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Antitumourigenic Polysaccharides Isolated from the Brown Algae, *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe

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ABSTRACT

Mannitol, other carbohydrates of low-molecular weight, fucose-containing polysaccharides, laminarin and alginic acid were isolated from Egyptian brown algae *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe. Fucose- containing polysaccharides were isolated with cold and hot water; glucuronic acid was the major hydrolysate in both extracts isolated from *Padina pavonia* (L.) Gaill. while glucuronic and galacturonic acids were the major hydrolysate in cold extract polysaccharides of *H. clathratus*; fucose was the major hydrolysate in hot extract polysaccharides. The hot extracts of both were achieved by fractional precipitation with ethanol. The hot water extract of both algae and their fractions exhibited antitumour properties against HepG2 human cell line *in vitro*.

Keywords: alginic acid, cytotoxicity, laminarin, mannitol, marine algae, mucilage

INTRODUCTION

Brown algae represent a rich and easily regenerated source of polysaccharides of structural and biological activity interests: laminarans, fucoidans, and also alginic acids (Percival and McDowell 1967; Painter 1983). Laminarans are known as anticancer substances (Bohn and BeMiller 1995; Shin *et al.* 2009). Fucoidans have antivirus activities, including against hepatitis viruses (Venkateswaran *et al.* 1989), anti-HIV infection (Shaeffer and Krylov 2000), herpes (Wang *et al*. 2007, 2008a), anticomplementary (Zvyagintseva *et al*. 2000), antitumour (Dias *et al.* 2005) and anticoagulant (de Azevedo *et al.* 2009). Alginic acids are successfully used in various areas of industry as gel-forming materials, in medicine and in cosmetology for preparation of non-fatted ointments, etc. (Lewis *et al*. 1988).

MATERIALS AND METHODS

Algal material

The thalli of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe were harvested in May, 2002 from the rocks at about 0.60-1.20 m under the water surface in Abo Minqar at Hurghada, Egypt and identified by Dr. S. A. Shaalan, Professor of Phycology, Faculty of Science, Alexandria University.

Material for analysis

Authentic sugars for paper chromatography (PC) and high performance liquid chromatography (HPLC) (glucuronic acid, galacturonic acid, sucrose, glucose, xylose, galactose, fucose, fructose, arabinose, mannitol and sorbitol) were obtained from Fluka, Switzerland. All solvents used were of AR grade.

Paper chromatography analysis

Descending paper chromatography analysis of the free sugars and mucilage hydrolysates were carried out on Whatman 3MM papers (Whatman Ltd., Maid Stone, Kent, England) using *n*-butanol: acetic acid: water (4: 1: 5, v/v) as the developing system. After development, the chromatograms were dried, sprayed with aniline hydrogen phthalate reagent (0.93 g aniline and 1.66 g *O*-phthalic acid were dissolved in 100 ml *n*-butanol saturated with water) and heated at 105°C for 5 min (Stahl 1969).

HPLC analysis

HPLC analysis of the free sugars and mucilage hydrolysates were carried out on HPLC Hewlett Packard series 1050, RI detector. The analysis was carried out using Aminex carbohydrate HPX-87c (300 mm × 78 mm) at 85°C, flow rate of 0.8 ml/min and mobile phase was deionised water. 10 mg of each residue of low-molecular weight carbohydrates and hydrolysate of cold and hot water extracts, as well as of the aforementioned individual authentic sugars was separately dissolved in 1 ml of deionised water. 75 µl of each sample was injected into the HPLC using an SGE syringe (Syringe Perfection, Australia). Quantitative determination was based on peak area measurement while qualitative identification was carried out by comparison of the retention times of the peaks with those of the authentic sugars.

Isolation and analysis of carbohydrate contents for *P. pavonia* and *H. clathratus*

1. Determination of total carbohydrate and soluble sugars contents

Determination of total carbohydrates as well as soluble sugars (as glucose) of *P. pavonia* and *H. clathratus* was carried out using the phenol-sulphuric acid method of Dubois *et al.* (1956).

