J. Black Sea/Mediterranean Environment
Vol. 25, No. 2: 232-257 (2019)

REVIEW ARTICLE

Anticoagulant and antilipaemic activities of polysaccharides from marine algae

Kasım Cemal Güven[1*], Burak Coban[2], Ekrem Sezik[3]

[1] Turkish Marine Research Foundation (TUDAV), PK 10, Beykoz, Istanbul, TURKEY
[2] Department of Chemistry, Bulent Ecevit University, Incivez, 67100 Zonguldak, TURKEY
[3] Institute of Health Sciences, Yeditepe University, 34755, Istanbul, TURKEY

***Corresponding author:** kcguven@yahoo.com.tr

Abstract

In this review the research on the anticoagulant and antilipaemic activity of sulfated and synthetically modified polysaccharides extracted from marine macro algae between 1913 and 2018 was summarized and the results were compared with a long known natural polysaccharide from animal sources, heparin which contain *N*-sulfate group. The highest anticoagulant activity of algal sulfated polysaccharide was obtained from *Dictiyota menstrualis*, red alga, found to be 4.8 times more active than heparin. Antilipaemic activity of some algal sulfated polysaccharides showed similar activity as heparin.

Keywords: Anticoagulant, antilipaemic, polysaccharides

Received: 05.05.2019, **Accepted:** 28.06.2019

Introduction

Anticoagulants are important therapeutic agents for thrombotic disorders. The first natural anticoagulant is glucosamine and galactose sulfate ester, which was isolated from liver, heart and bovine of animals and activities were determined (McLean 1916; Howell and Holt 1918). The well known anticoagulant heparin contains *O*-sulfate and *N*-sulfate groups in the carbohydrate skeleton, on the other hand, sulfate containing algal polysaccharides have various carbohydrate components without *N*-sulfate groups.

The assessment of anticoagulation properties of sulfate containing polysaccharides from marine algae has been conducted by several medical assays such as Heptest, Hepocloth, thrombin time (TT) (Pitney and Dacie 1953), the Prothrombin time (PT) (Quick 1945), the partial thromboplastin time (PTT) and activated partial thromboplastin time (aPTT) (Margolis 1958) to measure

how quickly blood clotting takes place and to compare with heparin. The fibrinolytic activity is measured with fibrin plat method (Astrup and Mullertz 1952) and platelet aggregation for blood clotting assays (Deacon-Smith and Rogers 1982).

Antilipaemic agents are lipid-lowering substances that are used in the treatments of hyperlipidemia. This activity is measured by Baker method (Baker 1957).

Algal polysaccharides were identified based on metachromasy (Lison 1935). This phenomenon was found by Ehrlich (1877). Agar, alginic acid and carrageenan (including iota-, lambda- and kappa-) were identified by this method (Güven and Güvener 1985a; 1985b). Sulfate content of polysaccharides were determined as inorganic sulfate liberated after acid hydrolysis (Dodgson and Price 1962).

In this paper, anticoagulant and antilipaemic activities of algal polysaccharides were reviewed in accordance with their taxa, that is, class order, family, and species as well as active compounds.

Anticoagulant activities of macro algal polysaccharides

Chlorophyta

Marine green algae especially *Monostroma*, *Ulva*, *Enteromorpha*, *Codium* and *Caulerpa* are very important sources of sulfate containing polysaccharides.

Maeda *et al.* (1991) examined 23 green algae for anticoagulant activity and the extract of *Monostroma nitidum* had 3.3-fold higher activity than that of standard heparin. Hayakawa *et al*. (2000) found the same activity for this alga. A high anticoagulant activity was found in *M. latissimum* (Maeda *et al*. 1991; Hayakawa *et al*. 2000; Zhang *et al*. 2008). Two fractions of *M. nitidum* extract including 1,2- linked 1-rhamnose substituted sulfate group at C-3/C-4 position exhibited a high activity (Mao *et al.* 2008; 2009).

Codium fragile also showed a high anticoagulant activity, >300 APTT (Athukorala *et al.* 2007). Strong anticoagulant activities of *C. dwarkense*, *C. tomentosum*, *C. indicum*, *C. iyengarii*, *C. tomentosum*, *C. tenue*, *C. decorticatum* and *C. geppei* (Siddhanta *et al*. 1999; Shanmugam *et al*. 2001a; 2001b; Shanmugam *et al*. 2002), *C. latum* (Uehara *et al*. 1992), *C. stranglatum* (Costa *et al.* 2010), *C. fragile* ssp. *tomentosoides* (Deacon-Smith *et al*. 1985; Rogers *et al.* 1990; Jurd *et al*. 1995) were shown. *C. pugniformis* and *C. cylindricum* showed lower anticoagulant activities than heparin tested by TT, AT and APT (Matsubara *et al*. 2000; 2001). A highly sulfated pyranosic *β*-arabinan from green seaweed *C. vermilara* exhibited some anticoagulant activity (Ciancia *et al*. 2007; Fernández *et al*. 2013). *Codium fragile*, *C. divaricatum*, *C. latum* possess weak anticoagulant activity with lower sulfate content (Maeda *et al*.

1991; Ciancia *et al*. 2007). Another study reported *C. divaricatum* possesses a high anticoagulant activity *in vitro* of which sulfated polysaccharide mainly composed of (1→3)-*β*-d-galactopyranose residues, branched by single (1→)-*β*-d-galactopyranose units attached to the main chain at C-4 positions (Li *et al*. 2015). *C. divaricatum* also had *in vitro* fibrinolytic enzyme with a high anticoagulant activity (Matsubara *et al.* 2000).

The anticoagulant activity of some extracts of *Caulerpa* species *C. cupressoides*, *C. prolifera*, *C. sertularioides* were assayed by PPT and PT, but only *C. cupressoides* was found active (Costa *et al*. 2010; Selim *et al*. 2015). A certain polysaccharide with high sulfate value from *C. cupressoides* was found with an anticoagulant activity (Rodrigues *et al.* 2011). *C. peltata* showed the highest activity among *Caulerpa* species with heparin unit of ~151 I.U./mg (Shanmugam *et al.* 2001c) but *C. okamurae* had a weak activity (Maeda *et al*. 1991). *C. cupressoides*, *Anadyomene stellata* presented potent anticoagulant activities same as heparin (De Lara-Isassi *et al.* 2004). *C. lentillifera* has a potential anticoagulant activity as heparin (Arenajo *et al.* 2017).

A water soluble polysaccharide isolated from *Enteromorpha clathrata* showed high activity (Qi *et al.* 2012). Two sulfated polysaccharides with anticoagulant activity were isolated from *E. linza* (Qi et al., 2013) and one was effective in *in vitro* APTT and TT assays (Wang *et al*. 2013). *E. compressa* and *E. intestinalis* exhibited weak activities (Maeda *et al*. 1991).

Ulva conglobate with high anticoagulant activity (Mao *et al.* 2006), *U. lactuca* crude extract with some anticoagulant properties (Elmegeed *et al.* 2014) a weak activity of *U. arasakii* (Maeda *et al.* 1991) and a prolonged APTT for a sulfated polysaccharide from *U. fasciata* have been reported (Shonima *et al.* 2012; Faggio *et al.* 2016; Ibrahim *et al*. 2016). *U. pertusa* showed good anticoagulant properties (Kang *et al.* 2016).

The other examined algae *Udotea indica*, *U. flabellum*, *Cladophora fascicularis*, *C. gracia*, *Boodlea composita*, *Chaetomorpha media*, *C. torta*, *Valoniopsis pachynema* and *Bryopsis plumosa* were found with moderate activities (Shanmugam *et al*. 2001c). *Cladophora flexuosa* var. *densa*, *Cladophora rugulosa*, *Chaetomorpha crassa* showed a potent activity whereas the extracts of *Chaetomorpha spiralis*, *Spongomorpha duriuscula*, *Bryopsis maxima* showed weak activities (Maeda *et al.* 1991). A sulfated polysaccharide containing rhamnose (49.7%), galacturonic and glucuronic acid (32% of total sugar) with 20% sulfate content with an anticoagulant activity mediated by heparin cofactor II was obtained from *Arthrospira platensis* (Majdoub *et al.* 2009).

Phaeophyta

Brown algae are rich sources of sulfated fucans (fucoidans) which are potent anticoagulant agents with heterogeneous structures that contain sulfate ester groups and *L*-fucose units. The most studied group of fucoidan was first isolated by Kylin (1913) named as fucoidin from *Laminaria digitata* and later anticoagulant activity of fucoidan isolated from green, red and especially brown algae: *Fucus serratus*, *F. platycarpus*, *Halidrys silicuosa*, *Laminaria saccharine*, *Himanthalia lerea*, *Ascophyllum nodosum* and *Cordaria flagelliformis* demonstrated by Elsner (1938). Crude and purified fucoidans possess antithrombin activities (Schuler and Springer 1957; Springer *et al*. 1957; Bernardi and Springer 1962). The fucoidans have the potent anticoagulant activity mediated by antithrombin and heparin cofactor II (Mourao 2004). The anticoagulant activity of fucoidan was found to be more potent than heparin for the first time by Springer *et al.* (1957).

