®, Sea-Nine 211®, chlorothalonil, dichlofluanid, tolyfluanid, and zinc pyrithiona (Voulvoulis et al., 1999; Konstantinou and Albanis, 2004; Bellas, 2005). However, these compounds have been found in relatively high concentrations in ports, marinas, and coastal zones (Martínez et al., 2001;
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Bearing in mind the aforementioned and due to the vast bioactive potential found in natural products obtained from marine organisms or which are synthesized by them, currently, studies are focused on optimizing methods that permit their incorporation onto anti-fouling paints.

The natural products are chemical entities synthesized by cells of plants, animals, and microorganisms that are not used in the primary metabolism, which has granted them the name of “secondary metabolites” (Pawlik, 2011). The functions of these metabolites are not as evident on the survival of the organisms as are the products of the primary metabolism; however, they play a fundamental ecological role in the development and survival of the species that synthesize those (Harper et al., 2001).

Currently, one of the most promising fields of search of natural products is that of marine organisms, given that they synthesize secondary metabolites that have proven to have great potential in the pharmaceutical, cosmetic, and agrochemical industries. Their potential has also been shown for development of molecular probes, enzyme technology, production of chemical precursors and nutritional supplements (Pomponi, 1999). Growing interest in substances produced by marine organisms is mainly because many of the compounds they produce are unique at structural level and have pharmacological properties (Jha and Zi-rong, 2004). This search for new natural products has resulted in the report of over 18,000 compounds isolated from 4,796 species from 24 different phyla, which is equivalent to 0.27% of the known species of marine organisms (Blunt et al., 2008).

Current research on the topic focuses on the search for environmentally friendly compounds. Emphasis has been made on identifying active principles existing in organisms that naturally do not present epibionts and which have not developed inhibition or repulsion mechanisms to protect their bodily surface (Harder, 2009) or rather in those that have developed chemical defenses against predators, which is why it is assumed that secondary metabolites are potential antifouling agents (Davis and Wright, 1989; Clare et al., 1992; Pawlik, 1992; Abarzua and Jakubowski, 1995; Clare, 1996; da Gama et al., 2002; Rao et al., 2007). The effect fouling provokes upon organisms is varied, numerous compounds act as anesthetics, others as repellents of the attachment or as inhibitors of the properties that determine the adhesion mechanisms without having biocide effects (Omae, 2003). However, although the antifouling action of these compounds has been widely proven, until now their incorporation onto paints has not been concrete.

This work evaluated the behavior of anti-fouling paints of soluble matrix based on extracts of marine invertebrates from the Colombian Caribbean, seeking to find environmentally friendly formulations with potential application on vessels and other submerged structures. For this, extracts were elaborated from Agelas tubulata, Myrmekioderma gyroderma, Oceanapia peltata, Aplysina lacunose, and Neopetrosia proxima sponges and from the Holothuria glaberrima sea cucumber.

Methodology

Study area

The study was developed near Santa Marta in the Colombian Caribbean and Mar del Plata in the Argentine Atlantic. The area for the collection of marine organisms includes different locations along the coastal zone of the Tourism, Cultural, and Historical District of Santa Marta, in the department of Magdalena to the north of the Colombian territory, between the bay of Neguange in the Tayrona Natural National Park and the beaches by the airport on the extreme south of the urban perimeter of the city of Santa Marta.

The study zone in Argentina is located in the port of Mar del Plata, province of Buenos Aires, situated to the south of Cabo Corrientes (38°08’S – 57°31’W). The sampling site used to carry out the experiences is the Club de Motonáutica located in the port.
Species evaluated

The species studied were Agelas tubulata, Myrmekioderma gyroderma, Oceanapia peltata, Aplysina lacunosa, Neopetrosia proxima sponges and the Holothuria glaberrima sea cucumber.

