

EXTRACTION OF POLYSACCHARIDES FROM THE LEAVES OF JEWS-MALLOW *Corchorus olitorius L.* AND THEIR POTENTIAL ANTICOAGULANT ACTIVITY

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ABSTRACT

Polysaccharide was extracted from the leaves of (Molokhia) Jew's-mallow by hot-water extraction using trichloro acetic acid(TCA) to removal of protein from the polysaccharide ,precipitation with ethanol, and obtained a polysaccharide yield from 4.2% based on wet weight source. The biochemical composition of the polysaccharide contains total carbohydrate,sulfateandprotein77.6%, 8.6%, 0.63%, respectively. Phytochemical tests were carried out for polysaccharide. The previous studies indicated the presence of glycoside, phenol and tannin in polysaccharide from Jew's-mallow. However, the presence of both alkaloids and saponins was not observed .The Fourier transform infrared (FTIR) spectral analysis of the polysaccharides consist of carboxyls and sulfate groups.The anticoagulant activity of polysaccharide was evaluated by activated partial thromboplastin time (APTT) and prothrombin time (PT) assays with respect to heparin The results obtained by APTT assay, through the increase in coagulation time, the increase in blood coagulation time with increased polysaccharide concentration, the 100 µg/ml polysaccharide concentration had the longest time of 210Sec and was compatible with the concentration of1000 (IU/ ml) of heparin.However the time of PT did not have any apparent effectiveness in increasing the time of coagulation at all concentration compared with heparin.

INTRODUCTION

Corchorus olitorius (Linn.) is an annual herb with slender stems, it belong to the family Tiliaceae and commonly known as “Juteor Jew Mallow”. It is found in the Mediterranean region,its tender leaves known as (Molokhia), are eaten in several

countries(1,2).It is considered as a common vegetable in Egypt, the Philippines, Australia, Senegal and Thailand (3). Jew's-mallow is native to Egypt and the Middle East.Plant stem is an important source of fibres, while its leaves are very rich in b-carotene, vitamin B and also contain water soluble miosilage(4).Molokhia leaves contain a significant amount of mu-cilaginous polysaccharide, which is rich in uronic acidwas approximately90% of total sugars, and consists of glucose, rhamnose, galacturonic acid, and glucuronic acid(5,6).Polysaccharides of water-soluble, long-chain polymers with high molecular weight, are generally present in cell walls of higher plants. In recent years, there has been an increasing interest in polysaccharides that have been isolated from higher plants that may have varied diversity in chemical structure and biological (7,8). The polysaccharides extracted from higher plants are widely used in diverse fields in the food, pharmaceutical and many other industries(9). Sulfated polysaccharides have diverse biological activities including antitumor (10), anti-inflammatory (11), immune-modulatory (12), anticoagulant (13), antioxidant (14), antimicrobial (15) and antiviral (16). In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of plants used in various pharmaceutical application such as diluents, binder, disintegrant in tablet formulation, thickeners in oral liquids, protective colloids in suspensions, gellingagents in gels and bases in suppository(17,18), Since these biopolymers are copious, come from renewable sources, are relatively inexpensive, are highly stable, safe, non-toxic and hydrophilic and gel forming in nature are amenable to both chemical and biochemical modification, it is not astonishing that they find pervasive and extensive use(19).Only recent studies have demonstrated the anticoagulant activity of plant polysaccharides, an activity that was associated to the presence of uronic acid residues (20).Now the diseases that involve heart and blood vessels and as a consequence thrombosis ,it causes of death. The anticoagulant action of heparin is achieved mainly through potentiation of antithrombin and heparin cofactor II, the major inhibitors of coagulation enzymes, in particular thrombin and factor Xa(21,22). Heparin has several side effects, such as development of thrombocytopenia, hemorrhagic effect, ineffectiveness in congenital or acquired antithrombin deficiencies, and incapacity to inhibit thrombin bound to fibrin. Furthermore, the incidence of prion-related diseases in mammals and the increasing need for antithrombotic therapy indicate that we should look for alternative sources of anticoagulant and antithrombotic compounds

(23,24). Thus, the aim of this study was to isolate, characterize and evaluate the anticoagulant effects of the polysaccharides of Jew's-mallow (*Molokhia*).

