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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

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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 (EMEA), 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 (dehydrodidemnin 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 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 (Jordán-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 B_2 and astichoposide 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.

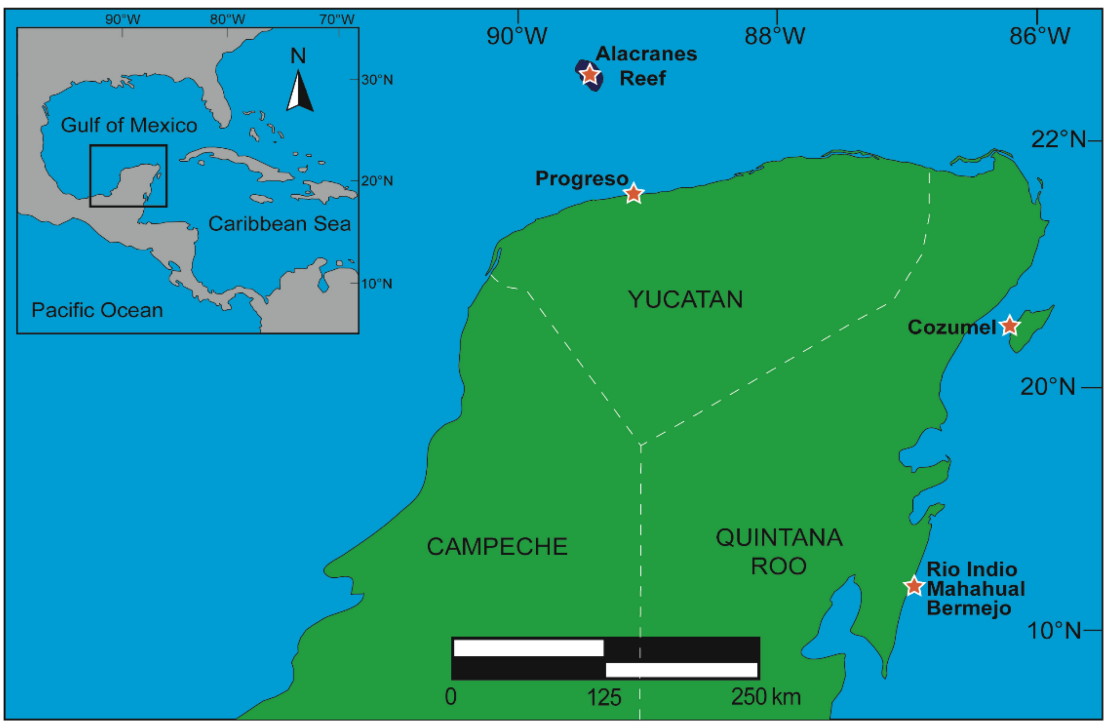

FIGURE 1 | Sites of collection of marine organisms in the Yucatan Peninsula, Mexico.

