

## Chemical characterization of polysaccharide-rich ingredients from *Aloe vera*, *Larix laricina*, *Larix occidentalis*, and *Undaria pinnatifida*

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### INTRODUCTION

Numerous medicinal plants used from early ages as healing remedies have kept their ancient use until today; many have become key ingredients for the dietary supplements and related industries (1). As a result of scientific efforts aimed to better understand their effects on human physiology, some plants have been thoroughly characterized and their mechanism of action is well understood (2). For many others, though, there's still a lot to learn. In the case of *Aloe vera*, larch arabinogalactans, and seaweed there is accumulating data from recent studies suggesting that carbohydrates are indeed the biologically active components (3 - 5). However, more work needs to be undertaken to fully characterize these compounds, to identify the molecules with bioactive roles and to understand their interaction with the human body.

### OBJECTIVE & METHODS

This study was aimed to determine the chemical profile and carbohydrate composition of *Aloe vera* gel powder (AVG), *Aloe vera* extract (AVE), larch arabinogalactans (*Larix* species bark extract, AGS), and seaweed fucoidans (*Undaria pinnatifida* extract, FCS).

The experiments were designed to provide a wide array of information on: elemental analysis (ICP-MS); chemical profiles (total carbohydrate – Dubois method; total protein - Bradford assay); total sugars after hydrolysis by HPLC-PAD, GC-MS of TMS derivatives, and GC-MS of alditol acetates; free sugars by GC-MS of alditol acetates; molecular weight (MW) profiles and sugar composition of the resulting fractions (SEC/GC-MS).

### Experimental Analysis

#### Elemental Analysis by ICP-MS

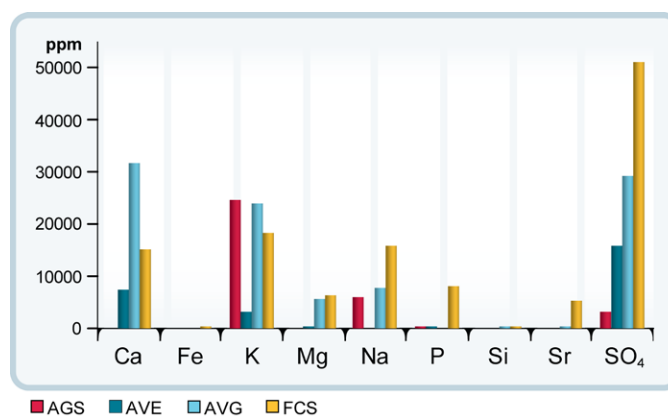
An aliquot of a well mixed, homogeneous aqueous or solid sample was accurately weighed or measured for sample processing. Analytes were first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample was made up to volume, mixed and centrifuged or allowed to settle overnight prior to analysis.

Sample material in solution was introduced by pneumatic nebulization into a radiofrequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions transmitted through the quadrupole were detected by an electron multiplier or Faraday detector and the ion information processed by a data handling system.

Table 1.

	AGS	AVE	AVG	FCS
50000 to 10000 ppm	K		Ca, K SO <sub>4</sub>	K, Ca, Na SO <sub>4</sub>
10000 to 1000 ppm	K SO <sub>4</sub>	Ca, Na SO <sub>4</sub>	Na, Mg P	Mg, Sr
1000 to 100 ppm	P	Mg P		Fe P, Si

Figure 1.



### Carbohydrate and protein content by colorimetric assays

#### Carbohydrate quantitation by Dubois method

Phenol/Sulfuric Acid Assay. A derivation of the microscale phenol sulfuric acid assay of Matsuka et al (Analytical Biochemistry 339 (2005) 69-72) was used. Briefly, 10  $\mu$ L of sample was added to microwell plates in triplicate with 40  $\mu$ L 5% phenol. 0.2 ml concentrated sulfuric acid

was then added, the plates were incubated at 50 C for 15 minutes and absorbance at 490 nm was read on a Molecular Devices Spectra Max plate reader. A standard curve was made using dilutions of 1mg/mL Arabinose.

#### ***Protein quantitation by Bradford assay***

A colorimetric assay for protein concentration was performed. Standards of 160 µL nanopure water containing a range of 0 to 8ug BSA were added to a microwell plate which also contained 280 µL of each sample in triplicate. Sample was incubated at room temperature for 15 minutes with 40 µL Bio-Rad protein assay dye reagent. Absorbance at 650 nm was read using a Molecular Devices Spectra Max plate reader.