2. Mannitol content

Mannitol was extracted from *P. pavonia* and *H. clathratus* according to the method by Black *et al.* (1951a) and identified by PC and melting point (Gallen Kamp, England).

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Original Research Paper

3. Low-molecular weight carbohydrates content

LMW carbohydrates were isolated from *P. pavonia* and *H. clathratus* according to Abdel-fattah and Hussein (1970) and analysed by both PC and HPLC.

4. Mucilage content

Mucilage was isolated from *P. pavonia* and *H. clathratus* from the marc after isolation of mannitol and LMW carbohydrates. The marc powdered was extracted by mechanical stirring with cold water followed by hot water. Each individual extracts was concentrated to about 50 ml under reduced pressure and absolute ethanol (250 ml) was added drop-wise till complete precipitation of mucilages. The residue obtained, in each case, was washed with absolute ethanol, dehydrated with acetone under suction and then saved in refrigerator. The isolated mucilage was hydrolysed (Awad *et al.* 2001) and analysed by PC and HPLC.

5. Laminarin content

Laminarin was extracted from *P. pavonia* and *H. clathratus* according to the method of Black *et al*. (1951b). On hydrolysis with 0.3 M HCl at 100°C for 3 h laminaran afforded mainly glucose which was identified by PC (Abdel-Fattah *et al.* 1978).

6. Alginic acid content

Alginic acid was extracted from *P. pavonia* and *H. clathratus* according to the method mentioned by Whyte (1988) and analysed by ^{13}CNMR. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{13}C spectra were run at 75.46 MHz (Ikeda *et al.* 2000).

7. Fractionation of hot aqueous extract

A solution of polysaccharide composed of 1 g of crude polysaccharide dissolved in 50 ml water was successively treated with different concentrations of ethanol (i.e. 20, 30, 40%) until its concentration reached 80%. The precipitate was dried in vacuum, and weighed according to Abdel-Fattah *et al.* (1978).

Antitumor activity

1. Cells

Authentic culture, HepG2 human cell line originated from liver and U251 originated from brain carcinoma human cell line, were obtained from The American Type Culture Collection, USA.

2. Culture media

HepG2 and U251 cells were suspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 1% antibiotic-antimycotic mixture (10.000 U/ml K-penicillin, 10.000 μg/ml streptomycin sulphate and 25 μg/ml amphotericin B) and 1% L-glutamine (all purchased from Lonza, Belgium).

3. Assay method for cytotoxic activity

The cytotoxicity against HepG2 and U251 were performed in the National Cancer Institute, according to the method by Skehan *et al.* (1990). Adriamycin® (Doxorubicin) 10 mg vials (Pharmacia, Sweden) were used as the reference drug.

HepG2 and U251 cell lines were plated in 96-multiwell plates (10^4cells/well) for 24 hrs before treatment with the tested sample to allow attachment of cells to the wall of the plate. Then, 50 μl aliquot of serial dilution of crude extract (1.0, 2.5, 5 and 10 μg/ml) was added and the plates were incubated for 48 hrs at 37°C in a humidified incubator containing 5% CO_2 in air. Triplicate wells were prepared for each individual dose. Cells were fixed, washed and stained with Sulforhodamine B stain (Sigma, USA). Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer (Sigma, USA). Colour intensity was measured in an ELISA reader spectrophotometer (Tecan Group Ltd.-Sunrise, Germany).

Statistical analysis

All values were expressed as the mean of percentage of inhibition cells of three replicates for each treatment. Data were subjected to paired-samples *t*-test using SPSS (ver. 9.0). $P < 0.05$ was regarded as significant.

RESULTS AND DISCUSSION

The carbohydrate contents of the brown algae *P. pavonia and H. clathratus* are listed in **Table 1**. The total carbohydrates (calculated as glucose) of the brown algae *P. pavonia* and *H. clathratus* were 19.20 and 25.60%, respectively, while soluble sugars of the tested algae constituted 1.34 and 2.45%, respectively.