The sponges belong to the Phylum Porifera and are fundamentally marine organisms, whose principal characteristic is multicellularity (they do not form true tissue). Their structure is constituted by pores and channels through which water currents flow used to carry out most of their functions. These are eminently sessile, filtering, and mostly asymmetric organisms; except for some less complex species with radial symmetry (Mille, 2008). Sea cucumbers, in turn, belong to the Phylum Echinodermata characterized for having a calcareous internal skeleton and complex organ systems.

**Agelas tubulata**

In the Agelas genus species are characterized for having styles with rows of spines with more or less regular spaces as the only type of spike present. *A. tubulata* (Fig. 1) forms clusters that can exceed 20 cm heights, with tubular shape, apertures 5-8 cm in diameter, yellow-orange coloring, with a smooth surface and of elastic and compressible consistency (Lehnert and Soest, 1996). It is broadly distributed in the Caribbean, being reported in Belize, Cuba, Caiman Islands, Greater Antilles, and Venezuela (Lehnert and Soest, 1996; Miloslavich et al., 2010).

**Myrmekioderma gyroderma**

The Myrmekioderma genus is characterized for generating encrusting or massive formations, presenting a hispid surface, with characteristic excavated meanders, sinuous or straight channels, and grooves. On occasion, it forms tuberculated polygonal plates (Hooper, 2002). The *M. gyroderma* species (Fig. 2) may or may not present lobed or cushion shape, often reaching high thickness, with osculate 4-10 cm without determined position, with coloring from yellow to bright orange, of irregular surface and firm, elastic consistency and somewhat lumpy on touch (Alcolado, 1984). It has broad distribution throughout the Caribbean, Greater Antilles, Colombia, Panama, Gulf of Mexico (Alcolado, 1984; Miloslavich et al., 2010).

**Neopetrosia proxima**

The Neopetrosia genus includes compact species with a finely hispid surface, which is smooth and velvety on touch; of hard, rock-like consistency; of irregular sizes and shapes (Desqueyroux-Faúndez and Valentine, 2002a). The *N. proxima* species (Fig. 3) is presented as encrustations, as fingerlike erect lobes with thickened apices, or flattened to sub-spherical masses. It has punctate, microhispid surface, in general full of detritus. Its coloring is commonly brown with light or dark purple or violet tones; unexposed sides and cream interior. The consistency is firm, hard, somewhat difficult to break and cut, exuding sticky mucus upon touch (Zea, 1987). It is distributed in Colombia (Urabá, Cartagena, and Santa Marta), Brazil, Puerto Rico, and the Virgin Islands (Zea, 1987).
Oceanapia peltata
The *Oceanapia* genus is constituted by species of massive, globular, or lamellar form, with long tubular processes or fistulas, open or close in its ends (Desqueyroux-Faúndez and Valentine, 2002b). *Oceanapia peltata* is a massive globular species that is usually embedded in the sand; it has fistular cylindrical projections that protrude from the sand and have smooth ends or projections with pagoda form, with 2-4 cm width and 1 mm thickness, with cream or grayish color (Fig.4). The consistency of the body globule is slightly compressible and quite fibrous; also, the fistulas on touch are smooth, softer, and less fibrous (Collin et al., 2005). The species is registered at 50-100 m depths in Colombia, Florida, Cuba, and Bocas del Toro (Panamá) (Collin et al., 2005).

Aplysina lacunosa
The *Aplysina* genus is characterized by the presence of fibers of a single class, without exogenous detritus and by the presence of a thick medullar component. The fibers form a regular reticulum with large polygonal meshes without specialized surface arrangement (Bergquist and Cook, 2002). The *A. lacunosa* species (Fig. 5) forms solitary tubes or in groups of up to 50 cm in height and 4-10 cm of diameter. It has thick walls from 1.5 to 2.3 cm and above. It is a species of firm consistency, not very compressible and in very big specimens these are more flexible and soft (Zea, 1987). It is distributed in Colombia (Urabá, Cartagena, Santa Marta, and Providencia), the Bahamas, Florida, Jamaica, Puerto Rico, the Dominican Republic, Curaçao and Bonaire (Zea, 1987).
The invertebrates studied were obtained through manual collection in natural substrates with autonomous diving equipment. Prior to the extraction process, the material is cleaned (removal of accompanying fauna and other elements); the tissue is cut into portions and deposited in jars and frozen at -15 °C. Thereafter, the samples are lyophilized between 36 and 48 hours at -45 °C and 0.120 mbar. The weight of the dry material obtained is recorded, followed by phase to obtain the extract.

