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Isolation and characterization of sulphated polysaccharides from *Codium tomentosum* (J. Stackhouse, 1797) collected from southeast coast of India

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ABSTRACT

A sulphated polysaccharide was isolated from Codium tomentosum by hot-water extraction, deproteinized, precipitation with ethanol, and obtained a crude polysaccharides yield from 24.2% based on dry weight source. The crude polysaccharides further fractionated by anion-exchange column chromatography using Q-Sepharose Fast Flow column, fractionation yielded was 17.31%, and purified by gel-permeation chromatography using Sephadex G-50 Fast Flow column they were yielded from 10.83% respectively. The biochemical composition of the sulphated polysaccharides contains total carbohydrate 88.7%, sulfate 14.7%, protein 0%, ash 15.9% and moisture 2.3% respectively. The FT-IR spectral analysis of the sulphated polysaccharides consist carboxyls and sulfate groups. The elemental analysis of sulphated polysaccharides possess higher amount of carbon at 25.86%, hydrogen 5.80%, nitrogen 6.50% and very less in sulfur at 0.16% respectively. The present study was concluded the sulphated polysaccharides would be in useful product development and bioprospecting approaches.

Keywords: Marine alga, Polysaccharides, Chemical analysis, FT-IR analysis, CHN/S analysis.

INTRODUCTION

Seaweed cell wall mainly consists of interconnecting polysaccharides, in the most abundant source of organic carbon in the biosphere. Seaweeds consist various cell wall polysaccharides and possess the higher amount and more active compound simplicity of diversity. Generally, cell walls are made up of highly complex large polymers such as cellulose, hemicellulose, lignin and pectin. Cellulose, the most abundant carbohydrate polymer in nature is the main structural component of plant cell walls and it is principally difficult to degrade, as it is impassable and is present as hydrogen-bonded crystalline fibers [1]. However, the seaweed cell walls and cuticles are chemically and structurally extra complex and heterogeneous than those of land plants. They are self-possessed of mixtures of sulphated and branched polysaccharides that are attached to proteins and a mixture of bounds ions together with calcium and potassium [2].

Sulphated polysaccharides (SPs) comprise a complex group of macromolecules with a wide range of critical biological activities. These polymers are chemically anionic and distributed extensively not only in marine algae but also in animals such as mammals and invertebrates [3]. However, the marine algae are the most important source of non-animal Sulphated polysaccharides and the chemical structure of the polymers varies according to the algae species [4]. They are found with a wide range of chemical structures, but some general characteristics have been identified [5].

The amount of Sulphated polysaccharides comprised in algae varies according to the different classes of marine algae, such as Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyta (brown algae). The chemical

structures of seaweed polysaccharides have been described extensively [6,7,8]. The production of different bioactive polysaccharides with lyases is required in order to increase the extraction efficiency of more functional ingredients from seaweeds. Therefore, enzyme-assisted extraction (EAE) technique can be employed as an alternative method to enhance the extraction efficiency of bioactive polysaccharides from seaweed sources for industrial use [9,10].

Many researchers employed, particularly in seaweed polysaccharides to different types of extraction method are using distilled water for hot water, cold water extraction and Microwave-assisted extraction and characterized by using FT-IR and NMR spectral [11]. Commonly these polysaccharides have been extracted using water or aqueous organic solvents. In practice, precipitation from aqueous solutions with 80%v/v ethanol is the step used in many carbohydrate analysis procedures to separate [12]. Keeping the importance of minds, the present study was the isolation and characterization of sulphated polysaccharides from *C. tomentosum* collected from Southeast coast of India.

MATERIALS AND METHODS

Collection of seaweed and processing

Marine red algae species *C. tomentosum* were collected by hand picking from the submerged in the rocks inland water of the Thiruchendur coast (Lat. 08°29'N; Long. 78°07'E) in Tamil Nadu, Southeast cost of India. Algal samples were thoroughly washed with seawater and then in fresh water and to remove the contamination. The seaweeds were transported to the laboratory on ice packed sterile polyethylene bags at 0°C temperature. In the laboratory, the sample was rinsed with sterile distilled water and was shade dried, cut into small pieces and powdered in a mixer grinder.