Mannitol white crystals (1.04 and 2.52% of dry weight yield, respectively), with melting point = 166-168°C. PC of aqueous solution of these crystals revealed a single bluish-violet spot (R_f =0.28) identical with authentic reference mannitol. This result confirms the reported data by Mian and Percival (1973).

From **Table 2**, PC of LMW carbohydrate revealed the presence of fucose, arabinose and glucuronic acid in *P. pavonia*, while PC of LMW of *H. clathratus* showed fucose, xylose, glucose, galactose and galacturonic acid.

From **Table 3**, we can conclude that glucuronic acid

Table 1 Carbohydrate contents of dried powdered thalli of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe.

Algae	% Dry weight ± SEM	
	P. pavonia	***Hydroclathrus clathratus***
Total carbohydrates	19.20 ± 0.03	25.60 ± 0.005
Total soluble sugars	1.34 ± 0.01	2.45 ± 0.01
Mannitol	1.04 ± 0.01	2.52 ± 0.02
Total mucilage	12.64 ± 0.05	10.49 ± 0.02
Alginic acid	1.748 ± 0.01	5.684 ± 0.05
Total laminarin	2.22 ± 0.05	3.58 ± 0.01

Each value represents the mean of three replicates ± SEM (Standard Error of Mean)

Table 2 Paper chromatographic investigation of free sugars and polysaccharide hydrolysates of dried powdered thalli of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe.

Spot No.	R_f with solvent system*	Free sugars of *P. pavonia*	Free sugars of *H. clathratus*	Mucilage hydrolysates of *P. pavonia*		Mucilage hydrolysates of *H. clathratus*		Colour with aniline phthalate	Comparable with
				Cold extract	Hot extract	Cold extract	Hot extract		
1	0.55	+	+	+	+	+	++	Reddish-brown	Fucose
2	0.53	-	+	+	+	+	+	Reddish-brown	Xylose
3	0.39	++	-	+	+	-	+	Brown	Arabinose
4	0.30	-	+	+	+	+	+	Brown	Glucose
5	0.29	-	+	-	+	+	+	Brown	Galactose
6	0.28	++	-	++	++	++	+	Pale brown	Glucuronic acid
7	0.27	-	-	+	+	+	-	Bluish violet	Mannitol
8	0.26	-	++	-	-	++	+	Pale brown	Galacturonic acid

* *n*-Butanol- acetic acid- water (4:1:5 v/v); ++: Appreciably present, +: Present, - : Absent or not observed.

Table 3 HPLC analysis of free sugars and polysaccharide hydrolysates of dried powdered thalli of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe.

Authentic sugars	R_t (min)	Relative percentage					
		Free sugars of *P. pavonia*	Free sugars of *H. clathratus*	Mucilage of *P. pavonia*		Mucilage of *H. clathratus*	
				Cold hydrolysate	Hot hydrolysate	Cold hydrolysate	Hot hydrolysate
Glucuronic acid	5.87	90.33	-	53.68	79.78	72.95	1.72
Galacturonic acid	5.91	-	73.56	-	-	10.83	1.94
Sucrose	6.20	-	-	-	-	-	-
Glucose	7.57	0.01	0.09	3.59	1.43	2.73	4.44
Xylose	8.37	0.03	0.18	4.85	0.85	2.15	3.04
Galactose	8.50	0.06	0.02	-	0.90	1.35	6.53
Fucose	9.57	0.82	0.08	6.83	1.24	4.61	38.55
Fructose	9.67	-	-	-	-	-	-
Arabinose	9.77	1.83	-	5.56	1.01	0.12	6.35
Mannitol	12.62	-	-	4.15	0.86	0.92	0.37
Sorbitol	15.67	0.03	0.24	-	1.71	-	-

Table 4 ^{13}CNMR of alginic acid isolated from *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe.