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 (Mangrove)	5.0	No	No	
Didemnidae	*Didemnum perlucidum*	E8-2	Rio Indio, Quintana Roo	1.8	No	No	
	Didemnum sp.	T18-M4	Progreso, Yucatan (Mangrove)	3.5	No	Pyrazin-2(1*H*)-ones **1**–**3** IC_{50} 1.5– > 50 μg/mL	Takeara et al., 2008; Shaala et al., 2016
	Didemnum sp.	E01	Bermejo, Quintana Roo	3.7			
	Tridemnum solidum	**E7-2**	Rio Indio, Quintana Roo	3.4	Didemnin A-C 0.05 μg/mL (HSV-2)	Didemnin B (**4**) ID_{50} 0.002 μg/mL	Rinehart et al., 1981a,b, 1988; Crampton et al., 1984; Sakai et al., 1995, 1996
	***Polysyncraton* sp.**	**EY18-8**	Progreso, Yucatan	3.6	No	No	
Polycitoridae	***Eudistoma amanitum***	**RIO18-T1**	Rio Indio, Quintana Roo	3.6	No	No	
	Eudistoma sp.	TY18-2	Progreso, Yucatan	2.9	Eudistomins such **5** : HSV-1 active	Eudistomin E (**5**) eudistalbin A (**6**) ED_{50} < 5.0 ng, 3.2 mg/mL	Rinehart et al., 1987; Adesanya et al., 1992
Polyclinidae	*Polyclinum* sp.	T18-M5	Progreso, Yucatan (Mangrove)	1.8	No	No	
Ascidiidae	*Phallusia nigra*	TY18-1	Progreso, Yucatan	5.5	No	No	
Perophoridae	*Ecteinascidia* sp.	T18-M2	Progreso, Yucatan (Mangrove)	9.0	Ecteinascidin 743 (**7**) SV40 (IC_{50} 2 μM)	ecteinascidin 743 (**7**) IC_{50} 0.16–0.68 nM	Takebayashi et al., 2001; Dziegielewska et al., 2004
Molgulidae	*Molgula* sp.	T18-M6	Progreso, Yucatan (Mangrove)	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	Briareolate ester L (**8**), EC_{50} values of 2.4 and 9.3 μM	Gupta et al., 2011; Hall et al., 2017
Agelasidae	***Agelas citrina***	**CZE56**	Cozumel, Quintana Roo	1.9	No	(–)-Agelasidine C (**9**) IC_{50} 10 mM	Stout et al., 2012
	Agelas clathrodes	E27-2	Cozumel, Quintana Roo	11.2	No	No	
	Agelas clathrodes	MA18-10	Mahahual, Quintana Roo	7.2			
	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	
	***Ectyoplasia* sp.**	**MA18-13**	Mahahual, Quintana Roo	2.2	No	No	
Chondrillidae	***Chondrilla caribensis f. hermatypica***	**MA18-6**	Mahahual, Quintana Roo	2.1	No	Thiocoraline (**10**) EC_{50} 0.0095 μM	Wyche et al., 2011
	***Chondrilla* sp.**	**RIO18-1**	Rio Indio, Quintana Roo	4.6	No	No	
Clathrinidae	*Clathrina* sp.	EY18-10	Progreso, Yucatan	1.4	No	No	
Leucettidae	***Leucetta floridana***	**E2-2**	Bermejo, Quintana Roo	1.3	No	No	

(Continued)

TABLE 1 | Conitnued

Family	Organism	Code	Site	Weight (g)	Antiviral activity reported	Antiproliferative activity reported	References
Clionaidae	*Cliona delitrix*	EY18-1	Progreso, Yucatan	5.2	No	No	
	Cliona varians	**EY18-3**	Progreso, Yucatan	1.8	No	No	
Dysideidae	***Dysidea* sp.**	**EY18-12**	Progreso, Yucatan	3.3	Sesquiterpenes hydroquinones (**11**–**13**) from *Dysidea arenaria* IC_{50} 16.4, 239.7, 176.1 μM (HIV-1 RT)	Scalarane sesterpenoid GI_{50} 6.4–14 μM	Qiu and Wang, 2008; Wang et al., 2020
Irciniidae	***Ircinia felix***	**E9-2**	Rio Indio, Quintana Roo	43.5	No	Felixins F and G IC_{50} 1.27–27.08 μM	Lai et al., 2015a,b
	Ircinia felix	MA18-11	Mahahual, Quintana Roo	1.7			
	Ircinia strobilina	E24-2	Cozumel, Quintana Roo	14.1	No	No	
	Ircinia strobilina	E52	Bermejo, Quintana Roo	4.9			
Spongiidae	***Spongia tubulifera***	**E11-2**	Rio Indio, Quintana Roo	29.8	No	3β-Hydroxyspongia-13(16),14-dien-2-one, spongian diterpene 17 and ambliol C IC_{50} 11.7–91.3 μM	Pech-Puch et al., 2019
Callyspongiidae	*Callyspongia longissima*	E28	Alacranes Reef, Yucatan	1.8	No	No	
	Callyspongia plicifera	E31	Alacranes Reef, Yucatan	1.2	No	No	
	Callyspongia vaginalis	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 IC_{50} 7.12–9.9 μg/mL	Lhullier et al., 2019
	Niphates digitalis	E15	Cozumel, Quintana Roo	2.5	No	No	
	Niphates erecta	E49	Alacranes Reef, Yucatan	1.6	Niphatevirin EC_{50} 12 nM (HIV-1)	Organic fractions CC_{50} 95.2 μg/mL (HeLa) SI > 4.20	O'Keefe et al., 1997; Mendiola et al., 2014
	Niphates erecta	MA18-7	Mahahual, Quintana Roo	2.8			
	Niphates erecta	MA18-12	Mahahual, Quintana Roo	5.5			
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**) IC_{50} 0.43–1.02 μM	Patil et al., 1992; Dung et al., 2019
Plakinidae	***Plakinastrella onkodes***	**E3**	Bermejo, Quintana Roo	4.9	No	Cyclic peroxide methyl capucinoate A (**17**) IC_{50} 12 μg/mL	Horton et al., 1994; Williams et al., 2001
Crambeidae	***Monanchora arbuscula***	**E35**	Alacranes Reef, Yucatan	29.8	No	Polycyclic guanidine alkaloids such as **18** GI_{50} 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			

