ISOLATION OF POLYSACCHARIDES, FUCOSE AND OTHER OPTICALLY ACTIVE COMPOUNDS FROM MARINE VEGETABLE EXTRACTS BY MEANS OF LIQUID CHROMATOGRAPHY WITH A LASER POLARIMETRIC DETECTOR

AISLAMIENTO DE POLISACÁRIDOS, FUCOSA Y OTROS COMPUESTOS ÓPTICAMENTE ACTIVOS DE EXTRACTOS VEGETALES MARINOS MEDIANTE CROMATOGRAFÍA LIQUIDA CON DETECTOR POLARIMÉTRICO LÁSER

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Recibido 16/3/2017; Aceptado 12/10/2017

PACS: Optical methods, 83.85.-c.Ei; Chromatography, 82.80.-d Bg; Light-emitting devices, 85.60.-q .Jb; Carbohydrates, 87.14.-g.Df; Structure of biomolecules,87.15.-v B; Biochemical separation processes, 87.80.-y.Qk

The combination of molecular exclusion liquid chromatography and laser polarimetric detection has made feasible the isolation of polysaccharides from seaweeds and marine angiosperms extracts. Computer programs, developed at CEADEN, made easy to display on line chromatograms and store electronic data. Marine vegetables are a source of natural bioactive compounds that can be used in pharmaceutical and nutraceutical industries. Polar extracts of *Stypopodium zonale* and *Sargassum fluitans* (Phaeophyceae) and *Thalassia testudinum* (Hydrocharitaceae), species that are highly represented in Cuban seashores, were studied and their pharmacological activity was tested by "in vivo" and "in vitro" assays [1] and [2]. A voucher specimen of each collected specie was deposited at the Cuban National Aquarium Collection. The aim of this work was to demonstrate the effectiveness of a continuous flow liquid chromatographic system using a combination of Sephadex G-10 and G-50 and polarimetric detection to recognize and isolate some optically active constituents from these marine vegetable extracts.

The size exclusion chromatography system [3] (Fig. 1) is basically composed of two interconnected columns (150 and $300 \pm 0,2$ mm long and $25 \pm 0,1$ mm inner diameter), packed with Sephadex G-10 (exclusion limit 0,7 kDa) and G-50 (exclusion limit 10 kDa), respectively, both from Amersham Pharmacia Biotech., Uppsala, Sweden. The whole system is equilibrated with Milli Q water, which is also used as mobile phase at a flow rate of 5 mL/min.

Extracts were prepared mixing dried and ground samples in ethanol:water (1 : 1), except for *S. zonale* which was mixed in acetic acid (1 mol L^{-1}). The extracts were filtered and concentrated using a vacuum rotoevaporator at temperatures lower than 45°C until waterless. Five mL of each diluted sample (2g/50mL) were injected into Sephadex G-10 column after being centrifuged at 24°C and 2000 x g and filtered through Whatman ♯ 1 filter paper.

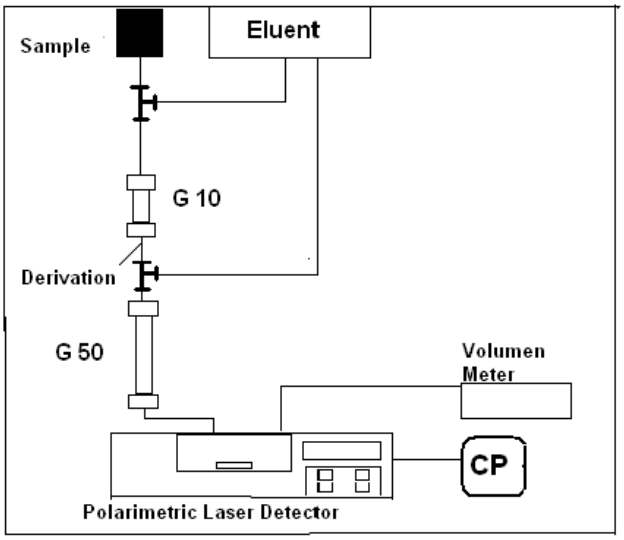

Figure 1. Size exclusion chromatography system and polarimetric laser detector.

The laser polarimetric detector with a He-Ne beam of 1 mm diameter and a measuring interval of one second has a continuous flow polarimetric tube of $100 \pm 0,02$ mm of length and a volume of 0,6 mL placed into the polarimeter chamber connected to the outlet of the second column (Sephadex G-50). The on line detector is also connected to a computer. The system (Fig. 1) includes programs for data acquisition (ADQUIPOL) and data processing and storing

(CROMAPOL).

Eluted fractions of 10 mL each, corresponding to relative maxima sugar constituents by HPLC (Column Eurokat Ca, (300 × 8 mm i.d.), Milli Q water was used as mobile phase, isocratic with a flow rate of 0.6 mL/min, a column temperature of 85°C and 20 μL injection volume). A refractive index detector was used. Standards of nistose (tetrasaccharide), kestose (trisaccharide), sucrose (disaccharide), glucose and fructose (monosaccharide) were used as reference standards (0.02 g/mL) (Data not shown).