Carbon no.	*P. pavonia*		*H. clathratus*
	Mannuronic acid	Guluronic acid	Guluronic acid
C2	δ 73.297	δ 67.338	δ 68.080
C3	δ 74.374	δ 72.176	δ 72.707
C4	δ 83.60	δ 81.070	δ 81.464
C5	δ 77.427	δ 70.450	δ 70.393
C6	δ 175.737	δ 175.949	δ 176.101

Table 5 Yield of fractions obtained from fractionation of hot aqueous crude polysaccharide extract of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe by ethyl alcohol.

Fractions	% Ethanol	Yields in mg	
		P. pavonia	*H. clathratus*
Fraction I	20%	752	680
Fraction II	30%	216	189
Fraction III	40%	11	60
Fraction IV	50%	13	21
Fraction V	60%	-	40

Table 6 Cytotoxic activity of cold and hot aqueous polysaccharide extracts of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe against cultured U251 human cells.

Drug / Conc. µg/ ml	% of inhibition cells ± SEM			
	1	2.5	5	10
Doxorubicin	67.1 ± 0.20*	85.2 ± 0.05*	85.8 ± 0.05*	92.8 ± 0.05*
Cold aqueous polysaccharide extract of *P. pavonia*	6.77 ± 0.19* **	3.54 ± 0.13* **	7.53 ±0.05* **	10.6 ± 0.15* **
Hot aqueous polysaccharide extract of *P. pavonia*	4.31 ± 0.11* **	6.42 ± 0.15* **	1.63 ± 0.08* **	19.81 ± 0.14* **
Cold aqueous polysaccharide extract of *H. clathratus*	-5.5 ± 0.32* **	9.81 ± 0.17* **	8.37 ± 0.12* **	6.1 ± 0.11* **
Hot aqueous polysaccharide extract of *H. clathratus*	7.45 ± 0.11* **	13.23 ± 0.07* **	12.24 ± 0.10* **	14.39 ± 0.13* **

Each value represents the mean of percentage of inhibition cells of three replicates ± SEM (Standard Error of Mean)
* Significantly different from control value at $p < 0.05$ according to paired-samples *t*-test
** Significantly different from Doxorubicin value at $p < 0.05$ according to paired-samples *t*-test

appeared in LMW, cold and hot hydrolysate of *P. pavonia* as a major component. On the other hand, galacturonic acid was present as a major component in the free sugar of *H. clathratus*, while glucuronic acid and fucose were considered as the major components in cold and hot hydrolysates, respectively of this alga.

Total laminarin content of *P. pavonia* and *H. clathratus* amounted to 0.11 g and 0.18 g, which represented 2.22 and 3.58%, respectively. Paper chromatographic analysis showed one brown spot with $R_f = 0.3$ which is identical to reference glucose. Variable quantity of laminarin contents of *P. pavonia* collected from various shores of Red Sea have been reported by Abdel-Fatah and Edrees (1977) and Khafaji (1986, 1992).

Alginic acid isolated from *P. pavonia* and *H. clathratus* yielded 0.437 and 1.421 g, respectively which represented 1.748 and 5.684%, respectively. Variable quantitative composition of alginic acid isolated from *P. pavonia* collected from various locations has been reported by many authors (e.g. Abdel-Fatah and Edrees 1977; Khafaji 1986, 1992). The ^{13}CNMR spectrum of crude alginic acid isolated from the brown alga *P. pavonia* recorded 4 peaks in the anomeric region. These signals (δ 98.36, δ 99.313, δ 100.352 and δ 101.733) correspond to MG, GG, MM and GM, respectively, i.e., to C1 of mannuronic and guluronic acids. On the other hand, ^{13}CNMR of alginic acid isolated from *H. clathratus* shows one peak in the anomeric region at δ100.39 which corresponds to GG, i.e., to C1 of guluronic acid. Other peaks are listed in **Table 4**. The obtained ^{13}CNMR data was consistent with the reported data (Ikeda *et al.* 2000).

Four and five fractions were obtained by fractionation of hot aqueous extracts of *P. pavonia* and *H. clathratus*, respectively and their yields are presented in **Table 5**.