MATERIALS AND METHODS

Plant material

Frozen (*Molokhia*) Jew's-mallow *Corchorus olitorius* used from the Al-wadi Al-akhdar produced in Egypt for DOVE PROCESSING SAL.

Chemicals and reagents

Bovine serum albumin (BSA) were purchased from Sigma. Heparin was obtained from Hipolabor. Kits for activated partial thromboplastin time (APTT) and prothrombin time (PT) were (BIOLABO SA, INDIA). All other chemicals and reagents were of analytical grade.

Human blood

Human blood (3.2% sodium citrate) was obtained from healthy volunteer donors of the Health Center at Basrah University.

Extraction, isolation and purification of polysaccharides

The water-soluble polysaccharide was extracted from the leaves of (*Molokhia*) Jew's-mallow (*Corchorus olitorius*) as described previously Sanya (25) Thirty grams of the leaves of *Molokhia* was heated with 100 ml water at 90°C on the magnetic stirrer for one hour. After cooling and filtration with a cheesecloth, the residual material was further extracted under the same conditions. The resulted extract was mixed with the first one and then concentrated under vacuum 45°C to half volume to get purer polysaccharides using trichloroacetic acid (TCA) to removal of protein from the crude polysaccharides. The above solution was mixed with 10% TCA v/v (26), and allowed to stand for 1h at room temperature. The supernatant was harvested by decanting precipitate after being centrifuged for 20 min 5000 rpm. Then dialyzed against distilled water for 48 h MW cut-off 7 kDa. The dialyzed solution was then centrifuged 5000 rpm for 15 min and the supernatant containing the polysaccharide was precipitated with 3 volumes of ethanol 95% for 24 h at 4 °C. After another

centrifugation for 20 min at 5000 rpm. The resulted precipitate was then dried by vacuum freezing dryer (Denmark. Hetosicc), weighed and kept tightly refrigerated. The polysaccharide yield (%) based on wet weight source was then calculated using the following equation:

Weight of polysaccharides (g)

$$\text{Yield of polysaccharide (\%)} = \frac{\text{Weight of polysaccharides (g)}}{\text{Weight of raw material (g)}} \times 100$$

Weight of raw material (g)

Primary qualitative tests of polysaccharide

Phytochemical tests were carried out for polysaccharide. Qualitative estimation of phenol was done as described previously by (27). Presence of glycosides and saponins were qualitatively evaluated as described by (28). Presence of tannins were confirmed as described by (29).

Chemical analysis of polysaccharide (total sugar, sulphate and protein)

Total sugars were assayed by the phenol-sulfuric method using glucose as a standard (30). Proteins were estimated as described previously by (31) and the protein content was calculated from a standard curve of pure bovine albumin. Sulfate content was determined by the gelatin-barium method using sodium sulfate (1 mg/ml) as standard and after acid hydrolysis of the polysaccharides by 6 N HCl at 100°C for 6 h (32).

FTIR analysis

The IR spectra of the polysaccharides were determined using a Fourier transform infrared spectrometer (FTIR) (JASCO Japan). The polysaccharide was ground with spectroscopic grade potassium bromide (KBr) powder and then pressed into 1 mm pellets for FTIR measurement in the wave number range of 400 and 4000 cm^{-1} using 2 scans.

Anti-coagulation assay

Activated partial thromboplastin time (APTT)

APTT assay was carried out as described previously by (33). All clotting assays were carried out using normal citrated human plasma according to the manufacturers' specifications. For this, 90 µl of citrated normal human plasma was mixed with 10 µl of a solution of different 10,25,50,75 and 100 µg/ml polysaccharide amounts before adding 100 µl of APTT reagent. The mixture was then incubated for 3 minutes at 37°C. Then, 100 µl of 0.025 M calcium chloride reagent was added to the mixture to trigger the coagulation cascade. The clotting time was recorded in a coagulometer. Heparin with 50,100 international units per mg (IU/ml) of polysaccharide was used as the standard. All the tests were performed in triplicate.