(Continued)

TABLE 1 | Conitnued

Family	Organism	Code	Site	Weight (g)	Antiviral activity reported	Antiproliferative activity reported	References
Scopalinidae	***Scopalina ruetzleri***	**DNY**	Rio Indio, Quintana Roo	29.8	No	IC_{50} 10.51–18.35 μg/mL	Biegelmeyer et al., 2015
	Scopalina ruetzleri	E53	Cozumel, Quintana Roo	1.8			
	Scopalina ruetzleri	**EY18-7**	Progreso, Yucatan	5.5			
Halichondriidae	*Halichondria melanadocia*	E18-M1	Progreso, Yucatan (Mangrove)	14.1	No	No	
Suberitidae	***Aaptos* sp.**	**E38**	Alacranes Reef, Yucatan	4.9	4-methylaaptamine (**19**) EC_{50} 2.4 μM (HSV-1)	Aaptamine (**20**) IC_{50} (NT2) 50 μM	Souza et al., 2007; Dyshlovoy et al., 2012
Tethyidae	***Tethya* sp.**	**E20**	Cozumel, Quintana Roo	29.8	HSV-1, EC_{50} 425 mg/mL, adenovirus EC_{50} 230 mg/mL	No	Aswell et al., 1977; da Silva et al., 2006
Geodiidae	*Melophlus 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			
	Aplysina cauliformis	E36	Alacranes Reef, Yucatan	6.3	No	No	
	Aplysina fistularis	E46	Alacranes Reef, Yucatan	2.7	No	11-deoxyfistularin-3 (**21**) LD_{50} 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			
	Aplysina muricyana	E47	Alacranes Reef, Yucatan	4.4	No	No	

No, no previous reports for this genus or species.

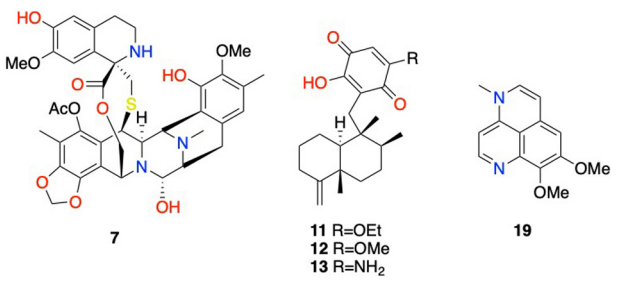

FIGURE 2 | Selected structures of compounds with reported antiviral activities displayed in **Table 1**. **7**: ecteinascidin 743; **11**: isosemnonorthoquinone; **12**: ilimaquinone; **13**: smenospongine; **19**: 4-methylaaptamine.

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). The 293β5 stable cell line overexpressing the human β5 integrin subunit was kindly provided by Dr. Glen Nemerow (Nguyen et al., 2010). Both cell lines were propagated in Dulbecco's modified Eagle medium (DMEM, 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). Wild-type HAdV5 was obtained from ATCC. The HAdV5-GFP showed in this work is a replication-defective virus with a CMV promoter-driven enhanced green fluorescent protein (eGFP) reporter gene cassette in place of the E1/E3 region (Nepomuceno et al., 2007). HAdV were propagated in 293β5 cells and isolated from the cellular lysate by cesium chloride (CsCl) density gradient combined with ultracentrifugation. Virus concentration, in mg/mL, was calculated with the Bio-Rad protein assay (Bio-Rad Laboratories) and converted to virus particles/mL (vp/mL) using 4×10^{12} vp/mg.

Plaque Assay

Natural extracts were tested using low multiplicity of infection (MOI) (0.06 vp/cell) and at concentration of 10 μg/mL in a plaque assay. Organic extracts that showed HAdV inhibition greater than 70% were tested in a dose-response assay ranging from 10 to 0.625 μg/mL in plaque assay. Briefly, 293β5 cells were seeded in 6-well plates at a density of 4×10^5 cells per well in duplicate for each condition. When cells reached 80–90% confluency, they were infected with HAdV5-GFP (0.06 vp/cell) and rocked 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.

