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# The Developement of a New Method to Detect the Adulteration of Commercial Aloe Gel Powders

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Simple and accurate methods to detect the adulteration of commercial aloe gel powder were developed. Crude polysaccharide in aloe gel powder was isolated by precipitating with excess ethyl alcohol and total hexose in isolated polysaccharide was determineded by Dubois assay. After hydrolysis of non-dialysable polysaccharides, resultant free sugar was determined by gas chromatography for sugar recognition and ash contents was considered simultaneously. In some products, the content of ash was very low while the content of total hexose was very high. And polysaccharides of these products revealed typical dextran pattern, therefore, these products could be identified that adulterated with commercial maltodextrin. The content of maltodextrin in adulterated product was determined by HPLC and TLC analysis which could be adopted as a part of a certification process.

**Key words :** Aloe gel powder, Adulteration, Maltodextrin

## INTRODUCTION

Aloe has long been used in folk medicine for the treatment of diarrhea, burns and dermatitis. Generally, several anthraquinones, anthrones, chromones and their C-glycosyl derivatives was known to have various biological activities, such as wound-healing effect, anti-inflammatory effect, antibacterial and immuno-modulating effect, antigastric ulcer effect, hypoglycemic and antidiabetic effect and so on (Heggers *et al.*, 1995; Hikino *et al.*, 1986; Hirata *et al.*, 1978; Obata *et al.*, 1993; Saito *et al.*, 1989; Yamamoto *et al.*, 1991). Therefore, in recent years considerable attention has been directed to the use of aloe species as healthy foods and new drugs and sales of products derived from aloe species are growing rapidly. Large numbers of aloe products are commercial in various fields of industry, and therefore many kinds of aloe gel powders are merchandised. But as in all food industries, where opportunities exist to realize large margins, unscrupulous people will exploit the situation. For protecting the image of Aloe as a safe and pure product, quality control of aloe products is necessary.

After L-malic acid was proposed as a marker of aloe products (Pelley *et al.*, 1992; Pelley *et al.*, 1993), artificial addition of synthetic D-malic acid to the adulterated aloe products could be found out, but it was impossible to find out the adulteration if L-malic acid was added. We tried the quality control with phenolic compounds in aloe species such as aloesin, methylaloesol, aloeresinD, barbaloin and aloe-emodin etc., but failed because the contents of them were varied significantly according to the processes of maufacturing.

In order to detect the adulteration of aloe product with polysaccharide, many researchers have developed various methods. Carbohydrate pattern in aloe plant was studied (Mandel *et al.*, 1980a, Mandel *et al.*, 1980b). Polysaccharides were isolated by alcohol precipitation method and determined by the phenol-sulfuric acid colorimetric method (Hodge *et al.*, 1962; Whistler *et al.*, 1962). The isolated crude polysaccharids were purified by dialysis across a membrane with a cut-off approximately 5,000 MW for removal of small molecular interfering substances. They were hydrolyzed and the resultant free sugars were determined (Gowda *et al.*, 1979; Waller *et al.*, 1994). But the analysis of polysaccharide in aloe products cannot reveal the adulteration completely.

We have tried many methods and found that commercial aloe products were adulterated with maltodextrin. The qualitative TLC method and quantitative HPLC method were developed for the tranditional polysaccharide analysis.

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## MATERIALS AND METHODS

### Materials and equipments

Commercial aloe gel products 21 samples and maltodextrin were gifts from Namyang Aloe Co. (Jincheon, Korea) and these materials were coded in a manner such as to conceal their nature and origin. As a laboratory control, authentic Aloe vera gel powder were prepared as described below. Cellulase (cellulase 4000, Valley Research) acquired from Namyang Aloe Co. (Jincheon, Korea) was used for standard sample preparation. Glucose, mannose and galactose were purchased from Sigma (St. Louis, USA). Trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) for trimethylsilylation of monosaccharides in GC analysis were purchsed from Fluka Chemie AG (Buchs, Switzerland). Deionized water was prepared with Millipore Milli-RO 15 water purification system and all other chemicals were reagent grade.