Extraction of the metabolites present in the tissues of the organisms selected is carried out by using a methanol/dichloromethane mix (1:1) that permits the dissolution of a broad range of compounds according to the methodology used by Castellanos (2007). The dry tissue is subjected to extraction through maceration at room temperature (20 °C) during 24 hours by submerging it in the solvent mixture and maintaining continuous agitation at 120 rpm. After this time, gravity filtering is carried out by employing slightly compact degreased cotton as filter; the leftover tissue is again extracted under the same conditions until completing 72 hours of extraction, filtering every 24 hours. To obtain a mixture free of solvents, the solution resulting from each filtration is concentrated by using a rotavapor with a heating bath at 38 °C; under pressure of 600 mbar during one hour the dichloromethane is removed and at 100 mbar during three hours the methanol is removed. If necessary, the extract is lyophylized to bring the sample to complete dryness; lastly, the resulting material is decanted to a clean jar to register what will be denominated “total extract”. A portion of the total extract is destined for the fractionating process according to the scheme shown in Fig. 7.

The portion of the extract denominated organic phase was reserved to prepare anti-fouling paints.

**Preparation of soluble matrix paints**

In soluble matrix paints, the toxic and the vehicle are solubilized jointly, with diminished film thickness. For the formulation of the vehicle rosin resin is used, which is the principal film forming material. Rosin resin is of natural origin; it is extracted from conifers and it is soluble or erosional by water. It is of acidic nature and composed of 90% resin acids, mainly abietic and levopimaric acid (Rascio et al., 1988). The free carboxyl groups (-COOH) allow it to react with alkaline sea water (pH 8) forming soluble resinates and facilitating the release of the toxic substance. However, the colophony generates an excessively soluble film, which is not very adherent and brittle, which is why it is plasticized by incorporating oils or varnishes; its solubility in sea water is affected by the drying time (Rascio et al., 1977).

The paints were prepared at the CIDEPINT pilot plant (Argentina). For this, a porcelain ball mill was used constituted by a 1-l capacity cylindrical container (jar) that rotates according to a horizontal axis on cylindrical bearings powered by a motor (Fig. 8). The porcelain jar used has an adjustable lid and it is loaded with the paint components. The dispersion is achieved fundamentally through the cascade effect produced by the motion of the spheres. The pigment particles are subjected to impact and shear effort (Giúdice, et al., 1980).

During a first stage, the ligand of the paint was elaborated by dissolving rosin resin and oleic acid (plasticizer) in a xylene/mineral turpentine mix in a high-speed disperser. The jar was loaded with the ligand and the pigments (zinc oxide and chalk) and these were dispersed for 24 hours. The
Fig. 7.

Samples Entered

Weigh

Cleaning fabric wet fractionation

Lyophilized tissue (36-48h, 0.120mbar, -45°C)

Weigh

Tissue dry

Marinate tissue (24h, 120rpm)

Methanol / dichloromethane (1:1)

Filtration by gravity

Solution

Remove the solvent in a rotary evaporator (4h, 80rpm, 38°C)

Amber bottle recover extract

Lyophilized tissue (36-48h, 0.120mbar, -45°C)

Dry extract

No

Liquid-liquid fractionation

Weigh

600 mbar dichloromethane
100 mbar methanol

Water / dichloromethane (1:1)

Bioassays

Dichloromethane fraction (FO)

Lost in ball

Remove the solvent in a rotary evaporator (80rpm, 38°C)

Dry fraction

Yes

Amber bottle recover extract

Bioassays

Aqueous fraction (FA)

Re-split twice

No

Yes

FO

FA

Lost in ball

Rotavapor 15 minutes to remove residual dichloromethane

Lyophilized tissue (36-48h, 0.120mbar, -45°C)

