Antiviral Potential of Polysaccharides Extracted from Marine Macroalgae of the El Jadida Atlantic Coast (Morocco).

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Abstract. Herpes simplex virus type 1 (HSV-1) is a globally widespread pathogen, and its increasing prevalence, associated complications, and emerging drug resistance underscore the need for novel antiviral agents. In this study, polysaccharide fractions were extracted from seven marine algae species collected from the El Jadida Atlantic Coast, Morocco, using aqueous and Soxhlet extraction methods. The antiviral activity of these extracts against HSV-1, as well as their cytotoxicity, was evaluated. All tested extracts demonstrated notable antiviral activity. Among them, the polysaccharide from *Chondracanthus acicularis* exhibited the strongest effect (IC_{50}= 17.78 ± 2.5 µg/mL), followed by the extract *from Ellisolandia elongata* (IC_{50} = 18,12 ± 4,8 µg/mL). Importantly, none of the extracts showed cytotoxicity at the tested concentrations. These results highlight the potential of marine algal polysaccharides as antiviral agents and provide a basis for further studies on the chemical characterization and bioactivity of polysaccharides from marine macroalgae.

1 Introduction

Herpes simplex virus type 1 (HSV-1) is a type of virus that is an enveloped, double-stranded DNA virus that is classified as the *Alphaherpesvirinae* subfamily of the *Herpesviridae* family [1]. It has a genome that is approximately 152 kb in size which codes over 80 viral proteins that participate in viral replication and interaction with the host [2]. HSV-1 is one of the most widespread human pathogens, infecting more than two-thirds of the global population under the age of 50 [3–5]. Although infection is often asymptomatic or limited to mild oral lesions, HSV-1 can cause severe clinical manifestations, including keratitis, meningitis, eczema herpeticum, and, in rare cases, fatal encephalitis [6].
HSV-1 is a neurotropic virus capable of invading the central nervous system and establishing lifelong latency in the trigeminal ganglia following primary infection, with periodic

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reactivation throughout life [7]. While immunocompetent individuals generally experience mild disease, reactivation or infection in vulnerable populations can result in serious complications reactivations [6]. Despite extensive research efforts, there is currently no effective vaccine against HSV-1 or HSV-2. Antiviral treatment relies mainly on nucleoside analogues such as acyclovir and related compounds [8]. however, long-term administration has led to the emergence of drug-resistant viral strains, underscoring the urgent need for alternative antiviral strategies.

Natural products have emerged as an important reservoir of structurally diverse molecules with antiviral potential [9]. In this regard, marine macroalgae represent a particularly promising source of bioactive compounds. Among the metabolites produced by marine algae, sulfated polysaccharides have attracted increasing attention due to their structural complexity and broad spectrum of biological activities, including antiviral, anti-inflammatory, and immunomodulatory effects [10].

Morocco benefits from an extensive coastline of approximately 3,500 km and hosts remarkable marine biodiversity, including a wide variety of macroalgal species [11]. In this context, the present study aims to investigate two types of extracts aqueous extracts and polysaccharide fractions obtained from seven marine macroalgal species collected along the Sidi Bouzid – El Jadida coast. The cytotoxicity and antiviral activity of these extracts were evaluated against herpes simplex virus type 1 (HSV-1), with the objective of identifying natural algal derived compounds with potential antiviral properties.

2 Material and Methods

2.1 Harvest Site

The samples of macroalgae were collected in the coastal zone of Sidi Bouzid, El Jadida, Morocco (33 o 16 09 N, 8 o 30 845 W) in spring 2019 (March to April). The collection was undertaken manually at a low tide. The location was selected because it was easily accessible and the species under study were naturally abundant in it (Fig. 1).

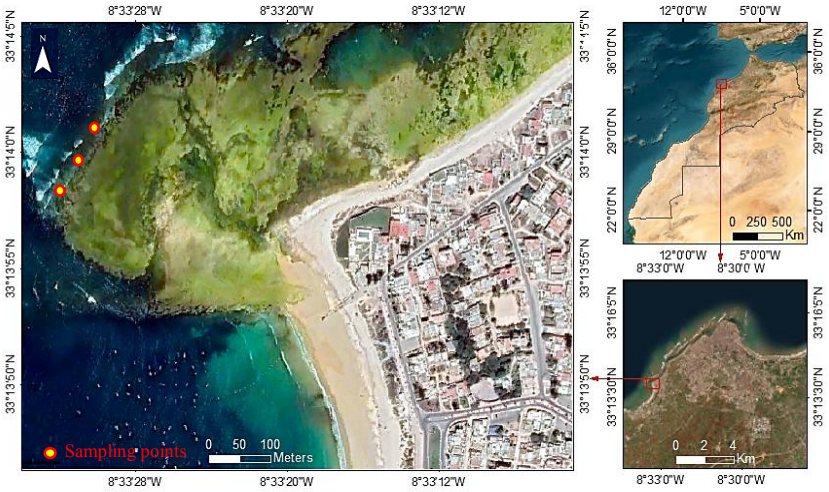

Fig. 1. Geographical position of the sampling site.

