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Full Length Research Paper

Antiviral activity of the extracts of *Rhodophyceae* from Morocco

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Fifty-five aqueous, methanolic, chloroforme-methanolic and dichloromethanolic extracts derived from sixteen species of marine Rhodophyta from the coast of Morocco have been screened for the presence of inhibitory compounds against Herpes simplex virus type 1 (HSV-1) by cell viability method. The aqueous extracts of *Asparagopsis armata*, *Ceramium rubrum*, *Gelidium pulchellum*, *Gelidium spinulosum*, *Halopitys incurvus*, *Hypnea musciformis*, *Plocamium cartilagineum*, *Boergeseniella thuyoides*, *Pterosiphonia complanata* and *Sphaerococcus coronopifolius* were capable of inhibiting the replication of HSV-1 in vitro at an EC50 (Effective Concentration 50%) ranging from <2.5 to 75.9 µg mL-1. No cytotoxic effect of the aqueous extracts on the Vero cells was observed in the range of the concentrations assayed for all extracts. The results corroborate that marine algae from Morocco can be a rich source of potential antiviral compounds.

Keywords: Antiviral, Aqueous extracts, Organic extracts, *Rhodophyceae*, Herpes simplex virus

INTRODUCTION

More than 150 species of marine algae are commercially important food sources and over $2 billion worth of seaweed is consumed each year by humans, mostly in Japan, China and Korea. Algae have long been recognized as rich and valuable natural resources of bioactive compounds because of their various biological properties (Mayer, 2002; Mayer et al., 2009). Since the finding of antimicrobial (antibacterial, antifungal or antiviral) activities in many species of marine algae and the isolation of some active compounds from them, marine algae have become recognized as potential sources of antibiotic substances (Fenical and Paul, 1984; Moreau et al., 1984; Gonzalez et al., 2001; Selvin and Lipton, 2004; Kornprobst, 2005; Salvador et al., 2007; Karabay-yavasoglu et al., 2007; Mayer et al., 2009). Substances that currently receive the most attention from pharmaceutical companies for use in drug development, or from researchers in the field of medicine-related research include generally: sulphated polysaccharides as antiviral substances, halogenated furanones from *Delisea pulchra* as antifouling compounds and depsipeptides kahalalide F from a species of *Bryopsis* as a possible treatment of lung cancer, tumours and acquired immune deficiency syndrome (AIDS). Other substances such as macroalgal lectins, fucoidans, kainoids and aplysiatoxins are routinely used in biomedical research and a multitude of other substances have known biological activities (Smit, 2004; Kornprobst, 2005).

Recently, screening assays of the antiviral activity of many extracts have led to the identification of a number of polysaccharide (Chattopadhyay et al., 2007; Mandal et al., 2008; Harden et al., 2009; Yasuhara-Bell and Lu, 2010) and the diterpenes (El Gamal, 2010) having potent inhibitory effects against *Herpes simplex* virus (HSV) type 1.

Except for the studies of Abourriche et al. (1999), Etahiri

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Abbreviations: HSV-1, *Herpes simplex* virus type 1; **EC_{50},** effective concentration 50%; **AIDS,** acquired immune deficiency syndrome; **DMSO,** dimethylsulphoxide; **MEM,** minimal essential medium; **FCS,** fetal calf serum; **OD,** optical densities; **CC_{50},** 50% cytotoxic concentration; **%D,** percentage of cell destruction; **MOI,** multiplicity of infection; **CPE,** cytopathic effect; **% P,** percentage of cell protection; **SI,** selectivity index.