Prothrombin time (PT)

Prothrombin time was determined as described by (13). Prothrombin time was examined through the standard kit for prothrombin time determination (BIOLABO SA, INDIA). The reaction mixture containing both different concentration of polysaccharide at the concentration of 10,25,50,75 and 100 µg/ml in saline solution was incubated with 90 µl of citrated normal human plasma for 3 min at 37°C, then adding 200 µl PT reagent and the time for clot formation was recorded. The reaction mixture containing Water and heparin were used as negative and positive controls, respectively.

RESULTS AND DISCUSSION

Primary qualitative tests of polysaccharide

The phytochemical analysis for the presence of glycosides, phenol, tannin, alkaloids and saponins were carried out in polysaccharide from Jew's-mallow. The studies indicated the presence of glycoside, phenol and tannin in polysaccharide from Jew's-mallow (Table 1). While the presence of both alkaloids and saponins was not observed in polysaccharide from Jew's-mallow. The role of tannin and phenol (34,35) as antioxidants have been reported. However, no report on anticoagulant activity by these molecules is found.

Table 1. Primary qualitative tests of polysaccharide from Jew's-mallow

Glycoside	Phenol	Tannins	Alkaloids	Saponins
+	+	+	-	-

Estimation of total sugar, sulphate and protein

Some of the components (sugar, sulphate and protein) and yield of polysaccharide from Jew's-mallow are shown in Table2. The data indicates that total sugar, protein and sulphate content was 77.6, 0.63 and 8.6% respectively, As a rule, molecular size and sulfate content are among the most important prerequisites for polysaccharide to have anticoagulant

Table 2. Analysis of total polysaccharide(sugar, sulphate and protein) and Yield

Composition		%
1	total sugar	77.6
2	total sulphate	8.6
3	Protein	0.63
4	Yield	4.2

activity (36), while the yield of polysaccharide from Jew's-mallow was obtained with 4.2% (w/w) from the wet weight source.

FT-IR structural characterization of polysaccharides

Fourier transform infra-red (FT-IR) spectroscopy was performed in the 4000–400 cm^{-1} region to further characterize the data so far obtained for the polysaccharides from Jew's-mallow (Figure1). The bands at 3392.17 cm^{-1} represented the stretching of the hydroxyl groups and that at 2921.63 cm^{-1} corresponds to a weak C-H stretching vibration (37). Furthermore, the bands at 1649.8 cm^{-1} were attributed to the bond

stretching vibrations of C–O bonds in the acylamino group(38).The most important bands were found at 1042.34cm^{-1} indicated D-glucose and 1262.06cm^{-1} corresponding to ester sulfate groups(S=O)(39).The absorption peak at 1151.29cm^{-1} indicated a pyranose unit. The region around 936cm^{-1} can be attributed to the(C–O–S) group of 3,6-anhydro- α -L-galactopyranose and the absorption around 867cm^{-1} indicates the presence of sulfate groups on the C-4 of galactose (40).