**Table 2.**

	Carbohydrate (%)	Protein (%)
<b>AGS</b>	73	<1
<b>AVE</b>	35	2
<b>AVG</b>	100	1.7
<b>FCS</b>	83	2

#### **Total sugar content by instrumental analysis**

##### ***GC-MS of the derivatives***

Glycosyl composition analysis was performed by combined GC-MS of the TMS derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis. An aliquot was taken from the sample and added to separate tubes with 40 ug and 60 ug of inositol as the internal standard. Methyl glycosides were then prepared from the dry sample following the mild acid treatment by methanolysis in 1 M HCl in methanol at 80 C (16 hours), followed by re-N-acetylation with pyridine and acetic anhydride in methanol (for detection of amino sugars). The sample was then per-O-trimethylsilylated by treatment with Tri-Sil (Pierce) at 80 C (0.5 hours). These procedures were carried out as previously described in Merkle and Poppe (1994) *Methods Enzymol.* 230: 1-15; York, et al. (1985) *Methods Enzymol.* 118:3-40. GC/MS analysis of the TMS methyl glycosides was performed on an AT 6890N GC interfaced to a 5975B MSD, using a Supelco EC-1 fused silica capillary column (30m × 0.25 mM ID).

##### ***GC-MS of Alditol Acetates, with hydrolysis***

The samples were hydrolyzed, reduced, and acetylated, and the resultant Alditol Acetates were analyzed by GC-MS. An aliquot was taken from each sample and added to separate tubes with 40 ug and 60 ug of Inositol as the internal standard. The samples were then hydrolyzed in a solution of 2 M TFA at 121 C for 2 hours. Once cooled and dried the samples were reduced by mixing with a solution of 10 mg/mL Sodium Borodeuteride in 1 M Ammonium Hydroxide for overnight. The samples were neutralized with glacial Acetic Acid and dried down. Acetylation of the samples

was accomplished by mixing each with Acetic Anhydrous and concentrated TFA and sitting for 25 minutes at 50 C. The samples were then removed and dried down. 2 mL of Sodium Carbonate was added to each sample along with 2 mL DCM. The samples were agitated and centrifuged; removal of the aqueous layer followed. The samples were then washed 3 times with water and the organic layer was extracted and dried down. The samples were then dissolved in a small amount of DCM and analyzed on a Hewlett Packard 5890 GC interfaced to a 5970 MSD (mass selective detector, electron impact ionization mode); separation was performed on a 30 m Supelco 2330 bonded phase fused silica capillary column.

##### ***HPLC-PAD with hydrolysis***

Monosaccharide contents of the hydrolyzates and standards were determined by AEC-IPAD using the DX 600 chromatographic system with a CarboPac PA20 guard column coupled to a CarboPac PA20 analytical column by injecting 20 µL of the sample or the standard. The eluent flow rate was 0.4 mL/min, and the column temperature was 30 C. The monosaccharides were separated using eluent gradients consisting of Eluent A: DI water (18 MW/cm), Eluent B: 250 mM sodium acetate in 100 mM sodium hydroxide, Eluent C: 10 mM sodium hydroxide, and Eluent D: 200 mM sodium hydroxide. Saccharide detection was by quadruple integrated pulse amperometry on disposable gold working electrode. A postcolumn addition of 300 mM sodium hydroxide was introduced into the detector cell at a flow rate of 0.8 mL/min using the pneumatic PC 10 controller. The sodium hydroxide was added through a 3-way-T, used to combine liquid flows from the analytical column and PC 10 tube. The blended liquids were then directed to the detector cell through a 375 mL knitted reaction coil. The added base compensated for the low base concentration in the initial period of the gradient program.