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 5×10^3 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 manufacture's indications. The cytotoxic concentration 50 (CC_{50}) 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% CO_2. Infection, measured as HAdV5-mediated GFP expression, was analyzed using a Typhoon 9410 imager (GE Healthcare Life Sciences) and quantified with ImageQuantTL (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

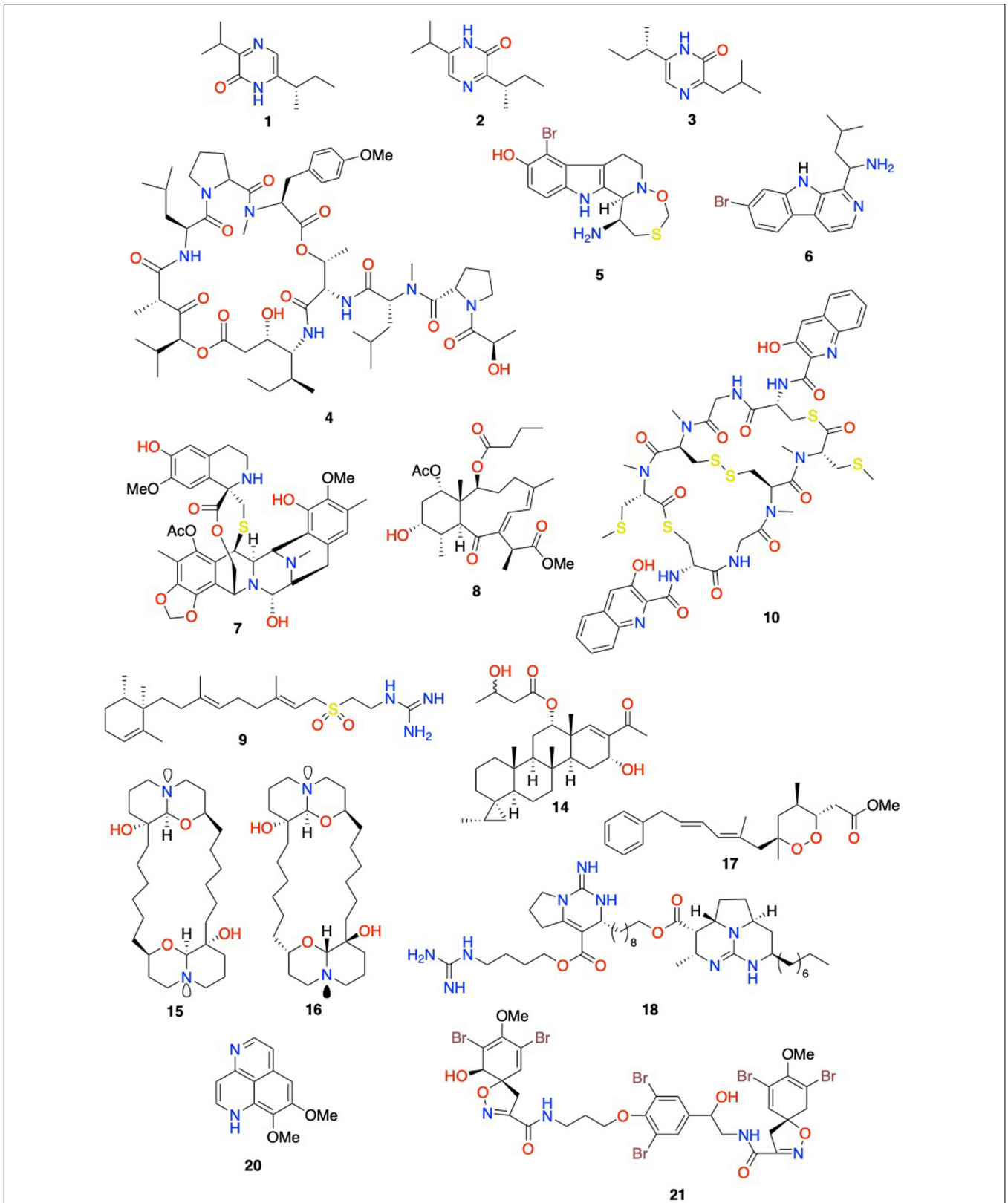

FIGURE 3 | Selected structures of compounds with reported antiproliferative activities displayed in **Table 1**. **1**: (S)-6-(sec-butyl)-3-isopropylpyrazin-2(1*H*)-one; **2**: (S)-3-(sec-butyl)-6-isopropylpyrazin-2(1*H*)-one; **3**: (S)-6-(sec-butyl)-3-isobutylpyrazin-2(1*H*)-one; **4**: didemnin B; **5**: eudistomin E; **6**: eudistalbin A; **7**: ecteinascidin 743; **8**: briareolate ester L; **9**: (−)-agelasidine C; **10**: thiocoraline; **14**: scalarane sesterpenoid; **15**: araguspongine C; **16**: meso-araguspongine C; **17**: cyclic peroxide methyl capucinoate A; **18**: norbatzelladine A; **20**: aaptamine; **21**: 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., *Ircinia 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 (IC_{50}) in plaque assay and their cytotoxicity (CC_{50} 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 IC_{50} 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 IC_{50} 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 CC_{50} values were 19.84 μg/mL for *S. tubulifera*, 19.46 μg/mL for *Dysidea* sp., 6.45 μg/mL for *M. arbuscula*, 5.35 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 IC_{50} 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 CC_{50} 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 IC_{50} values between 2.15 and 10 μg/mL. The CC_{50} 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 *Tridemnum solidum*, *Eudistoma* sp., *Amphimedon compressa*, or *Aaptos* sp., which extracts display significant anti-HSV or anti-SV40 activities with IC_{50} 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, its 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 IC_{50} values of the entry assay with those of CC_{50}

