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Chemical and pharmacological investigations of red algae *Hypnea musciformis*

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Abstract

Objective: *Hypnea musciformis* belongs to the family Hypneace, are often in clumps or masses 10-20 cm high of loosely intertwined cylindrical axes and branches. *H. musciformis* is easily distinguished by the presence of flattened, broad hooks at the tips of the branches. The study was designed to investigate the phytochemical potential and biological activity of *H. musciformis*.

Methods: *H. musciformis* was successively extracted with petroleum ether, chloroform and ethyl acetate by maceration and was subjected to the phytochemical test. The fractions of ethyl acetate were analyzed by ultraviolet (UV) spectroscopy and infrared (IR) spectral methods. The petroleum ether extract, chloroform extract, ethyl acetate extract and ethyl acetate fractions were subjected to antibacterial and antifungal activity.

Results: The antiviral activity and cytotoxicity of the extracts were tested in human embryonic lung (HEL) cells (herpes simplex virus-1, herpes simplex virus-2, vaccinia virus, vesicular stomatitis virus and herpes simplex virus-1 TK- KOS ACVr), human epithelial (HeLa) cells (vesicular stomatitis virus and coxsackie virus B4) and Vero cells (parainfluenza-3 virus, reovirus-1, sindbis virus coxsackie virus B4 and punta toro virus). Among the extracts and fractions, fractions of ethyl acetate exhibited remarkable results. This potentiality demonstrates that it could be used to treat bacterial and fungal infections.

Conclusion: The results of this study had brought to light the effective utilization, chemical constituents and antibacterial, antifungal and antiviral activity of *H. musciformis* for further references for researchers.

Keywords: *Hypnea musciformis*, Seaweed, Red algae, Antibacterial activity, Antifungal activity, Antiviral activity

Introduction

Seaweeds grow in the intertidal as well as in the subtidal area up to a certain depth where 0.1% photosynthetic light is available; they are one of the ecologically and economically important living resources of the world ocean. They are able to biosynthesize secondary metabolites that can mediate a broad range of intra and inter specific ecological interactions between marine organisms, including chemical defenses against herbivores [1, 2]. They form one of the important living resources grouped under three divisions namely, Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae). About 624 species have been reported in India with a potential of 77,000 tons (wet weight) per annum. The red seaweeds contribute 27.0 %, brown 0.2 % and others 72.8 %.

Seaweeds serve as an important source of bioactive natural substances. Red algae are mainly used as human food, feed and medicine [3, 4]. Seaweed has been used as medicine and food stuff in the Asian diet for centuries as it contains carotenoids, low calorie food, vitamins, minerals and dietary fibers [5]. In addition to these seaweeds are potentially good sources of protein, polysaccharides and fibers [6, 7]. Many metabolites isolated from marine algae have been shown to possess bioactive effects and they show antibacterial activity [8-11]. Compounds with cytostaic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in red algae [12, 13].

H. musciformis belongs to the family Hypneace, whose plants are bushy, spreading, cylindrical, 10-30 cm high, purplish green in color, cartilaginous, much branched, branches irregular, giving a bushy look to the plant [14]. There are 18 species of red seaweeds belonging to 13 families and 6 orders. Family hypneaceae contains a species *H. musciformis*, whose plants are bushy, spreading, cylindrical, 10-30 cm high, purplish green in color, cartilaginous, much branched, branches irregular, giving a bushy look to the plant [14]. The hooked and swollen tendrils are the characteristic feature of this species. It has been reported to possess k-carrageenan [15, 16]. Carrageenan is extensively used as a food additive in a wide range of products including cheese, cream, chocolate and ice creams. Its chief use is as a suspending and stabilizing agent and has a number of pharmacological properties [17]. There are reports of antispasmodic and anti inflammatory activity of *H. musciformis* by Salimabi and Das [18]. The active compounds isolated in several studies include novel mono, bi and tri

cyclic diterpenoids. Despite this fact, research into the use of marine natural products as pharmaceutical agents is still in its infancy in many countries around the world, mainly in developing countries [19]. This research work aims for the chemical investigation and pharmacological evaluation of red algae *H. musciformis* to generate measurable and testable data, gradually adding to the accumulation of human knowledge.