**Table 3.**

	Glycosyl composition by GC-MS of TMS derivatives (%)	Alditol acetate analysis by GC-MS (%)	HPLC- PAD (%)
<b>AGS</b>	93	50	58
<b>AVE</b>	38	21	29
<b>AVG</b>	20	23	20
<b>FCS</b>	51	47	33

#### **Total sugars, Free Sugars, Oligo and Polysaccharides by GC-MS**

##### ***Alditol Acetate analysis of free sugar***

For Glycosyl free sugar analysis, the samples were reduced, then acetylated, and the resultant Alditol Acetates were analyzed GC-MS. An aliquot was taken from each sample

and added to separate tubes with 40 ug and 60 ug of Inositol as the internal standard. The samples were then reduced by mixing with a solution of 10 mg/mL Sodium Borodeuteride in 1 M Ammonium Hydroxide for 4 hours. The samples were neutralized with glacial Acetic Acid and dried down. Acetylation of the samples was accomplished by mixing them with Acetic Anhydrous and concentrated TFA and sitting for 25 minutes at 50 C. The samples were then removed and dried down. 2 mL of Sodium Carbonate were added to the samples along with 2 mL DCM. The samples were agitated and centrifuged, removal of the aqueous layer followed. The samples were then washed 3 times with water and the organic layer was extracted and dried down. The samples were then dissolved in a

small amount of DCM and analyzed on a Hewlett Packard 5890 GC interfaced to a 5970 MSD (mass selective detector, electron impact ionization mode); separation was performed on a 30 m Supelco 2330 bonded phase fused silica capillary column.

**Table 4.**

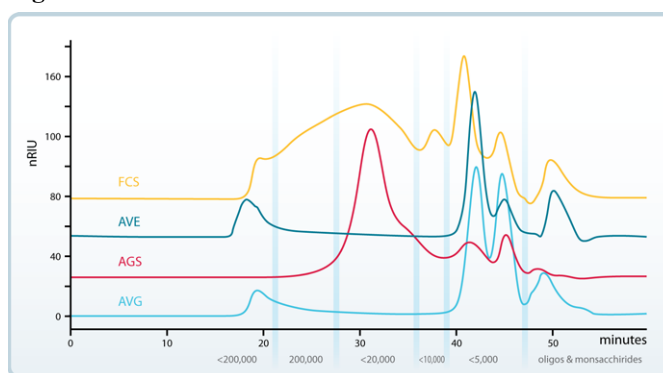
	Total Sugar Alditol acetate analysis by GC-MS (%)	Free Sugar Alditol acetate analysis by GC-MS (%)	Oligo and Polysaccharide estimate (%)
<b>AGS</b>	50	1	49
<b>AVE</b>	21	<1	~21
<b>AVG</b>	23	10	13
<b>FCS</b>	47	<1	~47

**MWs of the Oligo and Polysaccharides by HPLC-SEC**

**Size exclusion chromatography**

A 20 mg/mL solution of the dialyzed sample prepared in 50 mM Ammonium Acetate buffer was prepared and passed through a 0.45 um syringe filter, followed by 3 1 mL-injections into HPLC. The size exclusion chromatography was performed on Agilent 1100 HPLC system. Column: sephadex 75 16/700; Eluent: 50 mM; Ammonium Acetate; Flow rate: 0.5 mL/min; Detection: Refractive Index Signal.