Desalting of crude polysaccharides was accomplished using Spectra/Por cellulose ester (CE) membrane filter (Pittsburg, USA) and hydrolysis of polysaccharide was performed using dry bath incubator purchased from Fisher Scientific (Pittsburg, USA). Electrical muffle furnace used for determination of ash content was purchased from Seonjin Ltd. (Seoul, Korea). Spectrophotometer of Pharmacia Biotech Ltd. (Uppsala, Sweden) was used for determination of alcohol precipitable hexose.

The high-performance liquid chromatograph was a Shimadzu LC-9A pump (Kyoto, Japan) equipped with Shidmadzu RID-6A refractive index detector (Kyoto, Japan), GL-Science Inertsil ODS-2 (5 μm, 300×4.6 mm I.D., Tokyo, Japan) and Shimadzu C-R6A integrator (Kyoto, Japan). Mobile phase was 100% DI water filtered with 0.45 μm-microporous membrane filter prior to use.

Gas chromatograph for analysis of sugar composition in polysaccharides was a Hewlett-Packard GC 5890 Series II (USA) equipped with flame ionization detector (FID), Shimadzu C-R6A data processor (Kyoto, Japan) and J&W Scientific DB-1 capillary column (0.25 μm, 0.25 mm×30 m, USA).

### Preparation of standard samples

About 1010 g of Aloe vera gel which was acquired by peeling of Aloe vera whole leaves was stirred well with glass rod after adding 0.018 g of cellulase. The fresh thawed crude gel was filtered with sieve (pore size~180 μm). After adding 5 g of charcoal to a filtered gel solution, the mixture was heated at 70°C for 30 min and filtered with high vacuum. The filtered solution was lyophilized and about 5 g of aloe gel powder was acquired consequently. Differently, about 1080g of whole Aloe vera gel was lyophilized and 15 g of dried crude Aloe vera gel was obtained. The resultant Aloe vera gel powder and dried crude Aloe vera gel were coded as 'A' and 'B' respectively.

### Isolation of crude polysaccharide

A 5 ml aliquot of test material (10% w/v in DI water) was added to a 100 ml graduate cornical tube and mixed with 95 ml of ethyl alcohol. The tubes were capped and the mixed until homogenity. The tubes were placed at 4°C for precipitation overnight.

After precipitation, the contents of each tube were centrifuged at 2000 rpm for 10 min. After centrifugation, the supernatant was discarded and the each precipitation was lyophilized for 24 hrs. The weight of each precipitate was measured.

### Analysis of free sugars in hydrolysates of non-dialysable polysaccharides by GC

Crude polysaccharides (200 mg) was dissolved in 5 ml of DI water and dialysed. Dialysis was carried out using microporous membrane separating molecules at approximately a 5,000 MW cutoff. The lyophilized retained materials (5 mg) were placed into a hydrolysis tube and 6N-hydrochloric acid (2 ml) was added. After dissolution, the hydrolysis tube was evacuated of oxygen by high vacuum while chilled in an ice bath to minimize loss of hydrochloric acid. The tube was sealed and hydrolysis acomplished by incubation for 30 min at 120°C. The solution was neutralized with 2N-sodium hydroxide solution. 250 μl of each solution was transfered into 1 ml-reaction vial and was lyophilized.

The residue was dissolved in 100 μl of pyridin anhydrous, followed with adding 50 μl of hexamethyldisilazane (HMDS) and 25 μl of trimethylchlorsilane (TMCS). After mixing, the whole was kept at R.T. for 5 min and then each 2 μl aliquot of solution was injected into gas chromatography.

Standard calibration curves was generated as follows. Standard solution of mannose, galactose and glucose were prepared by dissolving in pyridin anhydrous. To a 1 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.05 mg/ml and 0.025 mg/ml of each standard solution, 50 μl of hexamethyldisilazane (HMDS) and 25 μl of trimethylchlorsilane (TMCS) were added After mixing, the whole was kept at R.T. for 5 min and then each 2 μl aliquot of solution was injected into gas chromatography.