Table 1. List of marine algae screened for antiviral activity

Scientific name	Orders	Collection localites	Position
Alsidium corallinum	Ceramiales	nador	35º11'11.98''N 2º55'30.75''O
Asparagopsis armata	Bonnemaisoniales	Ksar sghir	35º50'52.58''N 5º33'39.04''O
Ceramium rubrum	Ceramiales	Ksar sghir	35º50'52.58''N 5º33'39.04''O
Gelidium attenatum	Gelidiales	Dalya	35°54'24.20"N 5°28'18.84"O
Gelidium latifolium	Gelidiales	Kaa asrss	35°26'28.99"N 5°04'53.39"O
Gelidium pulchellum	Gelidiales	Sidi bouzid	33º13'36.23''N 8º33'12.52''O
		Ksar sghir	35º50'52.58''N 5º33'39.04''O
Gelidium pusillum	Gelidiales	Marina smir	35º45'56.81''N 5º20'58.04''O
Gelidium sesquipedale	Gelidiales	Ksar sghir	35º50'52.58''N 5º33'39.04''O
Gelidium spinulosum	Gelidiales	Ksar sghir	35º50'52.58''N 5º33'39.04''O
Halopitys incurvus	Ceramiales	Sidi bouzid	33º13'36.23''N 8º33'12.52''O
		Ksar sghir	35º50'52.58''N 5º33'39.04''O
Hypnea musciformis	Gigartinales	Ksar sghir	35º50'52.58''N 5º33'39.04''O
Plocamium cartilagineum	Giagartinales	Ksar sghir	35º50'52.58''N 5º33'39.04''O
Boergeseniella thuyoides	Ceramiales	Ksar sghir	35º50'52.58''N 5º33'39.04''O
Pterocladea capillacea	Gelidiales	Ksar sghir	35º50'52.58''N 5º33'39.04''O
Pterosiphonia complanata	Ceramiales	Ksar sghir	35º50'52.58''N 5º33'39.04''O
Sphaerococcus coronopifolius	Gigartinales	Belyounech	35°54'34,87"N 5°23'41.91"O

et al. (2003), Moujahidi et al. (2004) and Souhaili et al. (2004) on antibacterial activity, little is known about the antiviral activity of red seaweed extracts collected on the coast of Morocco. The present study led to the detection of potential antiviral activities from fifty-five extracts of sixteen *Rhodophyceae* collected on the Atlantic-Mediterranean coast of Morocco.

MATERIALS AND METHODS

The collection of seaweeds

Seaweeds were collected by hand using Scuba diving or snorkelling (1–4 m depth) and preserved on ice until further processing. Sixteen species were sampled between 2003 to 2007 at various sites along the northern Mediterranean (Ksar sghir, Marina smir, Dalya, Kaa asrss, Nador, Belyounech) and Atlantic (Sidi bouzid) coasts of Morocco. The taxonomic identification of species was done by experts in these fields using standard literature and taxonomic keys. Voucher specimens of all species tested are deposited in the herbarium of our Laboratory of Applied Algology-Mycology, Department of Biology, Faculty of Sciences at Abdelmalek Essâadi University, 93002 Tetouan, Morocco (Table 1). Seven species belong to the Gelidiales order, three to Gigartinales order, five to Ceramiales order and one to Bonnemaisoniales order.

Preparation of extracts

After collection, the samples were rinsed with sterile seawater to remove associated debris and necrotic parts. Epiphytes were removed from the algae and the surface microflora was removed by soaking the algal samples for ten minutes with 30% ethanol. The samples were shade dried, cut into small pieces and powdered in a mixer grinder. The powder obtained was preserved cold in - 12°C. Samples (5 g) were extracted with solvents of increasing polarity, chloroform-methanol (3: 2), dichloromethane (100%), methanol (100%) and water (100%) for 8 h using a soxhlet apparatus. The resulting organic extracts were concentrated to dryness under reduced pressure at 30-35C° with a rotary evaporator and aqueous extracts were lyophilised. Each residue was weighed and stored in sealed vials in a freezer until being tested. All extracts were stored at (-4°C) (Ozdemir et al., 2004).

Before antiviral screening, organic extracts were dissolved in 2% dimethylsulphoxide (DMSO) and then in Eagle's minimal essential medium (MEM, Eurobio). Aqueous extracts were diluted directly in Eagle's MEM. All the extracts were sterilized by filtration using 0.22 µm millipore membranes. Percent (W/W) yields of extracts determined in terms of dry starting material are listed in Table 2.