Variation in the quali- and quantitative composition of *P. pavonia* and *H. clathratus* polysaccharides from other cited data (Abdel-Fatah and Edrees 1977; Khafaji 1986, 1992) may be due to environmental variations such as season, month, water depth, age of algae, time and area of collection.

Cytotoxicity of the isolated polysaccharides of *P. pavonia* and *H. clathratus*

Neither cold nor hot aqueous polysaccharide extracted from *P pavonia* and *H. clathratus* exhibited a cytotoxic effect against cultured U251 (**Table 6, Fig. 1**).

On the other hand, the hot aqueous polysaccharide extract of *P. pavonia* showed cytotoxic activity against cultured HepG2 *in vitro* with a CD_{50} of 0.84 µg/ml (**Table 7, Fig. 2**).

Furthermore, both cold and hot water polysaccharide extracts isolated from *H. clathratus* exhibited cytotoxic activity with nearly same effect against cultured HepG2. However, hot water extract showed more potent activity at 1 and 2.5 µg/ml than the cold water extract (**Table 7, Fig. 2**).

The fractions isolated from hot aqueous polysaccharide extract of *P. pavonia* were cytotoxic against cultured HepG2 *in vitro* (**Tables 8, 9** and **Figs. 3, 4**). Fractions II and

Table 7 Cytotoxic activity of cold and hot aqueous polysaccharide extracts of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe against cultured HepG2 human cells.

Drug / **Conc. µg/ ml**	**% of Inhibition** ±SEM				**ED$_{50}$**
	1	**2.5**	**5**	**10**	
Doxorubicin	64.9 ± 0.11*	90.7 ± 0.11*	86.9 ± 0.15*	95 ± 0.10*	0.8
Cold aqueous polysaccharide extract of *P. pavonia*	-75.6 ± 0.23* **	-35.9 ± 0.05* **	-10.4 ± 0.15 * **	9.35 ± 0.02* **	-
Hot aqueous polysaccharide extract of *P. pavonia*	55.62 ± 0.10* **	56.62 ± 0.05* **	58.09 ± 0.02* **	58.07 ± 0.01* **	0.84
Cold aqueous polysaccharide extract of *H. clathratus*	18.8 ± 0.2* **	28.24 ± 0.05* **	41.46 ± 0.09* **	40.74 ± 0.05* **	> 10
Hot aqueous polysaccharide extract of *H. clathratus*	29.2 ± 0.08* **	42.4 ± 0.11* **	41.1 ± 0.15* **	41.9 ± 0.2* **	> 10

Each value represents the mean of percentage of inhibition cells of three replicates ± SEM (Standard Error of Mean)
* Significantly different from control value at $p < 0.05$ according to paired-samples *t*-test
** Significantly different from Doxorubicin value at $p < 0.05$ according to paired-samples *t*-test