### Quantification of total hexoses in polysaccharide

Ten mg of each alcohol precipitable solid was dissolved in 10 ml of DI water. A 10 μl aliquots of dissolved precipitate was placed into test tube. Each test tube then consecutively received 1 ml of DI water, 1 ml of 5% phenol solution, and 5 ml of concentrated sulfuric acid with mixture by vortex after each addition.

Then the test tubes was placed in a water bath (ambient temperature) until cool (approximately 20 min).

A reference standard curve was generated as follows. First, a stock solution of 1 mg/ml mannose was prepared. And volumes of 20, 40, 60, 80, and 100 μl was dispensed and treated with the phenol solution and sulfuric acid as above.

The absorbance of solutions was measured with UV/Visible spectrophotometer set at 490 nm.

### Quantification of ash contents of aloe gel powder

Each aloe gel powder (300 mg) was transfered into crucible and then heated at 500°C for 4 hrs. After cooling to R.T. in dessicator, the weights of residues were measured precisely.

### TLC analysis of aloe gel powder

In contamined Aloe gel powder with commercial polysaccharides, maltodextrin was detected using analytical TLC. Standard samples, maltodextrin and various commercial aloe gel powder was dissolved in DI water, and analysed on TLC using acetonitrile/ethyl acetate/iso-propyl alcohol/water (85/20/50/50) as a mobile phase. The plate was visualized with 5% sulfuric acid in ethanol.

### Determination of maltodextrin in aloe gel powder using HPLC

Various commercial aloe gel powder (100 mg) were dissolved in 1 ml of DI water, followed by filtration using 0.45 μm-membrane filter. And then 10 μl aliquot of samples were injected directly onto HPLC. And 100 mg of maltodextrin was dissolved in 1 ml of DI water, followed by injection onto HPLC as a standard solution. And standard calibration curve was generated by total area of maltodextrin peaks in