Many studies have been published on anticoagulant activity of fucoidans and it was found that the sulfate content/position (Dobashi *et al*. 1989; Nishino *et al.* 1989; Kitamura *et al.* 1991; Chevolot *et al.* 1999; Pereira *et al.* 1999; Haroun-Bouhedja *et al.* 2000; Pereira *et al.* 2002a; Albuquerque *et al.*, 2004; Mourao 2004; Cumashi *et al.* 2007; Zhang *et al.* 2014), and molecular weight (Chandía and Matsuhiro 2008) play an important role for the activity. The relationship between structure and anticoagulant activity of algal fucans is not simply a function of charge density, but depends critically on the pattern of sulfation and monosaccharide composition (Mourao 2004). Fucoidan expresses both antithrombin activity (Mourao 2004) and platelet aggregation (Cheng and Wang 2003; Yoon *et al.* 2007; Li *et al.* 2008). PT, TT test of fucoidans showed similar activity with heparin (Athukorala *et al.* 2006). A study demonstrated that fucoidan is not toxic in Sprague–Dawley rats (Kim *et al.* 2010). Fractionation of enzyme hydrolysate of fucoidan isolated from *Ascophyllum nodosum* brought out the structure may be responsible for anticoagulant activity (Mauray *et al.* 1995; Nardella *et al*., 1996; Chevolot *et al.* 1999; Millet *et al.* 1999; Chevolot *et al.* 2001; Trento *et al.* 2001; Colliec-Jouault *et al.* 2003; Cumashi *et al*. 2007; Durand *et al*. 2008). Heterofucans from the brown algae *Canistrocarpus cervicornis* with its anticoagulant activities were reported (Camara *et al.* 2011). Fucan isolated from *Cordaria firma* has an anticoagulant activity (Takemori 1957a; 1957b). A galactofucan fraction of *Dictyota menstrualis* polysaccharide was 4.8 times more active than the low molecular weight heparin (Albuquerque *et al.* 2004). The sulfated heterofucans of *D. cervicornis*, *D. deliculata*, *D. mertensis* showed important activities (Costa *et al.* 2010). Fucoidan, laminaran and mannuronan mixture from *Dictyopteris polypodioides* has a significant activity (Karaki *et al.* 2013). Enzymatic hydrolysate of sulfated polysaccharide from *Ecklonia cava* was found as active as heparin (Athukorala *et al.* 2006;

Jung *et al.* 2007; Wijesinghe *et al.* 2011). A high anticoagulant activity of sulfated fucoidans and their fractions from *E. kurome* (Nishino *et al.* 1989; 1999) and *Eisenia bicyclis* (Usui *et al.* 1980) was reported.

Fucoidan from *Fucus vesiculosus* showed similar anticoagulant activity to heparin (Soeda *et al.* 1992; Nishino *et al.* 1994; Trento *et al.* 2001; Kuznetsova *et al.* 2003; Costa *et al.* 2010). Highly purified fucoidan fraction obtained from *F. vesiculosus* showed potent anticoagulant and fibrinolytic properties with only minor platelet activating effect (Durig *et al.* 1997). *F. evanescens*, *F. serratus* and *F. distichus* fucoidans (Cumashi *et al.* 2007) and *F. serratus* and *F. spiralis* extracts (Deacon-Smith and Rogers, 1982; Deacon-Smith *et al.* 1985a; 1985b) exhibited strong antithrombin activity in platelet aggregation. Anticoagulant activity of fucoidan from *Hizikia fusiforme* (Dobashi *et al.* 1989; Li *et al.* 2008) and *H. fusiformis* were reported (Athukorala *et al.* 2007).

Laminaria angustata var. *longissima* (Kitamura *et al.* 1991) showed high anticoagulant activity. The fucoidan with low sulfate content from *L. brasiliensis* has a very high activity (Pereira *et al.* 1999). Fucan containing 2,3-disulfated, 4-linked unit from *L. cichorioides* has a potent anticoagulant activity in APTT assay (Yoon *et al.* 2007). *L. digitata*, *L. hyperborea* and *L. saccharina* extracts completely inhibited platelet aggregation of Ristocetin (Deacon-Smith and Rogers 1982; Deacon-Smith *et al.* 1985a; 1985b; Cumashi *et al.* 2007). The fucoidan fraction from *L. bongardiana* had a very strong anticoagulant activity (Bilan *et al.* 2016). Low molecular weight fucoidan fractions from *L. japonica* and *L. ochotensis* showed high activities (Athukorala *et al.* 2007; Wang *et al.* 2010). Fucans from *L. saccharina* (Ushakova *et al.* 2009) and *Lessonia vadosa* presented a good inhibition of coagulation (Chandía and Matsuhiro 2008).

Padina gymnospora contains heterofucans which are compounds of glucuronic acid, L-fucose and D-xylose units with sulfation at C-3 positions of L-fucose and presented anticoagulant activity (Silva *et al.* 2005). Anticoagulant and antithrombin activities of low molecular weight fucoidan extracted from *Pelvetia canaliculate*, xylofucan sulfate and *Punctaria plantaginea* were reported (Colliec *et al.* 1991; Ustyuzhanina *et al.* 2016).

Sargassan is a sulfated polysaccharide with a high anticoagulant activity and isolated from *Sargassum linifolium* (Abdel-Fattah *et al.* 1973; 1974). Fermentation process of *S. fulvellum* increased the anticoagulant activity of its sulfated polysaccharide (de Zoysa *et al.* 2008). Sulfated polysaccharide fucan isolated from *S. vulgare* exhibited high antithrombin action and prolonged APTT (Dore *et al.* 2013). Low molecular weight (LMW) polysaccharides from *S. fusiforme* possessed anticoagulant activity (Sun *et al.* 2018). Hot water extracts of *S. horneri* and *S. siliquastrum* showed high activities. On the other

hand, hot water extracts of *S. thunbergii*, *S. fulvellum*, *S. coreanum* and *Scytosiphon lomentaria* presented weak anticoagulant activity (Athukorala *et al*. 2007). Antithrombotic features of algal sulfated galactofucan from the *Spatoglossum schröederi* have been reported (Rocha *et al*. 2005; Almeida-Lima *et al*. 2010; 2011).

Orally administrated fucoidan from *Undaria pinnatifida* had also significant effect on coagulation assays (Irhimeh *et al*. 2009; Faggio *et al*. 2015).

Rhodophyta

Activity mechanisms and potency of red algal polysaccharides were discussed as follows. A sulfated galactan extracted from *Botryocladia occidentalis* with 2,3-disulfated and 2-sulfated of each 1/3 ratio were found that its anticoagulant activity was attributed mostly to the 2,3-disulfated units (Farias *et al*. 2000; Pereira *et al.* 2002a). Investigation on the mechanisms of anticoagulant activity of sulfated galactans is achieved mainly through potentiation of plasma cofactors which are the natural inhibitors of coagulation proteases. Anticoagulant activity of sulfated galactan was attributed to the sulfation at *O*-2 and *O*-3 position of the *α*-D-galactopyranosyl residues requiring molecular size between 16 and 46 kDa (Melo *et al.* 2004).

Sulfated polysaccharides contained in red algae are galactans, carrageenan, agar, porphyrans, funoran and other specific polysaccharides (Percival and McDowell 1967). These polysaccharides contain –O–SO_3H group which is important for inhibition of blood clotting (Demole and Reinert 1930; Fischer 1931; Chargaff *et al.* 1936). Earlier red algal anticoagulant activity studies showed galactans from *Iridae laminarioides* (Chargaff *et al.* 1936), carrageenan, agar and galactan (Elsner *et al.* 1937).

Galactan was first isolated from *Iridae laminarioides* (Hassid 1933). Carrageenan of *Corallina rubens* was more active than that of galactan (Guven *et al.* 1974b). A sulfated galactan of *B. occidentalis* has the same anticoagulant potency with heparin (Farias *et al.* 2000; 2001; Melo *et al*. 2004).

Carrageenan is a linear sulfated polysaccharide built up of alternative 1-3 and 1-4 linked *β*- or *α*-galactopyranosyl units. The former contains 2- and 4-sulfates or not, 1-4 linked contains 2- and 6- mono or disulfates, 2,6-anhydride and 3,6-anhydride 2-sulfate. It has various types depending on the sulfate content, gelling properties and substitution as: *λ*- (lambda), *κ*- (kappa), *ι*- (iota), *μ*- (mu), *ν*- (nu), *θ*- (theta), *ζ*- (xi). Carrageenan from *Delesseria sangiinea* (Elsner, 1938), *Chondrus crispus* and *I. laminarioides* (Springer *et al.* 1957) expressed anticoagulant activity. *Furcellaria festigiate*, *Eucheuma spinosum*, *Gigertina acicularis*, *G. pistillata*, *G. radula* extracts were compared and the highest

anticoagulant activity was shown by the extract from *G. acicularis* which was more active than heparin (Houck *et al.* 1957). Anticoagulant activity of *Phyllophora nervosa* extract was demonstrated by Howell time test on rabbit (Güven and Aktin 1962). A degraded carrageenan from *Eucheuma spinosum*, *λ*- and *κ*-carrageenan from *C. crispus*, *Polyides rotundus* showed anticoagulant activity in rabbits. The degraded carrageenan was less toxic and *λ*-carrageenan was more toxic than *κ*-carrageenan (Anderson *et al.* 1965). *Corallina rubens* extract and its fraction showed high anticoagulant and fibrinolytic activity (Güven *et al.* 1973). In contrary to these findings, anticoagulant and fibrinolytic activities of carrageenan rank as *λ*-, *κ*-, *ι*-carrageenan (Sigma), carrageenan from *Grateloupia dichotoma* and alginic acid (Roth) were tested and *G. dichotoma* carrageenan and *λ*-carrageenan (Sigma) showed similar anticoagulant and fibrinolytic activity in all biological tests applied (Güven *et al.* 1991). Sulfated polysaccharides from the clonned *G. filicina* had a good potential as anticoagulant agents (Chen 2015).