Cells and viruses

Vero cells (African green monkey kidney cell line, ATCC CCL81) were used for culturing HSV-1.The cells were grown in MEM supplemented with 8% fetal calf serum (FCS, Eurobio) and 1% of antibiotics PCS (10 000 IU Penicillin mL^{-1}, 25 000 IU Colimycin mL^{-1}, 10 mg Streptomycin mL^{-1}, Sigma). All the cells were cultured at 37°C in a humidified atmosphere supplied with 5% CO_2. HSV-1 (Wild type strain 17, sensitive to acyclovir) was obtained from Pr. Billaudel (Institute of virology of Nantes, France). The virus titter was estimated from cytopatogenicity according to the Reed and Muench dilution method (Reed and Muench, 1938) and expressed as 50% infectious doses per millilitre (ID_{50} mL^{-1}). The HSV-1 stock had a titter of $2 \times 10^{8.6}$ ID_{50} mL^{-1}.

Cytotoxicity assay based upon cell viability

To evaluate the cytotoxic activities of extracts, Vero cellular suspensions (3.5×10^5 cells mL^{-1}) were cultivated in 96-well culture

Table 2. Anti-HSV activities of extracts of seaweeds tested by cell viability with the HSV/Vero model

Algae species	ES	EY (%) [a]	D% [b]	CC_{50} [c]	P % [d]	EC_{50} [e]	SI [f]
Asparagopsis armata	M	14.37	67.6±0.4	142.4	0.0	7.7	18.3
	Ch/M	15.2	56.8±0.7	157.1	100±1.4	40.9	3.8
	DM	0.28	62.3±0.4	119.1	0.0	22.8	5.2
	W	43.40	**18.4±0.2**	**>250**	**90.5±0.6**	**<2.5**	**>100**
Alsidium corallinum	M	1.04	86.6±0.3	36.6	100±0.3	24.0	1.5
	Ch/M	0.27	81.2±0.9	71.5	91.1±0.5	24.5	2.9
	DM	0.12	45.2±0.7	>250	-	-	-
	W	-	-	-	-	-	-
Ceramium rubrum	M	3.22	66.2±0.2	134.0	100±0.2	<2.5	>53.6
	Ch/M	1.49	57.4±0.5	178.4	0.0	>250	<0.7
	DM	0.31	66.2±0.9	61.8	100±0.4	111.4	0.5
	W	13.80	**14.2±0.3**	**>250**	**100±0.5**	**12.4**	**>20.1**
Gelidium attenatum	M	0.39	89.2±0.2	23.8	100±0.7	9.3	2.5
	Ch/M	0.80	87.1±0.4	36.9	88.8±0.6	29.9	1.2
	DM	0.09	-	-	-	-	-
	W	-	-	-	-	-	-
Gelidium latifolium	M	0.89	93.2±1.2	29.8	-	-	-
	Ch/M	-	-	-	-	-	-
	DM	0.16	-	-	-	-	-
	W	-	-	-	-	-	-
Gelidium pulchellum (S.B)	M	0.68	91.5±0.3	15.7	95.8±0.8	16.1	0.9
	Ch/M	1.80	81.5±0.5	65.0	15.0±0.9	>250	<0.2
	DM	0.48	78.6±0.8	100.3	50.8±0.2	245.4	0.4
	W	17.60	47.3±0.6	**>250**	**100±1.6**	31.7	>7.8
Gelidium pusillum	M	5.69	90.5±0.4	35.8	86.4±0.7	36.4	0.9
	Ch/M	0.66	37.8±0.5	>250	-	-	-
	DM	0.32	**33.0±0.3**	**>250**	**86.0±0.5**	**60.6**	>4.1
	W	-	-	-	-	-	-
Gelidium sesquipedale	M	0.31	22±0.7	>250	-	-	-
	Ch/M	-	-	-	-	-	-
	DM	0.03	79.5±0.2	71.6	100±0.4	51.4	1.3
	W	2.00	3.7±0.6	>250	0.0	>250	>1
Gelidium spinulosum	M	2.05	83.1±0.5	40.1	68.9±0.5	70.5	0.5
	Ch/M	1.2	-	-	-	-	-
	DM	0.14	61.0±0.3	197.0	100±0.3	22.7	8.6
	W	26.40	**25.2±0.2**	**>250**	**100±0.2**	**37.6**	>6.6
Halopitys incurvus (S.B)	M	2.43	89.3±0.4	26.7	64.8±0.5	83.5	0.3
	Ch/M	-	71.9±0.5	98.8	86.6±0.6	36.0	2.7
	DM	0.18	67.9±0.6	99.8	65.5±0.9	87.7	1.1
	W	20.80	**28.7±0.8**	**>250**	**67.2±0.2**	**75.9**	>3.2
Hypnea musciformis	M	2.04	61.4±0.9	140.1	69.7±0.3	6.5	21.3
	Ch/M	2.20	57.0±0.8	205.5	100±0.7	51.4	3.9
	DM	3.2	49.48	>250	32.94	>250	>1
	W	32.6	**10.6±0.7**	**>250**	**100±1.8**	**23.5**	>10.5
Pterocladea capillacea	M	0.46	18.5±0.4	>250	0.0	>250	>1