TABLE 2 | IC_{50}, CC_{50}, 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_{50} (μg/mL)	Inhibition HAdV (%) at 10 μg/mL	Entry assay IC_{50} (μg/mL)	Inhibition HAdV (%) at 10 μg/mL	CC_{50}	SI[a]
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 perlucidum*	nt	22.06 ± 20.69	nt	nt	nt	nt
T18-M4	*Didemnum* sp.	nt	47.32 ± 1.38	nt	nt	nt	nt
E01	*Didemnum* sp.	nt	16.67 ± 0.00	nt	nt	nt	nt
E7-2	*Tridemnum solidum*	nt	0.00 ± 0.00	nt	nt	nt	nt
EY18-8	*Polysyncraton* sp.	nt	0.00 ± 0.00	nt	nt	nt	nt
RIO18-T1	*Eudistoma amanitum*	nt	0.00 ± 0.00	nt	nt	nt	nt
TY18-2	*Eudistoma* sp.	nt	27.47 ± 3.11	nt	nt	nt	nt
T18-M5	*Polyclinum* sp.	nt	38.54 ± 1.38	nt	nt	nt	nt
TY18-1	*Phallusia nigra*	nt	4.64 ± 10.18	nt	nt	nt	nt
T18-M2	*Ecteinascidia* sp.	nt	37.56 ± 4.14	nt	nt	nt	nt
T18-M6	*Molgula* sp.	nt	42.72 ± 17.85	nt	nt	nt	nt
E41	*Polycarpa* sp.	nt	23.07 ± 10.87	nt	nt	nt	nt
BA-3	*Briareum asbestinum*	nt	0.00 ± 0.00	nt	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	nt
MA18-10	*Agelas clathrodes*	nt	6.25 ± 16.51	nt	nt	nt	nt
E25-1	*Agelas dilatata*	nt	0.00 ± 0.00	nt	nt	nt	nt
E26-2	*Agelas sceptrum*	nt	0.00 ± 0.00	nt	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	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	*Chondrilla caribensis f. hermatypica*	nt	0.00 ± 0.00	nt	nt	nt	nt
RIO18-1	***Chondrilla* 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	nt
E2-2	*Leucetta floridana*	nt	0.00 ± 0.00	nt	nt	nt	nt
EY18-1	*Cliona delitrix*	nt	0.00 ± 0.00	nt	nt	nt	nt
EY18-3	*Cliona varians*	nt	0.00 ± 0.00	nt	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 ± 0.65	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	nt
E24-2	*Ircinia strobilina*	nt	0.00 ± 0.00	nt	nt	nt	nt
E52	*Ircinia strobilina*	nt	0.00 ± 0.00	nt	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)

