Antiviral and Antiproliferative Potential of Marine Organisms From the Yucatan Peninsula, Mexico

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Viral infections are one of the main human health problems in recent decades and the cancer remains one of the most lethal diseases worldwide. The development of new antiviral drugs for the treatment of human adenovirus (HAdV) infections continues to be a challenging goal for medicinal chemistry. There is no specific antiviral drug approved to treat infections caused by HAdV so far and the off-label treatments currently available show great variability in their effectiveness. In relation to cancer, most of the available drugs are designed to act on specific targets by altering the activity of involved transporters and genes. Taking into account the high antiviral and antiproliferative activity against tumor cell lines displayed by some marine natural products reported in the literature, sixty five marine organisms were selected: 51 sponges (Porifera), 13 ascidians (Chordata), and 1 gorgonian (Cnidaria), collected from Yucatan Peninsula, Mexico, to evaluate their antiviral activity against human adenovirus type 5 (HAdV5) and their anticancer properties against five human tumor cell lines, namely human lung carcinoma (A549), human skin melanoma (A2058), hepatocyte carcinoma (HepG2), breast adenocarcinoma (MCF7), and pancreas carcinoma (MiaPaca-2). Eleven extracts displayed anti-HAdV activity being the organic extracts of Dysidea sp., Agelas citrina, Chondrilla sp., Spongia tubulifera, and Monanchora arbuscula the five most active ones. On the other hand, 24 extracts showed antiproliferative activity against at least one tumor cell line, being the extracts of the ascidian Eudistoma amanitum and the sponge Haliclona (Rhizoniera) curacaoensis the most active ones. This work constitutes the first wide antiviral and antiproliferative screening report of extracts from the marine sponges, ascidians, and a gorgonian collected from the Yucatan Peninsula, Mexico.

Keywords: antiviral, antiproliferative, Yucatan Peninsula, marine organisms extracts, sponges, ascidians, gorgonian
INTRODUCTION

Human adenoviruses (HAdV) are non-enveloped viruses with an icosahedral capsid containing a linear double-stranded DNA whose size ranges from 34 to 37 kb in size (Lion, 2014). Currently, more than 100 serotypes have been identified and grouped into 7 HAdV species (HAdV-A to -G) in *Mastadenovirus* genus (Qiu et al., 2018; HAdV Working Group, 2019). HAdV infections are common in the human population, as indicated by the high seroprevalence of anti-adenovirus antibodies (ranging from 80 to 90% in sub-Saharan Africa, and from 30 to 70% in Europe and North America), but in otherwise healthy adults, these infections are generally mild and self-limited (Grosso et al., 2017; Inturi et al., 2018). On the other hand, with the advances in molecular techniques of diagnosis, HAdV have been found to be increasingly involved in occasional cases and outbreaks of community-acquired pneumonia (CAP) in healthy population (Yu et al., 2015; Kajon and Ison, 2016; Tan et al., 2016; Jonnalagadda et al., 2017; Yoon et al., 2017). In immunocompromised patients, HAdV infections occur with a wide clinical symptomatology including pneumonia, colitis, hepatitis, hemorrhagic cystitis, tubule-interstitial nephritis or encephalitis, which could result in disseminated disease with high morbidity and mortality in this population especially in pediatric units (Lion, 2014; Sulejmani et al., 2018).

Despite HAdV significant clinical impact, there is currently not an approved drug to treat these infections and the off-label antiviral drugs currently available such as ribavirin, ganciclovir and cidofovir show high variability in their clinical efficacy and their use is also limited by their poor bioavailability and side effects (nephrotoxicity or bone marrow suppression). Brincidofovir (CMX001), a lipidic conjugate of cidofovir, that finished a phase III clinical trial in 2016 with no reported results so far (ClinicalTrials.gov Identifier: NCT02087306) and is now being evaluated for the treatment of serious HAdV infection or disease (ClinicalTrials.gov Identifier: NCT02596997), represents the only potential alternative to be used for the treatment of HAdV infections (Toth et al., 2008; Paolino et al., 2011). Based on this scenario, the research on additional drugs with increased anti-HAdV efficacy is thus necessary.

On the other hand, cancer remains one of the most life-threatening disease and an economic burden worldwide (Bray et al., 2018). Cancer is an abnormal growth of cells and tissues, mainly influenced by the environmental and genetic factors of each individual. More than 277 types of cancer have been identified and diagnosed among which prostate, breast, lung, colon, rectum, bronchus, and urinary bladder cancers are the predominant ones (Wogan et al., 2004; Kumar and Adki, 2018; Khalifa et al., 2019). In 2018, approximately 18 million new cases of cancer were reported globally, resulting in approximately 10 million deaths (Vogelstein and Kinzler, 2004; Bray et al., 2018). Currently four cancer treatments are available, which include: surgery, radiotherapy, chemotherapy, and immunotherapy (Topalian et al., 2012; Bray et al., 2018; June et al., 2018). Unlike surgery and radiotherapy, which are treatment methods mainly indicated for solid tumors, chemotherapy is a treatment that interferes with the process of growth and cell division in tumor cells (Ma and Wang, 2009). Although tumor recurrence and metastasis are usual in some cases, several drugs for cancer chemotherapy are currently in use with a considerably high therapeutic success (Kuczynski et al., 2013; Widmer et al., 2014).

In this regard, society has become more and more reliant upon the availability of safe and efficacious pharmaceutical products with fewer side effects. Considering that the marine world provides approximately half of the total biodiversity on earth (Aneiros and Garateix, 2004; Vo and Kim, 2010), and of course the vast expanse of the ocean, this underwater environment would represent an exceptional opportunity for the search of new chemical compounds (Bhadury et al., 2006) with biological activities for the development of new anticancer and antiviral therapies. Today, around 29,000 new compounds have been reported from marine species, such as sponges, ascidians, corals, and bacteria, and they represent a huge structural diversity of secondary metabolites with very promising candidates to be developed as new drugs (Blunt et al., 2017; Pye et al., 2017). Up to date, agencies such as United States Food and Drug Administration (FDA), European Medicines Agency (EMA), Japanese Ministry of Health or Australia’s Therapeutic Goods Administration have approved only 8 compounds from marine origin as therapeutic drugs, and 22 drug candidates are in phases I, II, or III clinical trials (Pereira, 2019). Five out of the approved drugs are used in cancer therapies, namely Cytarabine (*ara-C*), Trabectedin, Eribulin mesylate, Brentuximab vedotin, and plitidepsin (dehydroididemnin B), while just one is used to treat viral infections, which is the Vidarabine (*ara-A*) (Jiménez, 2018). In addition, due to the current SARS-CoV-2 pandemic situation, marine compounds have acquired special interest as a potential source of antiviral candidates (Gentile et al., 2020; Khan et al., 2020).

The coasts of Mexico extend along 11,122 km of maritime littorals from the Pacific Ocean to the Caribbean Sea and the Gulf of Mexico, where a rich marine flora and fauna can be found (Morales et al., 2006). Even so, the underwater Mexican ecosystems remains largely unexplored. Particularly, the Yucatan Peninsula (YP), with 1,500 km of coastline, which includes the Mexican States of Campeche, Yucatan and Quintana Roo (Herrera-Silveira et al., 2004), that extends along approximately 14% of total Mexican coast and it harbors a great biological diversity in the shore and the ocean (Bye et al., 1995). All along the western and northern coasts of the YP, extends a region known as the Campeche Bank (CB) with abundant coral reef ecosystems remains largely unexplored. Particularly, the Yucatan Peninsula (YP), with 1,500 km of coastline, which includes the Mexican States of Campeche, Yucatan and Quintana Roo (Herrera-Silveira et al., 2004), that extends along approximately 14% of total Mexican coast and it harbors a great biological diversity in the shore and the ocean (Bye et al., 1995). All along the western and northern coasts of the YP, extends a region known as the Campeche Bank (CB) with abundant coral reef ecosystems either well offshore (>100 km, such as Alacranes reef, Cayo Arenas, Cayo Arcas, among others) or closer to the shore of the Yucatan state (such as Sisal, Madagascar, and Serpiente); both have been recognized as important biodiversity hotspots (Jordan-Dahlgren, 2002; Tunnell et al., 2007; Ortiz-Lozano et al., 2013; Zarco-Perelló et al., 2013). Additionally, the eastern coast of YP is part of the Mesoamerican Reef, which contains the largest barrier reef in the Western Hemisphere, stretching nearly 700 miles from the northern tip of the YP down through the Honduran Bay Islands (Villela et al., 2003). The potential of Mexican marine resources along the coasts of the YP has not been intensively investigated. Most of the few reports are limited to the
evaluation of the biological activity of their organic extracts and there are very few studies on the chemistry of the natural products (Pech-Puch et al., 2020).