Carrageenans prolonged the clotting time, inhibited amidolytic activity of thrombin coagulation factor x-(Xa)-catalyzed amidolysis potentiated the *in vitro* inactivation of thrombin and Xa by antithrombin III (Kindness *et al*. 1979a; 1979b). Carrageenan does not have an antithrombin effect, it inhibits fibrin aggregation or polymerization and its effect can be blocked by protamine (Schimpf *et al.* 1969). It activates Hageman factor in human plasma and promotes blood coagulation (Schwartz and Kellermeyer 1969). According to the activity degrees of carrageenan *λ*-type was the most potent followed by *ι*-type and *κ*-type (Hawkins and Leonard 1962; 1963; Anderson *et al.* 1965). Other studies proposed that the potency of the types were as *ι*>*λ*>*κ* (Winter *et al.* 1962; McMillan *et al.* 1979). In contrary to these findings, anticoagulant and fibrinolytic activities of carrageenan rank as *λ*>*κ*>*ι* (Güven *et al.* 1991).

Purified sulfated polysaccharide isolated from *Grateloupia indica* possesses potent PT, CT, BT and hemostatic activities (Sen *et al.*, 1994). *G. elliptica*, *G. lanceolate*, *Sinkoraena lancifolia*, *Halymenia dilatata*, *Lomentaria catenata*, *Martensia denticulate*, *Schizymenia dubyi*, *Chondrus crispus* showed the highest anticoagulant activities than the other examined algae species *Pterocladiella capillacea*, *Prionitis cornea*, *Capopeltis affinis*, *Gloiopeltis furcata*, *Laurencia okamurae*, *Gelidium amansii*, *Ahnfeltiopsis flabelliformis*, *Gracilaria textorii*, *Chondria cassicaulis*, and *Acrosorium flabellatum*. Enzymatic digestion of *S. dubyi* and *L. catenata* polysaccharide fractions exhibited the most potent anticoagulant activity (Lee *et al.* 2008). A sulfated polysaccharide of *Botryocladia occidentalis* was found more potent than *Gelidium crinale* extract (Pereira *et al.* 2005). A fraction of *Pterocladia capillacea* (*G. latifolium*) extract showed anticoagulant activity by RT, PT, ELT assays (Güven *et al.* 1979b; Abou Zeid *et al.* 2014).

The fraction obtained by depolymerization of the sulfated galactan from *Schizymenia binderi* showed anticoagulant activity (Zúñiga *et al.* 2006). A degraded fraction of porphyran isolated from *Porphyra haitanensis* showed anticoagulant activity (Zhao *et al.* 2006). Porphyran isolated from *P. haitanensis* after alkali treatment and resulfation showed anticoagulant activity (Zhang *et al.* 2010; Liu *et al.* 2015). The other red algae reported possessing anticoagulant activity were *Lomentaria catenata* (Pushpamali *et al.* 2008), *Gigartina skottsbergii* (Carlucci *et al.*, 1997), *Schizymenia binderi* (Zuniga *et al.* 2006), *P. haitanensis* (Zhang *et al.* 2010) and *Nothogenia fastigiata* (Kolender *et al.* 1997). *Hypnea esperi* methanol extract effectively prevented the blood clotting time (Selim *et al.* 2015).

Anticoagulant activity of synthetically sulfated algal polysaccharides

Synthetically sulfated polysaccharides were first prepared by Bergström in 1935 and 1936 (Bergström 1935; 1936). A sulfated alginate had much lower anticoagulant activity and much more toxic than heparin (Molho and Cotte 1951). When containing two sulfate groups per glucose units the alginate gave the highest anticoagulant activity (Dewar 1956). High sulfate content and low molecular weight sulfated alginates has a good anticoagulant activity (Fan *et al.* 2011). Many studies were published on synthetic laminarin sulfate which was extracted from *Laminaria* sp. and preparations with the highest sulfate groups per glucose residue had anticoagulant activities of 25-30% that of a standard heparin (O'Neill 1955) and sulfonic acid derivatives were more active than the sulfate esters (Hawkins and O'Neill 1955). A derivative of laminarin contained 1.83 sulfate groups per glucose unit showed third as potent as heparin in rabbit test and it was extremely toxic to guinea pigs (Adams and Thorpe 1957). Laminarin sulfate s.c. and i.m. applications showed some anticoagulant activity (Hawkins and Leonard 1958). Enhancement of anticoagulant properties of polysaccharides upon sulfation was shown by several authors (Doctor *et al.* 1991). Laminarin sulfates were synthesized and increasing content of sulfate groups increased the anticoagulant activity, highest activity was obtained with 1.49 sulfate groups per glucose unit (Alban *et al.* 1992). Synthetically sulfated polysaccharide from *Enteromorpha linza* showed anticoagulant activity and it was found that the activity was related to degree of sulfation and molecular weight (Wang *et al.* 2013). Sulfated alginate has 8.1 and 2.9 folds activity (Pulsawat and Tongmalee 2014).

On the contrary, native alginate did not demonstrate anticoagulant activity (Güven *et al.* 1991). After structural modifications, such as sulfonation, oxidation, and reduction, laminarans exhibited anticoagulant activity (Kraan *et al.* 2012).

Antilipaemic activity of macro algal polysaccharides

Antilipaemic activity is clearing of visible lipemia and used in the treatment of atherosclerosis. The origin of this subject was due to realize lipase from heparin hydrolyzes triglycerides finalize with free fatty acids liberation (Shore *et al.* 1953) and free acids were determined (Duncombe 1963; 1964; Güven *et al.* 1979a).

The antilipaemic activity of fucoidan was first demonstrated and found as active as heparin and probably acts by the release of the clearing factor (Schuler and Springer 1957). Fucoidan fraction with MW 5000-15000 possesses antilipaemic activity (Springer *et al.* 1957).

This review is not only focused on the sulphated polysaccharides but synthetically sulfated algal polysaccharides as well (Besterman and Evans 1957). Laminaran is a non-sulfated polysaccharide found in brown algae. There are two forms of laminaran as soluble and non-soluble. A low level of sulfation gave laminarin antilipaemic properties similar to those of heparin (Besterman and Evans 1957). Total fat, *β*-lipoprotein, esterified cholesterol levels decreased after parenteral administration of laminarin sulfate or of heparin (Mookerjea and Hawkins 1958). Sulfated alginic acid showed some antilipaemic potency as heparin (Constantinides *et al.* 1954). Clearing effects of various algae *Furcellaria festigiate*, *Eucheuma spinosum*, *Gigartina acicularis*, *G. pistillata*, *G. radula* and *Iridae* sp. were examined and found no activity (Houck *et al*. 1957). Synthetically sulfated polysaccharide polymannourides depresses hyperlipidemia in human (Constantinides *et al*. 1960). Laminaran sulfate prepared by sulfation of polysaccharides of the brown alga, *Eisenia bicyclis*, *Laminaria digitata* and carrageenan obtained from *Chondria crispus* showed antilipaemic and anti atherogenic activities in rabbit (Murata 1961; 1962). Degraded polysaccharides isolated from *L. cloustoni* were sulfated and examined their antilipaemic activities (Adams *et al*. 1962). Sulfated laminarin prepared from *L. cloustoni* is valuable in the treatment of lipemia and atherosclerosis (Gollin *et al.* 1965). Zha *et al.* (2012) reported that crude polysaccharides from *L. japonica* at a dose of 400 mg/kg/day caused a reduction in total serum cholesterol, TG, high density lipoprotein (HDL)-cholesterol, and LDL in serum. Lipolytic activity of algal extracts: some fractions of *Phyllophora nervosa* (Güven *et al.* 1972), *Corallina rubens* (Güven *et al.* 1973; 1974b; 1975), *Pterocladia capillacea* (*Gelidium latifolium*) (Güven *et al.* 1979b), *Sargassum vulgare*, *Polisiphonia subuliera* (Aktin and Güven 1965), *Halopytisin curvus* (Güven and Kızıl 1986), *Cystoseira barbata* (Güven and Aktin 1964; Güven *et al*. 1974a), *C. crinite* (Ben-Gara *et al.* 2017), *Gracilaria verrucosa* and *Gelidium latifolium* (Aktin and Güven 1969) extracts showed activities. Various algae (1 green, 21 brown and 4 red) were investigated for

their antilipaemic activities and found appreciable suppression were observed (Ren *et al.* 1994). The polysaccharide Ulvan from *Ulva pertussa* has been utilized in old China for various medicinal purposes and this polysaccharide decreased LDL-cholesterol level and has antilipaemic effect (Pengzhan *et al.* 2003). The acetylation of ulvans from *U. pertusa* by acetic anhydride showed higher antihyperlipidemic activity than natural ulvans, especially with regard to causing a decrease in TG and LDL-cholesterol levels (Yu *et al.* 2017). Polysaccharides extracted from *U. prolifera* exhibited pancreatic lipase inhibition activity (Yuan *et al.* 2018). Cao *et al.* (2016) reported that porphyran from *Pyropia yezoensis* at a dose of 200 mg/kg/day can decrease the percentage of body weight gain and serum lipid profiles of mice, similar to the effect of a hypolipidemic drug.

