Extraction of Sulphated Polysaccharides (SPs) from Different Species of Marine Macroalgae and Studying their Role as Natural Anticoagulant

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Abstract:

Marine algae are the most important source of non-animal sulphated polysaccharides which possess important pharmacological activities such as anticoagulant, antioxidant, anti-inflammatory, antiviral, antibacterial agents. Therefore, marine algae derived sulphated polysaccharides have great potential to be further developed as medicinal products. Marine macroalgae were collected seasonally for one year (September 2013 to August 2014) from the intertidal zone of site I: Ras El-Adabiya which located on the western shore of Suez Bay and site II: Ras Sedr which located north–east of the Gulf of Suez. The purpose of this study was to extract sulphated polysaccharides from marine macroalgae (*Ulva lactuca, Codium dwarkense, Hypnea cornuta*, *Hormophysa triquetra*, *Sargassum denticulatum* and *Cystoseira myrica*) by two different methods (hot and cold water extracts) and chemical analysis also done (protein content, total sugar , sulphate content , sulfer and nitrogen and uronic acid). The results of the physico-chemical parameters for sea water samples showed that temp ranged between 15-30 °C, pH 7.8-8.7, salinity 38-42 **‰** , DO 6-7 mg/L, NO_3 0.021-4.6 mg/L, NO_2 0.004 -0.032 mg/L, NH_4 0.019-0.3 mg/L and PO_4 0.005-0.015 mg/L. The potential of use sulphated polysaccharides as natural anticoagulant was tested by the activated partial thromboplastin time (APTT) and the prothrombin time (PT) tests. The results showed that higher blood anticoagulant activity of SPs is proportional to the carbohydrate and sulphate contents and inversely proportional to the protein and uronic acid contents. So, The significant highest value of prothrombin time was **26.50±0.10** sec at conc. of 20% SPs cold extract from the brown alga *Hormophysa triquetra*, while the lowest value was **1.13±0.06** sec at conc. of 5% SPs hot extract of the red alga *Hypnea cornuta*. Also, the results indicated that the highest value of activated partial thromboplastin time **42.20±0.10** sec was recorded at conc. of 20% SPs cold extract from the brown alga *Hormophysa triquetra*, On the other hand, the lowest value **5.63±0.25** sec was recorded at conc. of 5% SPs hot extract from the red alga *Hypnea cornuta* .

Keywords: Ras sedr, Ras El-adabiya, Marine Macroalgae, Sulphated polysaccharides , blood anticoagulation activity.

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1. Introduction and Review of Literature:

Marine algae are ecologically important and have been used as food and medicines for centuries. Different species of marine algae provide not only food but also produce extracts are used in numerous food, dairy, pharmaceutical, cosmetic, and industrial applications **(Raja *et al*.,2013).**

Marine algae are considered as valuable sources of structurally diverse bioactive compounds.Sulfated polysaccharides (SPs) in marine algae such as carrageenans in red algae, fucoidans in brown algae and ulvans in green algae exhibit many health beneficial nutraceutical effects such as antioxidant, anti-allergic, anti-human immunodeficiency virus, anticancer, antibacterial and anticoagulant activities. Therefore, marine algae derived SPs have great potential to be further developed as medicinal food products or nutraceuticals in the food industry **(Ngo and Kim , 2013).).**

Ulvan is the major water-soluble polysaccharide found in green seaweed of the order Ulvales (*Ulva* spp. and *Enteromorpha* spp.) that has sulfate, rhamnose, xylose, iduronic and glucuronic acids as main constituents **(Lahaye and Ray ,1996)** .

Costa *et al.*(2011) obtained five sulfated heterofucans from *Sargassum filipendula* by proteolytic digestion followed by sequential acetone precipitation, which displayed considerable antioxidant potential**.**

The most widely studied bioactivity of marine sulfated polysaccharides is the heparin-like anticoagulant. The blood coagulation system consists of intrinsic and extrinisic pathways, where a series of factors involve in the mechanism. Blood coagulation is proceeded by coagulation factors in order to stop the flow of blood though the injured vessel wall whenever, an abnormal vascular condition and exposure to non-endothelial surfaces at sites of vascular injury occurred. As endogenous or exogenous anticoagulants interfered with the coagulation factors by inactivate or restrict, the blood coagulation can be prolonged or stopped (**Jung *et al*.,2001).**

Patel (2012) reported that batteries of assays for assessment of anticoagulation properties of SPs from seaweeds have been conducted in recent times. Tests ranging from activated partial thromboplastin time (APTT), thrombin time (TT) and prothrombin time (PT).

Cold water extracts of all *Codium* species contain higher sugar and sulphate contents and lower protein and uronic acid contents. Also, cold water extracts exhibited higher blood anticoagulant activity than that of hot water extract. In *Udotea* spp. hot water extracts showed relatively higher blood anticoagulant activity which contained higher sugar and sulphate contents and lower protein and uronic acid contents. Hence, it can be inferred that higher blood anticoagulant activity is associated with relatively higher contents of sugar and sulphate and lower protein and uronic acid contents **(Shanmugam *et al.*, 2002)**.

The anticoagulant activity of SPs has been identified from several brown algae such as *Padina gymnospora* **(Silva *et al*.,2005),** *Dictyota menstrualis* **(Albuquerque *et al*.,2004)** and *Sargassum stenophyllum* **(Duarte *et al.*, 2001).** Also, SPs from green algae such as *Monostroma nitidum* were potent thrombin inhibitors **(Mao *et al*.,2008).** Moreover, **Pushpamali *et al.*(2008)** isolated a highly sulfated (21.76 %) anticoagulant from fermented red seaweed *Lomentaria catenata*.

Mao et *al* .*(*2006) mentioned a sulfated polysaccharide from *Ulva conglobata* with high rhamnose content and 35% sulfate ester that prolonged clotting time through what appeared to be direct inhibition of thrombin **.**

The hot water extract of green alga *Monostroma latissimum* gives a sulfated rhamnan polysaccharide with an anticoagulant activity. The anticoagulant activity as evaluated by assays of the APTT and thrombin time promises that it can be a potential source of anticoagulant **(Li *et al*. 2011).**

It was observed that anticoagulant activity was higher in SPs samples with higher sulfate content. In this regard, *Codium vermilara* proved to be superior with a higher degree of sulfation and arabinose content **(Ciancia *et al.* 2007**).

In the prothrombin time (PT) test, which evaluates the extrinsic coagulation pathway, *Caulerpa cupresoides* showed aggression. *Codium fragile* and *Codium vermilara* water-soluble sulfated arabinogalactans prevented coagulation **(Ciancia *et al.* 2007**).

Hayakawa *et al.* (2000) tested sulfated polysaccharides from 23 green algae species for anticoagulant activity and discovered a high rhamnose-containing sulfated polysaccharide from *Monostroma nitidum*, the purified version of which was more potent than standard heparin

Pereira *et al.* (2002) reported that heparin has been identified and used for more than fifty years as commercial anticoagulant and it is widely used for prevention venous thromboembolic disorders. However, several side effects of heparin have been reported such as development of thrombocytopenia, hemorrhagic effect, and ineffectiveness in congenital or acquired antithrombin deficiencies and incapacity to inhibit thrombin bound to fibrin.

Therefore, six marine macro algae were selected to extract sulphated polysaccharides and emphasize the use of them as natural anticoagulants with high efficiency. This is considered as an attempt to get alternative sources for manufacture of novel and natural anticoagulant drugs.