As far as we know, the only study of the antiviral activity in extracts of marine organisms from the YP was the report about the high activity of the L-carrageenan polysaccharide obtained from the red algae Solieria filiformis (Peñuela et al., 2018). In relation to antiproliferative activity of the marine extracts of YP, there are only two reports corresponding to the evaluation of 30 extracts obtained exclusively from seaweeds (Moo-Puc et al., 2009; Caamal-Fuentes et al., 2014a) which yielded, so far, four compounds with antiproliferative activity: the diterpene dictyol B acetate, the steroid fucosterol (Caamal-Fuentes et al., 2014b) and the triterpenoid saponins stichloroside B2 and astichopside C (Graniel-Sabido et al., 2016).

In our constant search for new biological compounds, the decision to explore the marine biodiversity of YP waters was made. In this work, we report the most comprehensive study undertaken to date on antiviral and antiproliferative screening of marine invertebrate species collected along the coasts of the YP, including a total of 65 organic extracts from sponges, ascidians and gorgonians.

MATERIALS AND METHODS

Animal Collection and Identification

Sixty five marine organisms (51 sponges, 13 ascidians, and 1 gorgonian) were collected by snorkeling and scuba diving in two different ecosystems in the Yucatan Peninsula, coral reef and mangroves, during three different periods of time: September–December 2016, January–March 2017, and September 2018. The selected species were collected from two different regions of the Yucatan Peninsula: Mexican Caribbean (Cozumel Island, Rio Indio, Mahahual and Bermejo, Quintana Roo) and Campeche Bank (Alacranes Reef and Progreso, Yucatan) in areas that were chosen based on their rich biological diversity present in coral reefs, islands and mangroves (Figure 1).

The samples were labeled with a code according to the collection site, stored in plastic bags and chilled on ice during transport to the laboratory. Voucher specimens of sponges were deposited in the Phylum Porifera Gerardo Green National Collection of the Institute of Marine Sciences and Limnology (ICMyL) at the National Autonomous University of Mexico (UNAM), Mexico City, while voucher specimens of ascidians and gorgonian were deposited in the Marine Biology Collection at the Autonomous University of Yucatan (UADY) in Yucatan, Mexico.

The sponges were identified at the ICMyL-UNAM (Mexico) while the ascidians were identified at the University of Vigo (Spain), the Autonomous University of Yucatan (Mexico) and the University of A Coruña, Spain. Information about the taxonomic identification of all the selected marine organisms, code numbers, site of collection, weight of each organic extract along with the antiviral and antiproliferative activity previously reported for each studied species are shown in Table 1. Figures 2, 3 show the structures of compounds with antiviral and antiproliferative activities, respectively, previously reported from the marine organisms present in this study.
TABLE 1 | Taxonomic information, voucher numbers, site of collection, weight of the organic extract and previously activity reported for the species studied.

| Family            | Organism                        | Code    | Site                  | Weight (g) | Antiviral activity reported | Antiproliferative activity reported | References                                                                 |
|-------------------|---------------------------------|---------|-----------------------|------------|-----------------------------|-------------------------------------|--------------------------------------------------------------------------------|
| Clavelinidae      | *Clavelina* sp.                  | T18-M1  | Progreso, Yucatan     | 5.0        | No                          | No                                  |                                                                                 |
| Didemnidae        | *Didemnum perlucidum*           | E8-2    | Rio Indio, Quintana Roo | 1.8        | No                          | No                                  |                                                                                 |
| Didemnidae        | *Didemnum* sp.                  | T18-M4  | Progreso, Yucatan     | 3.5        | No                          | Pyrazin-2(1H)-ones 1–3 IC₅₀ 1.5–> 50 µg/mL | Takeara et al., 2008; Shaala et al., 2016                                       |
| Didemnidae        | *Trididemnum solidum*           | E01     | Bermejo, Quintana Roo | 3.7        | No                          | Didermin B (4) IC₅₀ 0.002 µg/mL     |                                                                                 |
| Didemnidae        | *Didemnum* sp.                  | E7-2    | Rio Indio, Quintana Roo | 3.4        | Dierminin A-C 0.05 µg/mL (HSV-2) |                                     | Takeara et al., 2008; Shaala et al., 2016                                     |
| Polysyncraton sp. | *Eudistoma amanitum*            | EY18-8  | Progreso, Yucatan     | 3.6        | No                          | No                                  |                                                                                 |
| Polyclinidae      | *Polyclinia* sp.                | T18-M5  | Progreso, Yucatan     | 1.8        | No                          | No                                  |                                                                                 |
| Asciidiidae       | *Phallusia nigra*               | TY18-1  | Progreso, Yucatan     | 5.5        | No                          | No                                  |                                                                                 |
| Perophoridae      | *Ecteinascidia* sp.             | T18-M2  | Progreso, Yucatan     | 9.0        | Ecteinascidin 743 (7) SV40 (IC₅₀ 2 µM) | ecteinascidin 743 (7) IC₅₀ 0.16–0.68 nM | Takebayashi et al., 2001; Dziegielewska et al., 2004                         |
| Molgulidae        | *Molgula* sp.                   | T18-M6  | Progreso, Yucatan     | 3.9        | No                          | No                                  |                                                                                 |
| Styelidae         | *Polycarpa* sp.                 | E41     | Alacranes Reef, Yucatan | 2.4        | No                          | No                                  |                                                                                 |
| Briareidae        | *Briareum asbestinum*           | BA-3    | Rio Indio, Quintana Roo | 3.9        | No                          | No                                  |                                                                                 |
| Agelasidae        | *Agelas citrina*                | CZE56   | Cozumel, Quintana Roo | 1.9        | No                          | No                                  |                                                                                 |
|                   | *Agelas clathrodes*             | E27-2   | Cozumel, Quintana Roo | 11.2       | No                          | No                                  |                                                                                 |
|                   | *Agelas clathrodes*             | MA18-10 | Mahahual, Quintana Roo | 7.2        | No                          | No                                  |                                                                                 |
|                   | *Agelas dilatata*               | E25-1   | Cozumel, Quintana Roo | 21.3       | No                          | No                                  |                                                                                 |
|                   | *Agelas sceptrum*               | E26-2   | Cozumel, Quintana Roo | 4.6        | No                          | No                                  |                                                                                 |
| Heteroxyidae      | *Myrmekioderma gyroderma*       | CZE18   | Cozumel, Quintana Roo | 7.5        | No                          | No                                  |                                                                                 |
| Raspailiidae      | *Ectyoplasia ferox*             | MA18-9  | Mahahual, Quintana Roo | 5.9        | No                          | No                                  |                                                                                 |
| Chondrillidae     | *Ectyoplasia* sp.               | MA18-13 | Mahahual, Quintana Roo | 2.2        | No                          | No                                  |                                                                                 |
| Chondrillidae     | *Chondrilla carpensis f. hermatypica* | MA18-6 | Mahahual, Quintana Roo | 2.1        | No                          | Thiocoraline (10) EC₅₀ 0.0095 µM | Wyche et al., 2011                                                             |
| Clathrinidae      | *Clathrina* sp.                 | RIO18-1 | Rio Indio, Quintana Roo | 4.6        | No                          | No                                  |                                                                                 |
| Leucettidae       | *Leucetta floridana*            | E2-2    | Bermejo, Quintana Roo | 1.3        | No                          | No                                  |                                                                                 |