Materials and methods

Collection authentification and extraction

H. musciformis (red seaweed) was collected from Krusadai Island, near Pamban (Rameswaram), Tamil Nadu, India. It was authenticated by Dr. K. Eswaran, Scientist, Marine Algal Research Institute, Tamil Nadu, India. The collection was performed during the month of January, when red algal diversity remains dominant. It was harvested manually and washed thoroughly in running water to remove epiphytes, animal castings, sand and calcareous and other adhering detritus matters. Cleaned seaweed materials were shade dried to prevent photolysis and thermal degradation. The completely dried material was weighed and ground coarsely in a mechanical grinder.

The powdered material 600 gm was successively extracted with 2 L of petroleum ether, chloroform and ethyl acetate each by maceration with occasional shaking at room temperature for 72 h. Petroleum ether extract (Extract 1), chloroform extract (Extract 2), ethyl acetate extract (Extract 3) were concentrated, percentage yield was calculated [Table 1] and was kept in dessicator for further investigations.

Preliminary phytochemical screening

A qualitative phytochemical test for different solvent extracts of *H. musciformis* was determined as per the standard protocol to decipher the presence or absence of various phyto-compounds such as alkaloids, glycosides, steroids, flavonoids etc by observing characteristic color changes [Table 2] [20-24].

Chromatographic studies

Column chromatography profile of ethyl acetate extract

Silica gel (60-120 mesh) was used as an adsorbent for column chromatography. The column was filled with slurry, prepared using silica gel and cyclohexane solvent to a suspension of free flowing consistency. It was poured slowly from the top of the column of the apparatus in little quantity allowing for the even and uniform packing. Then the ethyl acetate extract (Extract 3) (250 mg) was dissolved in minimum quantity of cyclohexane and chromatographed over silica gel. It was then eluted with the solvents of increasing polarity in the solvents of increasing polarity in the order i.e. cyclohexane: ethyl acetate 30:1(Fraction-I), cyclohexane: ethyl acetate 25:1 (Fraction-II), cyclohexane: ethyl acetate 20:1 (Fraction-III), cyclohexane: ethyl acetate 15:1 (Fraction-IV), cyclohexane: ethyl acetate 10:1 (Fraction-V), cyclohexane: ethyl acetate 5:1 (Fraction-VI), cyclohexane: ethyl acetate 3:1(Fraction-VII), cyclohexane: ethyl acetate 2:1(Fraction-VIII), cyclohexane: ethyl acetate 1:1 (Fraction-IX) these elutes were monitored by TLC [Table 3].

Thin layer chromatography (TLC)

50 gm of silica gel G was weighed out and shaken to form a homogeneous suspension with 100 ml of distilled water. This suspension was poured into TLC plate which was adjusted to 0.25 mm thickness. The plates were allowed to dry at room temperature and then dried at 110 ºC for 30 min in hot air oven. TLC of all the fractions of ethyl acetate was performed using cyclohexane: ethyl acetate (7:1) solvent and iodine vapour as detecting agent [Table 4].

UV spectroscopy

The isolated compounds were prepared at a concentration of 40µg/ml by dissolving in ethanol. Scanning of the isolated compound was performed at the wavelength range of 200-400 nm and compared with reference standard [Table 5] [22].

IR spectroscopy

The extracts of *H. musiformis* were mixed with KBr salt, using a mortar and pestle and compressed into a thin pellet. Infra red spectra were recorded on Shimadzu (Fourier transform infrared spectroscopy) FTIR spectrometer 8000 series, between 4,000-400 cm^{-1}[Table 6] [25].

Antibacterial and antifungal activity

Bacteria and fungi

The extracts were screened against a panel of microorganisms, including *Staphylococcus aureus* subsp. Aureus (MTCC 737), *Staphylococcus epidermidis* (MTCC 3615), *Escherichia coli* (MTCC 1687), *Psuedomonas aeruginosa* (MTCC 424), *Aspergillus niger* (MTCC 228) and *Candida albicans* (MTCC 183). The investigated microbial strains were procured from the Institute of Microbial Technology, Chandigarh, India.