Table 2. Continued

	Ch/M	0.24	9.0±0.3	>250	0.0	>250	>1
	DM	0.31	49.9±0.2	>250	42.3±1.9	>250	>1
	W	-	7.1±1.8	>250	21.7±1.6	>250	>1
Plocamium cartilagineum	M	8.39	85.2±0.2	28.7	100±0.4	23.2	1.2
	Ch/M	6.62	88.1±0.4	64.3	91.9±0.2	27.4	2.3
	DM	2.69	49.7±0.8	>250	-	-	-
	W	3.80	**4.81±0.7**	**>250**	**100±0.2**	**6.4**	**>39.0**
Pterosiphonia complanata	M	4.57	89.7±0.6	28.3	93.1±0.6	18.1	1.5
	Ch/M	3.34	61.3±0.3	148.0	100±0.3	53.8	2.7
	DM	0.35	87.3±0.2	47.7	95.3±0.7	23.9	1.9
Boergeseniella thuyoides	M	12.53	44.8±0.3	>250	-	-	-
	Ch/M	6.20	46.8±0.7	**>250**	100±1.9	**35.0**	>7.1
	DM	0.80	62.2±1.8	151.0	80.2±0.3	26.2	5.7
	W	27.40	**15.1±1.5**	**>250**	**100±1.4**	**12.6**	**>19.84**
Sphaerococcus coronopifolius	M	2.15	92.7±0.2	3.3	96.8±0.2	21.4	0.1
	Ch/M	1.35	85.3±1.9	48.5	86.2±1.3	25.4	1.9
	DM	0.61	45.0±0.5	>250	0.0	>250	>1
	W	26.00	**11.1±1.8**	**>250**	**100±0.7**	**4.4**	**56.8**
Acyclovir			**4.0±1.2[g]**	**>250**	**100±0.8[g]**	**0.3**	**>250**

[a] Percentage extract yield (w/w) was estimated as (dry extract weight/dry starting material weight)×100.
[b]D%: percentage of Vero cell destruction at concentration extract of 250 µg mL^{-1}, %D= [(ODc)C- (ODc)Mock/(ODc)C] x 100. (ODc)C and (ODc)MOCK were the OD values of the untreated cells and treated cells respectively. Results were expressed as: m±s.e.m.
[c]CC_{50}: the 50% cytotoxic concentration of extract in Vero cells (µg mL^{-1}).
[d]P%: percentage of infected cell protection at a concentration extract of 250 µg mL^{-1}, %P= [((ODt)virus - (ODc)virus)/((ODc)MOCK – (ODc)virus)] × 100, (ODt) virus was the OD of the test sample, (ODc) virus was the OD of the virus control (no extract) and (ODc)MOCK was the OD of the mock-infected control. Results were expressed as: m±s.e.m.
[e] EC_{50}: Concentration of compound in µg mL^{-1} producing 50% inhibition of virus-induced cytopathic effect.
[f]SI: selectivity index was determined by the ratio of CC_{50} to EC_{50}.
[g]Acyclovir at concentration of 5 µg mL^{-1}.
-: not determined
S.B: Sidi Bouzid
M: Methanol; Ch/M: chloroforme-méthanol; DM: dichloromethane; W: water
ES: Extracted solvent, EY: Extract yield.

