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Anti-diabetic Polysaccharide from Mangrove Plant, *Sonneratia alba* Sm.

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Abstract. Mangrove trees have high potential in marine natural products being abundant in many shorelines all over the tropical regions of the globe. A study was conducted on one of the mangrove species, *Sonneratia alba* leaf extract, to evaluate its antidiabetic property and identify the specific molecule that shows such property using several chromatographic techniques and proton-Nuclear Magnetic Resonance spectrometry (H-NMR). Based on the NMR data the molecule is a complex polysaccharide with peaks observed at 3.4 to 4.5 ppm. When subjected to anti-diabetic bioassay using standard Glucometer (SIMPDO), data showed that it has significantly high attenuating activity for blood glucose because it reduced blood sugar level by 19.2% during the first 6 hours and reduced further to 66.9% after 12 hours. Hence, this polysaccharide molecule obtained from *S. alba* leaf extracts has a very effective anti-diabetic property and it was effectively isolated using a reverse phase column C18 gel with highly polar eluent.

Keywords: alloxan, *Sonneratia alba,* diabetes, hypoglycemia, mangroves

1. Introduction

People today are being bombarded with thousands of unhealthy food products which caused several diseases. One of these is type 2 diabetes. Numerous hypoglycemic drugs have been introduced by several pharmaceuticals to treat diabetes but most of them are adipogenic (Klein, *et al*, 2007). Thus, many have been searching systematically for an agent to modify glucose transport activity and to find natural products useful as anti-diabetic agents. The study on anti-diabetic compound was conducted with intense interest using a mangrove species *Sonneratia alba* because its close relative terrestrial plant, *Lagerstromia speciosa* had previously shown many anti-diabetic properties (Liu, *et al*., 2005). A study on mangrove species, *Ceriops decandra* was conducted by Nabeel, *et al* (2010) and found to have antidiabetic property whose effect was comparable to glibenclamide. Molecular markers are said to be similar among closely related species belonging to the same family or taxon, hence, this concept was used as the framework of the study.

2. Methodology

2.1 Extraction of Crude Molecule

Isolation was done using several chromatographic techniques: (1) gravity column chromatography (GCC) both stepwise gradient and isocratic elution, and (2) high performance liquid chromatography (HPLC) with isocratic elution and (3) liquid chromatography (LC). First, the air dried leaf samples of *S. alba* (900 g) were obtained and were cut into smaller pieces and soaked with methanol (3.75 L) for a period of 4-7 days.

2.2 Column Chromatography

Supernatant liquid were dried *in vacuo* using a Rotary Evaporator (BUCHI R110) and residues were then reconstituted to a small volume (ca. 100 mL) and loaded to silica column (9.7 cm x 15 cm). The column was eluted following step-wise gradient with the following concentrations: 50%, 70%, 90% and 100% methanol: water, respectively. Elution is 2x its column volume. Each fraction was collected separately and was chemically assayed for the presence of tannins using colorimetric method. Tannins that were detected on

fractions were eluted with 50:50 methanol:water (v/v) and so this was then evaporated to dryness and loaded again to silica column with isocratic elution of 60:40 (water:methanol v/v ratio). Fractions that were positive for the presence of tannins were collected and then dried for further purification. To remove non-polar components, liquid partitioning followed using methanol:water:ethyl-acetate (5:25:70, respectively, v/v ratio). Tannins which were recovered in the polar fractions were loaded onto the High Performance Liquid Chromatograph (HPLC) apparatus detected at 254 nm using a Reverse Phase Column (*Synerg 4u Hydro-RP80A, 250 x 10 mm 4u SN: 348934-4).* The molecule was further singled out using Liquid Chromatography (LC).

2.3 Nuclear Magnetic Resonance Spectrometry

Several elutions were tested ranging from 20% to 70% acetonitrile in water moving along the Reverse Phase (C18) gel. The best chromatographic separation obtained was then subjected to proton-Nuclear Magnetic Resonance spectrometry (H-NMR) to identify the whole nuclear molecule.