(Continued)
| Family       | Organism                        | Code  | Site            | Weight (g) | Antiviral activity reported | Antiproliferative activity reported | References                                      |
|--------------|---------------------------------|-------|-----------------|------------|-----------------------------|-----------------------------------|------------------------------------------------|
| Clionaidae   | Cliona delitrix                 | EY18-1| Progreso, Yucatan| 5.2        | No                          | No                                | Pech-Puch et al., 2019                   |
|              | Cliona varians                  | EY18-3| Progreso, Yucatan| 1.8        | No                          | No                                | Qu and Wang, 2008; Wang et al., 2020     |
| Dysideidae   | Dysidea sp.                     | EY18-12| Progreso, Yucatan | 3.3        | Sesquiterpenes hydroquinones (11–13) from Dysidea arenaria IC₅₀ 16.4, 239.7, 176.1 µM (HIV-1 RT) | No                                 |                                               |
|              | Ircinia felix                   | E9-2  | Rio Indio, Quintana Roo | 43.5  | No                          | Felixins F and G IC₅₀ 1.27–27.08 µM | Lai et al., 2015a,b                      |
|              | Ircinia strobilina              | MA18-11| Mahahual, Quintana Roo | 1.7       | No                          | No                                |                                               |
|              | Ircinia strobilina              | E24-2 | Cozumel, Quintana Roo | 14.1      | No                          | No                                |                                               |
|              | Ircinia strobilina              | E52   | Bermejo, Quintana Roo | 4.9       | No                          | No                                |                                               |
| Spongiidae   | Spongia tubulifera              | E11-2 | Rio Indio, Quintana Roo | 29.8     | No                          | No                                | Pech-Puch et al., 2019                   |
|              | Callyspongia longissima         | E28   | Alacranes Reef, Yucatan | 1.8       | No                          | No                                |                                               |
|              | Callyspongia plicifera          | E31   | Alacranes Reef, Yucatan | 1.2       | No                          | No                                |                                               |
|              | Callyspongia vaginae            | E16   | Cozumel, Quintana Roo | 0.9       | No                          | No                                |                                               |
| Chalinidae   | Haliclona (Rhizoniera) curacaoensis | EY18-4| Progreso, Yucatan | 7.9       | No                          | No                                |                                               |
| Niphatidae   | Amphimedon compressa            | E29   | Alacranes Reef, Yucatan | 12.9     | 49.25% HSV-1 inhibition, 3.12 µg/mL | Ethanolic extract IC₅₀ 7.12–9.9 µg/mL | L’Hullier et al., 2019                    |
|              | Niphates digitalis              | E15   | Cozumel, Quintana Roo | 2.5       | No                          | No                                |                                               |
|              | Niphates erecta                 | E49   | Alacranes Reef, Yucatan | 1.6       | Niphatevirin EC₅₀ 12 nM (HIV-1) | Organic fractions CC₅₀ 95.2 µg/mL (HeLa) Si > 4.20 | O’Keefe et al., 1997; Mendola et al., 2014 |
|              | Niphates erecta                 | MA18-7| Mahahual, Quintana Roo | 2.8       | No                          | No                                |                                               |
|              | Niphates erecta                 | MA18-12| Mahahual, Quintana Roo | 5.5       | No                          | No                                |                                               |
| Petrosiidae  | Xestospongia muta               | EP    | Alacranes Reef, Yucatan | 14.1      | Brominated polyacetylenic acids IC₅₀ 6–12 µM (HIV-1) | Araguspongine C (15), meso-araguspongine C (16) IC₅₀ 0.43–1.02 µM | Patil et al., 1992; Dung et al., 2019     |
| Plakinitidae | Plakinastrella onkodes           | E3    | Bermejo, Quintana Roo | 4.9       | No                          | Cyclic peroxide methyl capucinoate A (17) IC₅₀ 12 µg/mL | Horton et al., 1994; Williams et al., 2001 |
| Crabeidae    | Monanchora arbuscula            | E35   | Alacranes Reef, Yucatan | 28.9     | No                          | Polycyclic guanidine alkaloids such as 18 IC₅₀ 1.6–14 µM | Laville et al., 2009; Ferreira et al., 2011 |
| Microcionidae| Clathria gomezae                | EY18-11| Progreso, Yucatan | 1.8       | No                          | No                                |                                               |
|              | Clathria virgultosa             | E7-E34| Alacranes Reef, Yucatan | 5.5       | No                          | No                                |                                               |
| Mycalidae    | Mycale laevis                   | MA18-1| Mahahual, Quintana Roo | 14.1      | No                          | No                                |                                               |
|              | Mycale laevis                   | MA18-5| Mahahual, Quintana Roo | 4.9       | No                          | No                                |                                               |

(Continued)
| Family          | Organism                    | Code  | Site                      | Weight (g) | Antiviral activity reported | Antiproliferative activity reported | References                                      |
|-----------------|-----------------------------|-------|---------------------------|------------|-----------------------------|------------------------------------|------------------------------------------------|
| Scopalinidae    | Scopalina ruetzleri         | DNY   | Rio Indio, Quintana Roo   | 29.8       | No                          | IC₅₀ 10.51–18.35 µg/mL              | Biegelmeyer et al., 2015                 |
|                 | Scopalina ruetzleri         | E53   | Cozumel, Quintana Roo     | 1.8        | No                          | 10.51–18.35 µg/mL                  |                                                 |
|                 | Scopalina ruetzleri         | EY18-7| Progreso, Yucatan         | 5.5        | No                          | 5.5 µg/mL                          |                                                 |
| Halichondriidae | Halichondria melanadocia    | E18-M1| Progreso, Yucatan (Mangrove) | 14.1       | No                          | No                                 |                                                 |
| Suberitidae     | Aaptos sp.                  | E38   | Alacranes Reef, Yucatan   | 4.9        | 4-methylaaptamine (19)      | EC₅₀ 2.4 µM (HSV-1)                | Aaptamine (20) IC₅₀ (NT2) 50 µM           |
|                 |                             |       |                           |            |                             |                                    | Souza et al., 2007; Dyshlovoy et al., 2012 |
| Tethyidae       | Tethya sp.                  | E20   | Cozumel, Quintana Roo     | 29.8       | HSV-1, EC₅₀ 425 mg/mL       | No                                 | Aswell et al., 1977; da Silva et al., 2006  |
| Geodiidae       | Metophlus hajdui            | E4    | Bermejo, Quintana Roo     | 4.4        | No                          | No                                 |                                                 |
| Tetillidae      | Cinachyrella kuekenthali    | MA18-2| Mahahual, Quintana Roo    | 2.1        | No                          | No                                 |                                                 |
| Aplysinidae     | Aiolochroia crassa          | E50   | Alacranes Reef, Yucatan   | 5.2        | No                          | No                                 |                                                 |
|                 | Aiolochroia crassa          | MA18-4| Mahahual, Quintana Roo    | 8.7        | No                          | No                                 |                                                 |
|                 | Aplysina cauliformis        | E36   | Alacranes Reef, Yucatan   | 6.3        | No                          | No                                 |                                                 |
|                 | Aplysina fistularis         | E46   | Alacranes Reef, Yucatan   | 2.7        | 11-deoxyfistularin-3 (21)  | LD₅₀ 17 µg/mL→50 µg/mL             | Gopichand and Schmitz, 1979; Gunasekera and Cross, 1992; Compagnone et al., 1999 |
|                 | Aplysina fulva              | E42   | Alacranes Reef, Yucatan   | 1.8        | No                          | No                                 |                                                 |
|                 | Aplysina fulva              | EY18-5| Progreso, Yucatan         | 2.9        | No                          | No                                 |                                                 |
|                 | Aplysina muricyana          | E47   | Alacranes Reef, Yucatan   | 4.4        | No                          | No                                 |                                                 |

No, no previous reports for this genus or species.
Preparation of the Organic Extracts

Sliced bodies of each species were exhaustively extracted with the mixture of dichloromethane-methanol (1:1), three times with 500 mL (1.5 L total volume) at 25°C for 24 h for each extraction. After filtration, the solvent was then removed by rotatory evaporator at 40°C and the crude extract stored at −20°C in tightly sealed glass vials.