2.2 Algal Materials

The identification of the algal species was based on the standard taxonomic methods, which combined the Determination Key to Common Algae of the Atlantic Coast and Species Identification Guide. Samples were collected in sterile plastic bags and refrigerated (4 °C) before being taken to laboratory. The algae were rinsed intensively with distilled water, at the arrival point to eliminate the debris and epiphytes, freeze-dried, powdered, to a fine specimen, and stored until performed with additional analyses. The investigated macroalgae belong to the classes Rhodophyceae and Phaeophyceae, corresponding to red and brown algae, respectively. Species identification was performed on freshly collected specimens using taxonomic identification keys. The identified species were *Sargassum muticum* (a), *Bifurcaria bifurcata* (b), *Halopithys incurva* (c), *Gelidium corneum* (d), *Chondracanthus acicularis* (e), *Pterosiphonia complanata* (f), and *Ellisolandia elongata* (g), as illustrated in Figure 2.

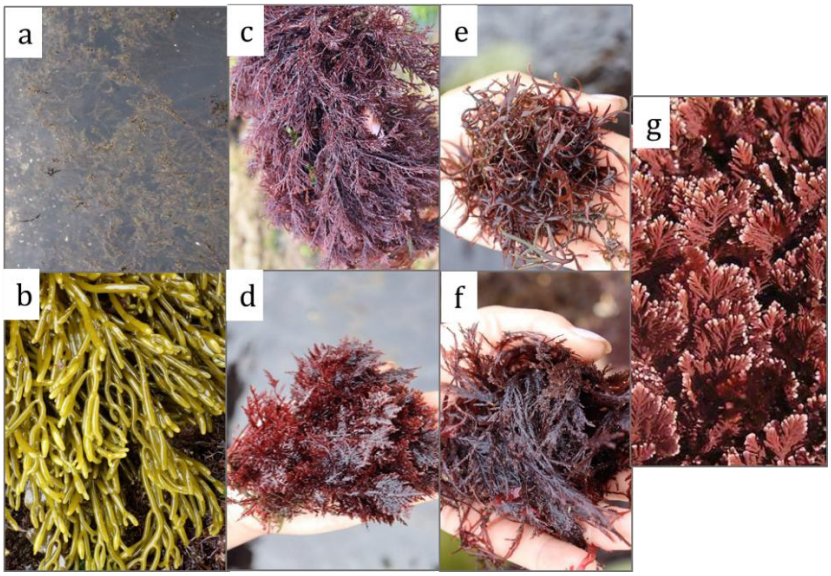

Fig.2. The various macroalgae species gathered by our study.

2.3 Extraction Procedure

2.3.1 Aqueous Extraction

Water-soluble compounds were extracted from the seven studied macroalgae. Briefly, 10 g of lyophilized algal powder were mixed with 500 mL of ultrapure water. The suspension was heated at 100 °C for 2 h with manual stirring every 30 min. After extraction, the mixture was filtered and centrifuged at 3000 rpm for 30 min to remove insoluble material. The recovered supernatant was stored at −24 °C for 24 h, freeze-dried, and stored in the dark until further use.

2.3.2 Extraction of Polysaccharides

The dried algal biomass was initially depigmented through successive Soxhlet extractions using organic solvents, after which hot water extraction was performed at 90 °C. The

resulting aqueous extract was filtered and concentrated, and polysaccharides were precipitated with ethanol, recovered by centrifugation, freeze-dried, and stored at 20 °C until further analyses.

2.4 Biological activities

The biological potential of the macroalgal extracts, including aqueous extracts and crude polysaccharides, was evaluated for both cytotoxic and antiviral activities. Cytotoxicity was assessed on Vero cells (ATCC CCL-81) to determine the 50% cytotoxic concentration (CC_{50}), while antiviral activity against herpes simplex virus type 1 (HSV-1) was quantified by the reduction of virus-induced cytopathic effects, allowing calculation of the 50% effective concentration (EC_{50}). Viral stocks and titers were prepared following established procedures [12], and antiviral efficacy was expressed as a percentage of protection according to the formula :

$$EC_{50}(\%) = \frac{[(ODt)HSV - (ODc)HSV]}{[(\mathrm{ODc})\mathrm{MOCK} - (\mathrm{ODc})\mathrm{HSV}]} \times 100$$

Where (ODt) HSV is the absorbance of the polysaccharide solution, (ODc)HSV is the absorbance of the virus-infected control without the sample and (ODc)MOCK is the absorbance of the mock-infected control. The percentage ratio of (ODc)HSV and (ODc)MOCK is given as a percentage of the control.