In conclusion, the first anticoagulant agent heparin was introduced for thrombosis therapy. The clearing of visible fat in the blood by heparin is accompanied by changes in lipoprotein metabolism. Chemically it is a sulfated polysaccharide and composed of sulfated uronic acid and *N*-sulfate-glucosamine. On the other hand, algal sulfated polysaccharides contain only -O-SO_3H groups and these groups were essential for the anticoagulant effect and may be important for antilipaemic effect, thus many studies have been undertaken on the algal polysaccharides. However, these experiments were only on animals, these polysaccharides have very high molecular weights (100,000-300,000) and they have high toxicities.

Deniz alglerinden elde edilen polisakkaritlerin antikoagülan ve antilipemik etkileri

Öz

Deniz makro alglerinin sülfatlı polisakkaritlerinin antikoagülan etkileri üzerindeki 1913-2018 yılları arasında yapılan çalışmalar bu derlemede toplanmıştır. Bu çalışmalara doğal olarak sülfat içermeyen fakat sentetik olarak sülfatlanan polisakkaritler de eklenmiştir. In vivo ve in vitro olarak yapılan çalışmalarda, sonuçlar hayvansal ürün olan ve tıpta 100 senedir kullanılan heparin ile karşılaştırılmıştır. En yüksek antikoagülan etki kırmızı alg *Dictiyota menstrualis*'ten elde edilen sulfatlı polisakkaritte bulunmuştur ve heparinden 4.8 kat daha etkindir. Bazı alg polisakkaritleri heparin seviyesinde antilipemik etki göstermişlerdir. Bu konudaki ülkemizde yapılan çalışmalar da derlemeye eklenmiştir.

Anahtar kelimeler: Antikoagülan, antilipemik, polisakkaritler

References

Abdel-Fattah, A.F., Hussein, M.M.-E., Salem, H.M. (1973) Sargassan: A sulphated heteropolysaccharide from *Sargassum linifolium*. *Phytochem* 12(8): 1995-1998.

Abdel-Fattah, A.F., Hussein, M.M.-D., Salem, H.M. (1974) Studies of the purification and some properties of sargassan, a sulphated heteropolysaccharide from *Sargassum linifolium*. *Carbohydr Res* 33(1): 9-17.

Abou-Zeid, A.H., Aboutabl, E.A., Sleem, A.A., El-Rafie, H.M. (2014) Water soluble polysaccharides extracted from *Pterocladia capillacea* and *Dictyopteris membranacea* and their biological activities. *Carbohydr Polym* 113: 62-66.

Adams, S.S., Heathcote, B.V., Walker, D. (1962) Sulphated degraded laminarin: an antilipaemic agent. *J Atheroscler Res* 2: 314-316.

Adams, S.S., Thorpe, H.M. (1957) The anticoagulant activity and toxicity of laminarine sulphate K. *J Pharm Pharmacol* 9(1): 459-463.

Aktin, E., Güven, K.C. (1965) Studies on antilipemic properties of the algae collected from Turkish coasts - III. *Sargassum vulgare* Ag., *Polysiphonia subulifera* (C. Agardh) Harvey. *Qual Plant Mater Veget* 12(2): 210-215.

Aktin, E., Güven, K.C. (1969) Studies on the lipase released by the substances obtained from *Gracilaria verrucosa* (Hunds.) and *Gelidium latifolium* (Greville). *Arzneimittel-Forsch* 19(2): 190-193.

Alban, S., Kraus, J., Franz, G. (1992) Synthesis of laminarin sulfates with anticoagulant activity. *Arzneimittel-Forsch* 42(8): 1005-1008.

Albuquerque, I.R., Queiroz, K.C., Alves, L.G., Santos, E.A., Leite, E.L., Rocha, H.A. (2004) Heterofucans from *Dictyota menstrualis* have anticoagulant activity. *Braz J Med Biol Res* 37(2): 167-171.

Almeida-Lima, J., Costa, L.S., Silva, N.B., Melo-Silveira, R.F., Silva, F.V., Felipe, M.B., Medeiros, S.R., Leite, E.L., Rocha, H.A. (2010) Evaluating the possible genotoxic, mutagenic and tumor cell proliferation-inhibition effects of a non-anticoagulant, but antithrombotic algal heterofucan. *J Appl Toxicol* 30(7): 708-715.

Almeida-Lima, J., Dantas-Santos, N.L., Gomes, D., Cordeiro, S.L., Sabry, D.A., Costa, L.S., Freitas, M.D.L., Silva, N.B., Moura, C.E.B., Lemos, T.M.A.M., Leite, E.L., Rocha, H.A.O. (2011) Evaluation of acute and subchronic toxicity of a non-anticoagulant, but antithrombotic algal heterofucan from the *Spatoglossum schröederi* in Wistar rats. *Rev Bras Farmacogn* 21: 674-679.

Anderson, W., Duncan, J.G.C., Harthill, J. E. (1965) The anticoagulant activity of carrageenan. *J Pharm Pharmacol* 17(10): 647-654.

Arenajo, A.R., Ybanez, A.P., Ababan, M.M.P., Villajuan, C. E., Lasam, M.R.M., Young, C.P., Reyes, J.L.A. (2017) The potential anticoagulant property of *Caulerpa lentillifera* crude extract. *Int J Health Sci* 11(3): 29-32.

Astrup, T., Mullertz, S. (1952) The fibrin plate method for estimating fibrinolytic activity. *Arch Biochem Biophys* 40(2): 346-351.

Athukorala, Y., Jung, W.-K., Vasanthan, T., Jeon, Y.-J. (2006) An anticoagulative polysaccharide from an enzymatic hydrolysate of *Ecklonia cava*. *Carbohydr Polym* 66(2): 184-191.

Athukorala, Y., Lee, K., Kim, S., Jeon, Y. (2007) Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. *Bioresour Technol* 98(9): 1711-1716.

Baker, S.P. (1957) Heparin-activated clearing factor; standardized test, agewise application, and clinical observations. *Circulation* 15(6): 889-896.

Ben Gara, A., Ben Abdallah Kolsi, R., Chaaben, R., Hammami, N., Kammoun, M., Paolo Patti, F., El Feki, A., Fki, L., Belghith, H., Belghith, K. (2017) Inhibition of key digestive enzymes related to hyperlipidemia and protection of liver-kidney functions by *Cystoseira crinita* sulphated polysaccharide in high-fat diet-fed rats. *Biomed Pharmacother* 85: 517-526.

Bergström, S. (1935) Über die wirkungsgruppe des heparins. *Naturwissenschaften* 23(41): 706-706.

Bergström, S. (1936) Über polysaccharides terschwefelsäuren mit heparin wirkung. *H-S Z Physiol Chem* 238(4-5): 163-168.

Bernardi, G., Springer, G.F. (1962) Properties of highly purified fucan. *J Biol Chem* 237: 75-80.

Besterman, E.M.M., Evans, J. (1957) Antilipaemic agent without anticoagulant action. *Brit Med J* 1: 310-312.

Bilan, M.I., Klochkova, N.G., Ustyuzhanina, N.E., Chizhov, A.O., Shashkov, A.S., Nifantiev, N.E., Usov, A.I. (2016) Polysaccharides of algae 68. Sulfated polysaccharides from the Kamchatka brown alga *Laminaria bongardiana*. *Russ Chem Bull* 65(11): 2729-2736.

Camara, R.B.G., Costa, L.S., Fidelis, G.P., Nobre, L.T.D.B., Dantas-Santos, N., Cordeiro, S.L., Costa, M.S.S.P., Alves, L.G., Rocha, H.A.O. (2011) Heterofucans from the brown seaweed *Canistrocarpus cervicornis* with anticoagulant and antioxidant activities. *Mar Drugs* 9(1): 124.

Cao, J., Wang, S.C., Yao, C.W., Xu, Z., Xu, X.M. (2016) Hypolipidemic effect of porphyran extracted from *Pyropia yezoensis* in ICR mice with high fatty diet. *J Appl Phycol* 28: 1315-1322.

Carlucci, M.J., Pujol, C.A., Ciancia, M., Noseda, M.D., Matulewicz, M.C., Damonte, E.B., Cerezo, A.S. (1997) Antiherpetic and anticoagulant properties of carrageenans from the red seaweed *Gigartina skottsbergii* and their cyclized derivatives: correlation between structure and biological activity. *Int J Biol Macromol* 20(2): 97-105.

Chandía, N.P., Matsuhiro, B. (2008) Characterization of a fucoidan from *Lessonia vadosa* (Phaeophyta) and its anticoagulant and elicitor properties. *Int J Biol Macromol* 42(3): 235-240.

Chargaff, E., Bancroft, F.W., Brown, M.S. (1936) Studies on the chemistry of blood coagulation: II. On the inhibition of blood clotting by substances of high molecular weight. *J Biol Chem* 115: 155-161.

Chen, X., Yang, S., Wang, J., Song, L., Xing, R., Liu, S., Yu, H., Li, P. (2015) Sulfated polysaccharides isolated from cloned *Grateloupia filicina* and their anticoagulant activity. *BioMed Res Int.* doi: 10.1155/2015/612352.