Antiviral Assays

Viruses and Cells

Human A549 (human lung carcinoma) and 293 (human embryonic kidney) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, United States). A549 cells were grown in minimum essential medium (MEM, Life Technologies/Thermo Fisher) supplemented with 10% fetal bovine serum (FBS) (Omega Scientific, Tarzana, CA, United States), 10 mM HEPES, 4 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.1 mM non-essential amino acids (complete DMEM). The anti-HAdV activity was initially measured in an entry assay. The cytotoxicity of the extracts was analyzed by the use of the commercial kit AlamarBlue® (Invitrogen, Ref. DAL1025). A549 cells at a density of 5 × 10^5 cells/well in 96-well plates were seeded. Decreasing concentrations of each extract (100, 80, 60, 40, 30, 20, 10, 5, 2.5, 1.25, and 0 µg/mL) were diluted in 100 µL of Dulbecco’s Modified Eagle Medium (DMEM). Cells were then incubated at 37°C for 48 h following the manufacturer’s indications. The cytotoxic concentration 50 (CC50) value was calculated using the statistical package GraphPad Prism. This assay was performed in duplicate.

Cytotoxicity Assay

The cytotoxicity of the extracts was analyzed by the use of the commercial kit AlamarBlue® (Invitrogen, Ref. DAL1025). A549 cells at a density of 5 × 10^5 cells/well in 96-well plates were seeded. Decreasing concentrations of each extract (100, 80, 60, 40, 30, 20, 10, 5, 2.5, 1.25, and 0 µg/mL) were diluted in 100 µL of Dulbecco’s Modified Eagle Medium (DMEM). Cells were then incubated at 37°C for 48 h following the manufacturer’s indications. The cytotoxic concentration 50 (CC50) value was calculated using the statistical package GraphPad Prism. This assay was performed in duplicate.

Entry Assay

The anti-HAdV activity was initially measured in an entry assay using human A549 epithelial cells (3 × 10^5 cells/well in corning black wall, clear bottom 96-well plates) infected with HAdV5-GFP (2,000 vp/cell) in the presence of 12.5 µg/mL of each extract and in a dose-response assay. A standard infection curve was generated in parallel by infecting cells in the absence of extracts using serial twofold dilutions of the virus. All reactions were done in triplicate. Cells, viruses, and extracts were incubated for 48 h at 37°C and 5% CO2. Infection, measured as HAdV5-mediated GFP expression, was analyzed using a Typhoon 9410 imager (GE Healthcare Life Sciences) and quantified with ImageQuant TL (GE Healthcare Life Sciences).

Statistical Analyses

Statistical analyses were performed with the GraphPad Prism 5 suite. Data are presented as the mean of duplicate/triplicate samples ± standard deviation (SD).

Antiproliferative Assays

Colorimetric MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays were carried out to assess the cell viability of the samples against a panel of five different tumor cell lines (i.e., human lung carcinoma A549 ATCC® CCL-185TM, human skin melanoma A2058 ATCC® CRL-11147TM, hepatocyte carcinoma HepG2 ATCC® HB-8065TM, breast adenocarcinoma MCF7 ATCC® HTB-22TM and pancreas carcinoma MiaPaca-2 ATCC® CRL-1420TM). All cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, United States). A549 cells were grown in Ham’s F12K medium with 2 mM Glutamine, 10% FBS, 100 U/mL and rocking for 2 h at 37°C. After incubation, the inoculum was removed and the cells were washed once with phosphate buffered saline (PBS). The cells were then carefully overlaid with 4 mL/well of equal parts of 1.6% (water/vol) Difco agar Noble (Becton, Dickinson and Co., Sparks, MD, United States) and 2× EMEM (Minimum Essential Medium Eagle, BioWhittaker) supplemented with 2× penicillin/streptomycin, 2× L-glutamine, and 10% fetal bovine serum (FBS). The mixture also contained the extracts in concentrations ranging from 10 to 0.625 µg/mL. Following incubation for 7 days at 37°C, plates were scanned with a Typhoon 9410 imager (GE Healthcare Life Sciences) and plaques were quantified with ImageJ (Schneider et al., 2012). This assay was performed in duplicate.

Statistical Analyses

Statistical analyses were performed with the GraphPad Prism 5 suite. Data are presented as the mean of duplicate/triplicate samples ± standard deviation (SD).
FIGURE 3 | Selected structures of compounds with reported antiproliferative activities displayed in Table 1. 1: (S)-6-(sec-butyl)-3-isopropylpyrazin-2(1H)-one; 2: (S)-3-(sec-butyl)-6-isopropylpyrazin-2(1H)-one; 3: (S)-6-(sec-butyl)-3-isobutylpyrazin-2(1H)-one; 4: didemnin B; 5: eudistomin E; 6: eudistalbin A; 7: ecteinascidin 743; 8: briareolate ester L; 9: (−)-agelasidine C; 10: thiocoraline; 11: scalarane sesterpenoid; 12: araguspongine C; 13: meso-araguspongine C; 14: cyclic peroxide methyl capucinoate A; 15: norbatzelladine A; 16: aaptamine; 17: 11-deoxyfistularin-3.
penicillin and 100 µg/mL streptomycin. A2058 and HepG2 were
grown in ATCC formulated Eagle's M essential medium (MEM)
with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and
100 µM MEM non-essential amino acids. MCF-7 cells were
grown in the previous medium supplemented with 0.01 mg/mL
of bovine insulin. MiaPaca-2 cells were grown in DMEM with
10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin
(Audoin et al., 2013). The antiproliferative activity was assessed
after 48 h of treatment with extracts at concentrations of 30, 15,
and 7.5 µg/mL.

RESULTS AND DISCUSSION

The antiviral and antiproliferative activity of the organic extracts
from 65 marine organisms, corresponding to 51 sponges,
13 ascidians, and 1 gorgonian, collected from two different
ecosystems in the Yucatan Peninsula, coral reef and mangroves,
were evaluated. Around 17% of the extracts showed antiviral
activity against HAdV and 37% of them displayed antitumor
activity against one or more tumor cell lines.

Antiviral Screening

Marine organisms are invaluable sources of bioactive natural
products, some of them being highly significant hits for drug
development against pathogenic bacteria, viruses, and fungi
(Sagar et al., 2010). A current interest in developing antiviral
drugs has been increased since viral diseases have become major
human health problems (Sagar et al., 2010).

The results of the antiviral evaluation of marine organic
extracts are shown in Table 2. Eleven extracts displayed significant in vitro antiviral activity against HAdV (Table 2), in particular extracts from the ascidian Clavelina sp. and 10 sponges
which include: Agelas citrina, Myrmekioderma gyroderma,
Ectyoplasia sp., Chondrilla sp., Dysidea sp., Incinia felix (collected from Rio Indio, Quintana Roo), Spongia tubulifera, Monanchora arbuscula, Aaptos sp., and Cinachyrella kuekenthali.

All the extracts were first screened in plaque assay at the
concentration of 10 µg/mL to quantify their ability to inhibit
HAdV plaque formation. The active extracts screened out
(inhibition >70%) were further evaluated to characterize their
antiviral activity (IC50) in plaque assay and their cytotoxicity
(CC50 values).

The extracts of the sponges Dysidea sp., A. citrina, Chondrilla sp., S. tubulifera, and M. arbuscula showed the higher activity
with an inhibition >97% at 10 µg/mL concentration and IC50 values between 0.53 and 2.15 µg/mL in the plaque assays
(Table 2). The same sponges species showed >98% inhibition in
the entry assay at 12.5 µg/mL and IC50 values of 5.24 µg/mL
for Dysidea sp., 4.74 µg/mL for A. citrina, 3.23 µg/mL for
M. arbuscula, 2.35 µg/mL for S. tubulifera, and 1.09 µg/mL
for Chondrilla sp. The CC50 values were 19.84 µg/mL for
S. tubulifera, 19.46 µg/mL for Dysidea sp., 6.45 µg/mL for
M. arbuscula, 5.33 µg/mL for A. citrina and 2.45 µg/mL for
Chondrilla sp. The sponge S. tubulifera displayed the best
selectivity index (SI = 37.43), followed by Dysidea sp. (SI = 17.38),
A. citrina (SI = 5.05), M. arbuscula (SI = 3.00), and finally
Chondrilla sp. (SI = 1.87) (Table 2).