Extraction of Polysaccharides

Extraction of polysaccharides was carried out by the procedure previously described Zhang *et al.* [13]. Briefly 50g of seaweed powder dipped into 20 volumes (1*litre*) of distilled water and kept at room temperature for 2 h, then homogenized and refluxed at 100^{o}C for 2 h. After cooling, the supernatant was separated from the algae residues by centrifugation. The residue was further extracted thrice with double-distilled water at 100°C for 2 h. All extracts were combined and concentrated under reduced pressure using rotary evaporator and dialyzed in cellulose membrane (molecular weight cut off 12,000), against distilled water for three successive days. The retained fraction was concentrated under reduced pressure using a rotary evaporator. Obtain to the mixture of polysaccharides.

Deproteinization

The polysaccharide mixture was deproteinized following the Sevag method as described by Staub [14] as previously described Luo and Fan [15]. Briefly, the polysaccharides was mixed with Sevag reagent, 5:1 (v: v) CHCl3: n-BuOH and stirred for 30 min. Then, the mixture was centrifuged and the upper polysaccharide solution was collected. The polysaccharide solution was further deproteinized with Sevag reagent for 5 times until there was no white layer between polysaccharide solutions and the polysaccharide was free of proteins as scan by UV Spectra in 260 nm and 280 nm. After removal of the Sevag reagent, the resulting polysaccharide solution was precipitated by adding ethanol; and the final mixture was kept in 4°C overnight for further processing. The precipitate was collected afterwards by centrifugation at 4000 rpm for 20 min, and was washed successively with petroleum ether, acetone and ethanol several times, then subjected to lyophilization get a crude polysaccharides which named as CPs. The polysaccharide yield (%) was then calculated using the following equation:

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

Fractionation of polysaccharides by anion exchange chromatography

The crude polysaccharides was dissolved in distilled water (10 mg/ml), centrifuged 6000 rpm at 10 min, and the supernatant was applied to a Q-Sepharose™ Fast Flow column (EX 150 mm×50 cm) equilibrated with distilled water. The column was eluted stepwise with distilled water, with a linear gradient of 0–3 mol/L NaCl at a flow rate of 0.55 ml/min. Fractions (15 ml) were collected, and polysaccharides were detected using the phenol-sulfuric acid method Dubois et al [16] Fractions corresponding to individual peaks were combined and dialyzed then lyophilized to get semi-purified polysaccharides.

Purification of fractionated polysaccharide by gel filtration chromatography

The semi-purified polysaccharides was further purified by gel permeation chromatography using a High-Resolution Sephadex G-50 column (EX 100 mm×25 cm) Fast Flow column, equilibrated with distilled water. The column was eluted stepwise with distilled water; with extend of 0.2 mol/L sodium acetate buffer at an elution rate of 0.33

ml/min. The elutions were pooled, concentrated, desalted and freeze-dried to obtain a purified polysaccharide. Further the subsequent analysis.

Analysis of the biochemical composition

Total carbohydrate and sulfate content

The total carbohydrate content was estimated calorimetrically by the phenol-sulfuric acid method using D- glucose as a standard [16]. The sulfate content was determined after acid hydrolysis (2 N HCl at 100°C for 2 h) of the polysaccharides, according to the gelatin-barium method, using potassium sulfate as the standard [17].

Total protein content

Protein content was measured by using the Bradford method [18], using bovine serum albumin as a standard.

Determination of ash content

The ash content of polysaccharides, which represents the total inorganic materials present, was quantified gravimetrically according the method of [19], 2 g of the dried polysaccharides taken in a porcelain crucible at 600^0C for 6 hrs in a muffle furnace. The weight of the residue, which represented the ash content, was recorded and the results are given as percentage of the dry weight of polysaccharides.