Dry fraction

No

Yes

Amber bottle recover extract

Bioassays

Weigh

Filtration by gravity

Tissue

Marinate tissue (24h, 120rpm)

Filtration by gravity

Tissue

Marinate tissue (24h, 120rpm)

Filtration by gravity

Tissue

Re-split twice

Dry fraction

No

Yes

Amber bottle recover extract

Bioassays

Weigh
paint obtained was filtered and fractionated into seven portions; one of these was used as negative control (P1) and the remaining ones were added to the extracts (organic phase) dissolved in the same solvent mixture used for the paint (Tables 1 and 2).

Table 1. Composition of the paints

<table>
<thead>
<tr>
<th>Components</th>
<th>% V/V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc oxide</td>
<td>16.2</td>
</tr>
<tr>
<td>Chalk</td>
<td>10.8</td>
</tr>
<tr>
<td>Rosin resin</td>
<td>27.0</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>6.0</td>
</tr>
<tr>
<td>Xylene/mineral turpentine (1:1)</td>
<td>40.0</td>
</tr>
<tr>
<td>Extracts (organic phase)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 2. Identification of the paints

<table>
<thead>
<tr>
<th>Identification</th>
<th>Extracts (organic phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 1</td>
<td>Control</td>
</tr>
<tr>
<td>P 2</td>
<td><em>Agelas tubulata</em> (Demospongia, Agelasidae)</td>
</tr>
<tr>
<td>P 3</td>
<td><em>Myrmekioderma gyroderma</em> (Demospongia, Heteroxydae)</td>
</tr>
<tr>
<td>P 4</td>
<td><em>Holothuria glaberrima</em> (Echinodermata, Holothuriidae)</td>
</tr>
<tr>
<td>P 5</td>
<td><em>Oceanapia peltata</em> (Demospongia, Phloeodictyidae)</td>
</tr>
<tr>
<td>P 6</td>
<td><em>Aplysina lacunosa</em> (Demospongia, Aplysinidae)</td>
</tr>
<tr>
<td>P 7</td>
<td><em>Neopetrosia</em> (Xestospongia) <em>proxima</em> (Demospongia, Petrosiidae)</td>
</tr>
</tbody>
</table>

The anti-fouling paints prepared were applied with a brush on 120 x 40 mm sanded acrylic panels previously degreased with toluene. Four coats of paint were applied with a 24-hour drying time between them until obtaining a final dry-film thickness of 75 ± 5 µm. The panels with each type of paint were placed on aluminum racks in series of six and were submerged in the port of Mar del Plata registering the behavior of each formulation after 45 and 90 days. The estimation of attachment of organisms on panels exposed with or without formulations in the sea in function of time was evaluated through a grid as a guide to observe the whole panel given its small size. The panels were removed and observed in the laboratory. Abundance was registered for each species from the macro and microfouling and percentages were estimated.

Results

The results from the first 45 days demonstrated that significant differences exist regarding the percentage of total coverage between the painted panels and the controls (p<0.05). The differences were basically due to settlement of the *Enteromorpha intestinalis* and *Ectocarpus* sp. algae, colonies of the *Bugula* sp. bryozoan, and to the sandy tubes of the *Corophium* sp. amphipod. The paints presenting the best antifouling behavior were: P4, P5, P6, and P7. Paint P3, in turn, did not present antifouling effect because the attachment registered did not differ statistically from the controls. However, reduced percentages of attachment of organisms could be noted, especially of the *Polydora, Bugula, Ectocarpus, Enteromorpha,* and *Corophium* genera (Fig. 9).

Coincidentally, after 90 days of exposure to the sea significant differences were observed in total coverage between the painted panels and the controls (p<0.05) (Fig. 10). The species that determined those differences were the same that were registered during the first sampling to which *Hydroides elegans* was added.