Preparation of inoculum

Active cultures for screening were prepared by transferring a loopful of cells from the stock to test tubes of nutrient broth for bacteria, yeast peptone dextrose broth for *Candida albicans* and Czapek yeast extract broth for *Aspergillus niger*. Moreover, they were incubated without agitation for 24 h at 37 ºC, 48 h at 30 ºC and 7 d at 30 ºC, respectively as per the guidelines specified by Institute of Microbial Technology, Chandigarh, India. The cultures were diluted with fresh broths to achieve optical densities corresponding to 106 colony-forming units (CFU/mL) for bacteria and 105 spores/mL for fungal strains.

Table 1: Percentage yield of extracts

Extract	% Yield
1	0.53
2	0.65
3	0.68

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Antibacterial and antifungal assay

Extracts 1, 2, 3 and Fractions I-IX were dissolved in 100 % dimethylsformamide (DMF) at a concentration of 1 mg/mL and used as working stocks. Ampicillin (30 µg) for bacteria and clotrimazole (30 µg) for fungi were used as reference agents. Susceptibility test was determined by disc diffusion method [26-28]. The nutrient agar plates were prepared by pouring 15 mL of molten media into sterile petriplates. The plates were allowed to solidify for 5 min, 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. Extracts 1, 2, 3 and Fractions I-IX were loaded on 6 mm discs. The loaded discs were placed on the surface of the medium and the extracts were allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 h for bacteria and 30 °C for 48 h for fungi with yeast peptone dextrose agar and Czaepak yeast extract agar media. At the end of incubation, inhibition zones formed around the discs were measured with a transparent ruler in millimeters.

Determination of minimal inhibitory concentration (MIC)

A broth dilution susceptibility assay was used for the determination of the MIC [29]. Briefly, bacterial strains were cultured overnight at 37 °C in nutrient agar; *Candida albicans* and *Aspergillus niger* were cultured overnight at 30 °C in yeast peptone dextrose agar and Czaepak yeast extract agar, respectively. Bacterial and fungal strains were suspended in their corresponding broths to give a final density of 10 6 and 105 organism/mL respectively. Dilutions of extracts 1, 2, 3 and 4 ranged from 1000 µg/mL to 0.05 µg/mL were prepared in capped tubes. A control was also served; 20 µL from each of the test organisms was used to inoculate the tubes. The tubes were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for fungi. Tubes containing broth (2 mL) were inoculated with organisms and kept at +4 °C in a refrigerator overnight to be used as standards. The MIC was recorded as the lowest concentration at which no microbial growth was observed [Table 7 and 8].

Table 2: Preliminary phytochemical screening

S No.	Chemical class	Test	Extracts		
			Extract 1	Extract 2	Extract 3
1	Carbohydrates	Molisch's test	–	–	–
		Fehling's test	–	–	–
2	Proteins	Biuret test	–	–	–
3	Amino acids	Ninhydrin test	+	–	+
4	Steroids	Salkowski test	–	+	+
		Liebermann-Burchard test	–	+	+
5	Alkaloids	Dragendorff's test	+	+	+
		Wagner's test	–	+	+
6	Glycosides	Legal's test	+	–	–
		Keller-Killiani test	+	–	+
7	Flavonoids	NaOH test	–	+	+
8	Tannins and Phenolic compounds	$FeCl_3$ test	–	–	–
		Br_2 water test	–	–	–

Present (+), Absent (-)

Antiviral activity

The origin of the viruses was as the following: herpes simplex virus-1 (strain KOS), herpes simplex virus-2 (strain G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK-KOS ACVr, coxsackie virus B-4, sindbis virus, punta toro virus, reovirus-1 (ATCC VR230) and parainfluenza virus-3 (ATCC VR-93) (American Type Culture Collection, Rockville, Md.). The virus stocks were grown in human embryonic lung (HEL) cells (herpes simplex virus-1, herpes simplex virus-2, vaccinia virus, vesicular stomatitis virus and herpes simplex virus-1 TK- KOS ACVr), human epithelial (HeLa) cells (vesicular stomatitis virus, coxsackie virus B4 and respiratory syncytial virus) and Vero cells (parainfluenza-3 virus, reovirus-1, sindbis virus, coxsackie virus B4, and punta toro virus).