plates and exposed to increasing concentrations of the extracts from 2.5 to 250 µg mL^{-1} using four wells for each concentration. The plates were incubated at 37°C in a humidified CO_2 atmosphere (5% CO_2) for 72 h. Each assay was done in triplicate. The cells were examined daily under a phase-contrast microscope to determine the minimum concentration of extract that induced alterations in cell morphology, including swelling, shrinkage, granularity and detachment (Olicard et al., 2005). Cytotoxicity was tested using the neutral red dye method (Langlois et al., 1986) and optical densities (OD) were measured at 540 nm using a spectrophotometer (SpectraCount TM, Packard). The 50% cytotoxic concentration (CC_{50}) was the concentration of the extract that inhibited actively replicating cells by 50% as compared to untreated group. Cytotoxicity was also expressed as the percentage of cell destruction (%D):

$$\%D = [(OD_c - OD_t) / OD_c] \quad X\ 100$$

Where, OD_c is the mean optical density of the cell controls at 540 nm and OD_t is the mean optical density of the test samples at 540 nm (Bourgougnon et al., 1994).

Antiviral assays based upon cell viability

To test the antiviral effect of extracts, 100 ml of Vero cellular suspension ($3.5x10^5$ cells mL^{-1}) was infected with HSV-1 suspension (50 µL) at a multiplicity of infection (MOI) of 0.001 ID_{50} mL^{-1} without or in the presence of different dilutions of the extracts (concentration from 2.5 to 250 µg mL^{-1}). Infected cell cultures were cultivated in 96-well culture plates at 37°C in a humidified CO_2 atmosphere (5% CO_2) for 72 h. Each assay was done in triplicate. After incubation, antiviral activity was evaluated by the neutral red dye method (Langlois et al., 1986). The antiherpetic compound acyclovir (9-(2- hydroxyethoxymethyl) guanine) was used as a reference drug with concentration ranging from 0.1 to 5 µg mL^{-1}. The 50% effective antiviral extract concentration (EC_{50}) was expressed as the concentration that achieved a protection of 50% of virus-infected cells. OD were measured at 540 nm and the OD was related directly to the percentage of viable cells, which was inversely related to the cytopathic effect (CPE). The linear regression was determined for each extracts and assay on the basis of cell controls (0% CPE) and virus controls (100% CPE).

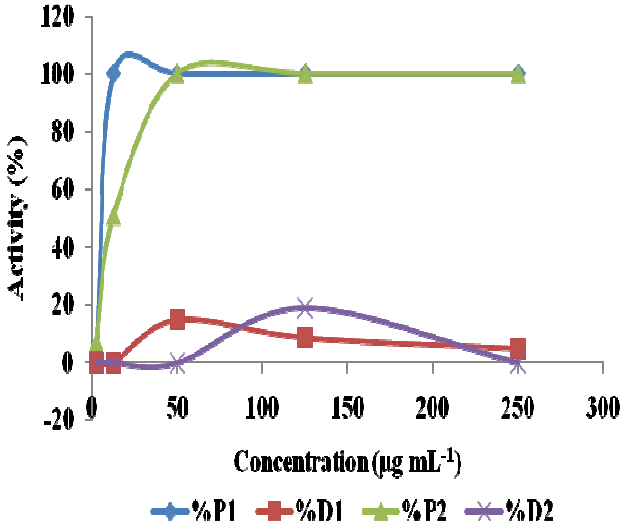

Figure 1. Anti HSV-1 activity of aqueous extracts from *P. cartilagineum* and *P. complanata* tested at various concentrations, after incubation for 72 h. Vero cells infected by HSV-1 (MOI 0.001 ID_{50} mL^{-1}) were mammalian fibroblastic cells and HSV-1 was *H. simplex* virus type 1. Antiviral activity was expressed as the percentage of protection from virus-infected cells (%P) with the aqueous extracts of *P. cartilagineum* (% P1) and *P. complanata* (%P2). Cytotoxic activity was observed as the percentage of destruction of mock-infected cells (%D) with the aqueous extracts of *P. cartilagineum* (% D1) and *P. complanata* (% D2). The 50% effective antiviral concentration of the aqueous extracts of *P. cartilagineum* and *P. complanata* (EC_{50}) were 6.4 and 10.4 µg mL^{-1}, respectively, but the 50% cytotoxic concentration (CC_{50}) was > 250 µg mL^{-1} for the two extracts. Values were presented as mean ± S.E.M. of three independent experiments.