Cheng, Z., Wang, S. (2003) Study on anticoagulant activities in vitro of fucoidan and fucoidan / collagen blends. *J Funct Polym* 2003(4): 557-560.

Chevolot, L., Foucault, A., Chaubet, F., Kervarec, N., Sinquin, C., Fisher, A.-M., Boisson-Vidal, C. (1999) Further data on the structure of brown seaweed fucans: relationships with anticoagulant activity. *Carbohydr Res* 319(1-4): 154-165.

Chevolot, L., Mulloy, B., Ratiskol, J., Foucault, A., Colliec-Jouault, S. (2001) A disaccharide repeat unit is the major structure in fucoidans from two species of brown algae. *Carbohydr Res* 330(4): 529-535.

Ciancia, M., Quintana, I., Vizcarguenaga, M.I., Kasulin, L., DeDios, A., Estevez, J.M., Cerezo, A.S. (2007) Polysaccharides from the green seaweeds *Codium fragile* and *C. vermilara* with controversial effects on hemostasis. *Int J Biol Macromol* 41(5): 641-649.

Colliec, S., Fischer, A.M., Tapon-Bretaudiere, J., Boisson, C., Durand, P., Jozefonvicz, J. (1991) Anticoagulant properties of a fucoidan fraction. *Thromb Res* 64(2): 143-154.

Colliec-Jouault, S., Millet, J., Helley, D., Sinquin, C., Fischer, A.M. (2003) Effect of low-molecular-weight fucoidan on experimental arterial thrombosis in the rabbit and rat. *J Thromb Haemost* 1(5): 1114-1115.

Constantinides, P., Cairns, A., Werner, A. (1954) Antilipemic activity of sulfated polysaccharides. *Arch Int Pharmacodyn Ther* 99(3-4): 334-345.

Constantinides, P., Johnson, C., Fahrni, B.M., Nakashima, R., Mc, I.H. (1960) Human hyperlipaemia and hypercholesterolaemia: rapid correction with a sulphated polymannuronide. *Brit Med J* 1(5172): 535-540.

Costa, L.S., Fidelis, G.P., Cordeiro, S.L., Oliveira, R.M., Sabry, D.A., Camara, R.B., Nobre, L.T., Costa, M.S., Almeida-Lima, J., Farias, E.H., Leite, E.L., Rocha, H.A. (2010) Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomed Pharmacother* 64(1): 21-28.

Cumashi, A., Ushakova, N.A., Preobrazhenskaya, M.E., D'Incecco, A., Piccoli, A., Totani, L., Tinari, N., Morozevich, G.E., Berman, A.E., Bilan, M.I., Usov, A.I., Ustyuzhanina, N.E., Grachev, A.A., Sanderson, C.J., Kelly, M., Rabinovich, G.A., Iacobelli, S., Nifantiev, N.E. (2007) A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiology* 17(5): 541-552.

de Lara-Isassi, G., Alvarez-Hernandez, S., Quintana-Pimentel, A. (2004) Screening for anticoagulant substances in some marine macroalgae. *Hydrobiologia* 14(1): 47-54.

de Zoysa, M., Nikapitiya, C., Jeon, Y.-J., Jee, Y., Lee, J. (2008) Anticoagulant activity of sulfated polysaccharide isolated from fermented brown seaweed *Sargassum fulvellum*. *J Appl Phycol* 20(1): 67-74.

Deacon-Smith, R.A., Lee-Potter, J.P., Rogers, D.J. (1985a) Anticoagulant activity in extracts of British marine algae. *Bot Mar* 28(8): 333.

Deacon-Smith, R.A., Lee-Potter, J.P., Rogers, D.J. (1985b) Platelet aggregation in the presence of extracts of British marine algae. *Med Lab Sci* 42(4): 404-405.

Deacon-Smith, R.A., Rogers, D.J. (1982) Antihaemostatic activities of British marine algae. *Brit Phycol J* 17: 231.

Demole, V., Reinert, M. (1930) Polyanetholsulfosaures Natrium, ein neues synthetisches Mittel zur Hemmung der Blutgerinnung. *N-S Arch Exp Pathol Pharmakol* 158(1): 211-218.

Dewar, E.T. (1956) Sodium laminarin sulphate as a blood anticoagulant. Paper read at 2nd Int. Seaweed Symposium, Trondheim.

Dobashi, K., Nishino, T., Fujihara, M., Nagumo, T. (1989) Isolation and preliminary characterization of fucose-containing sulfated polysaccharides with blood-anticoagulant activity from the brown seaweed *Hizikia fusiforme*. *Carbohydr Res* 194: 315-320.

Doctor, V.M., Lewis, D., Coleman, M., Kemp, M.T., Marbley, E., Sauls, V. (1991) Anticoagulant properties of semisynthetic polysaccharide sulfates. *Thromb Res* 64(4): 413-425.

Dodgson, K., Price, R. (1962) A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochem J* 84(1): 106-110.

Dore, C.M.P.G., Faustino Alves, M.G.D.C., Pofírio Will, L.S.E., Costa, T.G., Sabry, D.A., de Souza-Rêgo, L.A.R., Accardo, C.M., Rocha, H.A.O., Filgueira, L.G.A., Leite, E.L. (2013) A sulfated polysaccharide, fucans, isolated from brown algae *Sargassum vulgare* with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. *Carbohydr Polym* 91(1): 467-475.

Duncombe, W.G. (1963) The colorimetric micro-determination of long-chain fatty acids. *Biochem J* 88(1): 7-10.

Duncombe, W.G. (1964) The colorimetric micro-determination of non-esterified fatty acids in plasma. *Clin Chim Acta* 9: 122-125.

Durand, E., Helley, D., Zen, A.A.H., Dujols, C., Bruneval, P., Colliec-Jouault, S., Fischer, A.M., Lafont, A. (2008) Effect of low molecular weight fucoidan and low molecular weight heparin in a rabbit model of arterial thrombosis. *J Vasc Res* 45(6): 529-537.

Durig, J., Bruhn, T., Zurborn, K.H., Gutensohn, K., Bruhn, H.D., Beress, L. (1997) Anticoagulant fucoidan fractions from *Fucus vesiculosus* induce platelet activation in vitro. *Thromb Res* 85(6): 479-491.

Ehrlich, P. (1877) Beitrage zur kenntniss der anilinfarbungen und ihrer verwendung in der mikroskopischen technik. *Arch Mikrosk Anat* 13: 263-277.

Elmegeed, D.F.A., Ghareeb, D.A., Elsayed, M., El-Saadani, M. (2014) Phytochemical constituents and bioscreening activities of green algae (*Ulva Lactuca*). *IJAPR* 2(11): 373-378.

Elsner, H. (1938) Über das Vorkommen von hochwirksamen, die Blutgerinnung hemmenden Stoffen in Meeresalgen. II. *Hoppe-Seylers Z Physiol Chem* 252(3-4): 196-200.

Elsner, H., Broser, W. Bürgel, E. (1937) Über das Vorkommen von hochwirksamen, die Blutgerinnung hemmenden Stoffen in Rotalgen. *Hoppe-Seylers Z Physiol Chem* 246(5-6): 244-247.

Faggio, C., Morabito, M., Minicante, S.A., Piano, G.L., Pagano, M., Genovese, G. (2015) Potential use of polysaccharides from the brown alga *Undaria pinnatifida* as anticoagulants. *Braz Arch Biol Technol* 58: 798-804.

Faggio, C., Pagano, M., Dottore, A., Genovese G., Morabito M. (2016) Evaluation of anticoagulant activity of two algal polysaccharides. *Nat Prod Res* 30: 1934-1937.

Fan, L., Jiang, L., Xu, Y., Zhou, Y., Shen, Y., Xie, W., Long, Z., Zhou, J. (2011) Synthesis and anticoagulant activity of sodium alginate sulfates. *Carbohydr Polym* 83(4): 1797-1803.

Farias, W.R., Nazareth, R.A., Mourao, P.A.S.(2001) Dual effects of sulfated D-galactans from the red algae *Botryocladia occidentalis* preventing thrombosis and inducing platelet aggregation. *Thromb Haemost* 86(6): 1540-1546.

Farias, W.R., Valente, A.P., Pereira, M.S., Mourao, P.A.S. (2000) Structure and anticoagulant activity of sulfated galactans: Isolation of a unique sulfated galactan from the red algae *Botryocladia occidentalis* and comparison of its anticoagulant action with that of sulfated galactans from invertebrates. *J Biol Chem* 275(38): 29299-29307.

Fernández, P.V., Quintana, I., Cerezo, A.S., Caramelo, J.J., Pol-Fachin, L., Verli, H., Estevez, J.M., Ciancia, M. (2013) Anticoagulant activity of a unique sulfated pyranosic (1→3)-*β*-l-arabinan through direct interaction with thrombin. *J Biol Chem* 288(1): 223-233.

Fischer, A. (1931) Inhibition of blood coagulation. *Biochem Z* 240: 364-380.

Gollin, R.A., Michaclis, R., Waker, D., Adams, S.S. (1965) Process for the preparation of laminarin sulfates. US PATENT 3, 200, 110 A.

Güven, K.C., Aktin, E. (1962) A study on the antilipemic and anticoagulant activities of *Phyllophora nervosa* (D. C.) Grev. *Eczacılık Bülteni* 4: 199-206.

Güven, K.C., Aktin, E. (1964) Studies on antilipaemic and anticoagulant properties of the algae collected from Turkish coasts. *Bot Mar* 7(1-4): 1-3.