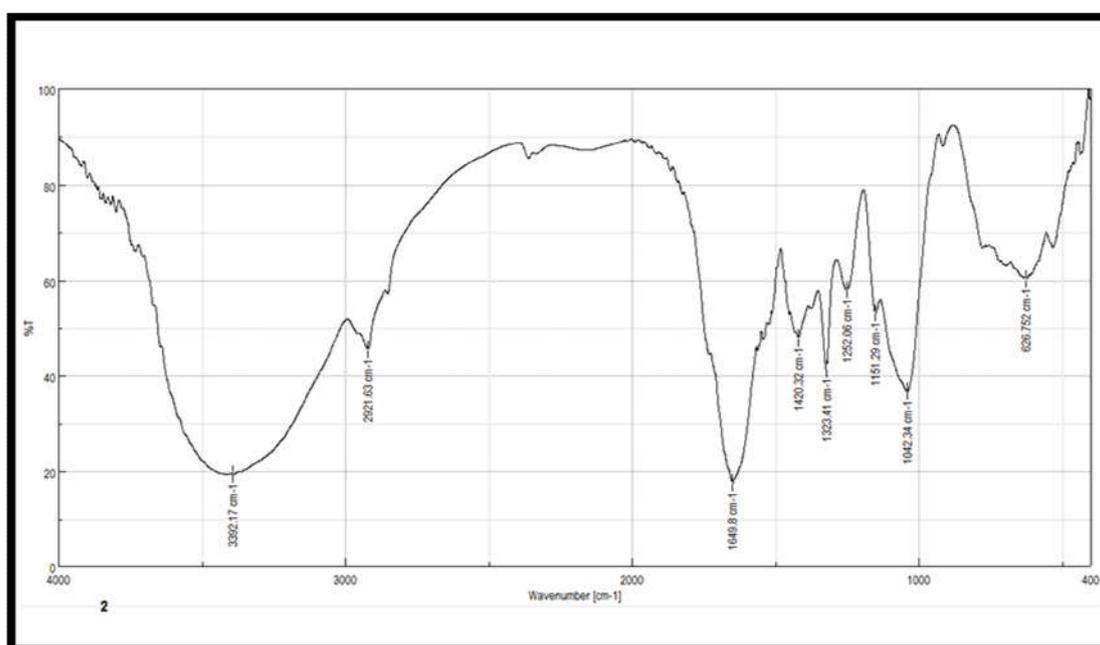


Fig. 1. FT-IR spectrometry of polysaccharides from Jew's-mallow

Anticoagulation activity of the polysaccharide

In vitro, anticoagulant activity of the polysaccharide from Jew's-mallow was evaluated by APTT and PT coagulation assays. The effect of polysaccharide as an anticoagulant by APTT is shown in figure 2. This assay was used to determine the action of the polysaccharide in intrinsic coagulation pathway and it is a very sensitive test to analyze all change in blood coagulation pattern and inhibition of many factors(36). Figure 2 showed that polysaccharide from Jew's-mallow have an anticoagulant effect prolonging coagulation time by APTT assay with respect to heparin, through the increase in coagulation time, the increase in blood coagulation time was with increased polysaccharide concentration. The polysaccharide concentration $100(\mu\text{g}/\text{ml})$ had the longest time of 210Sec and was compatible with the

concentration of 1000 (IU/ml) of heparin, which was 223 Sec, while the lowest time of coagulation 35Sec at concentration 10($\mu\text{g/ml}$). The prolongation of APTT indicates the inhibition of the intrinsic and/or extrinsic pathway (13). That anticoagulation property is related to the pattern of sulphation allowing interaction with target proteins of the coagulation cascade(36).