All the paints studied showed antifouling activity, except for P3 (*Myrmekioderma gyroderma*) whose...
behavior was similar to the controls, as occurred during the first sampling. However, it must be highlighted that the paints containing organic fractions of extracts of *Agelas tubulata*, *Holothuria glaberrima* and *Neopetrosia proxima* presented the best behavior, significantly inhibiting attachment of species present in the fouling from Mar del Plata except for Botryllus sp. (p<0.05) (Table 3) (Fig. 11). It is worth mentioning that all the organic fractions tested completely inhibited attachment of *Hydroides elegans*, calcareous tube forming polychaete considered among the most aggressive species of local fouling.

### Discussion

Porifera are among the most studied groups in function of the vast amount of secondary metabolites they produce and that they represent...
an important source of new structures. In this sense, the biggest flow of information on the theme corresponds to this group (33% of the articles reported), followed by chordates (8%), and echinoderms (5%). The number of compounds isolated from these phyla is 6668 for sponges, 1047 for echinoderms, and 977 for chordates (Blunt et al., 2008).

The most common biogenesis routes of secondary metabolites in sponges from the Demospongiae class (group to which the sponges studied in this group belong) are isoprenoids (50%), amino acids (25%), and acetogenins (22%) (Harper et al., 2001). These metabolites present diverse biological activities like, for example, anti-predator and anti-epibiotic (Becerro et al., 2003; Tsoukatou et al., 2007; Hellio et al., 2005; Clavico et al., 2006; Thakur and Anil, 2000; Harper et al., 2001; Faulkner, 2002; Blunt et al., 2004).

Also, studies carried out on isolating and identifying natural products extracted from echinoderms have focused fundamentally on sea cucumbers that have shown antifungal, antiviral, and antitumor activity (De Marino et al., 1997; Bryan et al., 1996; Haug et al., 2002; Bryan et al., 1992; Villasin and Pomory, 2000; Hua et al., 2009); in some species like Holothuria leucospilota, it was proven that compounds extracted from their tissues avoided development of biofilms formed by diatoms (Moshake et al., 1994; Gonsalves, 1997).

The huge potential of marine organisms as producers of secondary metabolites remains evident in this work, given that of six species evaluated five presented antifouling activity. More so, if we bear in mind that only the organic fraction of the extracts has been used, it could be assumed that bioactive metabolites could be present in the aqueous fraction and even synergic interactions among metabolites from both fractions that until now have not been contemplated. This point of view does not dismiss, then, that the Myrmekioderma gyroderma sponge, which in this focused work did not show antifouling activity, could be of use.

Due to the excellent results obtained in this series of experiences, it would be of interest to continue with these types of studies, conducting immersion tests with painted panels for more prolonged

Table 3. Antifouling activity of extracts on organisms from biofouling

<table>
<thead>
<tr>
<th>Components</th>
<th>P₁</th>
<th>P₂</th>
<th>P₃</th>
<th>P₄</th>
<th>P₅</th>
<th>P₆</th>
<th>P₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteromorpha</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ectocarpus</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bugula</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydroides</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Corophium tubes</td>
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<td>-</td>
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<tr>
<td>Botryllus spp.</td>
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(+): Positive antifouling activity; (-): negative antifouling activity.
periods to verify the effect of the extracts on other species present during distinct times of the year. Combinations of extracts should also be studied in the laboratory and at sea to detect synergic effects to maximize the antifouling activity.

It is also transcendental to conduct bio-guided tests to establish what molecule or molecules from the organic fraction of the extracts are responsible for this activity and, thus, offer a concrete alternative for their use, by obtaining the product through chemical synthesis or through organism or tissue cultures.

Conclusions

1. All the extracts included in anti-fouling paints of soluble matrix showed antifouling activity, except for those from Myrmekioderma gyroderma whose behavior was similar to the controls.
2. The best antifouling behavior was presented by paints containing the organic fractions of Agelas tubulata, Holothuria glaberrima, and Neopetrosia proxima extracts.
3. All the extracts tested completely inhibited attachment of Hydroides elegans, calcareous tube forming polychaete considered among the most aggressive species of local fouling.

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