Güven, K.C., Aktin, E., Koyuncuoglu, H., Bergisadi, N. (1974a) Lipolytic and hypoglycemic compounds obtained from *Cystoseira barbata. J Ag Arzneimittel-Forsch* 24(2): 144-147.

Güven, K.C., Bora, A., Aktin, E., Öker, C., Hatemi, H., Çetin, E.T. (1972) Investigations on *Phyllophora nervosa* (D. C.) Grev. *Bot Mar* 15(1): 46-51.

Güven, K.C., Bergişadi, N., Güler, E. (1979a) A modification of Duncombe's Method and its application to the lipolytic activity assay of heparin. *Fett Wiss Technol* 81(4): 152-154.

Güven, K.C., Güler, E., Aktin, E., Koyuncuoğlu, H. (1979b) Studies on *Pterocladia capillacea* (Gmel.) Born et Thur. Part II. In: Marine Algae in Pharmaceutical Science (eds. Hoppe, H. A., Levring, T., Tanaka, Y.) Walter de Gruyter, Berlin, New York, pp 693-710.

Güven, K.C., Güvener, B. (1985a) Metachromatic identification of (iota-, Kappa-, lambda-) carragenans. *Bot Mar* 28: 221-222.

Güven, K.C., Güvener, B. (1985b) A metachromatic method for identification of alginic acid, agar and carragenan. *Fett Wiss Technol* 87: 172-176.

Güven, K.C., Kızıl, Z. (1986) Pharmacological studies of *Halopytys incurvus* (Huds.) Batters (Rhodophyceae). *Acta Pharm Turc* 28: 47-50.

Güven, K.C., Mutluay, E., Aktin, E., Koyuncuoglu, H. (1975) Chemical and pharmacological investigations on *Corallina rubens*. *Planta Med* 27(1): 5-13.

Güven, K.C., Ozsoy, Y., Ulutin, O.N. (1991) Anticoagulant, fibrinolytic and antiaggregant activity of carregeenans and alginic acid. *Bot Mar* 34(5): 429-432.

Güven, K.C., Ulutin, S.B., Mutluay, E., Ulutin, O.N. (1973) Anticoagulant-antithrombin and fibrinolytic actions of extract of marine alga *Corallina rubens* L. *Pathophysiol. Haemost Thromb* 2(6): 260-268.

Güven, K. C., Ulutin, S. B., Mutluay, E., Ulutin, O. N. (1974b) Anticoagulant antithrombin and fibrinolytic actions of extract of marine alga *Corallina rubens* L. *Haemostasis* 2(6): 260-268.

Haroun-Bouhedja, F., Ellouali, M., Sinquin, C., Boisson-Vidal, C. (2000) Relationship between sulfate groups and biological activities of fucans. *Thromb Res* 100(5): 453-459.

Hassid, W.Z. (1933) The isolation of a sodium sulfuric acid ester of galactan from *Irideae Laminarioides* (Rhodophyceae). *J Am Chem Soc* 55(10): 4163-4167.

Hawkins, W.W., Leonard, V.G. (1958) The physiological activity of laminarin sulphate. *Can J Biochem Physiol* 36(1): 161-170.

Hawkins, W.W., Leonard, V.G. (1962) Antipeptic and antithrombic properties of carrageenin. *J Lab Clin Med* 60: 641-648.

Hawkins, W.W., Leonard, V.G. (1963) The antithrombic activity of carrageenin in human blood. *Can J Biochem Physiol* 41: 1325-1327.

Hawkins, W.W., O'Neill, A.N. (1955) The anticoagulant action in blood of sulphated derivatives of laminarin. *Can J Biochem Physiol* 33(4): 545-552.

Hayakawa, Y., Hayashi, T., Lee, J.-B., Srisomporn, P., Maeda, M., Ozawa, T., Sakuragawa, N. (2000) Inhibition of thrombin by sulfated polysaccharides isolated from green algae. *Biochim Biophys Acta - Prot Struct Mol Enzymol* 1543(1): 86-94.

Houck, J.C., Morris, R.K., Lazaro, E.J. (1957) Anticoagulant, lipemia clearing and other effects of anionic polysaccharides extracted from seaweed. *Exp Biol Med* 96(2): 528-530.

Howell, W.H., Holt, L.E. (1918) Two new factors in blood coagulation-heparin and pro-antithrombin. *Am J Physiol* 47: 328-341.

Ibrahim, E.A., Aly, H.F., and El-Baz, F.K. (2016) Anticoagulant and anti-inflammatory potentials of some Egyptian marine algae. *Int J Pharma Bio Sci Blood Coagul Fibrinolysis* 7(2): 89-94.

Irhimeh, M.R., Fitton, J.H., Lowenthal, R.M. (2009) Pilot clinical study to evaluate the anticoagulant activity of fucoidan. *Blood Coagulation & Fibrinolysis* 20(7): 607-610.

Jung, W.-K., Athukorala, Y., Lee, Y.-J., Cha, S. H., Lee, C.-H., Vasanthan, T., Choi, K.-S., Yoo, S.-H., Kim, S.-K., Jeon, Y.-J. (2007) Sulfated polysaccharide purified from *Ecklonia cava* accelerates antithrombin III-mediated plasma proteinase inhibition. *J Appl Phycol* 19(5): 425-430.

Jurd, K.M., Rogers, D.J., Blunden, G., McLellan, D.S. (1995) Anticoagulant properties of sulphated polysaccharides and a proteoglycan from *Codium fragile* ssp. *atlanticum*. *J Appl Phycol* 7(4): 339.

Kang, S.-R., Choi, J.-H., Kim, D.-W., Park, S.-E., Sapkota, K., Kim, S., Kim, S.-J. (2016) A bifunctional protease from green alga *Ulva pertussa* with anticoagulant properties: partial purification and characterization. *J Appl Phycol* 28(1): 599-607.

Karaki, N., Sebaaly, C., Chahine, N., Faour, T., Zinchenko, A., Rachid, S., Kanaan, H. (2013) The antioxidant and anticoagulant activities of polysaccharides isolated from the brown algae *Dictyopteris polypodioides* growing on the Lebanese coast. *J Appl Pharm Sci* 3(2): 43-51.

Kim, K.J., Lee, O.H., Lee, H.H., Lee, B.Y. (2010) A 4-week repeated oral dose toxicity study of fucoidan from the sporophyll of *Undaria pinnatifida* in Sprague-Dawley rats. *Toxicol* 267(1-3): 154-158.

Kindness, G., Long, W.F., Williamson, F.B. (1979a) Enhancement of antithrombin III activity by carrageenans. *Thromb Res* 15(1): 49-60.

Kindness, G., Long, W.F., Williamson, F.B., Boyd, J. (1979b) Effects of carrageenans on the aggregation of human blood platelets. *Thromb Res* 15(1-2): 3-15.

Kitamura, K., Matsuo, M., Yasui, T. (1991) Fucoidan from brown seaweed *Laminaria angustata* var. *longissima*. *Agric Biol Chem* 55(2): 615-616.

Kolender, A.A., Pujol, C.A., Damonte, E.B., Matulewicz, M.C., Cerezo, A.S. (1997) The system of sulfated alpha-(1-->3)-linked D-mannans from the red seaweed *Nothogenia fastigiata*: structures, antiherpetic and anticoagulant properties. *Carbohydr Res* 304(1): 53-60.

Kraan, S. (2012) Algal polysaccharides, novel applications and outlook. In: Carbohydrates –Comprehensive Studies on Glycobiology and Glycotechnology, (ed. Chang, C-F.), InTech, Rijeka, pp. 489-532.

Kuznetsova, T.A., Besednova, N.N., Mamaev, A.N., Momot, A.P., Shevchenko, N.M., Zvyagintseva, T.N. (2003) Anticoagulant activity of fucoidan from brown algae *Fucus evanescens* of the Okhotsk Sea. *Bull Exp Biol Med* 136(5): 471-473.

Kylin, H. (1913) Zur biochemie der meeresalgen. *H-S. Z. Physiol. Chem*. 83(3): 171-197.

Lee, S.-H., Athukorala, Y., Lee, J.-S., Jeon, Y.-J. (2008) Simple separation of anticoagulant sulfated galactan from marine red algae. *J Appl Phycol* 20(6): 1053-1059.

Li, N., Mao, W., Yan, M., Liu, X., Xia, Z., Wang, S., Xiao, B., Chen, C., Zhang, L., Cao, S. (2015) Structural characterization and anticoagulant activity of a sulfated polysaccharide from the green alga *Codium divaricatum*. *Carbohydr Polym* 121: 175-182.

Li, B., Zhao, R.-X., Wei, X.-J. (2008) Anticoagulant activity of fucoidan from *Hizikia fusiforme*. *Agro Food Industry Hi-Tech* 19(1): 22-24.

Lison, L. (1935) Etudes sur la metachromasie colorants metachromatiques et substances chromotropes. *Arch Biol* 46: 599-668.

Liu, J., Zhan, X., Wan, J., Wang, Y., Wang, C. (2015) Review for carrageenanbased pharmaceutical biomaterials: favourable physical features versus adverse biological effects. *Carbohydr Polym* 121: 27-36.