Antiviral assays

Confluent cell cultures in microtiter trays were inoculated with 100 CCID50 (1 CCID50 corresponding to the virus stock dilution that proved infective for 50% of the cell cultures). After 1 h of virus adsorption to the cells, residual virus was removed and replaced by cell culture medium (eagle minimal essential medium) containing 3% fetal calf serum and various concentrations of the test extracts (200, 100, 40, 20, 10, 4 µg/mL). Viral cytopathogenicity was recorded as soon as it reached completion in the untreated virus-infected cell cultures, i.e., at 1 to 2 days for vesicular stomatitis; at 2 days for coxsackie; at 2 to 3 days for herpes simplex types 1 and 2 and vaccinia; and at 6 to 7 days for reo and para-influenza viruses. Brivudin, ribavirin, acyclovir, gancyclovir and (S)-9-(2, 3-dihydroxypropyl) adenine were used as reference agents.

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Antiviral activity was expressed as minimal inhibitory concentration (MIC) required reducing virus induced cytopathogenicity by 50% (within the micro tray well) [30].

Cytotoxicity

Although confluent cell cultures had not been infected, they were treated with various concentrations of the test extracts, which were incubated in parallel with the virus-infected cell cultures and examined microscopically at the same time as the viral cytopathogenicity was recorded for the virus-infected cell cultures. A disruption of the cell monolayer, e.g. rounding up or detachment of the cells was considered as evidence for cytotoxicity. Cytotoxicity was expressed as minimal cytotoxic concentration (MCC) required causing a microscopically detectable alteration of normal cell morphology of the confluent cell cultures that were exposed to the test extracts.

Table 3: Yield of fractions

S. No.	Isolated fraction	% Yield
1	I	4
2	II	1.2
3	III	1.6
4	IV	3.2
5	V	2.0
6	VI	2.0
7	VII	2.4
8	VIII	4.8
9	IX	1.2

Table 4: TLC profile of fractions

S. No.	Isolated fraction	Rf Value
1	I	0.53
2	II	0.43
3	III	0.6
4	IV	0.3
5	V	0.7
6	VI	0.5
7	VII	0.4
8	VIII	0.38
9	IX	0.53

Table 5: UV spectral data of fractions

S.No.	Isolated fraction	λmax (nm)
1	I	223.6
2	II	227.2
3	III	233.2
4	IV	235.6
5	V	235.6
6	VI	234.4
7	VII	238.0
8	VIII	241.6
9	IX	239.2

Table 6: IR spectral data of fractions

S. No.	Isolated fraction	IR bands (cm^{-1})
1	I	2923, 2851, 2360,1735, 770
2	II	2924, 2851, 2361, 772
3	III	2923, 2851, 2360, 770
4	IV	3421,2919, 2849, 2359,1670, 1463,1378, 761
5	V	2360, 2343,1385, 771
6	VI	2919, 2850, 2361, 1712, 1464, 1379, 760
7	VII	2919, 2850, 2359, 1389, 774
8	VIII	29188, 2849, 2360, 1725, 772
9	IX	3434, 2360, 2342, 1219, 772

Results and discussions

The yields of *H. musiformis* extracts are detailed in Table 1. It is clear from the tabular representation that ethyl acetate extract had better yield than others. Phytochemical screening was carried out to investigate the presence of phyto-constituents [Table 2]. The phytochemical analysis reveals the presence of some phyto-compounds such as amino acids, alkaloids and glycosides in petroleum ether extract. Chloroform extract showed the presence of steroids, alkaloids and flavonoids and amino acid, steroids, alkaloids, glycosides and flavonoids were found present in ethyl acetate extract.

Tables 3 and 4 represents the chromatography profile of ethyl acetate extracts. Percentage yield of isolated fraction of ethyl acetate fraction (I-IX) was found to be 4%, 1.2%, 1.6%, 3.2%, 2.0%, 2.0%, 2.4%, 4.8% and 1.2% respectively where as Rf values 0.53, 0.43. 0.6, 0.3, 0.7, 0.5, 0.4, 0.38 and 0.53 resulted by the TLC profile of ethyl acetate fraction (I-IX).