2.4 Bioassay on Experimental Mice

The molecule was then subjected to bioassay after it was reconstituted at 32 mg/ml of distilled water. Induction of mice to hyperglycemic condition was done using a modified procedure used by Taquilut, *et al.* (2009). There were 27 white mice and were divided into three equal groups following a complete randomized design (CRD):

Group 1 (Control). The mice in this group have normal blood glucose levels and was not given the polysaccharide from the sample extract.

Group II. This group of experimental animals have normal blood glucose levels and was given the polysaccharide from plant extract.

Group III. (Diabetic/Hyperglycemic Group). Polysaccharide extracted from the leaves of *S. alba* was given the same dose and intervals as applied to Group II but the mice was given single intraperitoneal injection of alloxan in saline solution (.9% NaCl) to become hyperglycemic.

All experimental animals were acclimatized for one week in their respective cages made of metal screen with 27 rooms with 1 mouse each, for every plant assay prior to the experimental run. Weight (in grams) was measured using the triple beam balance. They were fed normally with pellets (PDP) and water was given *ad libitum*. Blood sugar levels was monitored using glucometer (SIMPDO) starting from initial levels (start time) and at six (6) hours interval thereafter up to the 12th hour period from the start of the experimentation.

3. Results and Discussions

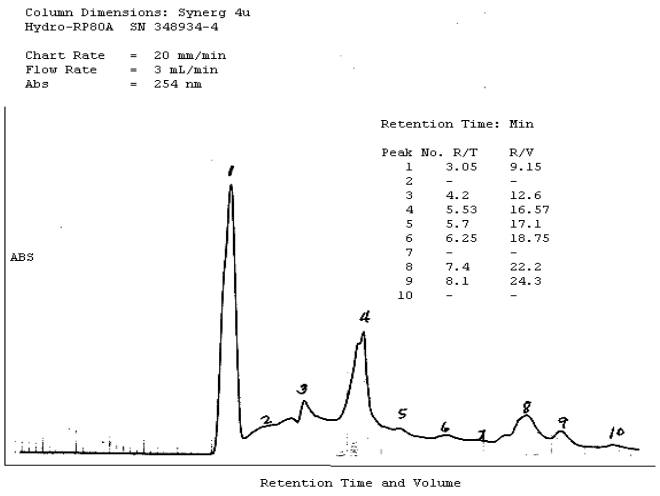

Figure 1. HPLC Chromatogram of polar fractions obtained from isocratic aqueous sub-fraction of S. alba extract ran under silica gravity column developed with 50:50 (methanol: water) at GCC Column dimension 15 cm x 9.

Peak 1 was singled out for analysis. It has a retention time of 3.05 minutes or a retention volume of 9.15 mL. Based on this chromatographic movement, the molecule is moving too fast along the C18 gel hence frontal in character and doubtful about its purity. However, when it was subjected to LC Chromatograph it was found out to be 99% pure as evidenced by a single peak shown in Fig. 2.

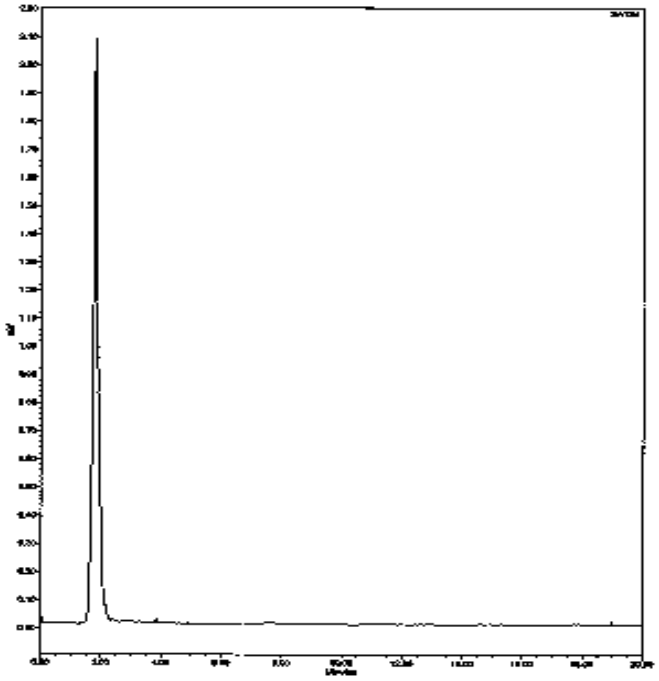

Fig. 2. LC- data of Peak No. 1 (active fraction) showing homogenous and pure fraction after running at Reverse Phase Column with Acetonitrile:Water (30:70 w/w).