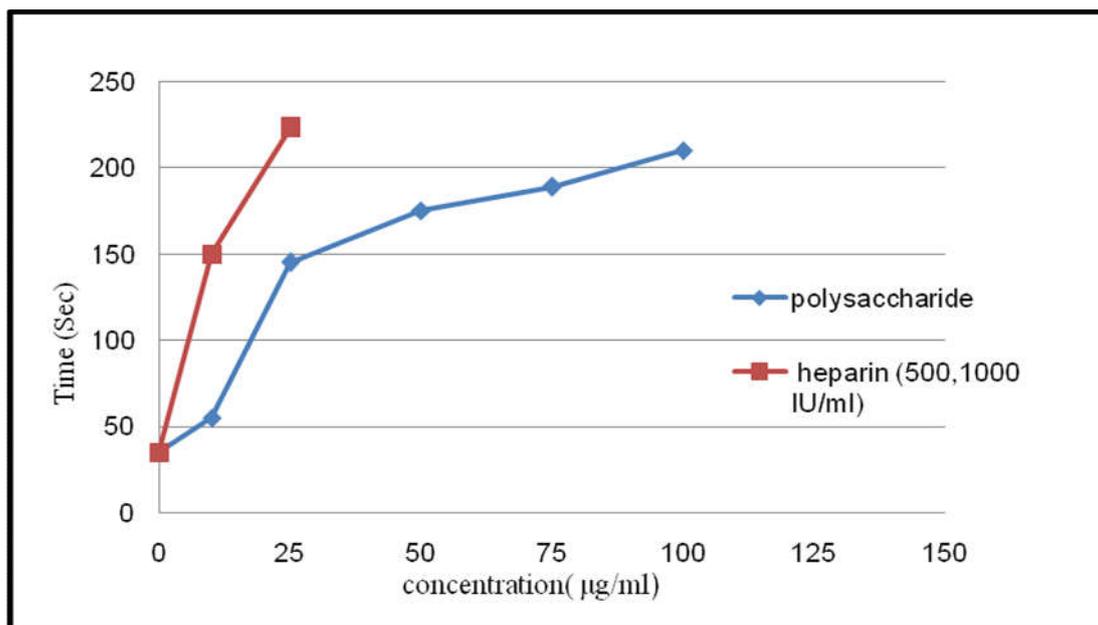


Fig. 2. Analysis of the anticoagulant activity by APTT on the polysaccharide

Figure 3 showed a prothrombin time (PT) evaluation. The current study revealed that the time of prothrombin (PT) did not have any apparent effectiveness in increasing the time of coagulation compared with heparin, which had the effect of increasing coagulation time to 44 seconds at a concentration of 1000 (IU/ml). This finding is in agreement with previous study(41). However, working on the green seaweed *Caulerpa cupressoides*, there is no anticoagulant activity in the measurement of prothrombin time(PT).

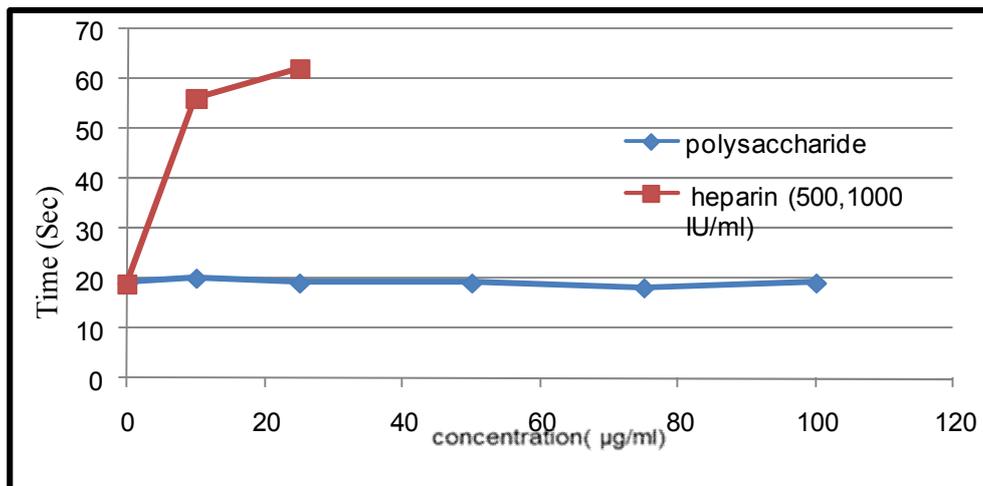


Fig. 3. Analysis of the anticoagulant activity by PT on the sulfated polysaccharide

استخلاص السكر المتعدد من الملوخية *Corchorus olitorius L.* والنشاط المحتمل المضادة للتخثر