2. Materials and Methods:

The marine macroalgae were collected from the intertidal zone of site I: Ras El-Adabiya which located on the western shore of Suez Bay at latitude, longitude (29.681737, 32.508970) respectively and site II: Ras Sedr which located north–east of the Gulf of Suez at latitude, langitude (29.622328, 32.687970) respectively and identified according to **Papenfuss (1989)** as the following (*Ulva lactuca*, *Codium dwarkense* **(Naser)** (Chlorophyceae), *Sargassum denticulatum* **(BØrgesen)** , *Hormophysa triquetra* (**Kützing**) and *Cystoseira myrica* (**C.Agardah)** (Phaeophyceae) and *Hypnea cornuta* **(Rayss and Dor)** (Rhodophyceae), as shown in plate **(1).**

A- Physico-chemical parameters for sea water samples were done:

1) Temperature
2) Hydrogen – ion concentration (pH): pH was measured in situ by a pH meter.

3) Dissolved oxygen (DO): DO was determined by Winkler method **(Strickled and parson ,1968).**

4) Water Salinity : Water salinity was measured in the laboratory using an inductive salinometer (S.C.T. meter) Model 33.

5**)** Determination of ammonium (NH_4) and nitrate (NO_3): Ammonium and nitrate were determined calorimetrically by using UV/Visible spectrophotometer at wave length 400 and 430 nm respectively **(ASTM, 2002)**.

6) Determination of nitrite (NO_2**)** and phosphate (PO_4): Nitrite and phosphate determined colorimetrically by using UV/Visible spectrophotometer at wave length of 430 and 880 nm respectively **(ASTM, 2002).**

B- Biological parameters (Photosynthetic pigments):

1) Chlorophyll's calculations: Calculations of chlorophyll (a), (b) and (c) concentrations were measured according to **APHA (1995)**

2) Carotenoids calculations: The spectrophotometric measurements at 510 and 480 were used to calculate carotenoids according to **(Timothy *et al.*,2013) .**

Extraction of sulphated polysaccharides by method of Subash *et al.*(2010) from six marine algal species .

1) Hot water extract :

-Algal powders were depigmentated and defatted by methanol in a soxhlet apparatus .

-100 gm of defatted and depigmentated powder of each algae was extracted with 300 ml of distilled water (90-95) °C for 3 hr.

-The coloured syrup filtered through whatman no.3 paper.

-Then concentrated to 1/4 th of the original volume .

-Cooled and precipitated with 100 ml of ethanol .

-The precipitate was collected by centrifugation for 30 min then dehydrated with diethyl ether and then dried at 37°C until diethyl ether free.

-Add 30 ml ethyl acetate then filter and take the residue .

2) Cold water extract:

The same procedure without heating but the 100 gm of defatted and depigmentated powder of each algae was soaked with 300 ml of distilled water at 4°C over night. The yield of sulphated polysaccharide (SPs) extracts was calculated on the basis of the dry weight of algal sample (100gm). The pH of SPs extracts was determined using pH meter, Orion 210 .

***Sargassum denticulatum* (BØrgesen)**

***Cystoseira myrica* (C.Agardah)**

***Hormophysa triquetra* (Kützing)**

Ulva lactuca

***Codium dwarkense* (Naser)**

***Hypnea cornuta* (Rayss and Dor)**

Plate (1)

Analysis of the sulphated polysaccharides extracts :

1) Protein content: Protein content was measured spectrophotometically using the folin –phenol reagent according to the method described by **Lowry *et al.* (1951)** using albumin as a standard .
2) Total sugar: The spectro-photometric method described **by Dubois *et al.*(1956)** was used to determine carbohydrates **.**
3) Sulphate content: Sulphate content of the SPs sample was estimated by hydrolysing the sample with 1.0 N HCL and measured turbidimetrically by $BaCl_2$ gelatin method **(Dodgson and price, 1962).**
4) Sulfer and nitrogen analysis: were done on Perkin-Elemer Series II-2400 CHNS/O Analyzer .
5) Uronic acid content: Uronic acid content was determined by the method of **Knutson and Jeans (1968).**

Blood anticoagulation activity of the sulphated polysaccharides:

The *in vitro* anticoagulant activity of the SPs extracts was evaluated by the activated partial thromboplastin (APTT) and prothrombin time (PT) coagulation assays with coagulometer coatron M1.

1) Normal blood plasma was made from individual healthy donor ,without history of bleeding or thrombosis with PT 13 sec and APTT 30 sec.

2) Algal SPs samples were dissolved in normal saline (0.85%) **(Shanmugam *et al.*, 2001)** and three different concentrations (5% ,10% and 20%) from each extract were used for assays. All coagulation assays were performed with three replicates

3. Results and Discussion

3.1 Results

A-Physico-chemical parameters of sea water samples:

The seasonal variations of physico-chemical characteristics of sea water samples are (Temp , pH, DO ,water Salinity, NO_3,NO_2, NH_3 and PO_4) for Site I: Ras El-Adabiya and Site II: Ras Sedr. The results showed that temp ranged between 15-30 °C , pH 7.8 - 8.7,salinity 38- 42 **‰** , DO 6-7 mg/L, NO_3 0.021 - 4.6 mg/L, NO_2 0.004 - 0.032 mg/L, NH_4 0.019 - 0.3 mg/L and PO_4 0.005 - 0.015 mg/L.

B-Biological parameter (Photosynthetic pigments):

1) Chlorophyll (a) content (Fig. 1)

The highest concentration of chlorophyll (a) was **17.46** µg/L in *Codium dwarkense* , while the lowest value of **6.9** µg/L in *Hypnea cornuta.*

2) Chlorophyll (b) content (Fig. 2)

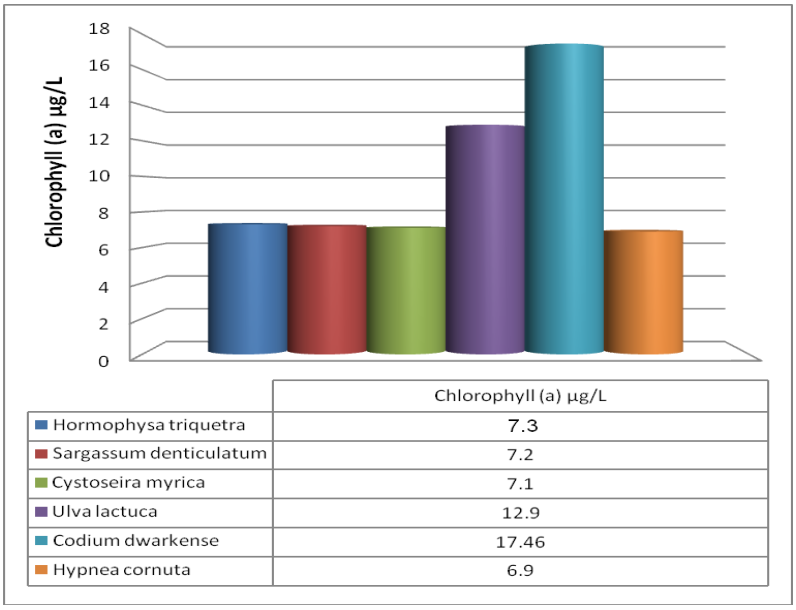
The highest concentration of chlorophyll (b) was **24.9** µg/L in *Ulva lactuca* , while the lowest value of **9.1** µg/L in *Sargassum denticulatum* .

3) Chlorophyll (c) content (Fig. 3)

The highest concentration of chlorophyll (c) was **12.9** µg/L in *Cystoseira myrica* , while the lowest value of **11.3** µg/L in *Hormophysa triquetra* .