Extraction of red alga, *H. musciformis* was done with petroleum ether, chloroform and ethyl acetate. The yields of *H. musiformis* extracts are detailed in [Table 1] thus obtained extracts were subjected to phytochemical analyses which revealed the presence of alkaloids, amino acids, flavonoids, glycosides and steroids [Table 2].The ethyl acetate extracts yielded nine fractions [Table 3]. TLC of all the fractions of ethyl acetate was performed using cyclohexane: ethyl acetate (7:1) solvent and iodine vapour as detecting agent [Table 4]. They were characterized by UV [Table 5] and IR [Table 6] spectral analyses.

UV and IR absorption spectra of fractions are characteristic of the functional group present. Spectral analyses revealed the presence of 1, 2-double bond and α, β-unsaturated carbonyl group as they are steroidal compounds.

UV maximum absorption of isolate II at 227.2 nm was found to have 1, 2-double bond. UV absorption at 238 indicated the presence of a α, β-unsaturated carbonyl group in isolate VII. The presence of the 4-ene-3-carbonyl structural moiety was supported by a strong absorption at 242 nm for isolation VIII. IR spectrum of isolation IV showed frequencies at 3421 and

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1670 cm^{-1}, indicating the presence of hydroperoxyl group and a conjugated carbonyl group, respectively. All the extracts and fractions were screened for antimicrobial activity and the fractions was screened for cytotoxicity and antiviral activity.

In the antimicrobial study, extracts exhibited moderate to mild activity at 1mg/ml concentration against Staphylococcus aureus, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger* [Table 7]. From the extent of zone of inhibition, the activities were compared. The isolate VI showed better activity than the extracts against *Pseudomonas aeruginosa* also I, II, VI and IX were found to be more effective than extracts against *Staphylococcus aureus* but the isolates were less effective than extracts against *Escherichia coli* and *Staphylococcus epidermidis* [Table 8]. Ethyl acetate extract was as active as I and IV against *Candida albicans*. Nevertheless I and VI possessed appreciable activity against *Aspergillus niger* and they were found to be more remarkable than extract.

The results of antiviral screening isolated fraction of ethyl acetate fraction (I, II, III, V,VI, VII, VIII, X) against a broad panel of DNA and RNA viruses , herpes simplex virus-1 (KOS) (HSV-1, KOS), herpes simplex virus-2 (G) (HSV-2, G), vaccinia virus (VV), vesicular stomatitis virus (VSV) and thymidine kinase-deficient (TK-) (KOS) of HSV-1 resistant to acyclovir (ACVr) (HSV-1 TK- ACVr) in human embryonic lung (HEL) cell cultures [Table 9], vesicular stomatitis virus (VSV), coxsackie virus B4 (CV-B4) and respiratory syncytial virus (RSV) in HeLa cell cutures [Table 10], parainfluenza-3 virus (PI-3V), reovirus-1 (RV-1), sindbis virus (SV), coxsackie virus B4 (CV-B4) and punta toro virus (PTV) in vero cell cultures [Table 5], influenza A [H1N1 subtype (A/PR/8/34) and H3N2 subtype (A/HK/7/87)] and influenza B (B/HK/5/72) in Madin Darby canine kidney (MDCK) cell cultures [Table 11] and Feline corona virus (FIPV) and feline herpes virus in Crandell-Rees Feline kidney (CRFK) cell cultures [Table 12], were determined by using cytopathicity (CPE) reduction assay.

The antiviral activities were compared with the reference antiviral drugs (ganciclovir, cidofovir, acyclovir, brivudin, DS-5000, (S)-DHPA, ribavirin, oseltamivir carboxylate, amantadine, rimantadine, Hippeastrum hybrid agglutinin (HHA) and *Urtica dioica* agglutinin (UDA) [Table 9-Table 13]. Antiviral activity was expressed as the minimum effective concentration (EC_{50}) required reducing virus-induced cytopathogenicity by 50% and cytotoxicity was expressed as the minimum cytotoxic concentration required causing a microscopically detectable alteration of normal cell morphology. None of the isolated fractions displayed antiviral activity against any of the viruses except for fraction IX against herpes simplex virus-1 (KOS) (HSV-1, KOS) and herpes simplex virus-2 (G) (HSV-2, G), (EC_{50} = 59 and 45 µM respectively) [Table 9].