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الخلاصة

تم استخلاص السكر المتعدد من أوراق الملوخية بواسطة الماء الساخن، استعمل TCA لإزالة البروتين من السكر المتعدد، بعدها رسب بالإيثانول، وكان الحاصل من السكر المتعدد 4.2% على أساس مصدر الوزن الرطب. احتوي التركيب الكيميائي للسكر المتعدد على الكربوهيدرات الكلي، الكبريت والبروتين 8.6%، 0.63% على التوالي، أجريت اختبارات النوعية للسكر المتعدد، وبينت الدراسة ايضاً وجود الكليكو سيداتو الفينولاتو التانينات، في حين لم يلاحظ وجود فلويداتو الصابونيات. وعند التحليل الطيفي بالأشعة تحت الحمراء FTIR للسكر المتعدد لوحظ وجود مجاميع الكربوكسيل والكبريت. تم تقييم النشاط المضاد للتخثر من خلال قياس وقت التخثر لجزء الثرموبلاستين (APTT) وقياس وقت البروثرومبين (PT) مقارنة بالهيبارين، أظهرت النتائج التي من خلال فحص APTT زيادة وقت التخثر، كانت الزيادة في زمن تخثر الدم متناسب طردياً مع زيادة تركيز السكر المتعدد، إذ أعطى تركيز 100 ميكروغرام / مل أطول وقت 210 ثانية وهو قريب مع تركيز 1000 (وحدة دولية / مل) من الهيبارين، ولكن أظهرت النتائج ان فحص البروثومبين (PT) لم يكن له أي فعالية واضحة في زيادة وقت التخثر عند كل تركيز مقارنة مع الهيبارين.

REFERENCES

- 1-**Abu-Hadid, A. F.; El-Shinawy, M. Z.; El-Bethagy, A. S.;Gaafer, S. A. and Medany,M.** (1994). Studies on the production of off-season jew's mallow (Molokhia) in Egypt, *Egyptian Journal of Horticulture*; 21: 187-193.
- 2-**Tulio, J. A.; Ose, K.; Chachin, K. and Ueda, Y.**(2002). Effects of storage temperatures on the postharvest quality of jute leaves (*Corchorus olitorius* L.). *Postharvest Biology and Technology*; 26: 329-338.
- 3-**Zeghichi, S., Kallithkara, S. and Simopoulus, A.P.**(2003).Nutritional composition of molokhia (*Corchorusolitorius*) and stamnagathi (*Cichoriumspinosum*). *World Rev Nutr Diet*; 91: 1-21.
- 4-**Hussein, M.M.; Hassan F.A.M.; Abdel Daym,H.H.; Salama,A.;Enab,A.K.andAbd El-Galil,A.A.**(2011).Utilization of some plant polysaccharides for improving yoghurt consistency.*Annals of Agricultural Science*; 56(2): 97–103.
- 5- **Ohtani, K.; Okai K.; Yamashita U.; Yuasa I. and Misa- ki A.**(1995). “Characterization of an Acidic Polysaccharide Isolated from the Leaves of *Corchorusolitorius*(Moroheiya),” *Bioscience, Biotechnology\and Biochemistry*; 59, (3): 378-381.
- 6- **Yamazaki, E., Kurita, O., Matsumura, Y.,** (2008). Hydrocolloid from leaves of *C. olitorius* and its synergistic effect on r-carrageenan gel strength. *Food Hydrocolloids*; 22:819–825.
- 7-**Kardošová, A. andMachová, E.** (2006). Antioxidant activity of medicinal plant polysaccharides, *Fitoterapia*; 77: 367-373.
- 8-**Srivastava, R. andKulshreshtha, D.K.** (1989). Bioactive polysaccharides from plants.*Phytochemistry*; 28 (11), 2877-2883.
- 9- **Ridley, B.L.; O'Neill, M.A. and Mohnen, D.** (2001).Pectins: structure, biosynthesis, and oligogalacturonide-related signaling.*Phytochemistry*; 57: 929-967.
- 10-**Wang, P. Y.; Zhu, X. L. and Lin, Z.-B.**(2012). Antitumor and immunomodulatory effects of polysaccharides from broken-spore of *Ganodermalucidum*,*Front. Pharmacol.*; 3 (135):1-8.