The ascidian Clavelina sp. displayed more than 80% inhibition
of HAdV5 infection at 10 µg/mL and an IC50 of 3.65 µg/mL in
the plaque assay. In the entry assay, it showed 39% of inhibition
at 12.5 µg/mL and in addition, it showed a CC50 value of
39.69 µg/mL and the third best selectivity index (10.87).

The sponges M. gyroderma, Ectyoplasia sp., C. kuekenthali,
Aaptos sp., and M. arbuscula showed an inhibition of HAdV5
infection ranging from 71 to 98.97% at 10 µg/mL, and IC50
values between 2.15 and 10 µg/mL. The CC50 values were
22.26 µg/mL for M. gyroderma, 39.77 µg/mL for Ectyoplasia sp.,
30.06 µg/mL for C. kuekenthali, 22.72 µg/mL for Chondrilla sp.
and 6.45 µg/mL for M. arbuscula. The selectivity index for the
five sponges showed values between 2.27 and 3.97.

For some of the organisms included in this work, the antiviral
activity of the extract had been previously described (Table 1)
as well as the activity of some of their constituent compounds
(Figure 2). That is the case of the species Trididemnum solidum,
Eudistoma sp., Amphimedon compressa, or Aaptos sp., which
display significant anti-HSV or anti-SV40 activities with
IC50 ranging between 0.05 and 3.12 µg/mL (Table 1). However,
the antiviral activity showed for the former extracts did not
always correlate with their corresponding anti-HAdV activity.
Indeed, T. solidum, Eudistoma sp., and A. compressa extracts
showed very low or any anti-HAdV activity (Table 2). Moreover,
although the Thetya sp. extract displayed anti-HSV-1 activity,
it’s antiviral activity against HAdV was only reached at high
concentrations (Tables 1, 2).

On the contrary, the extract of the species Aaptos sp. showed
both potent anti-HSV-1 (Table 1) and significant anti-HAdV
activity (Table 2 and Figure 2). On the other hand, the anti-
HAdV activity showed by the extract from Dysidea sp. was
significantly higher than the anti-HIV activity, however, the
Niphates erecta extract was significantly more active against HIV-
1 than against HAdV (Tables 1, 2). The anti-HIV activity of both
eXtracts from Dysidea sp. and N. erecta was previously reported.

These data are in line with previous studies from other groups
which showed a wide variability in virus inhibition of extracts
from marine sponges and cnidarian products (Cheung et al.,
2014). Despite the fact that many authors published results of
screening of marine organisms for antiviral activity (Donia and
Hamann, 2003), there are no many screenings of marine extracts
centered on detecting anti-HAdV activity, thus these results
highlight the importance of studying further marine organisms
extracts against HAdV as sources of new antiviral drugs.

Regarding the possible mechanism of action for the extracts
from Dysidea sp. and S. tubulifera, depending on the HAdV
entry inhibition assay and cytotoxic concentrations, it may be
related to the first steps during HAdV entry into the cell host.
On the other hand, the antiviral activity of the extracts from
Clavelina sp., Aaptos sp. and Cinachyrella kuekenthali would be
associated with later steps after the entry of HAdV genomes into
the nucleus. The significant antiviral activity showed by Aaptos
sp. against both HSV-1 and HAdV suggests a potential broad-
spectrum mechanism of activity that will require further study.
The very similar IC50 values of the entry assay with those of CC50
TABLE 2 | IC<sub>50</sub>, CC<sub>50</sub>, SI, % inhibition of HAdV infection and % inhibition of HAdV entry of organic extracts of marine organisms from the Yucatan Peninsula.

| Code    | Organism                  | Plaque assay IC<sub>50</sub> (µg/mL) | Inhibition HAdV (%) at 10 µg/mL | Entry assay IC<sub>50</sub> (µg/mL) | Inhibition HAdV (%) at 10 µg/mL | CC<sub>50</sub> | SI<sup>a</sup> |
|---------|---------------------------|--------------------------------------|---------------------------------|--------------------------------------|---------------------------------|----------------|--------------|
| T18-M1  | Clavelina sp.             | 3.65 ± 1.56                         | 80.95 ± 1.04                    | nt                                   | 38.82 ± 2.37                    | 39.69 ± 1.30  | 10.87        |
| E8-2    | Didemnum perfollicum      | nt                                   | 22.06 ± 20.89                   | nt                                   | nt                              | nt             |              |
| T18-M4  | Didemnum sp.              | nt                                   | 47.32 ± 1.38                    | nt                                   | nt                              | nt             |              |
| E01     | Didemnum sp.              | nt                                   | 16.67 ± 0.00                    | nt                                   | nt                              | nt             |              |
| E7-2    | Trididemnum solidum       | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| EY18-8  | Polysyncraton sp.         | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| RIO18-T1| Eudistoma amanitum        | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| T18-M5  | Polyclinum sp.            | nt                                   | 38.54 ± 1.38                    | nt                                   | nt                              | nt             |              |
| T18-1   | Phallus nigra             | nt                                   | 4.64 ± 10.18                    | nt                                   | nt                              | nt             |              |
| T18-M2  | Ectenascidia sp.          | nt                                   | 37.56 ± 4.14                    | nt                                   | nt                              | nt             |              |
| T18-M6  | Molgula sp.               | nt                                   | 42.72 ± 17.85                   | nt                                   | nt                              | nt             |              |
| E41     | Polycarpa sp.             | nt                                   | 23.07 ± 10.87                   | nt                                   | nt                              | nt             |              |
| BA-3    | Briareum asbestinum       | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| CZE56   | Agelas citrina            | 1.06 ± 0.41                         | 97.24 ± 0.02                    | 4.74 ± 0.53                         | 100.00 ± 0.00                   | 5.35 ± 2.45    | 5.05         |
| E27-2   | Agelas clathrodes         | nt                                   | 4.92 ± 6.95                     | nt                                   | nt                              | nt             |              |
| MA18-10 | Agelas clathrodes         | nt                                   | 6.25 ± 16.51                    | nt                                   | nt                              | nt             |              |
| E25-1   | Agelas dilatata           | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| E26-2   | Agelas sceptrum           | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| CZE18   | Myrmekioderma gyroderma   | 7.48 ± 1.69                         | 71.03 ± 5.85                    | nt                                   | 0.00 ± 0.00                     | 22.26 ± 2.23   | 2.98         |
| MA18-9  | Ectyoplasia ferox         | nt                                   | 35.00 ± 11.21                   | nt                                   | nt                              | nt             |              |
| MA18-13 | Ectyoplasia sp.           | 10.00 ± 0.00                        | 85.68 ± 7.35                    | nt                                   | 0.00 ± 0.00                     | 39.77 ± 7.88   | 3.97         |
| MA18-6  | Chondrella canibensis f. hemmatypica | 0.00 ± 0.00 | nt | nt | nt | nt |
| RIO18-1 | Chondrella sp.            | 1.31 ± 0.10                         | 97.24 ± 3.90                    | 1.09 ± 0.79                         | 99.72 ± 0.29                    | 2.45 ± 0.48    | 1.87         |
| EY18-10 | Clathrina sp.             | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| E2-2    | Leucetta floridana        | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| EY18-1  | Cliona delitrix           | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| EY18-3  | Cliona varians            | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| EY18-12 | Dysidea sp.               | 1.12 ± 0.02                         | 98.17 ± 0.52                    | 5.24 ± 0.50                         | 98.24 ± 0.72                    | 19.46 ± 0.30   | 17.38        |
| E9-2    | Ircinia felix             | 8.42 ± 0.85                         | 80.10 ± 20.00                   | nt                                   | 0.00 ± 0.00                     | 73.51 ± 33.54  | 8.73         |
| MA18-11 | Ircinia felix             | nt                                   | 51.05 ± 10.25                   | nt                                   | nt                              | nt             |              |
| E24-2   | Ircinia strobilina        | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| ES2     | Ircinia strobilina        | nt                                   | 0.00 ± 0.00                     | nt                                   | nt                              | nt             |              |
| E11-2   | Spongia tubulifera        | 0.53 ± 0.03                         | 99.09 ± 1.29                    | 2.35 ± 1.02                         | 100.00 ± 0.00                   | 19.84 ± 2.41   | 37.43        |