TABLE 2 | Continued

Code	Organism	Plaque assay IC_{50} (μg/mL)	Inhibition HAdV (%) at 10 μg/mL	Entry assay IC_{50} (μg/mL)	Inhibition HAdV (%) at 10 μg/mL	CC_{50}	SI[a]
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	*Haliclona* (*Rhizoniera*) *curacaoensis*	nt	51.65 ± 14.50	nt	nt	nt	nt
E29	*Amphimedon compressa*	nt	35.26 ± 29.17	nt	nt	nt	nt
E15	*Niphates digitalis*	nt	46.66 ± 9.99	nt	nt	nt	nt
E49	*Niphates erecta*	> 10.00	41.00 ± 8.64	nt	0.00 ± 0.00	nt	nt
MA18-7	*Niphates erecta*	nt	27.50 ± 12.68	nt	nt	nt	nt
MA18-12	*Niphates 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	nt	0.00 ± 0.00	22.72 ± 2.89	2.27
E20	*Tethya* sp.	nt	52.89 ± 5.18	nt	nt	nt	nt
E4	*Melophlus hajdui*	nt	48.71 ± 2.32	nt	nt	nt	nt
MA18-2	***Cinachyrella kuekenthali***	10.0 ± 0.00	73.08 ± 3.26	nt	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 muricyana*	nt	16.00 ± 12.62	nt	nt	nt	nt
CONTROL	**Cidofovir**	nt	6.7 ± 1.6	nt	nt	**13.9 ± 2.7**	**2.07**

[a]*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[a]			A2058[b]			HepG2[c]			MCF7[d]			MiaPaca2[e]		
		30	15	7.5	30	15	7.5	30	15	7.5	30	15	7.5	30	15	7.5
T18-M1	***Clavelina* sp.**	93	93	84	98	95	92	98	96	93	91	79	50	94	90	85
E8-2	*Didemnum perlucidum*	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	8	4	2	8	11	12	8	7	3
E7-2	***Tridemnum solidum***	73	51	25	87	69	29	92	71	52	52	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	100	97	99
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	*Briareum asbestinum*	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
CZE56	***Agelas citrina***	49	26	6	100	100	100	100	69	21	100	100	46	100	1	3
E27-2	*Agelas clathrodes*	21	17	8	20	9	6	53	26	21	0	8	3	4	2	3
MA18-10	*Agelas clathrodes*	8	8	1	19	1	3	29	6	6	18	5	12	4	1	2
E25-1	*Agelas dilatata*	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
CZE18	***Myrmekioderma gyroderma***	29	12	3	74	13	1	100	100	40	73	11	1	80	0	3
MA18-9	*Ectyoplasia ferox*	16	18	3	6	3	4	82	1	0	4	9	3	3	6	5
MA18-13	*Ectyoplasia* 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
RIO18-1	*Chondrilla* sp.	1	9	1	4	2	3	24	8	1	15	9	1	3	2	3
EY18-10	*Clathrina* sp.	7	15	3	9	0	2	33	10	7	14	6	5	5	1	2
E2-2	***Leucetta floridana***	100	66	6	100	98	16	100	99	36	100	51	7	100	23	2
EY18-1	*Cliona delitrix*	7	13	4	16	2	2	43	23	9	13	3	7	7	8	5
EY18-3	***Cliona varians***	69	32	15	22	9	2	80	66	47	2	2	0	29	5	1
EY18-12	***Dysidea* sp.**	52	12	2	64	15	1	96	65	18	86	41	4	27	3	4
E9-2	*Ircinia felix*	9	17	9	30	16	11	61	41	39	4	10	1	21	4	3
MA18-11	*Ircinia felix*	5	7	4	7	6	0	35	20	17	3	10	1	14	6	1
E24-2	*Ircinia strobilina*	20	22	24	39	37	38	66	47	44	10	13	3	3	1	17
E52	*Ircinia strobilina*	7	10	1	3	2	2	10	3	1	5	4	1	1	0	1
E11-2	***Spongia tubulifera***	13	12	0	35	9	0	84	55	24	31	20	1	51	16	4
E28	*Callyspongia longissima*	21	17	4	24	6	0	60	35	29	2	4	5	2	1	1
E31	*Callyspongia plicifera*	36	33	21	25	11	5	65	47	31	3	10	1	20	7	5
E16	*Callyspongia vaginalis*	3	8	1	6	1	2	12	5	0	14	8	9	3	2	3
EY18-4	***Haliclona* (*Rhizoniera*) *curacaoensis***	100	100	99	100	100	100	100	100	100	100	100	100	100	100	100
E29	***Amphimedon compressa***	100	100	86	99	36	26	100	69	47	68	36	11	27	4	4
E15	*Niphates digitalis*	1	11	3	12	3	1	23	5	1	7	10	1	2	0	2
E49	*Niphates erecta*	7	12	2	13	3	1	35	15	4	24	6	12	2	0	0
MA18-7	*Niphates erecta*	11	15	5	9	0	2	47	26	17	31	5	4	4	0	0
MA18-12	*Niphates erecta*	25	16	4	51	7	1	77	49	31	2	17	5	2	4	1
EP	*Xestospongia muta*	2	7	0	1	3	3	44	18	12	16	2	1	1	1	1
E3	***Plakinastrella onkodes***	100	100	84	100	98	6	100	100	90	100	56	11	100	63	3
E35	***Monanchora arbuscula***	67	56	48	100	100	94	99	98	93	100	99	87	100	89	90
EY18-11	***Clathria gomezae***	39	21	1	84	8	4	76	37	10	22	9	2	3	3	2
E7-E34	*Clathria virgultosa*	22	10	9	21	10	4	55	34	27	4	3	0	2	1	2
MA18-1	***Mycale laevis***	9	17	9	29	18	9	68	50	36	3	10	8	26	11	4
MA18-5	*Mycale laevis*	8	13	1	10	2	1	29	2	5	5	8	9	2	2	1
DNY	***Scopalina ruetzleri***	66	36	21	97	34	5	94	76	46	42	1	3	4	0	2