Determination of moisture content

The moisture content was obtained by heating 0.5 g of sample using oven drying at 105°C for 24 h.

FT-IR analysis

The structural characteristics of the sulphated polysaccharides were determined by Fourier transform IR spectrophotometer (Perkin-Elmer Corp., USA). Briefly 10 mg of sample was milled in a mortar with 100 mg of dried spectroscopic grade potassium bromide (KBr) powder and then pressed into pellets in preparing as salt disc (10 mm/dm) for transforming IR spectral measurement in the frequency range of 4000-500 cm−1 for reading further.

CHN/S analysis

Elemental analysis on the carbon, hydrogen, nitrogen and sulfur weight percentages of sulphated polysaccharide of *C. tomentosum* was performed by using the (PE 2400 Series II CHNS/O) Analyser. Approximately 1 mg of sample was injected, eluted with He and detected by TCD (Thermal conductivity detector). 2,5-Bis (5 tert-butyl-2-benzoxazol-2-yl) thiophene (BBOT) and as-partic acid was used as a reference standard.

RESULTS AND DISCUSSION

Yield of polysaccharides

The yields of the polysaccharides obtained from the hot the hot water extraction process gave varying to usual yield of crude polysaccharides was obtained with 24.2% (w/w) from the dry weight source. The polysaccharide mixture was separated to ion-exchange chromatography on the Q-Sepharose FF column, a size and charge uniform fractionation yielded 17.31%. The semi purified polysaccharides submitted on the Sephadex G-50 FF column, a size and charge uniform purification was acquired with the yield 10.83% shown in (Fig.1).

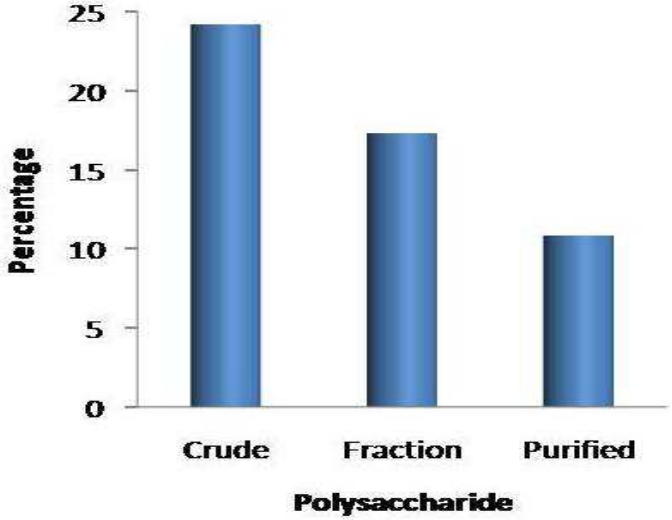

Fig. 1. Yield of polysaccharides from *C. Tomentosum*

Several studies detailed the sulphated polysaccharides, particularly in seaweeds posses great mount, when compare to land plants, as previously reported hot water used for the extraction of *A. Subcrenata Lischke* was precipitated in 80% ethanol, deproteinized by the Sevag method, washed with ethyl ether and acetone several times, and then dried in vacuum to obtain 9.32 g of crude polysaccharides [20]. XuJie et al [21] reported the extraction temperature is higher the extraction yield increased from 4.17% to 6.65%. As the way the present study, extraction temperature was increased at 100^0C, that are influenced a usual yield comparing to other studies. Souza et al [22] extracted the sulphated polysaccharides at room temperature and the residues was exhaustively extracted with hot water 90 ^{0}C, to obtained higher yield 27.2% than cold extraction, comparing the present study extraction yield also obtained 24.2%. According to Athukorala et al [23] obtained a polysaccharide yield of 41.52% from *Ecklonia cava*, through the hot water extraction.