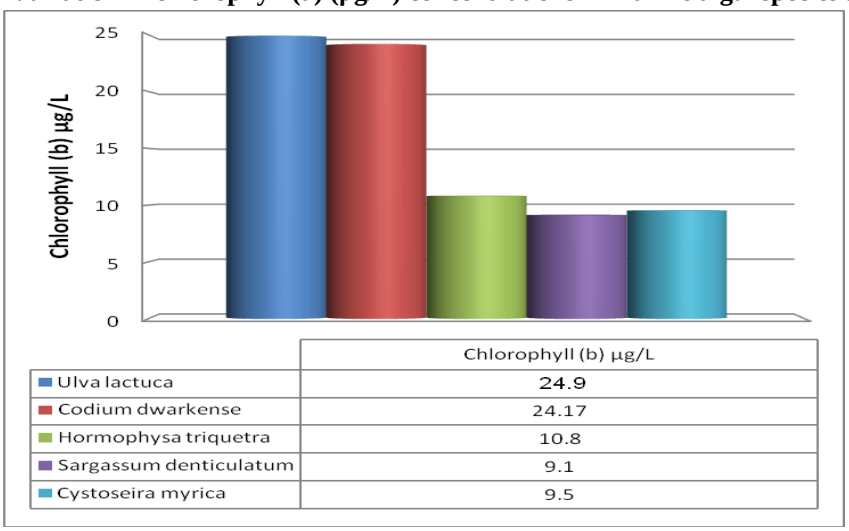
4) Carotenoids content (Fig. 4)

The highest concentration of carotenoids was **4.65** µg/L in *Codium dwarkense* , while the lowest value of **3.26** µg/L in *Cystoseira myrica* .



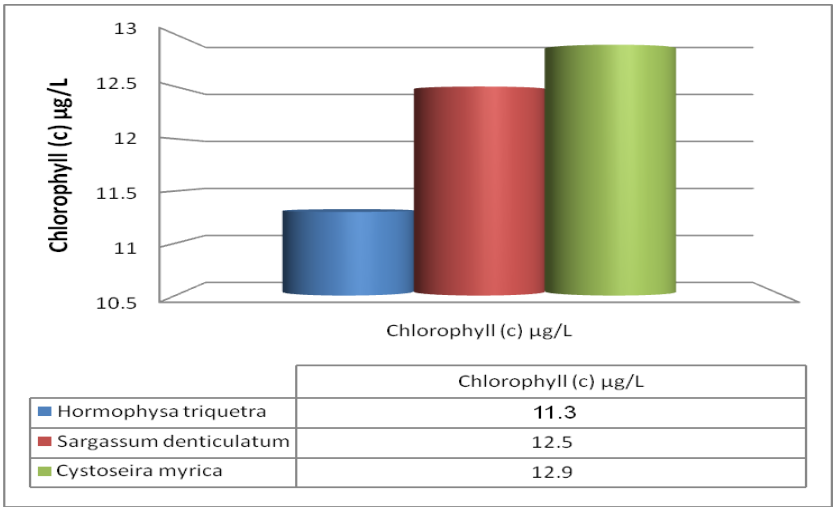
	Chlorophyll (a) µg/L
Hormophysa triquetra	7.3
Sargassum denticulatum	7.2
Cystoseira myrica	7.1
Ulva lactuca	12.9
Codium dwarkense	17.46
Hypnea cornuta	6.9

Fig.1: Variation in chlorophyll (a) (µg/L) concentrations in marine algal species .



	Chlorophyll (b) µg/L
Ulva lactuca	24.9
Codium dwarkense	24.17
Hormophysa triquetra	10.8
Sargassum denticulatum	9.1
Cystoseira myrica	9.5

Fig.2: Variation in Chlorophyll (b) concentrations (µg/L) in marine algal species .



	Chlorophyll (c) µg/L
Hormophysa triquetra	11.3
Sargassum denticulatum	12.5
Cystoseira myrica	12.9

Fig .3: Variation in chlorophyll (c) concentrations (µg/L) in brown marine algal species.

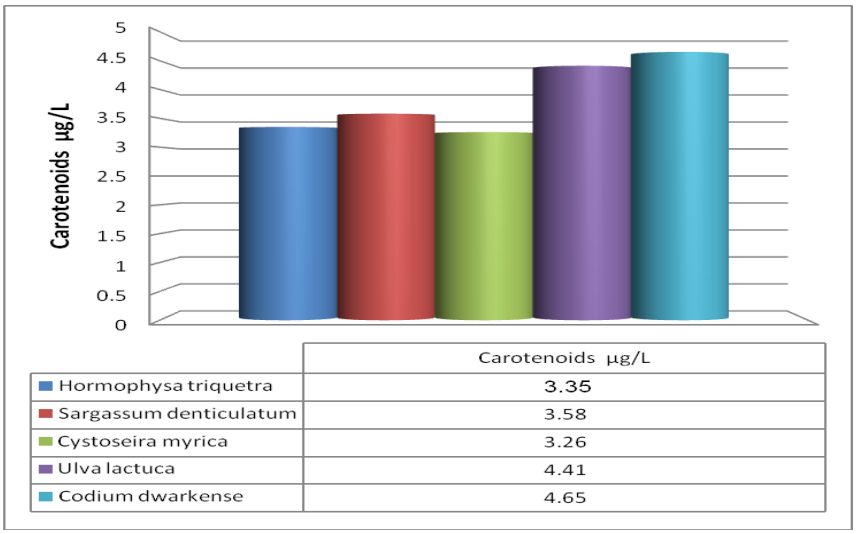


Fig .4: Variation in carotenoids concentrations (µg/L) in marine algal species.

Extraction of Sulphated polysaccharides (SPs) by hot and cold method :

1)The yield of SPs cold % (Fig. 5)

The yield of SPs cold extracts is relatively higher than hot extracts . In cold extracts the highest value was **7 %** in *Hormophysa triquetra* ,while the lowest value was **5 %** in *Hypnea cornuta.* On the other hand the maximum value in hot extracts was **6 %** in *Hormophysa triquetra* and the minimum value was **4.3 %** in *Hypnea cornuta* .

2)Chemical properties of Sps:

- Hydrogen ion concentration pH (Fig. 6)

pH values of SPs in cold and hot extracts were always in the alkaline side. . In cold extracts the maximum value was **7.7** in *Sargassum dentifolium,* while the minimum value was **7.1** in *Ulva lactuca.* On the other hand the maximum value in hot extracts was **7.9** in *Hormophysa triquetra* and the minimum value was **7.2** in *Codium dwarkense* .

- Total sugar % (Fig.7)

The cabohydrate concentrations of the SPs cold and hot extracts. In cold extracts the highest concentration value was **54.2 %** in *Hormophysa triquetra* ,while the lowest concentration value was **44.3 %** in *Hypnea cornuta.* On the other hand the maximum concentration value in hot extracts was **53.2 %** in *Hormophysa triquetra* and the minimum concentration value was **42.5 %** in *Hypnea cornuta* .

- Sulphate content % (Fig.8)

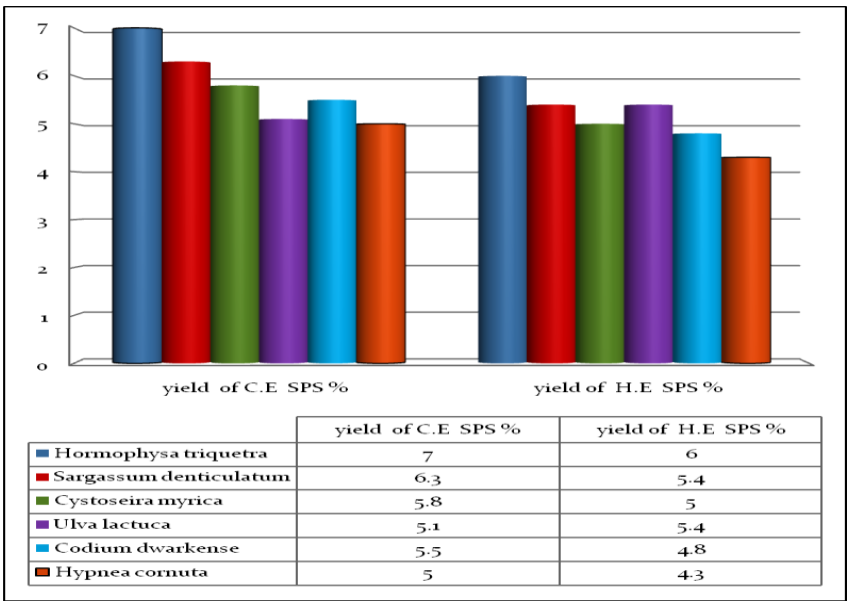
The sulphate concentrations of the SPs cold and hot extracts . In cold extracts the maximum concentration value was **25.7 %** in *Hormophysa triquetra* ,while the minimum concentration value was **20.4 %** in *Hypnea cornuta* .On the other hand the maximum concentration value in hot extracts was **24.4 %** in *Hormophysa triquetra* and the minimum concentration value was **19.1 %** in *Hypnea cornuta* .