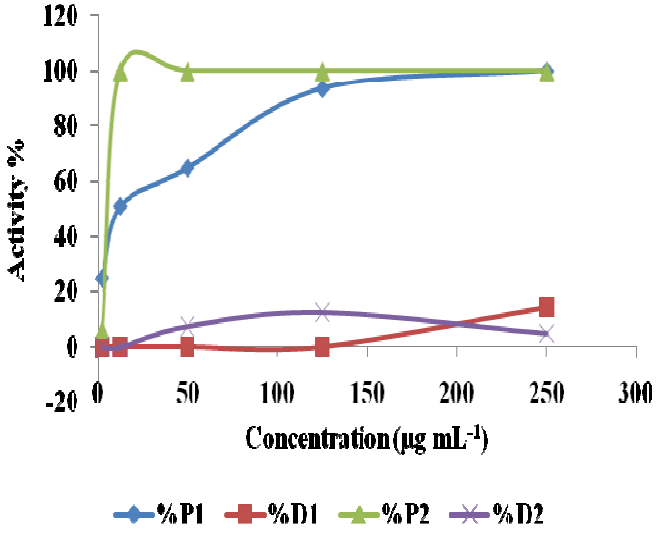

Figure 2. Anti HSV-1 activity of aqueous extracts from *C. rubrum* and *S. coronopifolius* tested at various concentrations, after incubation for 72 h. Vero cells infected by HSV-1 (MOI 0.001 ID_{50} mL^{-1}) were mammalian fibroblastic cells and HSV-1 was *H. simplex* virus type 1. Antiviral activity was expressed as the percentage of protection from virus-infected cells (%P) with the aqueous extracts of *C. rubrum* (% P1) and *S. coronopifolius* (%P2). Cytotoxic activity was observed as the percentage of destruction of mock-infected cells (%D) with the aqueous extracts of *C. rubrum* (% D1) and *S. coronopifolius* (% D2). The 50% effective antiviral concentration of the aqueous extracts of *C. rubrum* and *S. coronopifolius* (EC_{50}) were 12.4 and 4.4 µg mL^{-1}, respectively, but the 50% cytotoxic concentration (CC_{50}) was > 250 µg mL^{-1} for the two extracts. Values were presented as mean ± S.E.M. of three independent experiments.

Data were expressed as percentage of cell protection (% P):

$$\%P = [(OD_t \text{ virus} - OD_c \text{ virus}) / (OD_c \text{ MOCK} - OD_c \text{ virus})] \times 100$$

Where, OD_t virus is the OD of the test sample, OD_c virus is the OD of the virus control (no extract) and OD_c MOCK is the OD of the mock-infected control (Langlois et al., 1986).

Statistical analysis

The experiments were done in triplicate. The selectivity index (SI) was determined by the ratio of CC_{50} to EC_{50}. One-way analysis of variance (ANOVA) was used for comparison of mean values. All tests were considered to be statistically significant at $P < 0.05$.

RESULTS

The yields of the aqueous extracts were higher than that of the organic extracts. It appears that the yields of an extraction by the dichloromethane give a much weaker yield compared to that carried out by the solvents, methanol and chloroform-methanol (Table 2).

Inhibition of virus-induced cytopathic effect

The fifty-five crude extracts were studied for their inhibitory effect on HSV *in vivo* replication in Vero cells by measuring cell viability. The monolayers of Vero cells in 96-well plates were infected with HSV-1 at the MOI of 0.001 ID_{50} mL^{-1} in the presence of serially twofold diluted crude extracts. The CPE induced by the virus was scored on day 3 post-infection.