Chemical composition of sulphated polysaccharides

The chemical composition of the sulphated polysaccharides was determined as shown in Fig. 2. The sulphated polysaccharides were composed of total carbohydrate 88.7%, sulfate 14.7%, protein 0%, ash 15.9% and moisture 2.3%. Rioux et al [24] Isolated polysaccharides from *S. longicruris*, *A. nodosum* and *F. vesiculosus* to estimate the total sugar contents were measured on this fraction. These fractionated polysaccharides get a large amount of neutral sugars; *S. longicruris* contained 99.1% of neutral sugars while *A. nodosum* and *F. vesiculosus* showed lesser quantity about 89.6% and 84.1%, respectively.

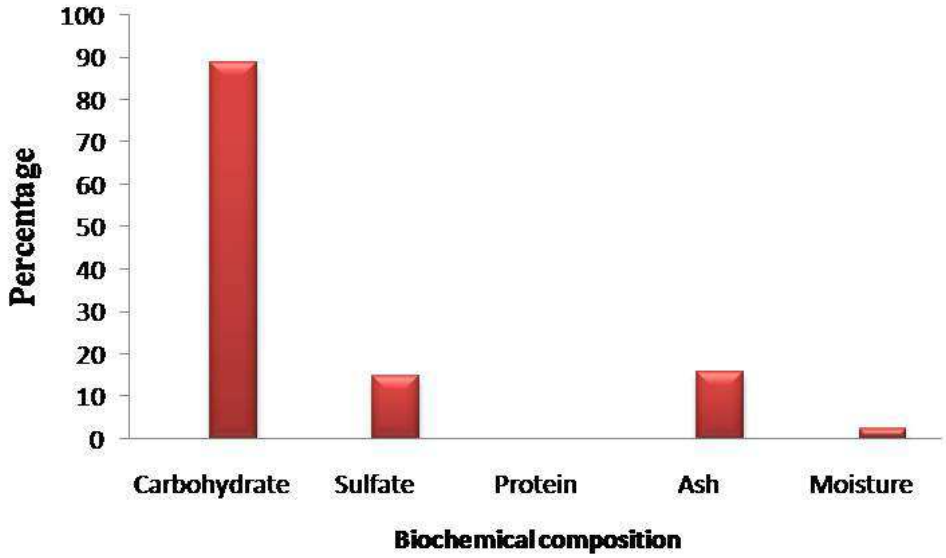

Fig. 2. Chemical analysis of sulphated polysaccharides from *C. Tomentosum*

Zhang et al [25] described the Polysaccharide from the Brown Seaweed of *Sargassum graminifolium* contain total sugar content of 75.68%, protein content as 0.12% and sulfate is 10.13%. Na et al [26] isolated water-soluble polysaccharide from *Capsosiphon fulvescens* were estimated the protein content from Bradford assay that can be clearly showed no protein, were mentioned the neutral sugar 49.8% and sulfate 5.7%. As agreed with the present study consist no protein in the presence sulphated polysaccharides.

FT-IR structural characterization of sulphated polysaccharides

In the FT-IR spectrum of sulphated polysaccharides (Fig. 3), the band in the region of 3560.00 cm^{-1}corresponds to the hydroxyl stretching vibration of the polysaccharide and that at 2924.09 cm^{-1} corresponds to a weak C-H stretching vibration, [27] broad stretching intense characteristic peak at around 3422 cm^{-1} for the hydroxyl group, and a weak C–H band at around 2929 cm^{-1} [28]. The strong band at 3422.76 cm^{-1} attributed to O–H stretching vibration and another band at 2926.42 cm−1 posses to C–H stretching vibration [29]. The most important bands were found at 1028.06 cm^{-1} indicated D-glucose and 1253.73 cm^{-1} corresponding to ester sulfate groups; the region around 1388.75 cm^{-1} to 1440.83 cm^{-1} are equivalent to the skeleton of galactans and the agar specific band, respectively. A characteristic absorption at 846.75 cm^{-1} was also observed, indicating the α-configuration of the sugar units. The absorption at 457.13 cm^{-1} to 671.23 cm^{-1} indicated sulfate ester groups, respectively.

