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RESEARCH ARTICLE

In-vitro anticoagulant activity of marine brown algae species *Padina tetrastromatica*

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ABSTRACT

The brown algae exhibit many health beneficial nutraceutical effects such as antioxidant, anti-allergic, anti-human immunodeficiency virus, anticancer and anticoagulant activities. To prevent abnormal bleeding and to maintain intravascular blood in a fluid state, in this study we aimed to evaluate the possible anticoagulant effect of marine algae extracts of Padina tetrastromatica. The algae was collected from different locations of Mandapam area, Tamilnadu, extracted with chloroform, methanol solvent the standard kit was purchased for the anticoagulant assay the APTT, PT, TT assay were performed and different concentration was used from 2mg/ml to 20mg/ml the result was tabulated and the methanol solvent possess more anticoagulant than chloroform as APTT value for 20mg/ml is 179sec, PT values is 54sec and TT values is 74 respectively.

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1. Introduction

The brown algae exhibit many health beneficial nutraceutical effects such as antioxidant, anti-allergic, anti-human immunodeficiency virus, anticancer and anticoagulant activities. To prevent abnormal bleeding and to maintain intravascular blood in a fluid state, in this study we aimed to evaluate the possible anticoagulant effect of marine algae extracts of Padina tetrastromatica. The algae was collected from different locations of Mandapam area, Tamilnadu, extracted with chloroform, methanol solvent the standard kit was purchased for the anticoagulant assay the APTT, PT, TT assay were performed and different concentration was used from 2mg/ml to 20mg/ml the result was tabulated and the methanol solvent possess more anticoagulant than chloroform as APTT value for 20mg/ml is 179sec, PT values is 54sec and TT values is 74 respectively.

2. Materials and Methods

Collection of marine algae:

Fresh algae of Padina tetrastromatica were collected from different locations of Mandapam area, Tamilnadu, South East Coast of India. In the early morning 5 am to 11.30am during which the tidal height was from 0.77 meter to 0.08 meter (lat 9° 15' N; long 79°E). Then the algae were washed thoroughly with sea water to remove extraneous materials and brought to the laboratory in plastic bag containing water to prevent evaporation. Samples were then shade dried until constant weight obtained and ground in an electric mixer. The powdered samples subsequently stored in refrigerator (Sellappa Sudha et al, 2014)

Preparation of Extracts: The shade dried material was extracted with analytical grade petroleum ether, chloroform and methanol for 8 hours by continuous hot percolation in Soxhlet apparatus (Harborne, 1984). The Extracts were dried under vacuum. The dry drug extract were dissolved in dimethyl formamide (DMF) the chloroform and methanol extract are used for further studies

Anticoagulation assay:

Blood was drawn from healthy people without history of bleeding disease (Wenjun Mao et al 2006, A.K Siddhanta et al, 2002). Nine parts of blood was collected by vein puncture and mixed with one part of 3.8% Tri Sodium Citrate. Blood was centrifuged at 5000rpm at 10mins. Supernatant was collected designated as plasma. Test samples were prepared at different concentrations as 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml, 10mg/ml, 12mg/ml, 14mg/ml, 16mg/m, 18mg/ml, and 20mg/ml

APTT assay: APTT assay was performed by using the standard APTT assay kit purchased from Diagnostic Enterprises, Himachal Pradesh. 100µl of plasma was mixed with 10µl of solution of different concentrations of sulphated polysaccharides and then 100µl of prewarmed APTT assay reagent was added and incubated for 3 minutes at 37°C and to this 100µl of calcium chloride is added and the time taken for formation of clot was recorded.

PT assay: Assay was performed by using the standard PT assay kit purchased from Diagnostic Enterprises, Himachal Pradesh. Prothrombin time (PT) assay was carried out using normal human plasma (100µl) mixed with solution of algal extract (10µl) then after 3 min incubation, clotting was induced by the addition of thrombo reagent (200µl) and clotting time was recorded.

Thrombin time (TT) assay: Thrombin time (TT) assay was performed with citrated normal human plasma (90µl) was mixed with 10µl of a solution of algal extract and incubated for 1min at 370c. Then TT assay reagent (200µl), prewarmed for 10min at 370c, was added and clotting time was recorded (Zhang et al, 2008).

3. Results and Discussion

Anticoagulation activity:

Crude extracts of different concentrations were taken and the anticoagulation activity profile was established by using Activated Partial Thromboplastin Time (APTT) Prothrombin time (PT) and Thrombin time assay. APTT assay of chloroform extract was found to have APTT activity of 156seconds at 20 mg/ml and 175 seconds at 20mg/ml for methanol extract. The results have been presented in Table 1and Table 2.

Prothrombin (PT) assay:

In PT various concentration was performed the chloroform extract of Padina tetrastromatica was 40 seconds at 20mg/ml and the methanol extract was 48 seconds at 20mg/ml The results have been presented in Table 1and Table 2.