16 methanolic, 13 chloroform-methanolic, 14 dichloromethanolic and 12 aqueous extracts were capable of inhibiting the replication of *H. simplex* virus type 1 *in vitro* in different concentrations. Ten water extracts of *Asparagopsis armata*, *Ceramium rubrum*, *Gelidium pulchellum*, *Gelidium spinullosum, Halopitys incurvus*, *Hypnea musciformis, Plocamium cartilagineum*, *Polysiphonia thuyoides*, *Pterosiphonia complanata* and *Sphaerococcus coronopifolius* exhibited anti-HSV-1 activity with EC_{50} ranging from < 2.5 to 75.9 µg mL^{-1} and ranging from > 3.2 to > 100. For a MOI of 0.001 ID_{50} mL^{-1}, 100% cellular protection was obtained for 250 µg mL^{-1} of extracts from *C. rubrum, G. pulchellum, G. spinullosum, H. musciformis, P. cartilagineum*, *P. thuyoides, P.*

complanata (Figure 1) and *S. coronopifolius* (Figure 2), 90% cellular protection for the extract from *A. armata* and 67% cellular protection for the extract from *Halopitys incurvus*. One organic extract (DM) from the *Gelidium pusillum* has shown anti-HSV for 250 µg mL^{-1} with a percentage protection of 86%.

Dose response effects were studied to define the EC_{50} and CC_{50}, as well as the corresponding SI of the potentially antiviral extracts (Table 2). Whereas antiviral activity and cytotoxicity may result from distinct compounds, we counterbalanced antiviral activity in relation to cytotoxicity using SI calculation to compare the antiviral potency of the extracts. The antiviral potency detected in the extracts of marine algae was classified using the selectivity index ($SI = CC_{50}/EC_{50}$). The aqueous extract from *A. armata* (with $EC_{50} < 2.5$ µg mL^{-1} and $CC_{50} > 250$ µg mL^{-1}) appeared to be the most efficient extract. This extract presented the highest SI values, superior to 250 µg mL^{-1}. The following extracts were from *S. coronopifolius* ($EC_{50} = 4.4$ µg mL^{-1} and $CC_{50} > 250$ µg mL^{-1}), *P. cartilagineum* ($EC_{50} = 6.4$ µg $mL^{-1}$and $CC_{50} > 250$ µg mL^{-1}), *P. complanata* ($EC_{50} = 10.4$ µg $mL^{-1}$and $CC_{50} > 250$ µg mL^{-1}), *C. rubrum* ($EC_{50} = 12.4$ µg $mL^{-1}$and $CC_{50} > 250$ µg mL^{-1}), *P. thyoides* ($EC_{50} = 12.6$ µg mL^{-1} and $CC_{50} > 250$ µg mL^{-1}) and *H. musciformis* ($EC_{50} = 23.5$ µg mL^{-1} and $CC_{50} > 250$ µg mL^{-1}).

DISCUSSION

H. simplex virus (HSV), a DNA enveloped virus, is a common human pathogen with between 60 and up to 95% of certain populations infected with HSV-1. Medications available for systemic treatment of HSV are acyclovir, famciclovir and alacyclovir. Acyclovir and penciclovir are available for topical use (Wyatt et al., 2001). The number of antivirals approved for clinical use has been increased from 5 to more than 30 drugs (De Clercq, 2004). However, as these drugs are not always efficacious or well-tolerated and drug-resistant virus strains are rapidly emerging, there is still a great demand for further drug development including novel modes of action (Pielop et al., 2000; Strand et al., 2002). A very promising approach is the antiviral screening of products derived from natural sources, such as marine flora and fauna, bacteria, fungi and higher plants (Mayer, 2002; Ben Sassi et al., 2008; Mayer et al., 2009).

Previous investigations have revealed antiviral activity in significant numbers of algae from various marine environments in the Mediterranean (Ballesteros et al., 1992; Bourgougnon et al., 1994; Haslin et al., 2001), Britain (Blunden et al., 1981), India (Premnathan et al., 1992), Korea (Hudson et al., 1999; Ahn et al., 2002), China (Zhu, 2002) and Japan (Hayashi et al., 1996). Moroccan Coasts are particularly rich in algal biodiversity. However, no study relates to antiviral activities of compounds extracted from Rhodophyta and to the upgrading of Moroccan species of Rhodophyta in pharmaceutical fields. In the study, the highest inhibitory effect on HSV showed by the hot water extract of *A. armata, C. rubrum, G. spinullosum, H. incurvus*, *H. musciformis*, *P. cartilagineum*, *P. thuyoides*, *P. complanata* and *S. coronopifolius*, followed by that from the dichloromethanolic extract of *G. pusillum.*