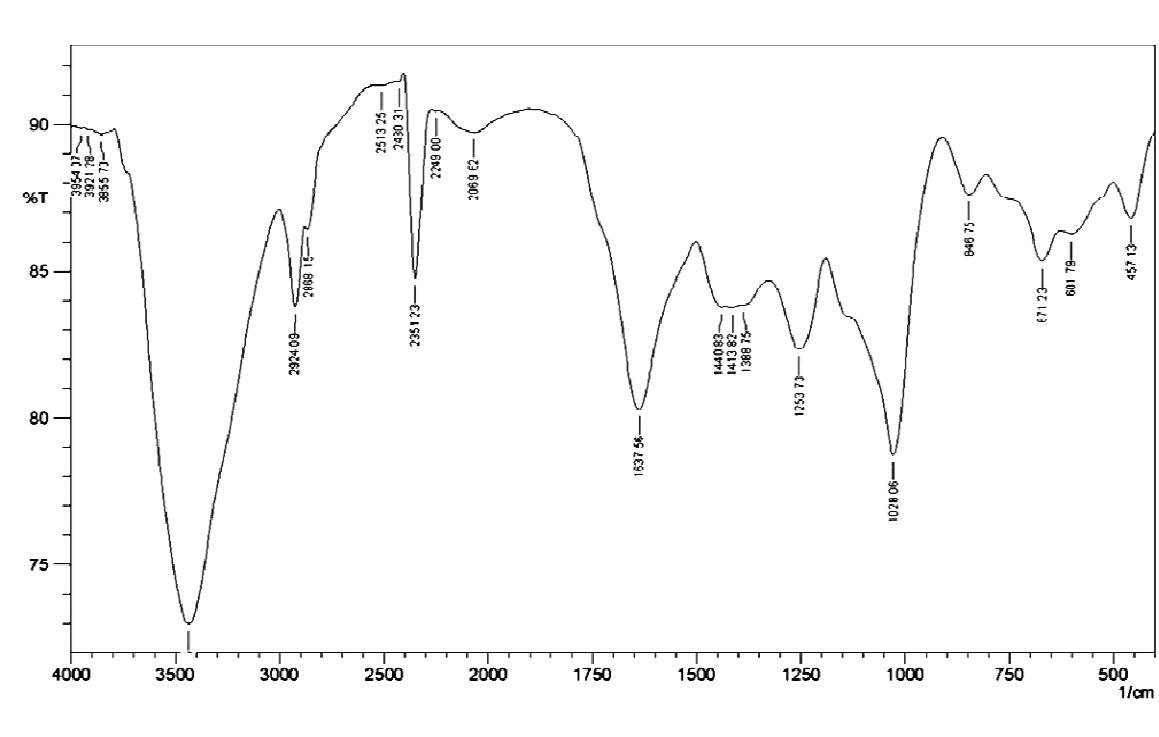

Fig. 3. FT-IR spectrum of sulphated polysaccharides from *C. tomentosum*

CHN/S composition of sulphated polysaccharides

The present study of the sulphated polysaccharides was analyzed the carbon, hydrogen, nitrogen and sulfur content contributed 25.86, 5.80, 6.50 and 0.16 respectively (Fig. 4). Na et al [26] determined carbon 31.9% w/w, hydrogen 4.9% w/w, nitrogen 0.6% w/w and sulfur 6.2% w/w from the sulphated polysaccharides of *Capsosiphon fulvescens.* In addition Okajima-Kaneko [30] extracted polysaccharides from *Aphanothece sacrum* using a hot alkaline solution which degraded other polymers such as proteins and nucleotides. The elemental analyses indicated carbon 36.04, hydrogen 5.91%, nitrogen 0.30% and sulfur 2.07%, the Polysaccharides contain carboxyls and sulfate groups. Agreed with the present study, sulphated polysaccharides contain carboxyls and sulfate groups.