- Sulphur content % (Fig. 9)

The sulphur concentrations of the SPs cold and hot extracts . In cold extracts the maximum concentration value was **7.92 %** in *Hormophysa triquetra* ,while the minimum concentration value was **5.11 %** in *Hypnea cornuta* .On the other hand the maximum concentration value in hot extracts was **6.92 %** in *Hormophysa triquetra* and the minimum concentration value was **4.65%** in *Hypnea cornuta*.

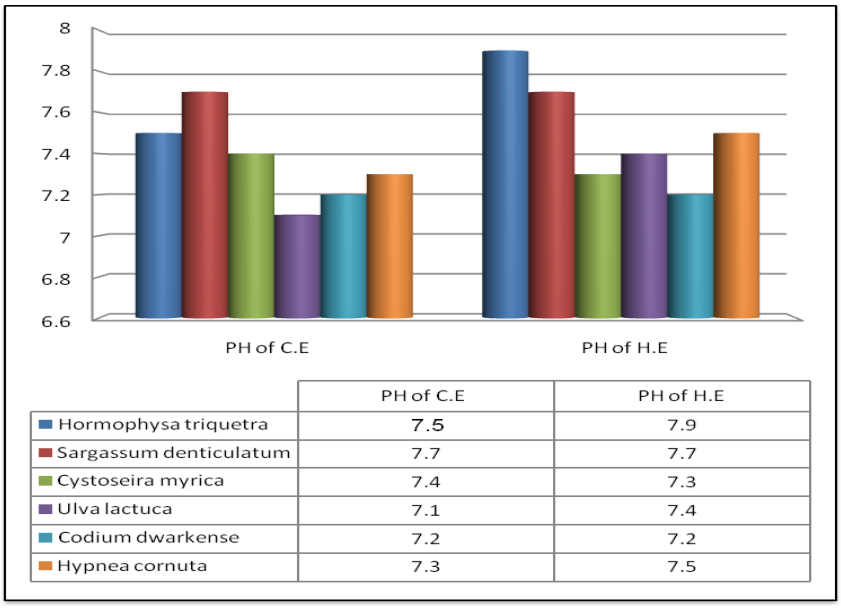
- Protein % (Fig. 10)

The protein concentrations of the SPs cold and hot extracts . In cold extracts the maximum concentration value was **10.2%** in *Hypnea cornuta*, while the minimum concentration value was **8.4 %** in *Hormophysa triquetra*. On the other hand the maximum concentration value in hot extracts was **8.1%** in *Hypnea cornuta* and the minimum concentration value was **6.3 %** in *Hormophysa triquetra* .



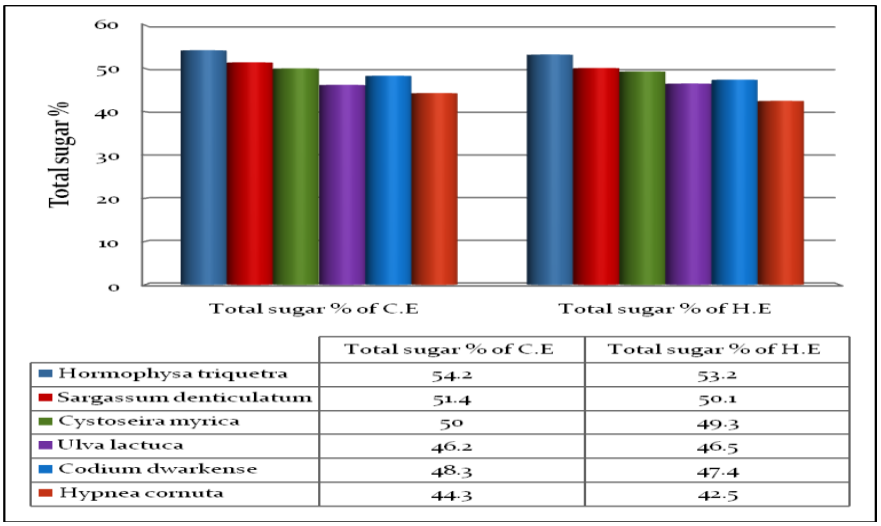
	yield of C.E SPS %	yield of H.E SPS %
Hormophysa triquetra	7	6
Sargassum denticulatum	6.3	5.4
Cystoseira myrica	5.8	5
Ulva lactuca	5.1	5.4
Codium dwarkense	5.5	4.8
Hypnea cornuta	5	4.3

Fig.5: Variations in the yield SPs of cold and hot extracts (%) for different marine algal species.



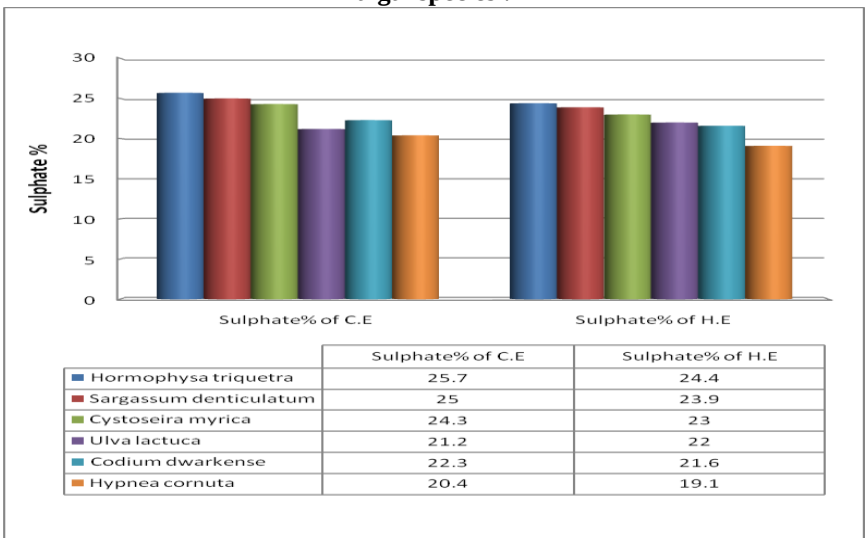
	PH of C.E	PH of H.E
Hormophysa triquetra	7.5	7.9
Sargassum denticulatum	7.7	7.7
Cystoseira myrica	7.4	7.3
Ulva lactuca	7.1	7.4
Codium dwarkense	7.2	7.2
Hypnea cornuta	7.3	7.5

Fig.6: pH variations in the yield SPs of cold and hot extracts for different marine algal species.