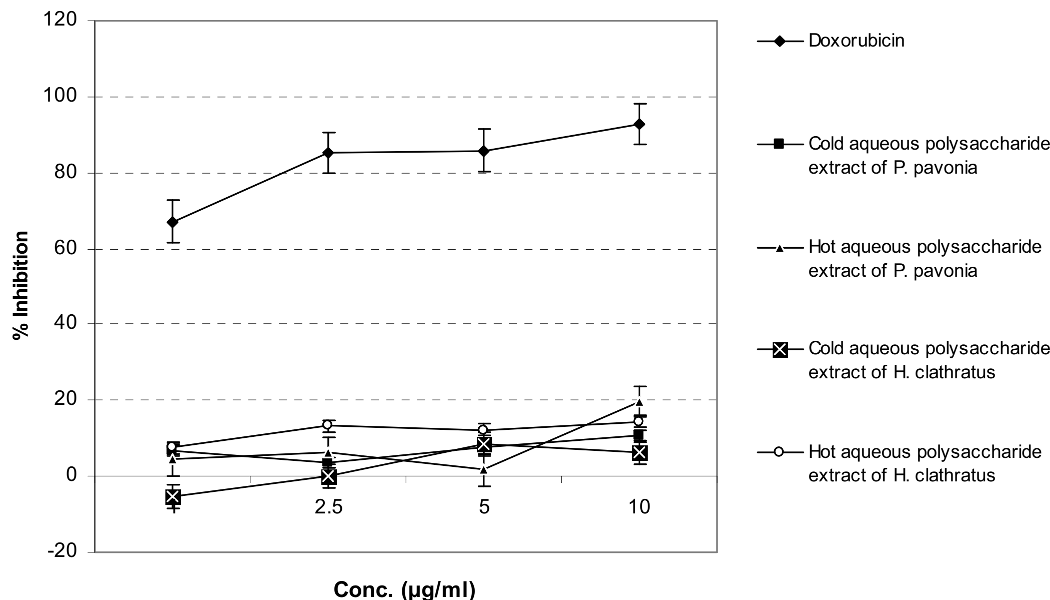

Fig. 1 Cytotoxic activity of cold and hot aqueous polysaccharide extracts of *P. pavonia* and *H. clathratus* against U251 *in vitro*.

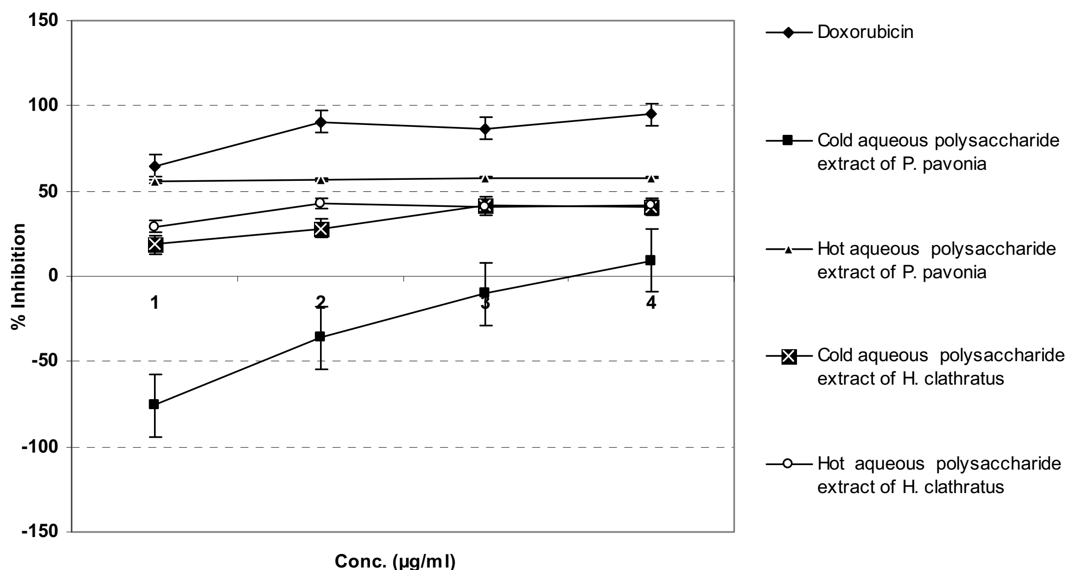

Fig. 2 Cytotoxic activity of cold and hot aqueous polysaccharide extracts of *P. pavonia* and *H. clathratus* against HepG2 *in vitro*.

III showed potent cytotoxicity in comparison with the reference Doxorubicin. Also, fraction I at 10 µg/ml and fraction IV at 5 and 10 µg/ml showed high cytotoxic activity while other tested concentrations exhibited good cytotoxic activity against cultured HepG2 *in vitro*. On the other hand, the survival of cells in fractions I-IV was reduced to 50% at 5.3, 0.9, 0.9 and 1.5 µg/ml, respectively.

Fractions I-V isolated from the hot water polysaccharide extract of *H. clathratus* were cytotoxic against cultured HepG2 *in vitro* (**Table 9, Fig. 4**). Fractions I, III and V at 5 and 10 µg/ml exhibited high cytotoxic activity in comparison with the reference Doxorubicin while fractions II and IV showed good cytotoxic activity. The survival of cells in fractions I-IV was reduced to 50% at 0.5, 3.5, 3.3, 2 and 0.5 µg/ml, respectively.