Table 7: Antibacterial and antifungal activity of extracts

Test organism	Zone of inhibition (in mm)				
	1	2	3	C	A
Staphylococcus aureus	11	8	8.5	-	12
Staphylococcus epidermidis	12	9	11	-	11
Escherichia coli	7	9	11.5	-	10
Pseudomonas aeruginosa	10	7.5	12	-	11
Aspergillus niger	10	11	12	28	-
Candida albicans	11	8	13	25	-

C= Clotrimazole, A= Ampicillin

Table 8: Antibacterial and antifungal activity of fractions of ethyl acetate extract

Test organism	Zone of inhibition (in mm)										
	I	II	III	IV	V	VI	VII	VIII	IX	C	A
Staphylococcus aureus	9	9.5	6.5	7	8	9	6	7	9.5	-	11
Staphylococcus epidermidis	7	6	6	6.5	7	8	7	6.5	8.5	-	12
Escherichia coli	6	6	9	11	6	5	5	5	9	-	10
Pseudomonas aeruginosa	7	6	6	6	12	12	13	11	8	-	11
Aspergillus niger	13	8	9	8	7	17	7	8	8	28	-
Candida albicans	13	12	11	13	9	10	12	8	9	25	-

C= Clotrimazole, A= Ampicillin

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Table 9: Cytotoxicity and antiviral activity of fractions (I-IX) in *HEL* cell cultures

Compound	Minimum cytotoxic concentration [a] (µg/ml)	Herpes-simplex virus-1 (KOS)	Herpes-simplex virus-2 (G)	EC_{50} [b] (µg/ml)		Herpes-simplex-virus-1 TK^- KOS ACV^r
				Vaccinia virus	Vesicular stomatitis virus	
1.	≥4	>4	>4	>4	>4	>4
2.	20	>4	>4	>4	>4	>4
3.	≥4	>4	>4	>4	>4	>4
4.	-	-	-	-	-	-
5.	100	>20	>20	>20	>20	>20
6.	>100	>100	>100	>100	>100	>100
7.	≥20	>20	>20	>20	>20	>20
8.	≥20	>20	>20	>20	>20	>20
9.	>100	59	45	100	>100	>100
Brivudin (µm)	>250	0.2	50	10	>250	250
Ribavirin (µm)	>250	>250	112	126	112	>250
Cidofovir (µm)	>250	1.2	2	29	>250	1.2
Ganciclovir (µm)	>100	0.16	0.03	100	>100	4

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 10: Cytotoxicity and antiviral activity of fractions (I-IX) in *HeLa* cell cultures

Compound	Minimum cytotoxic concentration [a] (µg/ml)	EC_{50} [b] (µg/ml)		
		Vesicular Stomatitis virus	Coxsackie virus B4	Respiratory Syncytical virus
1.	100	>20	>20	>20
2.	100	>20	>20	>20
3.	≥20	>20	>20	>20
4.	-	-	-	-
5.	>100	>100	>100	>100
6.	>100	>100	>100	>100
7.	>100	>100	>100	>100
8.	>100	>100	>100	>100
9.	>100	>100	>100	>100
DS-5000	>100	4	2	0.2
(S)-DHPA (µm)	>250	112	>250	250
Ribavirin (µm)	>250	10	50	17

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50%.

The results of the present study revealed that marine red alga are the potential producers of anti-inflammatory and antibacterial activity with some few exceptions. Therefore, it should be thoroughly investigated for natural sources bioactive compounds properties. Although the metabolites responsible for the antiproliferative action of algae species has not been chemically characterized, but the data suggest the occurrence of several secondary compounds with low polarity which are spread more easily in cell membranes than the more polar [31, 32] the crude extracts of dichloromethane, ethanol and chloroform fraction concentrated the substances responsible for the most significant cytotoxic activity.