- 11- Dorea, C.M.P.G.; Alvesa, M.G.C.F.; Willa, L.S.E.P.; Costa, T.G and Sabryb, D.A. (2012).** A sulfated polysaccharide, fucans, isolated from brown algae *Sargassumvulgare*with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. *Carbohydrate Polymers*; 91: 467-475.
- 12-Bhatia, S.;Rathea, P.;Sharmab, K.;Chaugulec, B.B. andKard, N.(2013).**Immuno-modulation effect of sulphated polysaccharide (porphyran) from *Porphyra vietnamensis*. *International Journal of Biological Macromolecules*; 57: 50-56.
- 13-Faggio,C. ;Morabito M.; Minicante, S.A. ; Piano, G.L. ; Pagano, M. and Genovese, G. (2015).** Potential use of polysaccharides from the brown alga *undariapinnatifida* as anticoagulants.*AN International Journal*; 58 (5): 798-804.
- 14-Tariq, A.; Athar, M.; Ara, J.; Sultana, V.; Ehteshamul-Haque, S. and Ahmad, M.(2015).** Biochemical evaluation of antioxidant activity in extracts and polysaccharide fractions of seaweeds, *Global J. Environ. Sci. Manage.*; 1 (1): 47-62.
- 15-Krichen, F.; Karoud, W.; Sila, A.; Abdelmalek, B. E.; Ghorbel, R.; Ellouz-Chaabouni, S. and Bougatef, A.(2015).**Extraction, characterization and antimicrobial activity of sulfatedpolysaccharides from fish skins. *International Journal of Biological Macromolecules*; 75 : 283–289.
- 16-Muschina, T.; Budragcha, D.;Kanamoto, T.; Nakashimac, H. and Ichiyamad, K. (2016).**Chemically sulfated natural galactomannans with specific antiviral and anticoagulant activities. *International Journal of Biological Macromolecules*; 89: 415-420.
- 17-Baveja, S. K.; Rao, K. V. and Arora J.(1989).** Examination of natural gums and mucilages as sustaining materials in tablet dosage forms. *Ind. J. of Pharma Sci.*; 51:115-119.
- 18-Pawar, H.(2004).** Isolation of seed gum from *Cassia tora* and preliminary studies of its application as a binder for tablets. *Indian Drugs.*; 41 (8): 465-468.
- 19- Shirwaikar, A., Shirwaikar, A., Prabu, L. S., and Arbindkumar, G. (2008).** Herbal excipients in novel drug delivery systems. *Ind J of Pharma Sci.*;70 (4):415-422.

- 20- Pawlaczyk, L.; Czerchawski W.; Pilecki E.; LamerZarawska,R. AND Gancarz, R., (2009).Polyphenolic-polysaccharide compounds from selected medicinal plants of Asteraceae and Rosaceae families: Chemical characterization and blood anticoagulant activity.*Carbohydrate Polymers*; 77 : 568-575.
- 21- Béguin, S; Lindhout, T. and Hemker, H.C.(1988). The mode of action of heparin in plasma. *ThrombHaemost*;60(3):457–462
- 22- Fareed, J.W.; Hoppensteadt, D. and Bick, R.L. (2000). An update of heparins at the beginning of the new millenium. *Seminars in Thrombosis and Hemostasis*; 26: 5-21.
- 23-Liaw, P.C.Y.; Becker, D.L.; Stafford, A.R.;Fredenburgh, J.C.and Weitz, J.I. (2001).Molecular basis for the susceptibility of fibrin-bound thrombin to inactivation by heparin cofactor II in the presence of dermatan sulfate but not heparin. *J. Biol. Chem.*; 276: 20959–20965.
- 24- Melo, F.R.; Pereira, M.S.; Foguel D. and Mourão, P.A.S. (2004). Antithrombin-mediated anticoagulant activity of sulfated polysaccharides: Different mechanisms for heparin and sulfated galactans. *J. Biol. Chem.*; 279:20824–20835.
- 25- Sanya, H.; Stephen, E.H.; Kari, I.;Kornelia, J.;Terje, E.M.; Thomas, H.; Andreas, K.;Berit, S.P. (2004). Bioactive polysaccharides from the stem of the Thai medicinal plant *Acanthus ebracteatus*: theirchemical and physical features. *Carbohydr. Res.*; 339: 753–762.
- 26- Rajalingam, D.; Loftis, C.; Xu, J.J. and Kumar, T.K.S.(2009).Trichloro acetic acid-induced protein precipitation involves the reversible association of a stable partially structured intermediate. *Protein Sci.*; 18: 980-993.
- 27-Akenga, T.; Orech F.O.; Ochora J.; Friis H. andAagaard H; (2005). Potential Toxicity of some Traditional Leafy Vegetables consumed in Nyang’oma Division, Western Kenya. *Afr. J. Food Nutr.; Sci.* 5(1):1-30.
- 28- Sofowora, A. (1982). Medicinal Plants and Traditional medicine in Africa. *John Wiley and Sons, Nigeria*; p. 66-79.