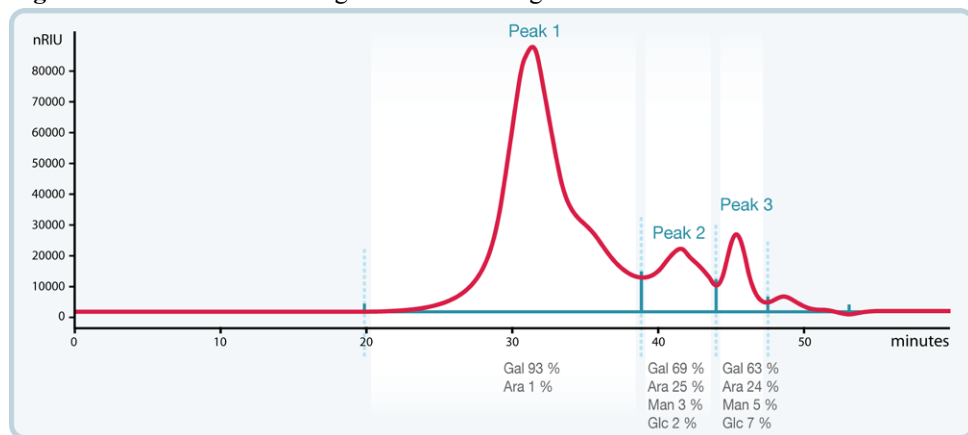
**Figure 2.**



**Carbohydrate composition of the HPLC-SEC fractions by GC-MS of TMS derivatives**

Experimental: same as described above

**Figure 3** The HPLC chromatogram of AGS along with the fractionation events



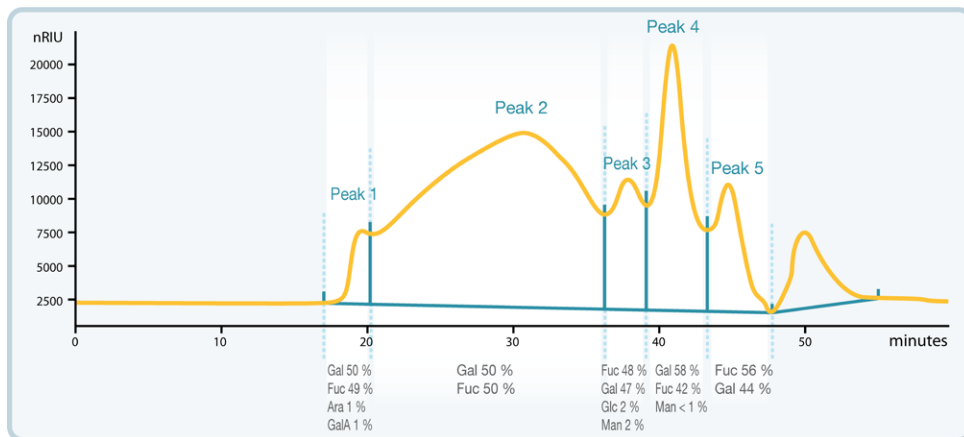
**RESULTS AND DISCUSSION**

Larch extract (AGS) was found to contain K in macronutrient amounts, smaller levels of Na, SO<sub>4</sub>, and P, and less than 1 % protein. The carbohydrate analysis showed that that it contains up to 93 % carbohydrate\*. Total sugar analysis found galactose to represent 90 % and arabinose 9 %, with smaller amounts of mannose and glucose. This ingredient appears to be a

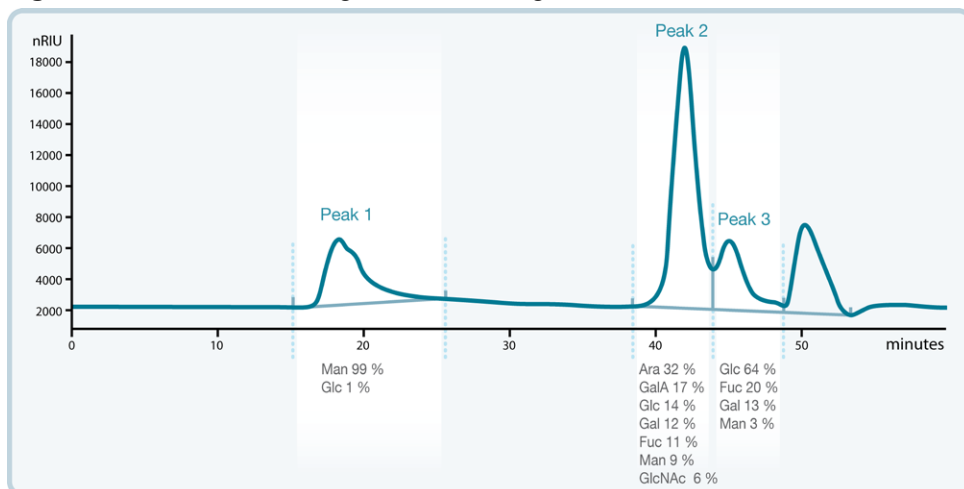
mixture of galactans having MWs within four major ranges up to 20,000 Da, and all based on a similar monosaccharide composition: Galactose was the major sugar in each fraction, followed by arabinose, mannose, and glucose.

Aloe vera extract (AVE) contained SO<sub>4</sub>, Ca, and K as major inorganic components. It showed 2 % protein and 38 % carbohydrate\* which appears to be in forms resistant to hydrolysis. The major sugars were galacturonic acid and mannose, followed by galactose, xylose, glucose, and smaller amounts of arabinose, rhamnose, and fucose.