Maeda, M., Uehara, T., Harada, N., Sekiguchi, M., Hiraoka, A. (1991) Heparinoid-active sulphated polysaccharides from *Monostroma nitidum* and their distribution in the Chlorophyta. *Phytochem* 30(11): 3611-3614.

Majdoub, H., Mansour, M.B., Chaubet, F., Roudesli, M.S., Maaroufi, R.M. (2009) Anticoagulant activity of a sulfated polysaccharide from the green alga *Arthrospira platensis*. *Biochim Biophys Acta* 1790(10): 1377-1381.

Mao, W.-J., Fang, F., Li, H.-Y., Qi, X.-H., Sun, H.-H., Chen, Y., Guo, S.-D. (2008) Heparinoid-active two sulfated polysaccharides isolated from marine green algae *Monostroma nitidum*. *Carbohydr Polym* 74(4): 834-839.

Mao, W., Li, H., Li, Y., Zhang, H., Qi, X., Sun, H., Chen, Y., Guo, S. (2009) Chemical characteristic and anticoagulant activity of the sulfated polysaccharide isolated from *Monostroma latissimum* (Chlorophyta). *Int J Biol Macromol* 44(1): 70-74.

Mao, W., Zang, X., Li, Y., Zhang, H. (2006) Sulfated polysaccharides from marine green algae *Ulva conglobata* and their anticoagulant activity. *J Appl Phycol* 18(1): 9-14.

Margolis, J. (1958) The kaolin clotting time a rapid one-stage method for diagnosis of coagulation defects. *J Clin Pathol* 11(5): 406-409.

Matsubara, K., Matsuura, Y., Bacic, A., Liao, M., Hori, K., Miyazawa, K. (2001) Anticoagulant properties of a sulfated galactan preparation from a marine green alga, *Codium cylindricum*. *Int J Biol Macromol* 28(5): 395-399.

Matsubara, K., Matsuura, Y., Hori, K., Miyazawa, K. (2000) An anticoagulant proteoglycan from the marine green alga, *Codium pugniformis*. *J Appl Phycol* 12(1): 9.

Mauray, S., Sternberg, C., Theveniaux, J., Millet, J., Sinquin, C., Tapon-Bretaudiere, J., Fischer, A. M. (1995) Venous antithrombotic and anticoagulant activities of a fucoidan fraction. *Thromb Haemost* 74(5): 1280-1285.

McLean, J. (1916) The tromboplastic action of cephalin. *Am J Physiol* 41: 250-257.

McMillan, R.M., MacIntyre, D.E., Gordon, J.L. (1979) Stimulation of human platelets by carrageenans. *J Pharm Pharmacol* 31(3): 148-152.

Melo, F.R., Pereira, M.S., Foguel, D., Mourao, P.A.S. (2004) Antithrombin-mediated anticoagulant activity of sulfated polysaccharides: different mechanisms for heparin and sulfated galactans. *J Biol Chem* 279(20): 20824-20835.

Millet, J., Jouault, S.C., Mauray, S., Theveniaux, J., Sternberg, C., Boisson Vidal, C., Fischer, A.M. (1999) Antithrombotic and anticoagulant activities of a low molecular weight fucoidan by the subcutaneous route. *Thromb Haemost* 81(3): 391-395.

Molho, D., Cotte, J. (1951) Blood coagulation and anticoagulants. II. Sulfuric esters of alginates: preparation and action in vitro on the factors involved in coagulation. *Bull Soc Chim Biol* 33(3-4): 312-320.

Mookerjea, S., Hawkins, W.W. (1958) The antilipaemic activity of laminarin sulphate. *Can J Biochem Physiol* 36(3): 261-268.

Mourao, P.A. (2004) Use of sulfated fucans as anticoagulant and antithrombotic agents: future perspectives. *Curr Pharm Des* 10(9): 967-981.

Murata, K. (1961) Effects of carrageenin on serum lipids and atherosclerosis in rabbits. *Nature* 191: 189-190.

Murata, K. (1962) The effects of sulfated polysaccharides obtained from seaweeds on experimental atherosclerosis. *J Gerontol* 17: 30-36.

Nardella, A., Chaubet, F., Boisson-Vidal, C., Blondin, C., Durand, P., Jozefonvicz, J. (1996) Anticoagulant low molecular weight fucans produced by radical process and ion exchange chromatography of high molecular weight fucans extracted from the brown seaweed *Ascophyllum nodosum. Carbohydr Res* 289: 201-208.

Nishino, T., Fukuda, A., Nagumo, T., Fujihara, M., Kaji, E. (1999) Inhibition of the generation of thrombin and factor xa by a fucoidan from the brown seaweed *Ecklonia kurome*. *Thromb Res* 96(1): 37-49.

Nishino, T., Nishioka, C., Ura, H., Nagumo, T. (1994) Isolation and partial characterization of a noval amino sugar-containing fucan sulfate from commercial *Fucus vesiculosus* fucoidan. *Carbohydr Res* 255: 213-224.

Nishino, T., Yokoyama, G., Dobashi, K., Fujihara, M., Nagumo, T. (1989) Isolation, purification, and characterization of fucose-containing sulfated polysaccharides from the brown seaweed *Ecklonia kurome* and their blood-anticoagulant activities. *Carbohydr Res* 186(1): 119-129.

O'Neill, A.N. (1955) Sulphated derivatives of laminarin. *Can J Chem* 33(6): 1097-1101.

Pengzhan, Y., Ning, L., Xiguang, L., Gefei, Z., Quanbin, Z., Pengcheng, L. (2003) Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from *Ulva pertusa* (Chlorophyta). *Pharmacol Res* 48(6): 543-549.

Percival, E., McDowell, R. (1967) Chemistry and Enzymology of Marine Algal Polysaccharides. Academic Press London, New York.

Pereira, M.G., Benevides, N.M., Melo, M.R., Valente, A.P., Melo, F.R., Mourao, P.A. (2005) Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? *Carbohydr Res* 340(12): 2015-2023.

Pereira, M.S., Mulloy, B., Mourao, P.A. (1999) Structure and anticoagulant activity of sulfated fucans. Comparison between the regular, repetitive, and linear fucans from echinoderms with the more heterogeneous and branched polymers from brown algae. *J Biol Chem* 274(12): 7656-7667.

Pereira, M.S., Melo, F.R., Mourão, P.A.S. (2002a) Is there a correlation between structure and anticoagulant action of sulfated galactans and sulfated fucans? *Glycobiology* 12(10): 573-580.

Pereira, M.S., Vilela-Silva, A.-C.E.S., Valente, A.-P., Mourão, P.A.S. (2002b) A 2-sulfated, 3-linked α-l-galactan is an anticoagulant polysaccharide. *Carbohydr Res* 337(21-23): 2231-2238.

Pitney, W.R., Dacie, J.V. (1953) A simple method of studying the generation of thrombin in recalcified plasma: Application in the investigation of haemophilia. *J Clin Pathol* 6(1): 9-14.

Pulsawat, W., Tongmalee, S. (2014) Synthesis and Anticoagulant activity of Sulfated alginate. *KKU Res J* 19(Suppl): 60-66.

Pushpamali, W.A., Nikapitiya, C., Zoysa, M.D., Whang, I., Kim, S. J., Lee, J. (2008) Isolation and purification of an anticoagulant from fermented red seaweed *Lomentaria catenata*. *Carbohydr Polym* 73(2): 274-279.

Qi, X., Mao, W., Chen, Y., Chen, Y., Zhao, C., Li, N., Wang, C. (2013) Chemical characteristics and anticoagulant activities of two sulfated polysaccharides from *Enteromorpha linza* (Chlorophyta). *J Ocean Uni China* 12(1): 175-182.

Qi, X., Mao, W., Gao, Y., Chen, Y., Chen, Y., Zhao, C., Li, N., Wang, C., Yan, M., Lin, C., Shan, J. (2012) Chemical characteristic of an anticoagulant-active sulfated polysaccharide from *Enteromorpha clathrata*. *Carbohydr Polym* 90(4): 1804-1810.

Quick, A.J. (1945) On the quantitative estimation of prothrombin. *Am J Clin Pathol* 15(12): 560-566.

Ren, D.L., Noda, H., Amano, H., Nishino, T., Nishizawa, K. (1994) Study on antihypertensive and antihyperlipidemic effects of marine-algae. *Fish Sci* 60(1): 83-88.

Rocha, H.A., Moraes, F.A., Trindade, E.S., Franco, C.R., Torquato, R.J., Veiga, S.S., Valente, A.P., Mourao, P.A., Leite, E.L., Nader, H.B., Dietrich, C.P. (2005) Structural and hemostatic activities of a sulfated galactofucan from the brown alga *Spatoglossum schroederi*. An ideal antithrombotic agent? *J Biol Chem* 280(50): 41278-41288.

Rodrigues, J.A.G., Vanderlei, E.D.S.O., Bessa, É.F., Magalhães, F.D.A., Paula, R.C.M.D., Lima, V., Benevides, N.M.B. (2011) Anticoagulant activity of a sulfated polysaccharide isolated from the green seaweed *Caulerpa cupressoides*. *Braz. Arch Biol Techn* 54: 691-700.

Rogers, D.J., Jurd, K.M., Blunden, G., Paoletti, S., Zanetti, F. (1990) Anticoagulant activity of a proteoglycan in extracts of *Codium fragile* ssp. *atlanticum*. *J Appl Phycol* 2(4): 357-361.