The described procedure gave information about the polarized light deviation of high, medium and low molecular mass polysaccharide composition of extracts of *S. zonale*, *S. fluitans* and *T. testudinum* in quite a short time and with minimum expenses (Fig. 2, 3 and 4). It also allowed the separation of their fractions for further analysis to disclose their partial biological characterization. An example of that disclosure is represented by one of the fractions of *S. zonale* extract (Fig. 2). Tri, di and monosaccharides (possibly responsible for leishmanicidal bioactivity [4]), contained in such a fraction, were detected by HPLC (data not shown).

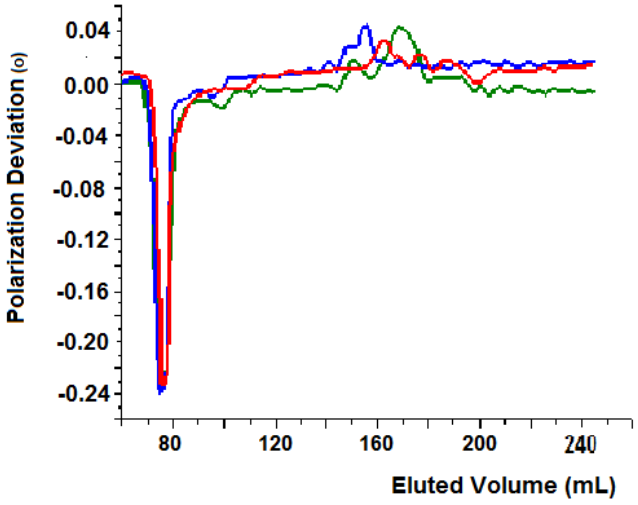

Figure 2. *S. zonale* extract chromatogram profile. Red, blue and green lines are replicates of the same sample.

The chromatographic profile of *S. fluitans* extract (Fig. 3), twice replicated (blue and green lines), showed a prevailing levorotatory fraction identified as L(-) fucose, according to signals of this standard (red line). Fucoidan is a sulphated polysaccharide mainly found in the cell-wall matrix of various brown seaweed species [5]. Fucose is an hexose deoxy-sugar which is the fundamental subunit of a fucoidan polysaccharide. Among the biological properties of this fucoidan polysaccharide have been found anticoagulant and antithrombotic [6], antitumor [7] and inmunomodulator [8] activities.

Besides, the chromatographic profile obtained from *T. testudinum* extract (Fig. 4) showed some replicated dextrorotatory relative maxima (blue and green lines). Precisely, in the extracts of this specie have been described some sulfated glycosilated flavonoids [9] which have an important biological activity in the repairing of UVB-damaged skin [10,11].

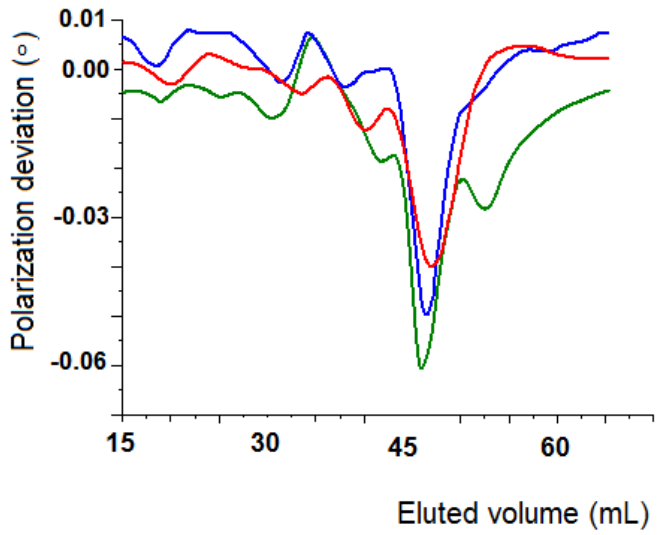

Figure 3. Chromatography separation of aqueous fraction of *S. fluitans* extract and the signal corresponding to the fucose standard (red line).

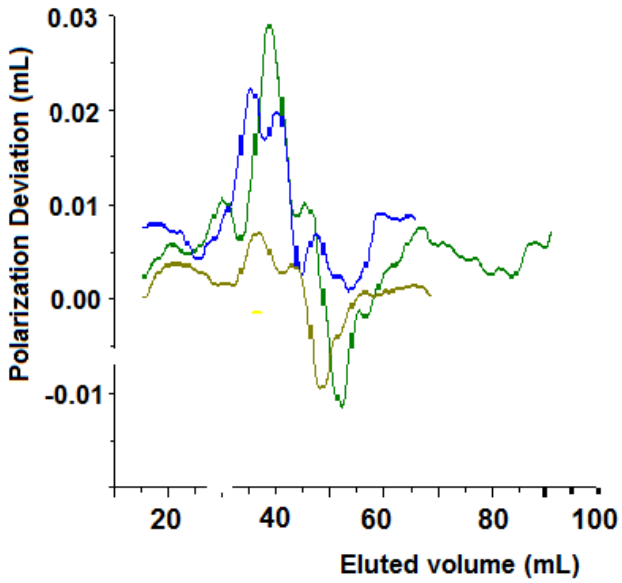

Figure 4. P Chromatographic profile of 3 *T. testudinum* sample extracts represented in different colors.

ACKNOWLEDGMENT

The authors are grateful for the assistance of Lorenzo Hernández.

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