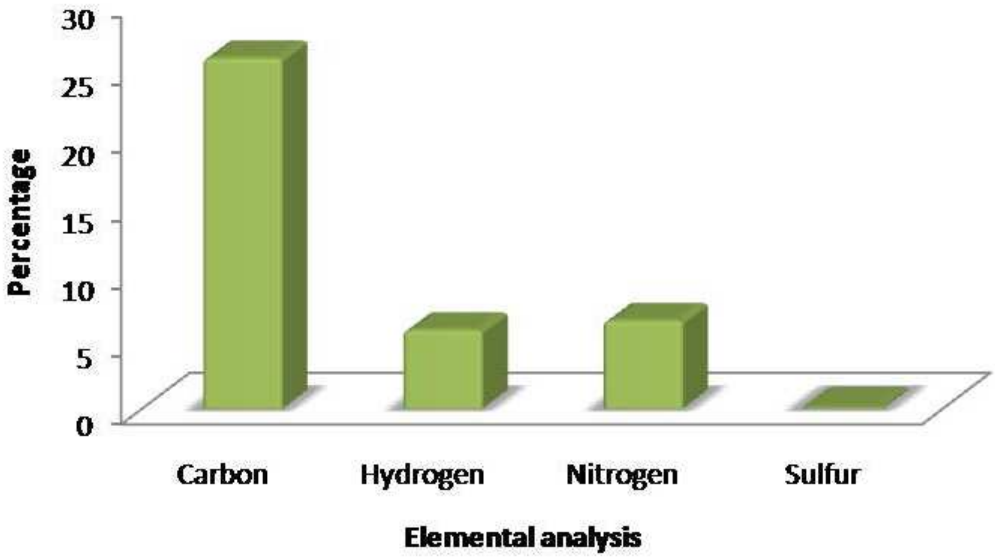

Fig. 4. Elemental analysis of sulphated polysaccharides from *C. tomentosum*

CONCLUSION

Based on the above results obtained, it could come to conclusion that the sulphated polysaccharides can be successfully isolated from *C. tomentosum*. The biochemical composition consists of higher amount of carbohydrate

and absence of protein, trace amount of ash and moisture content. Thus, the polysaccharide was structurally characterized through FT-IR. Thus the elemental analysis of sulphated polysaccharides possess higher amount of carbon followed by hydrogen, nitrogen and very less in sulfur content. The present study was concluded the sulphated polysaccharides would be in useful product development and bioprospecting approaches.