Schimpf, K., Lenhard, J., Schaaf, G. (1969) The influence of the polysaccharide carrageenin on the clotting mechanism of the blood in the rabbit in vitro and in vivo. *Thromb Diath Haemorrh* 21(3): 524-33.

Schuler, W., Springer, G.F. (1957) Experimentelle antilipämiewirkung von fucoidin. *Naturwissenschaften* 44(8): 265-265.

Schwartz, H.J., Kellermeyer, R.W. (1969) Carrageenan and delayed hypersensitivity. II. Activation of Hageman factor by carrageenan and its possible significance. *Proc Soc Exp Biol Med* 132(3): 1021-1024.

Selim, S., Amin, A., Hassan, S., Hagazey, M. (2015) Antibacterial, cytotoxicity and anticoagulant activities from *Hypnea esperi* and *Caulerpa prolifera* marine algae. *Pak J Pharm Sci* 28(2): 525-530.

Sen, A.K., Das, A.K., Banerji, N., Siddhanta, A.K., Mody, K.H., Ramavat, B. K., Chauhan, V. D., Vedasiromoni, J. R., Ganguly, D. K. (1994) A new sulfated polysaccharide with potent blood anti-coagulant activity from the red seaweed *Grateloupia indica*. *Int J Biol Macromol* 16(5): 279-280.

Shanmugam, M., Mody, K.H., Oza, R.M., Ramavat, B.K. (2001a) Blood anticoagulant activity of a green marine alga *Codium dwarkense* (Codiaceae, Chlorophyta) in relation to its growth stages. *Ind J Geo-Mar Sci* 30(1): 49-52.

Shanmugam, M., Mody, K. H., Ramavat, B. K., Murthy, A. S. K., Siddhanta, A. K. (2002) Screening of *Codiacean* algae (Chlorophyta) of the Indian coasts for blood anticoagulant activity. *Ind J Geo-Mar Sci* 31(1): 33-38.

Shanmugam, M., Mody, K.H., Siddhanta, A.K. (2001b) Blood anticoagulant sulphated polysaccharides of the marine green algae *Codium dwarkense* (Boergs.) and *C. tomentosum* (Huds.) Stackh. *Ind J Exp Biol* 39: 365-370.

Shanmugam, M., Ramavat, B. K., Mody, K. H., Oza, R. M., Tewari, A. (2001c) Distribution of heparinoid-active sulphated polysaccharides in some Indian marine green algae. *Ind J Geo-Mar Sci* 30(4): 222-227.

Shonima, G. M., Jiji, T., Pratheesh, P. T., Kurup, G. M. (2012) Ex vivo anticoagulant activity of the polysaccharide isolated form *Ulva fasciata*. *Int J LifeSc Bt and Pharm Res* 1(2): 194-197.

Shore, B., Nichols, A.V., Freeman, N.K. (1953) Evidence for lipolytic action by human plasma obtained after intravenous administration of heparin. *Proc Soc Exp Biol Med* 83(2): 216-220.

Siddhanta, A.K., Shanmugam, M., Mody, K.H., Goswami, A.M., Ramavat, B.K. (1999) Sulphated polysaccharides of *Codium dwarkense* Boergs. from the west coast of India: chemical composition and blood anticoagulant activity. *Int J Biol Macromol* 26(2-3): 151-154.

Silva, T.M., Alves, L.G., de Queiroz, K.C., Santos, M.G., Marques, C.T., Chavante, S.F., Rocha, H.A., Leite, E.L. (2005) Partial characterization and anticoagulant activity of a heterofucan from the brown seaweed *Padina gymnospora*. *Braz J Med Biol Res* 38(4): 523-533.

Soeda, S., Sakaguchi, S., Shimeno, H., Nagamatsu, A. (1992) Fibrinolytic and anticoagulant activities of highly sulfated fucoidan. *Biochem Pharmacol* 43(8): 1853-1858.

Springer, G.F., Wurzel, H.A., McNeal, G.M., Ansell, N. J., Doughty, M.F. (1957) Isolation of anticoagulant fractions from crude fucoidin. *Exp Biol Med* 94(2): 404-409.

Sun, Y., Chen, X., Liu, S., Yu, H., Li, R., Wang, X., Qin, Y., Li, P. (2018) Preparation of low molecular weight *Sargassum fusiforme* polysaccharide and its anticoagulant activity. *Chin J Oceanol Limn* 36(3): 882-891.

Takemori, S. (1957a) Isolation and composition of some seaweed polysaccharide sulfates and their anticoagulant activity I. *Hirasaki Igaku* 8: 749-759.

Takemori, S. (1957b) Isolation and composition of some seaweed polysaccharide sulfates and their anticoagulant activity II. *Hirasaki Igaku* 8: 760-769.

Trento, F., Cattaneo, F., Pescador, R., Porta, R., Ferro, L. (2001) Antithrombin activity of an algal polysaccharide. *Thromb Res* 102(5): 457-465.

Uehara, T., Takeshita, M., Maeda, M. (1992) Studies on anticoagulant-active arabinan sulfates from the green alga, *Codium latum*. *Carbohydr Res* 235: 309-311.

Ushakova, N.A., Morozevich, G.E., Ustyuzhanina, N.E., Bilan, M.I., Usov, A.I., Nifantiev, N.E., Preobrazhenskaya, M.E. (2009) Anticoagulant activity of fucoidans from brown algae. *Biochem (Moscow)* 3(1): 77-83.

Ustyuzhanina, N.E., Bilan, M.I., Gerbst, A.G., Ushakova, N.A., Tsvetkova, E.A., Dmitrenok, A.S., Usov, A.I., Nifantiev, N.E. (2016) Anticoagulant and antithrombotic activities of modified xylofucan sulfate from the brown alga *Punctaria plantaginea*. *Carbohydr Polym* 136: 826-833.

Usui, T., Asari, K., Mizuno, T. (1980) Isolation of highly purified “fucoidan” from *Eisenia bicyclis* and its anticoagulant and antitumor activities. *Agric Biol Chem* 44(8): 1965-1966.

Wang, X., Zhang, Z., Yao, Z., Zhao, M., Qi, H. (2013) Sulfation, anticoagulant and antioxidant activities of polysaccharide from green algae *Enteromorpha linza*. *Int J Biol Macromol* 58: 225-230.

Wang, J., Zhang, Q., Zhang, Z., Song, H., Li, P. (2010) Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from *Laminaria japonica*. *Int J Biol Macromol* 46(1): 6-12.

Wijesinghe, W.A.J.P., Athukorala, Y., Jeon, Y.J. (2011) Effect of anticoagulative sulfated polysaccharide purified from enzyme-assistant extract of a brown seaweed *Ecklonia cava* on Wistar rats. *Carbohydr Res* 86(2): 917-921.

Winter, C.A., Risley, E.A., Nuss, G.W. (1962) Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Exp Biol Med* 111(3): 544-547.

Yoon, S. J., Pyun, Y.R., Hwang, J.K., Mourao, P.A. (2007) A sulfated fucan from the brown alga *Laminaria cichorioides* has mainly heparin cofactor II-dependent anticoagulant activity. *Carbohydr Res* 342(15): 2326-2330.

Yu, Y., Li, Y.P., Du, C.Y., Mou, H.J., Wang, P. (2017) Compositional and structural characteristics of sulfated polysaccharide from *Enteromorpha prolifera*. *Carbohydr Polym* 165: 221-228.

Yuan, Y., Xu, X., Jing, C., Zou, P., Zhang, C., Li, Y. (2018) Microwave assisted hydrothermal extraction of polysaccharides from *Ulva prolifera*: Functional properties and bioactivities. *Carbohydr Polym* 181: 902-910.

Zha, X.Q., Xiao, J.J., Zhang, H.N., Wang, J.H., Pan, L.H., Yang, X.F., Luo, J.P. (2012) Polysaccharides in *Laminaria japonica* (LP): Extraction, physicochemical properties and their hypolipidemic activities in diet-induced mouse model of atherosclerosis. *Food Chem* 134: 244-252.

Zhang, H., Mao, W., Fang, F., Li, H., Sun, H., Chen, Y., Qi, X. (2008) Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from *Monostroma latissimum*. *Carbohydr Res* 71(3): 428-434.

Zhang, Z., Till, S., Jiang, C., Knappe, S., Reutterer, S., Scheiflinger, F., Szabo, C.M., Dockal, M. (2014) Structure-activity relationship of the pro- and anticoagulant effects of *Fucus vesiculosus* fucoidan. *Thromb Haemost* 111(3): 429-437.

Zhang, Z., Zhang, Q., Wang, J., Song, H., Zhang, H., Niu, X. (2010) Regioselective syntheses of sulfated porphyrans from *Porphyra haitanensis* and their antioxidant and anticoagulant activities in vitro. *Carbohydr Res* 79(4): 1124-1129.

Zhao, T., Zhang, Q., Qi, H., Zhang, H., Niu, X., Xu, Z., Li, Z. (2006) Degradation of porphyran from *Porphyra haitanensis* and the antioxidant activities of the degraded porphyrans with different molecular weight. *Int J Biol Macromol* 38(1): 45-50.

Zúñiga, E.A., Matsuhiro, B., Mejías, E. (2006) Preparation of a low-molecular weight fraction by free radical depolymerization of the sulfated galactan from *Schizymenia binderi* (Gigartinales, Rhodophyta) and its anticoagulant activity. *Carbohydr Polym* 66(2): 208-215.