Wang *et al.* (2008b) reported that the aqueous extracts of *H. clathratus* and *P. arborescens* showed a higher inhibitory effect on the growth of HL-60 and MCF-7 cell lines. In addition, the extract of *H. clathratus* caused morphological alteration of MCF-7 cells. Also some polysaccharide fractions isolated from *H. clathratus* had antiproliferative activity on both cell lines in *vitro* and suppressing tumour growth of Sarcoma 180 tumour-bearing BALB/C mice in *vivo*.

Table 8 Cytotoxic activity of fractions yielded from fractionation hot aqueous polysaccharide extract of *Padina pavonia* (L.) Gaill. against cultured HepG2 human cells *in vitro*

Conc.	Percentage of inhibition ± SEM					Relative inhibition			
µg/ml	Fraction I	Fraction II	Fraction III	Fraction IV	Doxorubicin	Fraction I	Fraction II	Fraction III	Fraction IV
1	38.9 ± 1.3* **	59.4 ± 0.08* **	61.4 ± 0.20* **	34.1 ± 0.11* **	64.9 ± 0.11*	59.9	91.5	94.6	52.5
2.5	31.2 ± 0.81* **	94.9 ± 0.11* **	75.3 ± 0.20* **	67.9 ± 0.11* **	90.7 ± 0.11*	34.3	104.6	83.0	74.8
5	44.9 ± 0.4* **	85.2 ± 0.05* **	70.7 ± 0.15* **	78.4 ± 0.05* **	86.9 ± 0.15*	51.6	98.0	81.3	90.2
10	87.2 ± 0.17* **	111.6 ± 0.05* **	64.8 ± 0.23* **	73.3 ± 0.11* **	95.0 ± 0.10*	91.7	117.4	68.2	77.1
ED_{50}	5.3 µg/ml	0.9 µg/ml	0.9 µg/ml	1.5 µg/ml	0.8 µg/ml	-	-	-	-

Each value represents the mean of percentage of inhibition cells of three replicates ±SEM (Standard Error of Mean)
* Significantly different from control value at $p < 0.05$ according to paired-samples *t*-test
** Significantly different from Doxorubicin value at $p < 0.05$ according to paired-samples *t*-test

The activity was evaluated according to the inhibition growth related to Doxorubicin. Activity > 75%: high, 75-50 %: good, 50-25 %: normal and <25%: weak activity.

Table 9 Cytotoxic activity of fractions yielded from fractionation hot aqueous polysaccharide extract of *Hydroclathrus clathratus* (C. Agardh) Howe against cultured HepG2 human cells *in vitro*

Conc.	Percentage of Inhibition*						Relative Inhibition				
(µg/ml)	Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V	Doxorubicin	Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V
1	84.9 ± 0.17* **	37.5 ± 0.10* **	31.8 ± 0.10* **	42.0 ± 0.15* **	64.2 ± 0.10*	64.9 ± 0.11*	130.8	57.7	48.9	64.7	98.9
2.5	84.7 ± 0.10* **	46.9 ± 0.17* **	41.5 ± 0.11* **	53.1 ± 0.05* **	69.0 ± 0.17* **	90.7 ± 0.11*	93.3	51.7	45.7	58.5	76.0
5	81.0 ± 0.20* **	52.6 ± 0.20* **	65.6 ± 0.20* **	48.0 ± 0.26* **	67.9 ± 0.20* **	86.9 ± 0.15*	93.2	60.5	75.4	55.2	78.1
10	85.2 ± 0.11* **	58.8 ± 0.20* **	98.9 ± 0.11* **	52.6 ± 0.11* **	74.7 ± 0.05* **	95.0 ± 0.10*	89.6	61.8	104.1	55.3	78.6
ED_{50}	0.5 µg/ml	3.5 µg/ml	3.3 µg/ml	2.0 µg/ml	0.5 µg/ml	0.8 µg/ml	-	-	-	-	-

Each value represents the mean of percentage of inhibition cells of three replicates ±SEM (Standard Error of Mean).
* Significantly different from control value at $p < 0.05$ according to paired-samples *t*-test
** Significantly different from Doxorubicin value at $p < 0.05$ according to paired-samples *t*-test

The activity was evaluated according to the inhibition growth related to Doxorubicin. Activity > 75%: high, 75-50 %: good, 50-25 %: normal and <25%: weak activity.