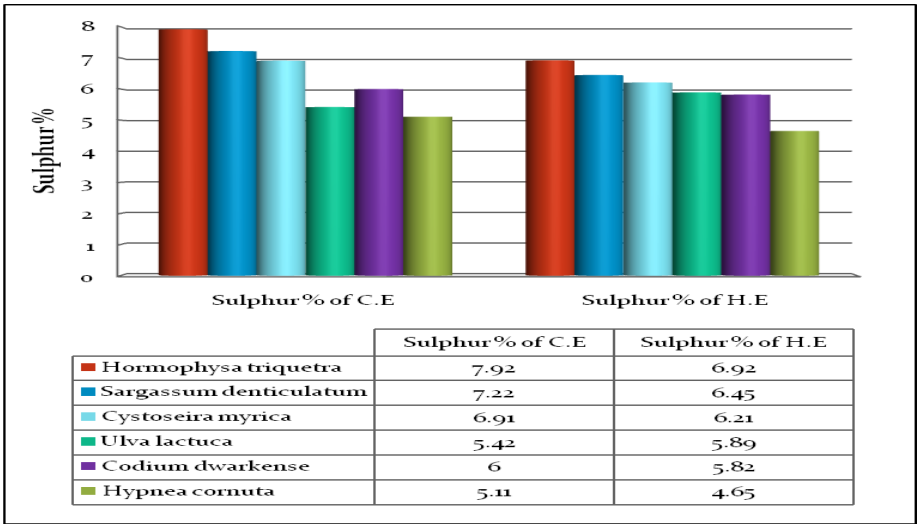
	Total sugar % of C.E	Total sugar % of H.E
Hormophysa triquetra	54.2	53.2
Sargassum denticulatum	51.4	50.1
Cystoseira myrica	50	49.3
Ulva lactuca	46.2	46.5
Codium dwarkense	48.3	47.4
Hypnea cornuta	44.3	42.5

Fig.7: Variations in total sugar content (%) in the SPs cold and hot extracts for different marine algal species .



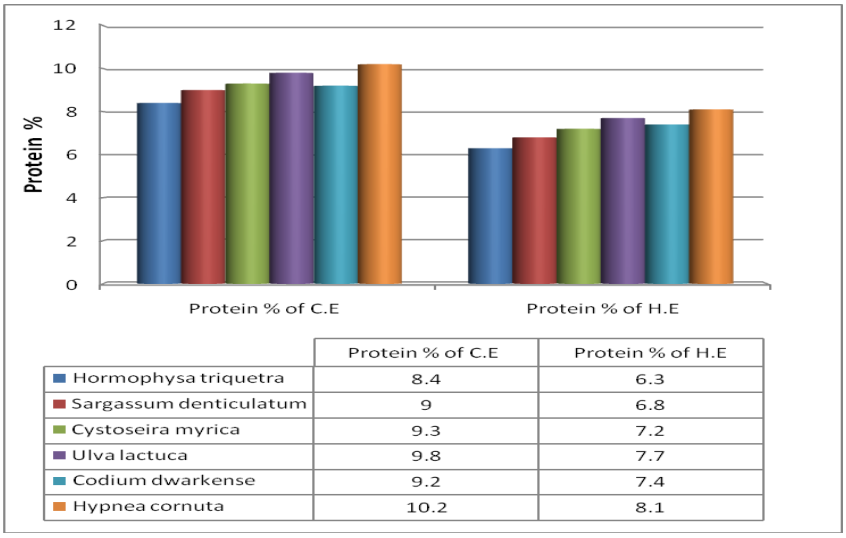
	Sulphate% of C.E	Sulphate% of H.E
Hormophysa triquetra	25.7	24.4
Sargassum denticulatum	25	23.9
Cystoseira myrica	24.3	23
Ulva lactuca	21.2	22
Codium dwarkense	22.3	21.6
Hypnea cornuta	20.4	19.1

Fig .8: Variations in sulphate content (%) in the SPs of cold and hot extracts for different marine algal species .



	Sulphur % of C.E	Sulphur % of H.E
Hormophysa triquetra	7.92	6.92
Sargassum denticulatum	7.22	6.45
Cystoseira myrica	6.91	6.21
Ulva lactuca	5.42	5.89
Codium dwarkense	6	5.82
Hypnea cornuta	5.11	4.65

Fig .9: Variations in sulphur content (%) in the SPs cold and hot extracts for different marine algal species .



	Protein % of C.E	Protein % of H.E
Hormophysa triquetra	8.4	6.3
Sargassum denticulatum	9	6.8
Cystoseira myrica	9.3	7.2
Ulva lactuca	9.8	7.7
Codium dwarkense	9.2	7.4
Hypnea cornuta	10.2	8.1

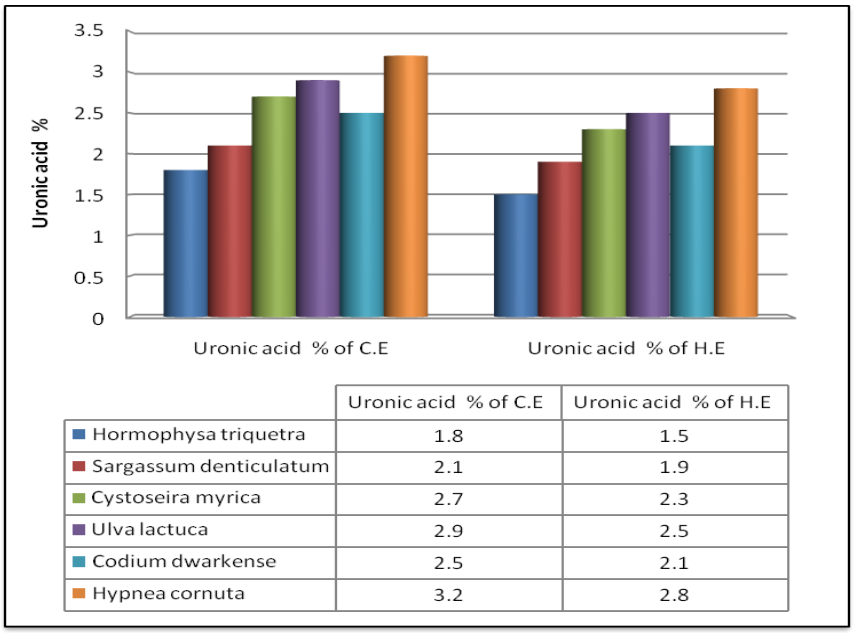
Fig .10: Variations in protein content (%) in the SPs cold and hot extracts for different marine algal species .

- Uronic acid % (Fig. 11)

The uronic acid concentrations of the SPs cold and hot extracts. In cold extracts the maximum concentration value was **3.2%** in *Hypnea cornuta* , while the minimum concentration value was **1.8%** in *Hormophysa triquetra*. On the other hand the maximum concentration value in hot extracts was **2.8%** in *Hypnea cornuta* and the minimum concentration value was **1.5 %** in *Hormophysa triquetra* .

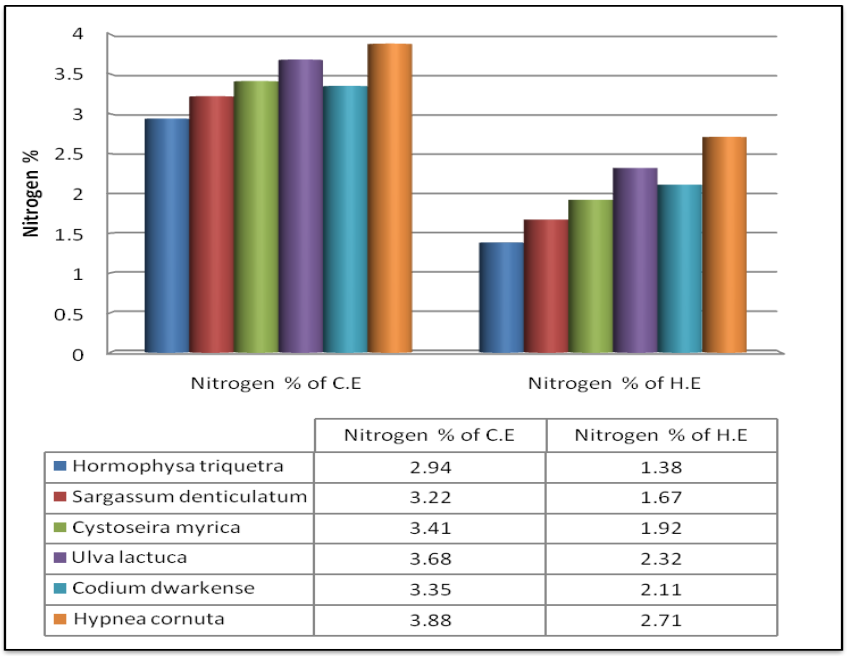
- Nitrogen content % (Fig. 12)