Thrombin time (TT) assay:

In TT various concentration was performed the chloroform extract of Padina tetrastromatica was 63 seconds at 20mg/ml and the methanol extract was 82 seconds at 20mg/ml. The results have been tabulated in table 1and 2 Anticoagulant activity associated with polysaccharides in marine algae was first reported in Iridae laminariodes by (Chargaff et al, 1936) due to the presence of galactan sulphuric acid. After this many seaweed species were reported to possess anticoagulant activity and they were Grateloupia filicina (Muruganantham, 2001) Grateloupia indica and etc.

Heparin has been widely used in anticoagulant therapy for more than 50 years. The major mechanism by which unfractionated heparin exerts its anticoagulant effect is by accelerating serine proteinase inhibitor plasma factor such as thrombin (factor IIa) and factor Xa. But being from animal origin, heparin can induce diseases in mammals, such as avian influenza and bovine spongiform encephalopathy (Mendes et al, 2009). These reasons reinforce the need to find a new anticoagulant and antithrombotic agent replacing heparin. The anticoagulant activity of the sulphated polysaccharides depends on their degree of substitution, their molecular weights and the position of the sulfate group (Jiraporn et al, 2009).

Thus, in the clotting cascade blocks the intrinsic pathway by inhibiting factors XII, XI, X, IX, VIII, prothrombin which identified by the results of APTT assay. Anticoagulant activity is largely dependent on the sugar composition, sulfate content, sulfate position and molecular weight of the compound. (Shanmugam and Mody, 2000) This anticoagulation activity may be due to the presence of uronic acids. The present results are in concomitant with (Toshihiko et al, 2003 and El-Baroty et al, 2005). Who explained that, the polysaccharides containing uronic acids, carrying a negative charge, have the ability for binding calcium ions and therefore prevent the formation of clot. In addition, variation in anti-coagulating activity of the different solvents is probably due to the quantity of uronic acids in polysaccharides and some conformational differences in the molecules of these polysaccharides While, (Mao et al, 2006) declared that the anticoagulant activities of the sulfated polysaccharides from the red algae Padina tetrastromatica. It also blocks extrinsic pathway by inhibiting factors X, V, prothrombin which was identified through PT and also by TT.

4. Conclusion

In this research work, Based on that different concentration of samples are prepared, tested for anticoagulant activity the

methanol extract possess more anticoagulant activity than chloroform extract and it would studied only *invitro* of anticoagulant assay in future in vivo study is possessed and the compound responsible for the anticoagulant is evaluated.

Table -1: *In-vitro* anticoagulant activity of APTT, PT, TT for Chloroform extract

Sample (mg/ml)	APTT (sec)	Sample (mg/ml)	PT (sec)	Sample (mg/ml)	TT (sec)
Control	32	Control	12	Control	22
2	48	2	13	2	26
4	68	4	13	4	34
6	79	6	14	6	38
8	74	8	16	8	40
10	92	10	20	10	45
12	108	12	24	12	53
14	116	14	30	14	56
16	122	16	35	16	58
18	136	18	36	18	62
20	142	20	42	20	66

Table -2: *In-vitro* anticoagulant activity of APTT, PT, TT for Methanol extract

Sample (mg/ml)	APTT (sec)	Sample (mg/ml)	PT (sec)	Sample (mg/ml)	TT (sec)
Control	32	Control	12	control	22
2	38	2	14	2	28
4	54	4	16	4	34
6	68	6	18	6	40
8	78	8	18	8	46
10	90	10	22	10	50
12	118	12	26	12	57
14	138	14	32	14	61
16	152	16	40	16	68
18	167	18	48	18	72
20	179	20	54	20	74

5. References

[1] Boergs. and C. tomentosum Huds. Stackh. Indian J Exp Biol 2001; 39:365–70. 2007; 17:541–52.

[2] A. K Siddhanta, A.M Goswami, M.Shanmugam, K. H. Mody, B.K.Ramavat & O.P Mairh. Sulphated galactans of marine red algae Laurencia sp. from the west coast of India. Indian J of Marine Science 2002; 31.4: 305-309

[3] Albuquerque IR, Queiroz KC, Alves LG, Santos EA, Leite EL, Rocha HA. Heterofucans from Dictyota menstrualis have anticoagulant activity. Braz J Med Biol Res 2004; 37:167–71.

[4] Azevedo TCG, Bezerra ME, Santos MD, Souza LA, Marques CT, Benevides NM, et al. Heparinoids algal and their anticoagulant, hemorrhagic activities and platelet aggregation. Biomed Pharmacother 2009; 63:477–83.

[5] Bilan MI, Usov AI. Structural analysis of fucoidans. Nat Prod Commun 2008; 3:1639–48.