REFERENCES

[1] Doi, R.H., and Kosugi, A.O., *Nat. Rev. Microbiol*, (**2004**),2, 541–51.
[2] Fuller, M.P., and Gibor, A., *Hydrobiologia*, (**1987**), 151/152, 405–9.
[3] Alban, S., Franz, Partial. G., *Biomacromole*, (**2001**), 2, 354–361.
[4] Costa, L.S., Fidelis, G.P., Cordeiro, S.L., Oliveira, R.M., Sabry, D.A., Camara, B.G., Nobre, L.T.D.B., Costa, M.S.S.P., Almeida-Lima, J., Farias, E.H.C., Leite, E.L., and Rocha, H.A.O, *Biomed. Pharmacother*, (**2010**), 64, 21–28.
[5] Rinaudo, M., Kamerling, J.P., Ed., *Elsevier: Amsterdam, The Netherland*, 2, (**2007**).
[6] Percival, E., and McDowell, R.H., *Academic Press: New York, NY, USA*, (**1967**).
[7] Percival, E., *Br. Phycol. J*, (**1979**), 14, 103–117.
[8] Rioux, L.E., Turgeon, S.L., and Beaulieu, M., (**2007**), 69, 530–537.
[9] Kanga, S.M., Kim, K.N., Lee, S.H., Ahn, G., Cha, S.H., and Kim, A.D., *Carbohydr. Polym*, (**2011**), 85, 80–85.
[10] Wijesinghe, W.A.J.P., Athukorala, Y., and Jeon, Y.J., *Carbohydr. Polym*, (**2011**), 86, 917–921.
[11] Rodriguez-Jassoa, R.M., Mussattoa, S.I., Lorenzo, P., Cristobal, N., and Aguilarc, J.A., *Carbohydr. Polym*, (**2011**), 86, 1137– 1144.
[12] Englyst, H.N., Quigley, M.E., and Hudson, G.J., *Analyst*, (**1994**), 119, 1497–1509.
[13] Zhang, H.J., Mao, W.J., Fang, F., Li, H.Y., Sun, H.H., Chen, Y., and Qi, X.A., *Carbohydr. Polym*, (**2008**), 71, 428–434.
[14] Staub, A.M., *Method. Carbohydr. Chem*, (**1965**), 5, 5–6.
[15] Luo, A., and Fan, Y., *Int. J. Mol. Sci*, 12, (**2011**), 4068-4079.
[16] Dubois, M., Gilles, K.A., and Hamilton, J.K., *Anal. Chem*, (**1956**), 28, 350.
[17] Craigie, J.S., Wen, Z.C., and van der Meer, J.P., *Bot. Mar*, (**1984**), 27, 55-61.
[18] Bradford, M.M. *Anal. Biochem*, (**1976**), 72, 248–254.
[19] Larson, B., *Cambridge university, press, London*, (**1978**), 182-188.
[20] He, Y., Liu, C., Chen, Y., Ji, A., Shen,Z., Xi, T., and Yao, Q., *J. Bio. Sci. and Bioeng*, (**2007**), 104, 111–116.
[21] XuJie H., Na, Z., SuYing, X., ShuGang, L., and BaoQiu, Y., *Carbohydr. Poly*, (**2008**), 73, 289–294.
[22] Souza, B.W.S., Cerqueira, M.A., Bourbon, A.I., Pinheiro, A.C., Martins, J.T., Teixeira, J.A., Coimbra, M.A., and Vicente, A.A., *Food Hydrocolloids*, (**2012**), 27, 287-292.
[23] Athukorala, Y., Jung, W.K., Vasanthan, T., and Jeon, Y.J., *Carbohydr. Polym*, (**2006**), 66, 184-191.
[24] Rioux, L.E., Turgeon, S.L., and Beaulieu, M., *Carbohydr. Polym*, (**2007**), 69, 530–537.
[25] Zhang, C.Y. Wen-Hui Wu, W.H., Wang, J., and Lan, M.B. (**2012**).
[26] Na, Y.S., Kim, W.J., Kim, S.M., Park, J.K., Lee, S.M., Kim, S.O., Synytsya, A., and Park, Y., *Int. Immunopharm*,(**2010**), 10 , 364–370.
[27] Fan, Y.J., He, X.J., Zhou, S.D., Luo, A.X., He, T., and Chun, Z., *Int. J. Biol. Macromol*, (**2009**), 45, 169–173.
[28] Santhiya, D., Subramanian, S., and Natarajan, K.A., *J. Colloid Inter. Sci*, (**2002**), 256, 237–248.
[29] Shi, Y., Zhaob, L., Liua, X., Hua, F., Cuia, F., Bia, Y., Maa, Y., and Fenga, S., *Carbohydr. Polym*, (**2012**), 87,160– 169.
[30] Okajima-Kaneko, M., Ono, M., Kabata, K., and Kanekol, T., *Pure Appl. Chem*, (**2007**), 79, 2039-2046.