The results showed that water extract seems to be more effective in comparison with methanolic, dichloromethane or chloroforme-methanol extracts. The aqueous extraction appears to be an effective technique for the research of antiherpetic compounds. Water extracts are known to be rich in effective polysaccharides (Carlucci et al., 1999; Duarte et al., 2001; De S.F-Tischer et al., 2006; Chattopadhyay et al., 2007). In recent years, a number of sulfated carbohydrate compounds from marine algae, cyanobacteria and animal sources were described showing potent inhibitory effects against several human and animal viruses (Luescher-Mattli, 2003; Damonte et al., 2004; Arad et al., 2006; Pujol et al., 2006). Sulfated polysaccharides of synthetic origin also have antiviral potency (Witvrouw and De Clercq, 1997; Wang et al., 2008). Some of these macromolecules are currently undergoing clinical evaluation (Kleymann, 2005; Ghosh et al., 2009). Haslin et al. (2001) mentioned that an aqueous extract from *A. armata* inhibits the human immunodeficiency virus (HIV-1) replication at 10 µg mL^{-1}. Serkedjieva (2000) reported that a water extract from *P. denudata* inhibited the reproduction of *Herpes virus* type 1 and type 2 in cell cultures ($EC_{50} = 8.7$ to 47.7 mg mL^{-1}), the inhibition affected adsorption, as well as the intracellular stages of viral replication. Harden et al. (2008) evaluated the antiviral activity of extract *P. cartilagineum* against HSV-1 and HSV-2 and concluded that this extract has non toxic and effective virucidal agents. The current screening showed positive results in orcinolsulfuric acid reaction (Tillmans and Philippi, 1929) from extracts of *A. armata* (Nemalionales), *C. rubrum*, *H. incurvus*, *P. complanata*, *P. thuyoides* (Ceramiales), *G. pulchellum*, *G. spinullosum* (Gelidiales) and *H. musciformis*, *P. cartilagineum*, *S. coronopifolius* (Gigartinales), suggesting that the main effective components in these extracts could be polysaccharides.

Serkedjieva (2004) reported that the chloroform-methanol (1: 1) from the red marine alga *C. rubrum* inhibited the reproduction of influenza viruses type A and B *in vitro* and *in vivo* with selectivity indices in the range of 9.5 - 68.3. The extract also inhibited the production of HSV-1 and HSV-2 in cell cultures. Ballesteros et al. (1992) tested the methanol-toluene extract (3: 1) of *S. coronopifolius* and *G. pusillum* on HSV-1 on the vesicular stomatitis virus (VSV), but observed no antiviral activity. Caccamese et al. (1981) showed that the methanol-toluene (3: 1) of *S. coronopifolius* gives a moderate inhibition against tobacco mosaic virus (TMV). Harden et al. (2009) reported that polysaccharide from gametophyte from *Gigartina atropurpurea* and *P. cartilagineum* had potent virucidal activity on the HSV-1and were active at very low concentrations (0.2 - 0.4 µg/ml, respectively)

against HSV-1.

According to our results, the orders of Ceramiales and Gigartinales seem to present more active extracts against the virus *H. simplex.* Aqueous extracts appear more effective and non cytotoxic on cell lines than methanolic, dichloromethanolic and chloroforme-methanolic extracts. In comparison to acyclovir antiherpetic evaluation (EC_{50} = 0.3 µg mL^{-1}), 7 extracts seem to be promising. These results mainly concern aqueous extracts of *A. armata, C. rubrum, H. musciformis, P. cartilagineum, P. thuyoides, P. complanata* and *S. coronopifolius.*

Conclusion

The water extract from red marine algae from Moroccan coast exhibits an antiherpetic virus effect *in vitro*. The presented results could be considered as encouraging; therefore, further studies should concentrate on the chemical characterization of the active principle (s) and reanalysis of their antiviral properties.

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