(Continued)

TABLE 3 | Continued

Code	Organism (μg/mL)	A549[a]			A2058[b]			HepG2[c]			MCF7[d]			MiaPaca2[e]		
		30	15	7.5	30	15	7.5	30	15	7.5	30	15	7.5	30	15	7.5
E53	*Scopalina ruetzleri*	8	11	1	9	1	2	53	26	14	5	2	5	6	2	1
EY18-7	***Scopalina ruetzleri***	13	24	16	31	22	13	71	51	42	3	5	2	1	3	4
E18-M1	*Halichondria melanadocia*	10	9	8	15	5	0	37	23	11	4	10	6	30	1	2
E38	***Aaptos* sp.**	75	71	63	99	99	98	93	89	86	67	69	56	95	91	77
E20	***Tethya* sp.**	81	72	58	99	91	96	90	87	83	75	72	50	97	86	8
E4	*Melophlus hajdui*	8	2	9	16	0	2	53	16	7	14	1	11	6	3	3
MA18-2	***Cinachyrella kuekenthali***	78	71	59	98	98	97	91	88	84	57	64	46	93	89	68
E50	***Aiolochroia crassa***	7	6	2	16	4	1	92	73	48	11	3	1	3	2	3
MA18-4	***Aiolochroia crassa***	11	10	4	31	13	4	95	62	36	27	6	14	2	1	1
E36	*Aplysina cauliformis*	20	14	3	18	11	7	52	39	28	22	7	10	3	3	4
E46	*Aplysina fistularis*	15	12	1	15	7	3	45	34	26	23	6	10	6	2	3
E42	*Aplysina fulva*	7	9	2	13	4	1	23	1	2	11	1	6	6	2	2
EY18-5	*Aplysina fulva*	32	24	14	23	10	4	60	46	35	1	4	3	9	1	4
E47	*Aplysina muricyana*	4	4	4	8	2	0	21	8	6	19	8	15	5	3	3
Reference	Doxorubicin 50 μM		90			100			99			100			100	
Reference	MMS 4 μM		100			100			100			100			100	

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.

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*, *Leucetta floridana*, *Cliona varians*, *Dysidea* sp., *S. tubulifera*, *Haliclona* (*Rhizoniera*) *curacaoensis*, *A. compressa*, *Plakinastrella onkodes*, *Monanchora arbuscula*, *Clathria gomezae*, *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.* (*Rhizoniera*) *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,

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 MiaPaca-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 MiaPaca-2 (30 μg/mL) and the sponge *Dysidea* sp. showed more than 51% of growth inhibition against A549, A2058, HepG2, and MCF7 cell lines to 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 asses 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 preliminar 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**) 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 didemnins 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_{50} values of these extracts were lower than those shown by cidofovir (IC_{50} = 6.7 ± 1.6 μg/mL; CC_{50} = 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 clatrin-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.* (*Rhizoniera*) *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.* (*Rhizoniera*) *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.* (*Rhizoniera*) *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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