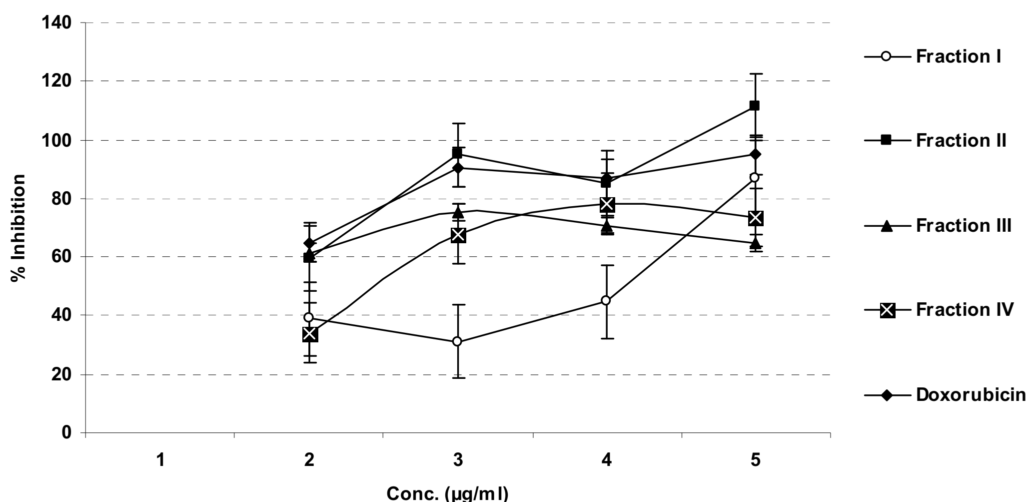

Fig. 3 Cytotoxic activity of fractions yielded from fractionation hot aqueous polysaccharide extract of *P. pavonia* against HepG2 *in vitro*.

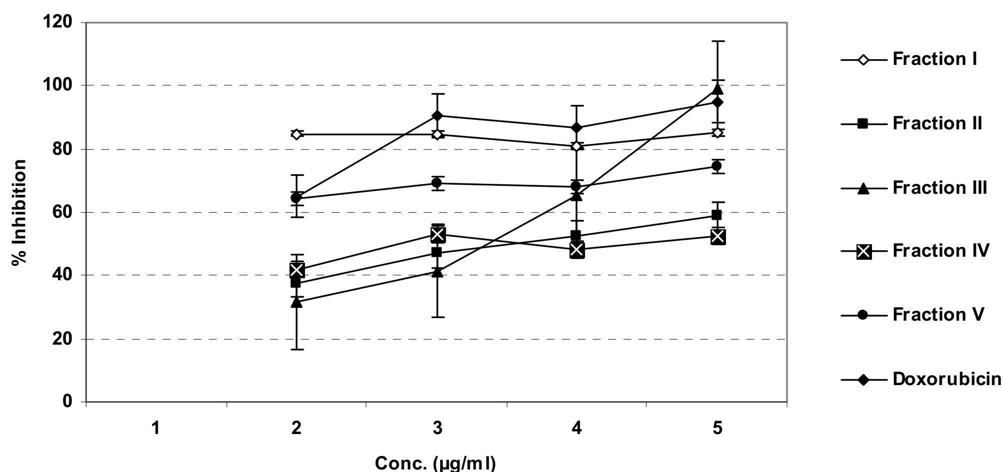

Fig. 4 Cytotoxic activity of fractions yielded from fractionation hot aqueous polysaccharide extract of *H. clathratus* against HepG2 *in vitro*.

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