The nitrogen concentrations of the SPs cold and hot extracts. In cold extracts the maximum concentration value was **3.88 %** in *Hypnea cornuta* ,while the minimum concentration value was **2.94 %** in *Hormophysa triquetra*. On the other hand the maximum concentration value in hot extracts was **2.71%** in *Hypnea cornuta* and the minimum concentration value was **1.38%** in *Hormophysa triquetra*



	Uronic acid % of C.E	Uronic acid % of H.E
Hormophysa triquetra	1.8	1.5
Sargassum denticulatum	2.1	1.9
Cystoseira myrica	2.7	2.3
Ulva lactuca	2.9	2.5
Codium dwarkense	2.5	2.1
Hypnea cornuta	3.2	2.8

Fig .11: Variations in uronic acid content (%) in the SPs cold and hot extracts for six marine algal species .



	Nitrogen % of C.E	Nitrogen % of H.E
Hormophysa triquetra	2.94	1.38
Sargassum denticulatum	3.22	1.67
Cystoseira myrica	3.41	1.92
Ulva lactuca	3.68	2.32
Codium dwarkense	3.35	2.11
Hypnea cornuta	3.88	2.71

Fig .12: Variations in nitrogen content (%) in the SPs cold and hot extracts for different marine algal species .

Blood anticoagulation activity of the sulphated polysaccharides by using different conc. (20, 10 and 5%) from cold and hot SPs extracts :

1) The prothrombin time (PT) test (Table 1)

The significant highest value of prothrombin time was **26.50±0.10** sec at conc. 20% SPs cold extract of the brown alga *Hormophysa triquetra*, while the significant lowest value was **1.13±0.06** sec at conc 5% SPs hot extract of the red alga *Hypnea cornuta* .

2) The prothrombin activity (Table 2)

The significant highest value of prothrombin activity was **1429.0** at conc.5 % SPs hot extract of the red alga *Hypnea cornuta*, while the significant lowest value was **47.63±0.35** at conc. 20 % SPs cold extract of the brown alga *Hormophysa triquetra.*

3) International normalization ratio (Table 3)

The significant highest value of the international normalization was **2.11±0.01** at conc. 20% SPs cold extract of the brown alga *Hormophysa triquetra*, while the lowest value was **0.08±0.02** at conc. 5% SPs hot extract of the red alga *Hypnea cornuta* .

4) Activated partial thromboplastin time (APTT) test (Table 4)

The significant highest value of activated partial thromboplastin time **(42.20±0.10** sec) was recorded at conc. 20 % SPs cold extract of the brown alga *Hormophysa triquetra*, On the other hand, the lowest value **(5.63±0.25** sec) was at conc. 5% SPs hot extract in the red alga *Hypnea cornuta* .

Table 1: Effect of different concentrations (20,10 and 5 %) from cold and hot SPs extracts for six marine algal species on prothrombin time(sec) (Mean ± SD)

Extract type	Algal species	Concentrations (%)		
		20	10	5
Cold extract	*Hormophysa triquetra*	26.50±0.10	16.20±0.10	8.33±0.15
	Sargassum denticulatum	25.43±0.25	15.03±0.15	7.50±0.20
	Cystoseira myrica	23.43±0.15	13.50±0.10	5.40±0.20
	Codium dwarkense	20.80±0.10	11.20±0.20	4.03±0.06
	Ulva lactuca	16.40±0.10	8.80±0.10	2.13±0.15
	Hypnea cornuta	17.63±0.06	9.63±0.15	2.83±0.06
Hot extract	*Hormophysa triquetra*	24.50±0.20	14.40±0.30	6.13±0.06
	Sargassum denticulatum	22.33±0.25	12.33±0.06	5.10±0.10
	Cystoseira myrica	20.40±0.30	11.33±0.15	4.03±0.06
	Codium dwarkense	17.50±0.20	9.73±0.06	2.63±0.15
	Ulva lactuca	18.50±0.10	10.20±0.10	3.23±0.06
	Hypnea cornuta	15.73±0.06	7.73±0.15	1.13±0.06
F-test	Extract type	2845.82	***	
	Conc	106984.42	***	
	Species	5936.44	***	
	Extract type * conc	32.83	***	
	Extract type * species	470.28	***	
	conc * species	159.43	***	
	extract type *conc*species	23.82	***	

The statistical analysis shows that there was significance difference between the two methods of extrations (cold and hot); the three concentrations; the studied species and the interaction between the investigated variables at $P < 0.001$.

Table 2: Effect of different concentrations (20,10 and 5 %) from cold and hot SPs extracts for six marine algal species on prothrombin activity (%) (Mean ± SD)

Extract type	Algal species	Concentrations (%)		
		20	10	5
Cold extract	*Hormophysa triquetra*	47.63±0.35	79.33±0.58	160.00±3.61
	Sargassum denticulatum	49.50±0.50	86.00±1.00	179.33±5.13
	Cystoseira myrica	53.90±0.46	95.67±1.53	254.00±10.15
	Codium dwarkense	61.30±0.40	117.33±2.52	340.33±6.35
	Ulva lactuca	78.70±0.60	151.67±2.52	668.33±44.55
	Hypnea cornuta	72.57±0.58	138.33±3.06	509.33±28.87
Hot extract	*Hormophysa triquetra*	51.80±0.30	90.00±2.00	220.33±2.89
	Sargassum denticulatum	56.57±0.97	106.33±1.15	270.00±7.00
	Cystoseira myrica	62.47±0.75	115.67±1.53	340.33±6.35
	Codium dwarkense	73.50±1.10	135.67±0.58	547.67±44.46
	Ulva lactuca	69.23±0.29	129.33±1.53	428.67±10.97
	Hypnea cornuta	82.13±0.40	174.00±4.58	1429.00±0.00
F-test	Extract type	852.7756	***	
	Conc	10112.64	***	
	species	1370.546	***	
	Extract type * conc	624.8703	***	
	Extract type * species	535.7639	***	
	conc * species	905.1469	***	
	Extract type * conc*species	432.6893	***	

The statistical analysis illustrates that there was significance variation between the two procedures of extractions (cold and hot); the three concentrations; the studied species and the interaction between the investigated variables at $P < 0.001$

Table 3: Effect of different concentrations (20,10 and 5 %) from cold and hot SPs extracts for six marine algal species on the international normalization ratio time(sec) (Mean ± SD)

Extract type	Algal species	Concentrations (%)		
		20	10	5
Cold extract	*Hormophysa triquetra*	2.11±0.01	1.26±0.01	0.62±0.02
	Sargassum denticulatum	2.02±0.02	1.16±0.02	0.56±0.02
	Cystoseira myrica	1.85±0.02	1.04±0.02	0.39±0.02
	Codium dwarkense	1.63±0.01	0.85±0.02	0.29±0.01
	Ulva lactuca	1.27±0.01	0.66±0.01	0.15±0.01
	Hypnea cornuta	1.38±0.01	0.72±0.02	0.20±0.01
Hot extract	*Hormophysa triquetra*	1.93±0.01	1.11±0.02	0.45±0.01
	Sargassum denticulatum	1.77±0.03	0.94±0.01	0.37±0.01
	Cystoseira myrica	1.60±0.02	0.86±0.01	0.29±0.01
	Codium dwarkense	1.36±0.02	0.73±0.01	0.18±0.02
	Ulva lactuca	1.44±0.01	0.77±0.01	0.23±0.01
	Hypnea cornuta	1.21±0.01	0.57±0.02	0.08±0.02
F-test	Extract type	2220.696	***	
	conc	81308.06	***	
	species	4536.951	***	
	Extract type * conc	40.75362	***	
	Extract type * species	362.029	***	
	conc * species	157.5478	***	
	Extract type *conc*species	20.36522	***	