2.5 Statistical Analysis

All tests were performed in triplicate and the findings were reported in the form of means and standard deviation (SD) (n = 3).

3 Results and Discussion

3.1 *Cytotoxicity and antiviral activity of macroalgal extracts.*

The antiviral activity of polysaccharide fractions of the seven macroalgal species studied was determined against the human pathogen, HSV-1 basing on cell viability, and no cytotoxic effects on Vero cells were noted in the concentrations used. The polysaccharide from *C. acicularis* exhibited the strongest effect (EC_{50} = 17.78 ± 2.5 µg/mL), followed by the extract from *E. elongata* (EC_{50}= 18,12 ± 4,8 µg/mL) at a Multiplicity Of Infection (MOI) of 0.001 ID50 /cell.

Overall, aqueous extracts exhibited moderate antiviral activity, whereas polysaccharide fractions consistently showed stronger inhibition of HSV-1, as reflected by lower IC_{50} values. For instance, *S. muticum* polysaccharides had an IC_{50} of 62.33 ± 12.9 µg/mL compared to 134.68 ± 82.7 µg/mL for its aqueous extract, while *C. acicularis* polysaccharides were the most active, with an IC_{50} of 17.78 ± 2.5 µg/mL versus 28.33 ± 25.0 µg/mL for the aqueous extract. Some species, such as *H. incurva* and *G. corneum*, displayed no antiviral activity in their aqueous extracts but were active in their polysaccharide fractions (IC_{50} = 132.29 ± 66.9 µg/mL and 103.53 µg/mL, respectively). *B. bifurcata* and *E. elongata* also followed this trend, confirming that polysaccharides generally contribute more to antiviral effects than aqueous extracts. While all macroalgal extracts showed lower activity than the reference drug

Zovirax (IC_{50} = 0.58 ± 0.23 µg/mL), these findings highlight the potential of macroalgal polysaccharides as bioactive agents with low cytotoxicity and moderate antiviral activity against HSV-1, These findings are consistent with previous studies reporting the antiviral properties of sulfated polysaccharides from macroalgae, which are known to interfere with early stages of HSV-1 infection, including viral attachment and entry, through interactions with cell surface heparan sulfate analogues [13]. The antiviral effectiveness of these compounds has been shown to depend on structural features such as sulfate content, molecular weight, and monosaccharide composition [14,15], supporting the relevance of the present results.

Table 1. Cytotoxicity and antiviral activity of aqueous extracts and polysaccharide fractions of various macroalgae.macroalgae.

Species	Extract type	Cytotoxicity (CC_{50}, µg/mL)	Antiviral activity (IC_{50}, µg/mL)
S. muticum	Aqueous extracts	>200	134,68 ± 82,7
	Polysaccharides	>200	62,325 ± 12,9
B.bifurcata	Aqueous extracts	>200	27,035 ± 6,1
	Polysaccharides	>200	21,1 ± 3,9
H. incurva	Aqueous extracts	Non cytotoxic	Non active
	Polysaccharides	>200	132,29 ± 66,9
G. corneum	Aqueous extracts	Non cytotoxic	Non active
	Polysaccharides	>200	103,53
C. acicularis	Aqueous extracts	>200	28,33 ± 25,0
	Polysaccharides	> 200	**17,775 ± 2,5**
P. complanata	Aqueous extracts	>200	145,715 ± 11,7
	Polysaccharides	>200	119,895 ± 74,5
E. elongata	Aqueous extracts	>200	34,75 ± 3,2
	Polysaccharides		**18,12 ± 4,8**
Zovirax (control)		>200	0,58 ± 0,23

4 Conclusion

This study highlights the antiviral potential of polysaccharides extracted from marine macroalgae collected along the Sidi Bouzid–El Jadida Atlantic Coast against HSV-1. Polysaccharide fractions exhibited stronger antiviral activity than aqueous extracts and showed no cytotoxic effects on Vero cells. Among the tested species, *C.acicularis* displayed the highest inhibitory activity (EC_{50}= 17.78 ± 2.5 µg/mL), followed by *E.elongata* (EC_{50} = 18.12 ± 4.8 µg/mL). These findings confirm that marine algal polysaccharides represent promising natural antiviral agents and support further investigations into their structural characterization and mechanisms of action.

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