[6] Chargaff, E F.W. Bancroft and M,Stanley-Brown, studies on the chemistry of coagulation II. On the inhibition of blood clotting by substance on high molecular weight. J.Biol.Chem, 1936 115,155-161

[7] Davie E. W., Fujikawa K., Kisiel W. The coagulation cascade: initiation, maintenance, and regulation. Biochemistry. (1991); 30:103 63–10370.

[8] El-Baroty G., Shallan M., Ali M.A., Abd El-Baky H.H., El-Baz F.K., Shalaby E.A. Antiviral activity of different extracts from Spirulina maxima. 3rd Conference on Recent Technologies in Agriculture, 2005, November 8-15:1-7.

[9] Fonseca RJC, Oliveira SNMCG, Melo FR, Pereira MG, Benevides NMB, Mourao PAS. Slight differences in sulfation of algal galactans account for differences in their anticoagulant and venous antithrombotic activities. Thromb Haemost 2008; 99:539–45.

[10] Harborne JB. Phytochemical methods 11 Edn.In Chapman &, Hall. New York, 1984; 4-5.

[11] H-J. Zhang, W-J. Mao, F. Fang, H-Y. Li, H-H. Sun, Y. Chen,.Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from Monostroma latissimum Carbohydrate Polymers, 71 (2008), pp. 428-434

[12] Hui-Juan Zhang,Wen-JunMao, Fang Fang, Hong-Yan Li, Hai-Hong Sun, Yin Chen, Xiao-Hui Qi.chemical characteristics and anticoagulant activities of a sulphated polysaccharide and its fragements from Mono stromala tissimum. Carbohydrate polymer, 71(2008) 428-434.

[13] Jiraporn S., Zhenqing Z., Boyangzi L., Preeyanat V., Puttinan M., Fuming Z.,. Sulfonation of papain-treated chitosan and its mechanism for anticoagulant activity, Carbohydrate Research. 2009; 344:1190–1196

[14] Li B, Lu F, Wei X, Zhao R. Fucoidan: structure and bioactivity. Molecules 2008; 13:1671–95

[15] Mao W., Zang X., Li Y., Zhang H. Sulfated polysaccharides from marine green algae Ulva conglobata and their anticoagulant activity. Journal of Applied Phycology,2006 18: 9-14.

[16] Medeiros VP, Queiroz KC, Cardoso ML, Monteiro GR, Oliveira FW, Chavante SF, et al. Sulfated galactofucan from Lobophora variegata: anticoagulant and antiinflammatory properties. Biochemistry (Mosc) 2008; 73:1018–24

[17] Mendes S.F. O., dos Santos Jr A.M., Barbosa A.F.D. Vasconcelos and G. Aranda-Selverio. Sulfonation and anticoagulant activity of botryosphaeran from Botryosphaeria rhodina MAMB-05 grown on fructose. Int. J. Biol. Macromol. 2009; 45: 305-309

[18] Mulloy B. The specificity of interactions between proteins and sulfated polysaccharides. An Acad Bras Cienc 2005; 77:651–64.

[19] Muruganantham, A . Heparin from seaweeds. A search in Grateloupia filicina J.Ag P 1-4

[20] Nader HB, Lopes CC, Rocha HA, Santos EA, Dietrich CP. Heparins and heparinoids: occurrence, structure and mechanism of antithrombotic and hemorrhagic activities. Curr Pharm Des 2004; 10:951–66.

[21] Painter, T.J. Algal polysaccharides. The Polysaccharide. Academic Press, New York, 1983 2: 195-285

[22] Quick, A.J., Bleeding problems in clinical medicine, Hemorrhagic Diseases and Thrombosis, W.B. Saunders Co., Philadelphia, 1970,225.

[23] Quick, A.J., Coagulation, Hemorrhagic Diseases and Thrombosis, Lea and Febiger, Philadelphia, 1966, 460.

[24] Sellappa Sudha, Palanisamy Senthilkumar, and Subramanian Prakash. Antidiabetic activity of aqueous extract of Padina boergesenii in Streptozotocin-induced diabetic rats. International Journal of Pharmacy and Pharmaceutical Sciences 2014 6(5), P 418-422

[25] Shanmugam M, Mody K.H. Heparinoid–active sulphated polysaccharides from marine algae as potential anticoagulant agents. Current Science, 2000, 79:1672-1682.

[26] Shanmugam M, Mody KH, Siddhanta AK. Blood anticoagulant sulphated polysaccharides of the marine green algae Codium dwarkense

[27] Silva TM, Alves LG, de Queiroz KC, Santos MG, Marques CT, Chavante SF, et al. Partial characterization and anticoagulant activity of a heterofucan from the brown seaweed Padina gymnospora. Braz J Med Biol Res, 2005, 38: 523–33.

[28] Toshihiko T., Amornut C., Robert L.J. Structure and bioactivity of sulfated polysaccharides. Trend in Glycosci and Glycotechnol, 2003, 5:29-46,