(Continued)
| Code | Organism                          | Plaque assay IC<sub>50</sub> (µg/mL) | Inhibition HAdV (%) at 10 µg/mL | Entry assay IC<sub>50</sub> (µg/mL) | Inhibition HAdV (%) at 10 µg/mL | CC<sub>50</sub> | SI<sup>a</sup> |
|------|----------------------------------|-------------------------------------|---------------------------------|-----------------------------------|--------------------------------|----------------|-------------|
| E28  | Callyspongia longissima          | nt                                  | 0.00 ± 0.00                     | nt                                | nt                             | nt            | nt          |
| E31  | Callyspongia plicifera           | nt                                  | 54.13 ± 6.43                    | nt                                | nt                             | nt            | nt          |
| E16  | Callyspongia vaginalis           | nt                                  | 47.00 ± 5.45                    | nt                                | nt                             | nt            | nt          |
| EY18-4| Haliociona (Rhizonia) curacaoensis| nt                                  | 51.65 ± 14.50                   | nt                                | nt                             | nt            | nt          |
| E29  | Amphimedon compressa             | nt                                  | 35.26 ± 29.17                   | nt                                | nt                             | nt            | nt          |
| E15  | Naphtes digitals                 | nt                                  | 46.66 ± 9.99                    | nt                                | nt                             | nt            | nt          |
| E49  | Naphtes erecta                   | > 10.00                             | 41.00 ± 8.64                    | nt                                | 0.00 ± 0.00                     | nt            | nt          |
| MA18-7| Naphtes erecta                  | nt                                  | 27.50 ± 12.68                   | nt                                | nt                             | nt            | nt          |
| MA18-12| Naphtes erecta                  | nt                                  | 48.96 ± 1.47                    | nt                                | nt                             | nt            | nt          |
| EP   | Xestospongia muta                | nt                                  | 18.58 ± 8.77                    | nt                                | nt                             | nt            | nt          |
| E3   | Plakinastrella onkodes            | nt                                  | 17.29 ± 0.00                    | nt                                | nt                             | nt            | nt          |
| E35  | Monanchora arbuscula             | 2.15 ± 0.37                         | 98.97 ± 1.46                    | 3.23 ± 1.04                       | 100.00 ± 0.00                   | 6.45 ± 2.41   | 3.00        |
| EY18-11| Clathria gomezae                 | nt                                  | 0.00 ± 0.00                     | nt                                | nt                             | nt            | nt          |
| E7-E34| Clathria virgultosa              | nt                                  | 0.00 ± 0.00                     | nt                                | nt                             | nt            | nt          |
| MA18-1| Mycale laevis                    | nt                                  | 0.00 ± 0.00                     | nt                                | nt                             | nt            | nt          |
| MA18-5| Mycale laevis                    | nt                                  | 0.00 ± 0.00                     | nt                                | nt                             | nt            | nt          |
| DNY  | Scopalina ruetzleri              | nt                                  | 0.00 ± 0.00                     | nt                                | nt                             | nt            | nt          |
| E53  | Scopalina ruetzleri              | nt                                  | 37.17 ± 7.51                    | nt                                | nt                             | nt            | nt          |
| EY18-7| Scopalina ruetzleri              | nt                                  | 0.00 ± 0.00                     | nt                                | nt                             | nt            | nt          |
| E18-M1| Halichondria melanadocia         | nt                                  | 25.86 ± 2.44                    | nt                                | nt                             | nt            | nt          |
| E38  | Aaptos sp.                       | 10.00 ± 0.00                        | 72.00 ± 10.97                   | 0.00 ± 0.00                       | 22.72 ± 2.89                   | 2.27          |             |
| E20  | Tethya sp.                       | nt                                  | 52.89 ± 5.18                    | nt                                | nt                             | nt            | nt          |
| E4   | Metophlus hajdui                 | nt                                  | 48.71 ± 2.32                    | nt                                | nt                             | nt            | nt          |
| MA18-2| Cinachyrella kuekenthali        | 10.00 ± 0.00                        | 73.08 ± 3.26                    | 0.00 ± 0.00                       | 30.06 ± 9.95                   | 3.00          |             |
| E50  | Aiolochroia crassa               | > 10.00                             | 35.29 ± 10.70                   | nt                                | 0.00 ± 0.00                     | nt            | nt          |
| MA18-4| Aiolochroia crassa              | nt                                  | 0.00 ± 0.00                     | nt                                | nt                             | nt            | nt          |
| E36  | Aplysina cauliformis             | nt                                  | 0.00 ± 0.00                     | nt                                | nt                             | nt            | nt          |
| E46  | Aplysina fistularis              | nt                                  | 18.00 ± 2.82                    | nt                                | nt                             | nt            | nt          |
| E42  | Aplysina fulva                   | nt                                  | 10.00 ± 3.26                    | nt                                | nt                             | nt            | nt          |
| EY18-5| Aplysina fulva                  | nt                                  | 0.00 ± 0.52                     | nt                                | nt                             | nt            | nt          |
| E47  | Aplysina muriycana               | nt                                  | 16.00 ± 12.62                   | nt                                | nt                             | nt            | nt          |
| CONTROL| Cidofovir                      | nt                                  | 6.7 ± 1.6                       | nt                                | nt                             | 13.9 ± 2.7    | 2.07        |

<sup>a</sup>Selective Index. nt, not tested.
**TABLE 3 | Antiproliferative activity in % of inhibition of organic extracts at different concentrations (in μg/mL) of marine organisms from Yucatan Peninsula.**

| Code     | Organism (μg/mL) | A549<sup>a</sup> | A2058<sup>b</sup> | HepG2<sup>c</sup> | MCF7<sup>d</sup> | MiaPaca2<sup>e</sup> |
|----------|------------------|------------------|------------------|------------------|---------------|-------------------|
|          |                  | 30   | 15   | 7.5 | 30   | 15   | 7.5 | 30   | 15   | 7.5 | 30   | 15   | 7.5 | 30   | 15   | 7.5 |
| T18-M1   | Clavellina sp.   | 93   | 93   | 84  | 98   | 95   | 92  | 98   | 96   | 93  | 91   | 79   | 90  | 94   | 90   | 85  |
| E8-2     | Didemnum perliecum | 17   | 17   | 13  | 19   | 10   | 11  | 67   | 51   | 45  | 0    | 10   | 3   | 2    | 2    | 3   |
| T18-M4   | Didemnum sp.     | 2    | 9    | 0   | 4    | 1    | 3   | 35   | 8    | 8   | 4    | 12   | 7   | 4    | 4    | 0   |
| E01      | Didemnum sp.     | 2    | 9    | 3   | 8    | 0    | 3   | 6    | 4    | 2   | 8    | 11   | 12  | 8    | 7    | 3   |
| E7-2     | Trididemnum solidum | 73   | 51   | 25  | 87   | 69   | 29  | 92   | 71   | 52  | 32   | 31   | 9   | 65   | 4    | 3   |
| EY18-8   | Polysyncraton sp. | 15   | 18   | 9   | 50   | 23   | 13  | 80   | 60   | 53  | 34   | 23   | 4   | 8    | 2    | 3   |
| RIO18-T1 | Eudistoma amanitum | 99   | 99   | 99  | 99   | 100  | 100 | 100  | 99   | 99  | 100  | 100  | 100 | 97   | 99   | 97  |
| TY18-2   | Eudistoma sp.    | 2    | 2    | 5   | 8    | 1    | 0   | 35   | 24   | 21  | 10   | 5    | 4   | 6    | 3    | 3   |
| T18-M5   | Polyclinum sp.   | 3    | 10   | 3   | 5    | 1    | 3   | 47   | 27   | 17  | 3    | 5    | 3   | 2    | 1    | 1   |
| TY18-1   | Phallusia nigra  | 13   | 10   | 1   | 2    | 3    | 4   | 25   | 7    | 4   | 8    | 3    | 1   | 4    | 0    | 1   |
| T18-M2   | Ecteinascidia sp. | 4    | 4    | 7   | 5    | 1    | 3   | 33   | 9    | 8   | 2    | 4    | 7   | 6    | 4    | 4   |
| T18-M6   | Molgula sp.      | 6    | 11   | 4   | 16   | 10   | 1   | 48   | 29   | 18  | 1    | 5    | 3   | 2    | 0    | 2   |
| E41      | Polycarpa sp.    | 1    | 10   | 0   | 24   | 7    | 1   | 67   | 47   | 32  | 14   | 20   | 14  | 1    | 3    | 4   |
| BA-3     | Bitreuroides asbestinum | nt | nt  | nt  | nt  | nt  | nt  | nt  | nt  | nt  | nt  | nt  | nt  | nt  | nt  | nt  |
| C2E56    | Agelas citrina   | 49   | 26   | 6   | 100  | 100  | 100 | 100  | 99   | 21  | 100  | 100  | 100 | 46   | 100  | 1   |
| E27-2    | Agelas clathODULES | 21   | 17   | 8   | 20   | 9    | 6   | 53   | 26   | 21  | 0    | 8    | 3   | 4    | 2    | 3   |
| MA18-10  | Agelas clathODULES | 8    | 8    | 1   | 19   | 1    | 3   | 29   | 6    | 6   | 18   | 5    | 12  | 4    | 1    | 2   |
| E25-1    | Agelas dilata    | 22   | 24   | 16  | 25   | 18   | 11  | 63   | 42   | 39  | 7    | 2    | 5   | 6    | 3    | 4   |
| E26-2    | Agelas sceptrum  | 22   | 16   | 4   | 39   | 27   | 20  | 66   | 55   | 48  | 6    | 17   | 12  | 3    | 2    | 3   |
| C2E18    | Myrmekioderma gyroderma | 29  | 12   | 3   | 74   | 13   | 1   | 100  | 100  | 40  | 73   | 11   | 1   | 80   | 0    | 3   |
| MA18-9   | Ectypaella ferox  | 16   | 18   | 3   | 6    | 3    | 4   | 82   | 1    | 0   | 4    | 9    | 3   | 3    | 6    | 5   |
| MA18-13  | Ectypaella sp.   | 43   | 20   | 10  | 37   | 18   | 5   | 86   | 47   | 22  | 41   | 20   | 5   | 26   | 4    | 2   |
| MA18-6   | Chondrilla caribensis f. hermatypica | 60   | 56   | 27  | 69   | 2    | 2   | 99   | 81   | 26  | 96   | 25   | 4   | 3    | 8    | 9   |