The statistical analysis illustrates that there was significance variation between the two procedures of extractions (cold and hot); the three concentrations; the studied species and the interaction between the investigated variables at $P < 0.001$

Table 4: Effect of different concentrations (20,10 and 5 %) from cold and hot SPs extracts for six marine algal species on activated partial thromboplastin time(sec) (Mean ± SD)

Extract type	Algal species	Concentrations(%)		
		20	10	5
Cold extract	*Hormophysa triquetra*	42.20±0.10	33.30±0.10	22.80±0.20
	Sargassum denticulatum	41.53±0.15	32.10±0.10	21.03±0.15
	Cystoseira myrica	39.70±0.10	30.43±0.06	17.50±0.20
	Codium dwarkense	37.53±0.25	28.43±0.15	14.73±0.06
	Ulva lactuca	33.80±0.10	23.40±0.10	8.10±0.10
	Hypnea cornuta	34.23±0.06	26.10±0.10	8.63±0.15
Hot extract	*Hormophysa triquetra*	40.43±0.15	31.80±0.20	19.20±0.10
	Sargassum denticulatum	38.23±0.15	29.73±0.15	17.00±0.10
	Cystoseira myrica	37.70±0.20	28.60±0.10	14.73±0.06
	Codium dwarkense	34.10±0.10	26.17±0.12	8.43±0.06
	Ulva lactuca	35.40±0.20	27.30±0.20	10.93±0.06
	Hypnea cornuta	32.43±0.06	21.20±0.10	5.63±0.25
F-test	Extract type		5747.445	***
	Conc		254639	***
	Species		15011.87	***
	Extract type * conc		222.7225	***
	Extract type * species		1394.637	***
	Conc * species		535.9263	***
	Extract type * conc *species		115.5722	***

The statistical analysis shows that there was significance difference between the two methods of extrations (cold and hot); the three concentrations; the studied species and the interaction between the investigated variables at $P < 0.001$

3.2 Discussion:

The present results of chlorophyll *(*a) concentrations (**Fig. 1)** were in agreement with that of **Kumar *et al.*(2009**) who mentioned that the highest chlorophyll *(*a) content was observed in the green alga *Cladophora fascicularis* followed by the green alga *Caulerpa seertulariodes* while the lowest chlorophyll *a* content was recorded in the red alga *Champia compressa*. Also, The results of the current study corroborated with the findings of **Francisco *et al*. (2006)** stating highest chlorophyll (*a)* content in green algae and lowest in red algae.

The current results of chlorophyll (b) concentrations (**Fig. 2**) were in agreement with that of **Kumar *et al.(*2009**) who observed that the richest in chlorophyll (b) content was the green alga *Cladophora fascicularis* ,While the lowest chlorophyll (b) content was noted in the brown alga *Sargassum polycustum* . Similar observations were incurred by **Chakraborty and Santra (2008)**.

The present results of chlorophyll (c) concentrations (**Fig. 3)**were in agreement with that of **Seely *et al.(*1972)** who reported that the brown algae of intertidal and subtidal zones of the shorelines are abundant source for chlorophyll (c).

The current results of carotenoids concentrations (**Fig. 4**) were corroborated with the findings of **Kumar *et al.(*2009)** who observed that the highest carotenoids were reported in *Sargassum polycustum* followed by *Padina gymnospora* both belonging to the Phaeophyta group. Also, **Fritsch (1971)** stated that there is high carotene content in brown algae .

The present results of the yield SPs cold and hot extracts (**Fig. 5)** were higher than found by **Shanmugam *et al.*(2001**) who reported that the yield ranged from **1.8 - 4.0**% because they used only green algae species. The present results for *codium dwarkense* in agreement with that of **Shanmugam *et al.*(2002)** who reported that the yields of cold water extract and hot water extract were **4.40 %** and **4.60 %** respectively. Also, **Siddhanta *et al.(*1999)** recorded that the yields of CWE and HWE were **4.4%** and **4.6%** respectively for the green marine alga *Codium dwarkense*.

The current results of pH for of the yield SPs cold and hot extracts (**Fig. 6**)were in agreement with that of **Shanmugam *et al.(*2001**) who recorded that all SPs products were slightly alkaline in nature (pH **7.1- 8.4**).

The present results of the total sugar for SPs cold and hot extracts (**Fig.7)** in agreement with that of **Siddhanta *et al.(*1999)** who reported that the total sugar contents in cold water extract were higher than hot water extract of *Codium dwarkense* . Also, **Shanmugam *et al.*(2001**) mentioned that the total sugar content in both cold and hot water extracts of some Indian marine green algae was >**20 %**. Furthermore, **Shanmugam *et al.(*2002**) recorded that the total sugar content was higher in all cold water SPs extracts of Codium species than in the hot water ones. Also, **Manoj *et al.*(2013)** mentioned that sugar content in SPs extracts of the brown algae *Turbinaria ornata* and *Padina tetrastromatica* were 56.42 and 50 % respectively.

The total sulphate content in all cold and hot water SPs extracts(**Fig. 8)** were in agreement with that of **Siddhanta et al.(1999)** who mentioned that the total sulphate content was higher in cold water extracts of *Codium dwarkense* species than in the hot water ones. Also, **Manoj *et al.(*2013)** corroborated the current results when recorded that *Sargassum wightii* and *Sargassum tenerrimum* showed high content of sulphate in SPs extracts.

Furthermore, **Wang *et al.(*2007)** reported that Sulphate content in SPs extracts from red algae *Grateloupia longifolia* and *Grateloupia filicin* were **18.5** and **25.7**% respectively. Also, **Siddhanta *et al.(*1999**) mentioned that sulphate were **26.50**% and 19.50% in SPs cold and hot extracts respectively. But these results were higher than that of **Vijayabaskar *et al .(*2012)** who recorded that sulphate content in SPs extract from the brown marine algae *Sargassum swartzii* was **5.3** %.

The present results of the sulfur content for SPs cold and hot extracts (**Fig. 9)** were in agreement with **Shanmugam *et al.*(2001)** who recorded that the sulphur content in cold and hot SPs extracts of some Indian marine green algae ranged between **2.61-6.18** %. But these results were higher than that obtained by **Vijayabaskar *et al .(*2012)** who recorded that sulphur content in SPs extract from the brown marine algae *Sargassum swartzii* was **0.98%** .

The current results of the protein content for SPs cold and hot extracts (**Fig. 10)** were in agreement with that of **Shanmugam *et al*.(2001)** who mentioned that species of *Ulva* and *Caulerpa* contained **7.0-10 %** protein in cold and hot SPs extracts. Moreover, **Siddhanta *et al*.(1999)** corroborated the current results when recorded that protein content in cold extract SPs of *Codium dwarkense* was **10.10**%. Also, **Shanmugam *et al.(*2002)** revealed that protein contents of cold water SPs extracts of all *Codium spp.* were higher than hot water ones.

The present results of uronic acid content for SPs cold and hot extracts (**Fig. 11)** were in agreement with that of **Shanmugam *et al.(*2001)** who mentioned that trace of uronic acid was found in SPs extracts of some Indian marine green algae ranged between **2-4** %. Also, **Shanmugam et al.(2002**) recorded that uronic acid content in *Codium* spp. ranged from **1.5** to **3.0** % .These results were lower than that found by **Karnjanapratum and You (2011)** who recorded that uronic acid content in the crude SPs of *Monostroma nitidum* was **18.6**% and also lower than that found by **Mao *et al.(*2006)** who reported that uronic acid contents in the sulfated polysaccharides extracted from *Ulva conglobata* ranged between **10.82**–**14.91**% this may be because they used different species of marine algae .