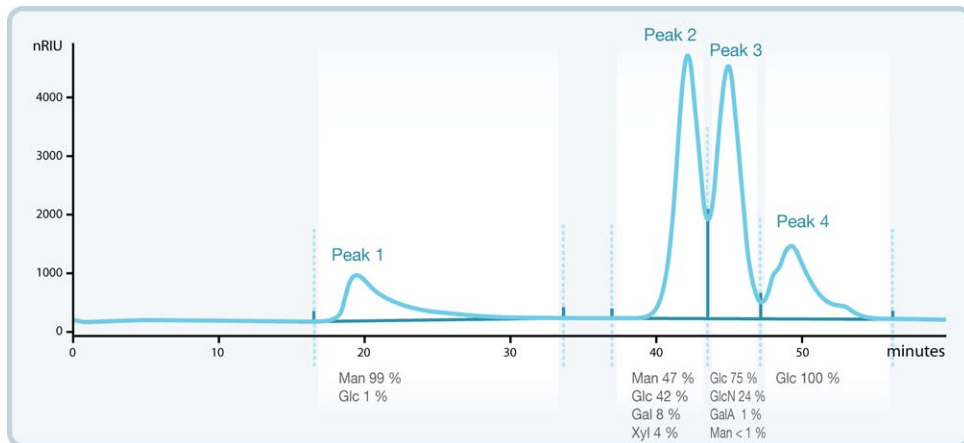
**Figure 4.** The HPLC chromatogram of FCS along with the fractionation events



**Figure 5.** The HPLC chromatogram of AVE along with the fractionation events



**Figure 6.** The HPLC chromatogram of AVG along with the fractionation events



AVE showed four MW fractions with the highest around 200,000 Da. Each fraction showed different monosaccharide constituents: the highest MW fraction was 99 % mannose with 1 % glucose; second fraction contained high amounts of arabinose, galacturonic acid, glucose, galactose, fucose, and mannose; the third fraction was mainly glucose, with smaller amounts of fucose, galactose, and mannose.

Aloe vera gel (AVG) had Ca, K, and SO<sub>4</sub>, as major inorganic components and was found to contain up to 1.7 % protein and 23 % carbohydrate\*, which appears to be in forms resistant to hydrolysis. It contained 52 % glucose, 40 % mannose, and 6 % galacturonic acid, followed by galactose, arabinose, rhamnose, and xylose. The four fractions had MW up to 200,000 Da and all of them contained different monosaccharide composition for each MW range: the highest MW peak contained 99 % mannose and 1 % glucose; the second peak was 47 % mannose and 42 % glucose, followed by galactose and xylose; the third peak contained 75 % glucose 24 % glucosamine, mannose and galactose.

Undaria fucoidans (FCS) had K, Ca, and Na, and SO<sub>4</sub> as major inorganic components and contained 2 % protein and 51 % carbohydrate\*. Most of its carbohydrates ranged from 20,000 to 200,000 Da, and contained primarily equal amounts of galactose and fucose, with small amounts of mannose and glucose. \*Based on the Total Sugars method resulting in the highest values

## CONCLUSION

The study revealed new information on the elemental constituents, chemical profile, carbohydrate composition, MW distribution and fraction composition. Chemical profiling confirmed a high carbohydrate content in all samples. Total sugar values were found to be dependent on the method, and the following main monosaccharides were determined: AGS – arabinose and galactose, AVG – mannose and glucose, AVE – mannose, and FCS – fucose and galactose. Free sugar

content ranged from 1 % (AGS, AVE, and FCS) to 10 % (AVG). MW distributions indicated five major groups: 200,000 Da (AVG, AVE, and FCS); 20,000 Da (AGS and FCS); 10,000 Da (FCS); > 5,000 Da (all samples); and oligos/monosaccharides (all samples). GC-MS sugar profiles of SEC fractions showed a more consistent sugar composition for AGS and FCS (AGS: arabinose and galactose; FCS: fucose and galactose) and wider profile for AVG and AVE (AVG: mannose and glucose; AVE: mannose, glucose, arabinose, and galacturonic acid). Future work will be directed towards further characterization of the polysaccharides in each fraction, aiming to identify the biologically active compounds and to determine their chemical structure.

### ACKNOWLEDGEMENTS

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