(Continued)
makes difficult to hypothesize the potential mechanism of action for *A. citrina*, *Chondrilla* sp., and *M. arbuscula* extracts.

### Antiproliferative Screening

The results of the antiproliferative evaluation of marine organic extracts are shown in Table 3 as well as the extracts (Table 1) and compounds (Figure 3) previously described for their antiproliferative activity. Twenty-four extracts showed growth inhibition for one or more tumor cell lines, namely those obtained from 4 ascidians (*Clavelina* sp., *T. solidum*, *Polysyncraton* sp., and *E. amanitum*) and 20 sponges (*A. citrina*, *M. gyroderma*, *Chondrilla caribensis* f. *hermatypica*, *Leucetia floridana*, *Cliona varians*, *Dysidea* sp., *S. tubulifera*, *Haliclona* (Rhizioniera) *curacaoensis*, *A. compressa*, *Plakinastrella onkodes*, *Monanchora arbuscula*, *Clathria gomezea*, *Mycale laevis* (collected from Mahahual, Quintana Roo), and *Scopalina ruetzleri* (collected from Progreso, Yucatan and Rio Indio, Quintana Roo), *Aaptos* sp., *Tethya* sp., *C. kuekenthali*, and *Aiolochroia crassa* (collected from Alacranes Reef, Yucatan and Mahahual, Quintana Roo). Interestingly, the extracts of two organisms, the ascidian *E. amanitum* and the sponge *H. (Rhizioniera) curacaoensis*, displayed the most potent antiproliferative activities with a complete growth inhibition in all the cell lines at all concentrations tested (Table 3). No previous studies reporting the antiproliferative activity in extracts from these two species have been published, highlighting the value of these two organisms as a potential source of new antiproliferative compounds (Table 1).

The extracts of seven additional organisms, the ascidian *Clavelina* sp. and the sponges *P. onkodes*, *M. arbuscula*, *Aaptos* sp., *Tethya* sp., *L. floridana*, and *C. kuekenthali*, also showed good activity against all the cell lines, but with variable potency according to the concentration tested (Table 3). Nonetheless, all of them except *L. floridana*, which only displays a 23% inhibition at 15 mg/mL against the MiaPaca-2 cell line, still meet the National Cancer Institute (NCI) guidelines to be considered as antiproliferative, i.e., inhibition higher than 50% at a concentration of 20 mg/mL. (Hostettman, 1991; Boik, 2001). Two extracts of the organisms, *Clavelina* sp. and *P. onkodes*, showed more than 84% growth inhibition of the A549 cell line at all concentrations while the six extracts displayed an almost complete growth inhibition of the A2058 cell line at all concentrations, except the extract of the sponge *P. onkodes* that did not show any antiproliferative activity at the lowest concentration tested. In the particular case of the cell line HepG2, the six extracts showed more than 83% growth inhibition at all concentrations. Regarding the MCF7 cell line, only the sponge *M. arbuscula* displayed an almost complete growth inhibition at all concentrations tested. Finally, extracts of all the organisms except *L. floridana* showed antiproliferative activity according to the NCI guidelines against the cell line MiaPaca-2. Out of this group of seven organisms, the sponges *C. kuekenthali* and *L. floridana* stood out as the most interesting ones due to the lack of previous reports on the chemical composition and antiproliferative bioactivity of their extracts. On the contrary, previous reports on the cytotoxic properties of compounds isolated from extracts from *P. onkodes*, *M. arbuscula*, and *Aaptos* sp. make these samples less interesting for the identification of new cytotoxic molecules, although chemical analyses should be performed to discard the presence of other bioactive components not previously reported in extracts of these species.

The extracts of the sponges *A. citrina*, *M. gyroderma*, *A. compressa*, *C. caribensis* f. *hermatypica*, and *Dysidea* sp., were active against four cell lines, being selective against some types of cancer. The three most active extracts were those of the sponge *A. citrina* that showed 100% of growth inhibition of the A2058 cell line at all concentrations tested, the HepG2 at 30 µg/mL, the MCF7 at 30 and 15 µg/mL, and the MiaPaca-2 at 30 µg/mL.

### Table 3 (Continued)

| Code | Organism (µg/mL) | A549 | A2058 | HepG2 | MCF7 | MiaPaca2 |
|------|------------------|------|-------|-------|------|---------|
|      |                  | 30   | 15    | 7.5   | 30   | 15      | 7.5   | 30 | 15 | 7.5 |
| E53  | Scopalina ruetzleri | 8    | 9     | 11    | 6    | 2       | 1     | 2  | 1   | 2   |
| EY18-7 | Scopalina ruetzleri | 13   | 13    | 21    | 7    | 11      | 7     | 2  | 1   | 2   |
| E18-M1 | Halichondria melanodacia | 10   | 6     | 18    | 37   | 11      | 10    | 6  | 2   | 1   |
| E38  | Aaptos sp.         | 75   | 71    | 63    | 99   | 99      | 99    | 99 | 99 | 99 |
| E20  | Tethya sp.         | 61   | 61    | 58    | 99   | 91      | 91    | 99 | 99 | 99 |
| E4   | Melophorus hajdui | 8    | 8     | 2     | 16   | 0       | 16    | 1  | 1   | 1   |
| MA18-2 | Cinachyrella kuekenthali | 78   | 78    | 59    | 98   | 98      | 98    | 98 | 98 | 98 |
| E50  | Aiolochroia crassa | 7    | 6     | 2     | 16   | 4       | 16    | 4  | 1   | 1   |
| MA18-4 | Aiolochroia crassa | 11   | 10    | 4     | 31   | 13      | 13    | 13 | 13 | 13 |
| E36  | Aplysina caulfurmis | 20   | 14    | 3     | 18   | 11      | 11    | 11 | 11 | 11 |
| E46  | Aplysina fistularis | 15   | 12    | 1     | 15   | 7       | 7     | 7  | 7   | 7   |
| E42  | Aplysina fulva     | 7    | 9     | 2     | 13   | 4       | 13    | 4  | 4   | 4   |
| EY18-5 | Aplysina fulva    | 32   | 24    | 14    | 23   | 10      | 4     | 10 | 10 | 10 |
| E47  | Aplysina municiyana | 4    | 4     | 4     | 8    | 2       | 0     | 2  | 2   | 2   |