The isolate was then subjected to proton-Nuclear Magnetic Resonance spectrometry (H-NMR) to identify the whole nuclear molecule. Based on the NMR data the molecule is a complex polysaccharide, a product of primary metabolism among mangroves (Bandaranayake, 2002), as shown in Fig. 3 with peaks observed at 3.4 to 4.5 ppm deuterated methanol as solvent matrix (Silverstein and Webster, 1998).

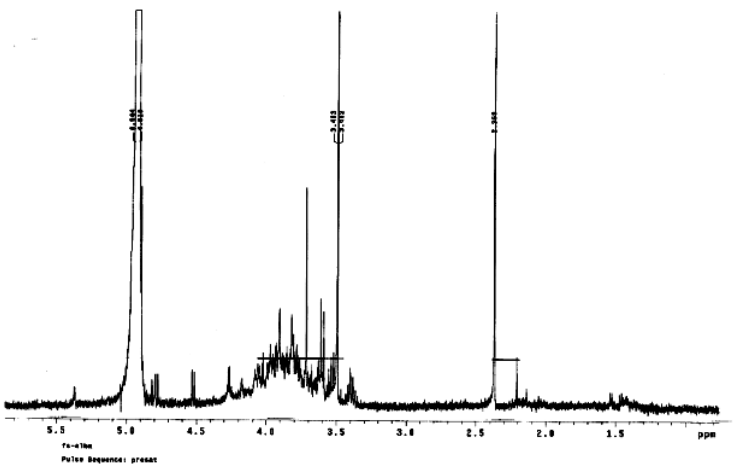

Fig. 3. Proton-NMR data showing peaks indicating that the pure substance from Peak No. 1 is a polysaccharide (peaks revealed from 3.4 tp 4.5 ppm)

Peak 1 was subjected to anti-diabetic bioassay using standard Glucometer (SIMPDO).

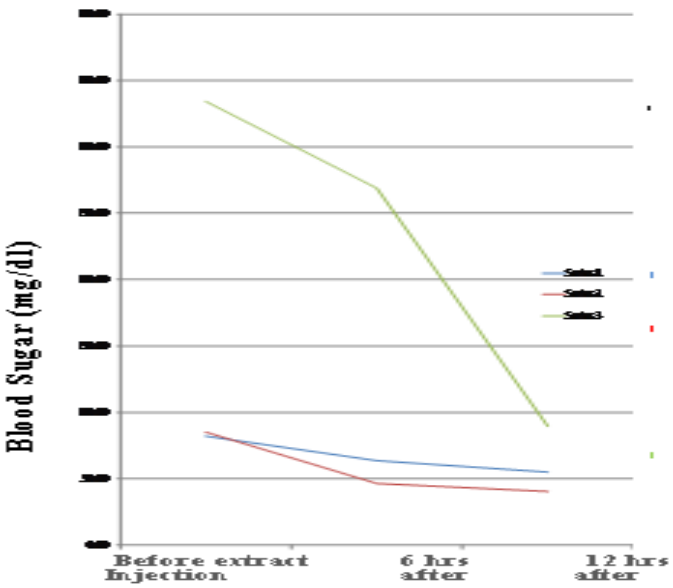

Figure 4. Graph of Peak 1 subjected to anti-diabetic bioassay using standard glucometer (SIMPDO). Legend: blue-group 1;red-group 2; green-group 3.

Data showed that it has tremendous blood glucose attenuating activity because it has reduced significantly the sugar level by about 19.2% during the first 6 hours and reduced further to 66.9% after 12 hours. Therefore, this polysaccharide molecule obtained from *S. alba* leaf extracts has a very effective anti-diabetic property and it was effectively isolated using a reverse phase column C18 gel with highly polar eluent.

4. Acknowledgment

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