- 29- Edeoga, H.O.; Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigeria medicinal plants. *Afr. J. Biotechnol*; 4(7):685-688.
- 30- Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*; 28:350-356.
- 31- Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*; 72:248-254.
- 32- Dodgson, K.S. (1961). Determination of inorganic sulphate in studies on the enzymic and nonenzymic hydrolysis of carbohydrate and other sulphate esters. *Biochem. J.*; 78:312-319.
- 33- Anderson, L.O.; Barrowcliffe, T.W.; Holmer, E.; Johnson, E.A. and Sims, G.E.C. (1976). Anticoagulant properties of heparin fractionated by affinity chromatography on matrix-bound antithrombin-3 and by gel-filtration. *Thromb. Res.*; 9(6):575-583.
- 34- Cox, S.; Abu-Ghannam, N. and Gupta, S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *Int. Food Res. J.*; 17:205-220.
- 35- Rastian Zahra, M. M.; Vahabzadeh, F. and Sartavi, K. (2007). Antioxidant activity of extract from a brown alga, *Sargassum boveanum*. *Afr. J. Biotechnol.*; 6(24):2740-2745.
- 36- Koch, E. and Biber, A. (2007). Treatment of rats with the Pelargonium sidoides extract EPs 7630 has no effect on blood coagulation parameters or on the pharmacokinetics of warfarin. *Phytomedicine*; 14(6): 40-45.
- 37- Fan, Y.J.; He X.J.; Zhou, S.D.; Luo, A.X.; He, T. and Chun, Z. (2009). Composition analysis and antioxidant activity of polysaccharide from *Dendrobium denneanum*. *Int. J. Biol. Macromol.*, 45: 169-173.
- 38- Wang, Y.M.; Wu, F.J.; Du, L.; Li, G.Y.; Takahashi, K.; Xue, Y. and Xue, C.H. (2014). Effects of polysaccharides from abalone (*Haliotis discus hannai* Ino) on HepG2 cell proliferation. *Int. J. Biol. Macromol.*; 66:354-361.
- 39- Amorim, R. N. S.; Rodrigues, J. A. G.; Holanda, M. L.; Quinderé, A. L. G.; Paula, R. C. M.; Melo, V. M. M. and Benevides, N. M. B.

(2012).Antimicrobial effect of a crude sulfated polysaccharide from the seaweed *Gracilariaornata*. *Brazilian Archives of Biology and Technology*; 55(2): 171-181.

40-Zhang, H.J.; Mao, W.J.; Fang, F.; Li, H.Y.; Sun, H.H.; Chen, Y. and Qi, X.H.

(2008). Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from *Monostromalatissimum*. *Carbohydr. Polym.*; 71:428-434.

41- Rodrigues, J.; Vanderlei, E.; Bessa, E.; Magalhaes, F.; Paula, R .; Lima, V.

and Benevides, N. (2011).Aanticoagulant activity of a sulated polysaccharide isolated from the green seaweed *Caulepacupressoides*.*Brazilian Archives of Biology and Technology*; 54(4): 691-700.