Conclusion

The seaweed *H. musciformis*, a red alga was analyzed for its biochemical and biological activity. The present study concluded that, marine red seaweed, *H. musciformis* proved to be the most potential seaweed for the development of pharmaceutical compounds. In conclusion, we isolated nine fractions of ethyl acetate extract of *H. musiformis* which were confirmed by UV and IR spectral analysis. Further, all the extracts and fractions (I-IX) were screened for antimicrobial

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activity and the fractions(I-IX) was screened for cytotoxicity and antiviral activity against a broad panel of DNA and RNA viral strains indicated that fraction IX showed significant antiviral activity against herpes simplex virus-1 (KOS) (HSV-1, KOS) and herpes simplex virus-2 (G) (HSV-2, G), (EC_{50} = 59 and 45 µM respectively) cell cultures could be selected as potential lead compounds for the development of novel antiviral agents against herpes simplex virus.

Table 11: Cytotoxicity and antiviral activity of fractions (I-IX) in *Vero* cell cultures

Compound	Minimum cytotoxic concentration [a] (µg/ml)	EC_{50} [b] (µg/ml)				
		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
1.	100	>20	>20	>20	>20	>20
2.	100	>20	>20	>20	>20	>20
3.	100	>20	>20	>20	>20	>20
4.	-	-	-	-	-	-
5.	>100	>100	>100	>100	>100	>100
6.	>100	>100	>100	>100	>100	>100
7.	>100	>100	>100	>100	45	>100
8.	>100	>100	>100	>100	>100	>100
9.	>100	>100	>100	>100	>100	>100
DS-5000	>100	>100	>100	59	12	20
(S)-DHPA (µm)	>250	112	>250	>250	>250	>250
Ribavirin (µm)	>250	112	250	146	>250	146

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 12: Cytotoxicity and anti-influenza virus of fractions (I-IX) in *MDCK* cell cultures

Compound	Concentration unit	Cytotoxicity		Antiviral EC_{50} [c]					
		CC_{50} [a]	Min. cytotoxic Conc[b].	Influenza A H1N1 subtype		Influenza A H3N2 subtype		Influenza B	
				Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
1.	µg/ml	6.4	4	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
2.	µg/ml	3.1	4	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
3.	µg/ml	9.6	20	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
4.	-	-	-	-	-	-	-	-	-
5.	µg/ml	15.7	20	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
6.	µg/ml	>100	≥100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
7.	µg/ml	8.8	20	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
8.	µg/ml	48.6	20	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
9.	µg/ml	29.2	≥4	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Oseltamivir carboxylate	µM	>100	>100	0.03	0.07	4	1.9	4	1.8
Ribavirin (µm)	µM	>100	100	9	7.6	12	10.4	9	7.4
Amantadin	µM	>100	>100	N.A.	N.A.	4	1.3	N.A.	N.A.
Rimantadin	µM	>100	>100	45	72.6	N.A.	N.A.	N.A.	N.A.

[a]50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan- based MTS assay.

[b]Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

[c]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

MDCK cells: Madin Darby canine kidney cells; N.A.: not active at the highest concentration tested, or at subtoxic concentration.

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Table 13: Cytotoxicity and antiviral activity [Feline Corona Virus (FIPV) and Feline Herpes Virus] in CRFK cell cultures

Compound	CC_{50} [a] (µg/ml)	EC_{50} [b] (µg/ml)	
		Feline Corona Virus (FIPV)	Feline Herpes Virus
1.	50.3	>20	>20
2.	30.7	>20	>20
3.	88.7	>20	>20
4.	-	-	-
5.	>100	50.2	>100
6.	>100	>100	>100
7.	>100	>100	>100
8.	>100	>100	>100
9.	57.5	>20	>20
HHA	>100	17.9	43.4
UDA	>100	3.0	33.7
Ganciclovir (µM)	>100	>100	1.8

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay; [b]50% Effective concentration or concentration producing 50% inhibition of virus induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay; CRFK cells: Crandell-Rees Feline kidney cells.

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