The current results of nitrogen content in SPs cold and hot extracts (**Fig. 12)** in agreement with that of **Shanmugam *et al.*(2001)** who reported that the concentration of nitrogen in cold and hot SPs extracts of some some Indian marine green algae ranged between **1.55- 4.25 %**. But these results were higher than that found by **Vijayabaskar *et al* .(2012)** who recorded that nitrogen content in SPs extract from the brown marine algae *Sargassum swartzii* was **0.15**%.

The present results (**Table 1)** in agreement with that of **Ferna´ndez *et al*. (2012)** who studied the anticoagulation efficacy of sulfated β-D-mannan extracted from green seaweed *Codium vermilara* and reported that higher sulfate content leads to more pronounced effect. As well, **Mulloy *et al*.(2000)** who reported that the high anticoagulant activity of algal fucans appears to correlate with the presence of sulphated fucose branches.

Qiu *et al*. (2006) reported that the anticoagulation activity improve with increase in sulphate content of the native fucoidan. Also, **Shanmugam *et al*. (2002)** corroborated the present results when reported that cold water SPs extract of *Codium tomentosum, Codium dwarkense* and *Codium tenue* containing comparatively lower uronic acid and higher sulphate showed stronger blood anticoagulant activity. As well, cold water SPs extracts of some of the species like *Codium geppei* and *Codium coronatum* containing less sulphate and more uronic acid showed relatively poor blood anticoagulant activity and that's what happened *Ulva lactuta* in the present study when the hot SPs extracts showed higher blood anticoagulant activity than the cold ones because they contain higher sulphate and sugar content.

Furthermore, **Uehara *et al* .(1992)** have reported that cold water extract from *Codium latum* of Japanese waters exhibited potent blood anticoagulant activity, whereas from the green alga *Monostroma nitidum* active fraction was obtained only by hot water extraction. This seems to be due to the difference in sugar and sulphate contents of the SPS. Similar finding was also reported by **Nishino and Nagumo (1992)** while working on *Ecklonia kurome*. Moreover, It was observed that the anticoagulant activity of *Codium dwarkense* is proportional to the carbohydrate and

sulphate contents and inversely proportional to the protein and uronic acid contents **(Siddhanta *et al.*,1999).**

The present results (**Table 2)** in agreement with that of **Croci *et al.* (2011)** who studied that the SPs from the brown seaweed *Laminaria saccharina* shows promising activity on thrombosis. Also, It was observed that anticoagulant activity was higher in SP samples with higher sulfate content. In this regard, *Codium vermilara* proved to be superior with a higher degree of sulfation and arabinose content **(Ciancia *et al*., 2007).**

The current results (**Table 3**) in agreement with that of **(Shanmugam *et al*., 2002)** who concluded that higher blood anticoagulant activity is associated with relatively higher contents of sugar and sulphate and lower protein and uronic acid contents. Also, **Cumashi *et al*.(2007**) reported that fucans from 10 brown seaweeds each prolonged the clotting time of human plasma; however, only five of these fucans had significant activity against thrombin induced platelet aggregation .

The present results(**Table 4)** in agreement with that of **Athukorala *et al*.(2007)** who mentioned that APTT activity of crude polysaccharide fraction increased with the sample concentration. Also, The high APTT values indicated that the crude SPs from *Sargassum tenerrimum* have high anticoagulation activity followed by *Sargassum wightii* > *Turbinaria ornata* > *Turbinaria conoides* > *Padina tetrastromatica* (**manoj *et al*.,2013)** .

Also, The hot water extract of green alga *Monostroma latissimum* gives a sulfated rhamnan polysaccharide with an anticoagulant activity. The anticoagulant activity as evaluated by assays of the APTT promises that it can be a potential source of anticoagulant **(Li *et al*. 2011).**

Furthermore**, Chevolot *et al*.(1999)** concluded that marine brown algae extracts exhibit higher anticoagulant activity than red and green algae extracts **.**

4.Conclusion:

Egyptian marine algae are still inexhaustible especially in the extraction of valuable medical materials.The results revealed that SPs extracts from brown algae exhibit higher anticoagulation activity than green and red algae. As well as, the anticoagulation activity is proportional to sulphate and sugar contents in SPs extracts and inversely proportional to protein and uronic acid content .

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الملخص باللغة العربية

استخلاص عديدات التسكر الكبريتية من انواع مختلفة من الطحالب البحرية ودراسة دورها كمضادات تجلط طبيعية

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الملخص العربى

الطحالب البحرية تعتبر أهم مصدر غير حيوانى لعديدات التسكر الكبريتية التى لديها الكثير من الفوائد فى مجال صناعة الأدوية حيث تستخدم كمضادات تجلط ومضادات أكسدة ومضادات التهاب ومضادات للفيروسات ومضادات للبكتيريا ومضادات التهاب ولذلك يمكن التطوير منها لاستخدامها فى المزيد من المجالات الطبية.تم تجميع العينات موسميا لمدة عام (سبتمبر 2013 إلى أغسطس 2014) من منطقة المد والجزر للموقع الأول وهو رأس الأدبية الذى يقع على الساحل الغربى لخليج السويس والموقع الثانى وهو رأس سدر الذى يقع شمال شرق خليج السويس. وقد كان الغرض من هذه الدراسة استخلاص عديدات التسكر الكبريتية من (الطحالب الخضراء *Codium dwarkense* , *Ulva Lactuca* ومن الطحالب الحمراء *Hypnea cornuta* ومن الطحالب البنية *Hormophysa triquetra*، *Sargassum denticulatum* و*Cystosira myrica*) بطريقتين مختلفتين (على الساخن والبارد) وتم التحليل الكيميائي لكل مستخلص (محتوى البروتين، السكريات، الكبريتات، الكبريت، النيتروجين، وحمض اليورونيك). أظهرت نتائج التحاليل الفيزيوكيميائية لعينات لمياة البحر أن درجة الحرارة تتراوح بين 15-30 درجة مئوية ، pH **7.8 -8.7** ، ملوحة **38- 42**٪، DO **6-7** ملغم / لتر، NO_3 0.021– **4.6** ملغم / لتر، NO_2 **0.004– 0.032** ملغم / لتر، NH_4 **0.019- 0.3** ملغم / لتر و PO_4 0.005 – 0.015 ملغم / لتر وبعد ذلك تم اختبار قدرة عديدات التسكر الكبريتية على العمل كمضادات تجلط طبيعية من خلال اختبار زمن الثرمبوبلاستين الجزئى المنشط (APTT) واختبار زمن البروثرومبين (PT). أظهرت النتائج أن قدرة عديدات التسكر على العمل كمضادات تجلط طبيعية تتناسب طردياً مع نسبة الكربوهيدرات والكبريتات وتتناسب تناسب عكسي مع البروتين وحمض اليورونيك . ولذلك كانت أعلى قيمة من اختبار PT هى **26.5**$\pm$**0.**10 ثانية عند تركيز 20 % من عديدات التسكر الكبريتية للمستخلص البارد من الطحلب البني *Hormophysa triquetra* بينما كانت أقل قيمة هى 1.13$\pm$0.06 ثانية عند تركيز **5** % من عديدات التسكر الكبريتية للمستخلص الساخن من الطحلب الأحمر *Hypnea cornuta* وأيضا أوضحت النتائج أن أعلى قيمة من اختبار APTT هي **42**$\pm$0.1 ثانية عند تركيز 20% من عديدات التسكر الكبريتية للمستخلص البارد من الطحلب البني *Hormophysa triquetra* وكانت أقل قيمة هى **5.63**$\pm$0.25 ثانية عند تركيز 5 % من عديدات التسكر الكبريتية للمستخلص الساخن من الطحلب الأحمر *Hypnea cornuta* .