Reference: Doxorubicin 50 µM, MMS 4 µM

nt, not tested. *a* Human lung carcinoma. *b* Human skin melanoma. *c* Hepatocyte carcinoma. *d* Breast adenocarcinoma. *e* Pancreas carcinoma. DMSO 0% inhibition in all concentrations.
followed by the sponge *M. gyroderma* extract that showed 100% of growth inhibition of the HepG2 at 30 and 15 µg/mL, and more than 73% of growth inhibition against A2058, MCF7, and MiaPac-2 cell lines at 30 µg/mL. Finally, the extract of the sponge *A. compressa* showed more than 86% of growth inhibition of the cell line A549 at all concentrations tested, it also showed more than 99% of growth inhibition of A2058 and HepG2 cell lines at 30 µg/mL and more than 68% growth inhibition of HepG2 and MCF7 cell lines at 15 µg/mL and at 30 µg/mL, respectively. The other two sponges, *C. caribensis f. hermatypica* showed more than 56% of growth inhibition of A549 (30 and 15 µg/mL), A2058 (30 µg/mL), HepG2 (30 and 15 µg/mL) and MiaPac-2 (30 µg/mL) and the sponge *Dysidea* sp. showed more than 51% of growth inhibition against A549, A2058, HepG2, and MCF7 cell lines at 30 µg/mL. Despite the moderate activity found in most extracts, *M. gyroderma* is perhaps the most interesting sponge of this group due to the lack of reports on its antiproliferative activity. In the cases of *A. citrina*, *A. compressa*, *C. caribensis f. hermatypica*, and *Dysidea* sp., once again chemical analyses of the extracts will be necessary to assess the novelty of their components and their potential interest for further studies.

The extracts of the rest of the organisms displayed bioactivity to a lesser extension, hitting only a few cell lines of the panel tested. Thus, the sponge *S. ruetzleri* showed more than 66% growth inhibition of A549, A2058, and HepG2 cell lines at 30 µg/mL. On the other hand, the extract of sponge *C. gomezae* showed more than 76% growth inhibition of A2058 and HepG2 cell lines at 30 µg/mL. *C. gomezae* seems to be the most interesting of these two sponges due to the lack of previous reports on cytotoxic activity of its extracts, although a preliminary chemical investigation by LC/MS should also be performed on the extract of *S. ruetzleri* before discarding the sample for further studies. Even though *Didemnum* sp. had been previous shown antiproliferative activity ([Table 1](#tab1){ref} in our experience it only showed very little activity at the highest concentration against the HepG2 cell line, perhaps indicating that the specimens collected by us do not contain didemmins or produce very low levels of these potent molecules. Finally, it is worth mentioning that more than 50% of the extracts tested showed antiproliferative activity against the cell line HepG2, 41 extracts exhibited at least more than 50% growth inhibition at 30 µg/mL concentration, and the organisms, *Polysyncraton* sp., *C. varians*, *S. tubulifera*, *M. laevis* (collected from Mahahual, Quintana Roo), *S. ruetzleri* (collected from Progreso, Yucatan) and *A. crassa* (collected from Alacranes Reef, Yucatan and Mahahual, Quintana Roo), only showed activity against the cell line HepG2.

**CONCLUSION**

Sixty-five marine organisms, corresponding to fifty-one sponges (Porifera), thirteen ascidians (Chordata) and one gorgonian (Cnidaria), were collected along the coast of Yucatan Peninsula in Mexico. They were selected on the basis of chemotaxonomical criteria. They were extracted with organic solvents and each extract was screened for its *in vitro* antiviral and antiproliferative activity against HAdV and five tumor cell lines, respectively. Evaluation through plaque assays showed a significant antiviral activity for 11 extracts corresponding to 10 sponges [*A. citrina*, *M. gyroderma*, *Ectyoplasia* sp., *Chondrilla* sp., *Dysidea* sp., *M. arbuscula*, *Aaptos* sp., *C. kuekenthali*, *I. felix* (collected from Rio Indio, Quintana Roo), and *S. tubulifera*] and one ascidian (*Clavelina* sp.). The extracts of the sponges *Dysidea* sp., *A. citrina*, *Chondrilla* sp., *S. tubulifera*, and *M. arbuscula* showed the best antiviral activity. The observed IC<sub>50</sub> values of these extracts were lower than those shown by cidofovir (IC<sub>50</sub> = 6.7 ± 1.6 µg/mL; CC<sub>50</sub> = 13.9 ± 2.7), which is the drug of choice to treat HADV infections. However, the high cytotoxicity displayed by *A. citrina* (5.35 ± 2.45 µg/mL; SI = 5.05) or *Chondrilla* sp. (2.45 ± 0.48 µg/mL; SI = 1.87) generated low SI values similar to those for cidofovir (SI = 2.07).

The high entry inhibition value registered for *Dysidea* sp. and *S. tubulifera* suggested that the antiviral action mechanism could be related with early steps in the HADV replicative cycle involving the binding, internalization by clathrin-mediated endocytosis, endosomal escape and microtubular transport of the viral particles to the nuclear pores of the host cell. In contrast, the mechanism of action for the extracts from *Clavelina* sp., *Aaptos* sp., and *C. kuekenthali* would be associated with later steps after the entry of HADV genomes into de nucleus which could be related with the transcription of the HADV immediate early gene E1A or the HADV DNA replication process, as in the case of cidofovir, a nucleoside analog that inhibit HADV DNA polymerase. *A. citrina*, *Chondrilla* sp., and *M. arbuscula* did not show clear data to suggest a potential mechanism of action.

Twenty-four extracts showed antiproliferative activity that corresponded to twenty sponges [*A. citrina*, *M. gyroderma*, *C. caribensis f. hermatypica*, *L. floridana*, *C. varians*, *Dysidea* sp., *S. tubulifera*, *H. (Rhizioniera) curacaoensis*, *A. compressa*, *P. onkodes*, *M. arbuscula*, *C. gomezae*, *M. laevis* (collected from Mahahual, Quintana Roo), *S. ruetzleri* (collected from Progreso, Yucatan and Rio Indio, Quintana Roo), *Aaptos* sp., *Tethya* sp., *C. kuekenthali* and *A. crassa* (collected from Alacranes Reef, Yucatan and Mahahual, Quintana Roo) and four ascidians (*Clavelina* sp., *T. solidum*, *Polysyncraton* sp. and *E. amanitum*)]. Two organisms, the ascidian *E. amanitum* and the sponge *H. (Rhizioniera) curacaoensis*, showed the best antiproliferative activity. Additionally, more than 50% of the extracts showed antiproliferative activity against the hepatocyte carcinoma cell line (HepG2). According to the results reported in this study, extracts of the tunicate *E. amanitum* and those of the sponges *H. (Rhizioniera) curacaoensis*, *C. kuekenthali*, and *L. floridana* proved to be the most interesting for future studies due to their high potency against most of the cell lines tested and the lack of previous reports on their chemical composition.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this article are not readily available because the raw data supporting the conclusions of this article will be made available by the authors upon request. Requests to access the datasets should be directed to JS-C, jsanchez-ibis@us.es; CJ, carlos.jimenez@udc.es; JR, jaime.rodriguez@udc.es.
AUTHOR CONTRIBUTIONS

DP-P and MP-P were responsible for the recollection of organisms and preparation of the marine extracts. JB-C and JS-C made the antiviral assays. FR and BC did the antiproliferative assays. PG, DP-P, HV-H, and SG-H performed taxonomic identification. DP-P, CJ, and JR wrote the original draft. DP-P, JR, CJ, FR, JP, and JS-C wrote, reviewed, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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