**Table 1.** Isolation and characterization of polysaccharides in various commercial aloe gel powders

| List of samples[a] | Ash contents (mg)[b] | Mass of alcohol precipitate (mg)[c] | Hexose in alcohol precipitate (mg)[d] | Mass retained upon dialysis (mg)[e] | Contents of maltodextrin (w/w, %)[f] | Relative contents (%)[g] | | | Mannose/ Glucose[h] | Adulteration with maltodextrin |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Mannose | Galactose | Glucose | | |
| A[1] | 115.90 | 231.80 | 22.50 | 18.60 | N.D. | 41.41 | 22.57 | 36.03 | 1.15 | ○ |
| B[2] | 93.80 | 322.40 | 53.40 | 197.30 | N.D. | 57.83 | 22.03 | 20.14 | 2.87 | ○ |
| Maltodextrin | 0.00 | 330.70 | 223.00 | 145.50 | * | 0.00 | 0.00 | 100.00 | 0.00 | * |
| K-1 | 82.80 | 320.00 | 96.10 | 163.80 | N.D. | 55.64 | 22.75 | 21.61 | 2.57 | ○ |
| A-1 | 126.70 | 271.10 | 18.50 | 14.90 | N.D. | 54.53 | 29.57 | 15.90 | 3.43 | ○ |
| A-2 | 188.70 | 333.30 | 10.10 | 6.20 | N.D. | 43.13 | 23.71 | 33.16 | 1.30 | ○ |
| A-3 | 185.40 | 343.00 | 10.20 | 7.50 | N.D. | 42.51 | 22.03 | 35.47 | 1.20 | ○ |
| A-4 | 126.50 | 334.40 | 9.10 | 7.00 | N.D. | 41.68 | 22.75 | 35.57 | 1.17 | ○ |
| A-5 | 158.30 | 304.50 | 11.10 | 5.70 | N.D. | 52.30 | 20.96 | 26.74 | 1.96 | ○ |
| A-6 | 167.80 | 315.30 | 13.90 | 6.60 | N.D. | 57.47 | 20.12 | 22.41 | 2.56 | ○ |
| A-7 | 162.30 | 313.90 | 9.60 | 5.90 | N.D. | 39.03 | 17.66 | 43.31 | 0.90 | ○ |
| A-8 | 188.80 | 347.40 | 6.70 | 3.10 | N.D. | 42.16 | 0.00 | 57.84 | 0.73 | ○ |
| A-9 | 170.10 | 185.30 | 10.30 | 1.50 | N.D. | 43.25 | 0.00 | 56.75 | 0.76 | ○ |
| G-1 | 0.00 | 357.80 | 285.40 | 176.50 | 57.10 | 18.65 | 0.00 | 81.35 | 0.23 | △ |
| G-2 | 39.80 | 352.20 | 196.60 | 129.00 | 94.60 | 0.00 | 0.00 | 100.00 | 0.00 | × |
| G-3 | 123.40 | 337.70 | 114.80 | 103.70 | 45.00 | 19.74 | 0.00 | 80.26 | 0.25 | △ |
| T-1 | 148.20 | 272.40 | 38.70 | 45.50 | N.D. | 57.03 | 24.79 | 18.19 | 3.14 | ○ |
| T-2 | 33.20 | 359.50 | 246.90 | 190.60 | 53.00 | 20.50 | 0.00 | 79.50 | 0.26 | △ |
| T-3 | 22.80 | 369.70 | 250.40 | 141.20 | 57.80 | 24.12 | 0.00 | 75.88 | 0.32 | △ |
| T-4 | 0.00 | 372.00 | 287.10 | 193.80 | 56.90 | 16.71 | 0.00 | 83.29 | 0.20 | △ |
| F-1 | 130.70 | 108.90 | 34.30 | 140.30 | N.D. | 63.51 | 26.20 | 10.29 | 6.17 | ○ |
| M-1 | 135.80 | 152.30 | 33.80 | 109.10 | N.D. | 64.24 | 25.09 | 10.68 | 6.02 | ○ |
| D-1 | 10.00 | 383.60 | 304.90 | 170.70 | 52.50 | 0.00 | 0.00 | 100.00 | 0.00 | △ |
| P-1 | 155.50 | 354.60 | 111.60 | 90.50 | 46.60 | 18.36 | 0.00 | 81.64 | 0.22 | △ |

[1),2)]: standard samples A and B respectively
[a]commercial aloe gel powder except A, B and maltodextrin
[b-e]weight (mg) in 500 mg of aloe gel powder
[f]weight of maltodextrin (mg) / weight of aloe gel powder (mg)
[g]relative sugar contents in non-dialysable polysaccharides isolated from aloe gel powder
[h]content of mannose/content of glucose
○ : free from maltodextrin contamination
△: adulterated with maltodextrin partially
× : consisted of almost maltodextrin

chromatogram.

## RESULTS AND DISCUSSION

Table I shows the general values for the major parameters examined. For the gross alcohol precipitate, three factors were determined: (1) the mass of solids precipated, (2) hexose content by the Dubois assay (phenol sulfuric acid method) and (3) sugar composition of polysaccharide. Contents of alcohol precipitable solid, total alcohol precipitable hexose and ash in aloe gel powder of standard sample A were appeared to be 46.4% (w/w), 4.5% (w/w) and 23.2% (w/w) respectively. Standard sample B, Product K1, A1~A9, T1, F1 and M1 showed similar pattern with standard sample A. However, in cases of product G1~G3, T2~T4, D1 and P1, the amounts of ashes were very low while the amounts of total alcohol precipitable hexoses were very high. So, these products were considered to be adulterated with commercial plant polysaccharides.

Relative sugar compositions in alcohol precipitable polysaccharides were investigated using gas chromatograph. Contents of mannose, galactose and glucose in standard sample A were appeared to be 41.4% (w/w), 22.6% (w/w) and 36.0% (w/w) respectively. The results showed that the sugar composition of these products revealed glucomannan pattern which usually observed for native Aloe vera gel polysaccharide. But, in cases

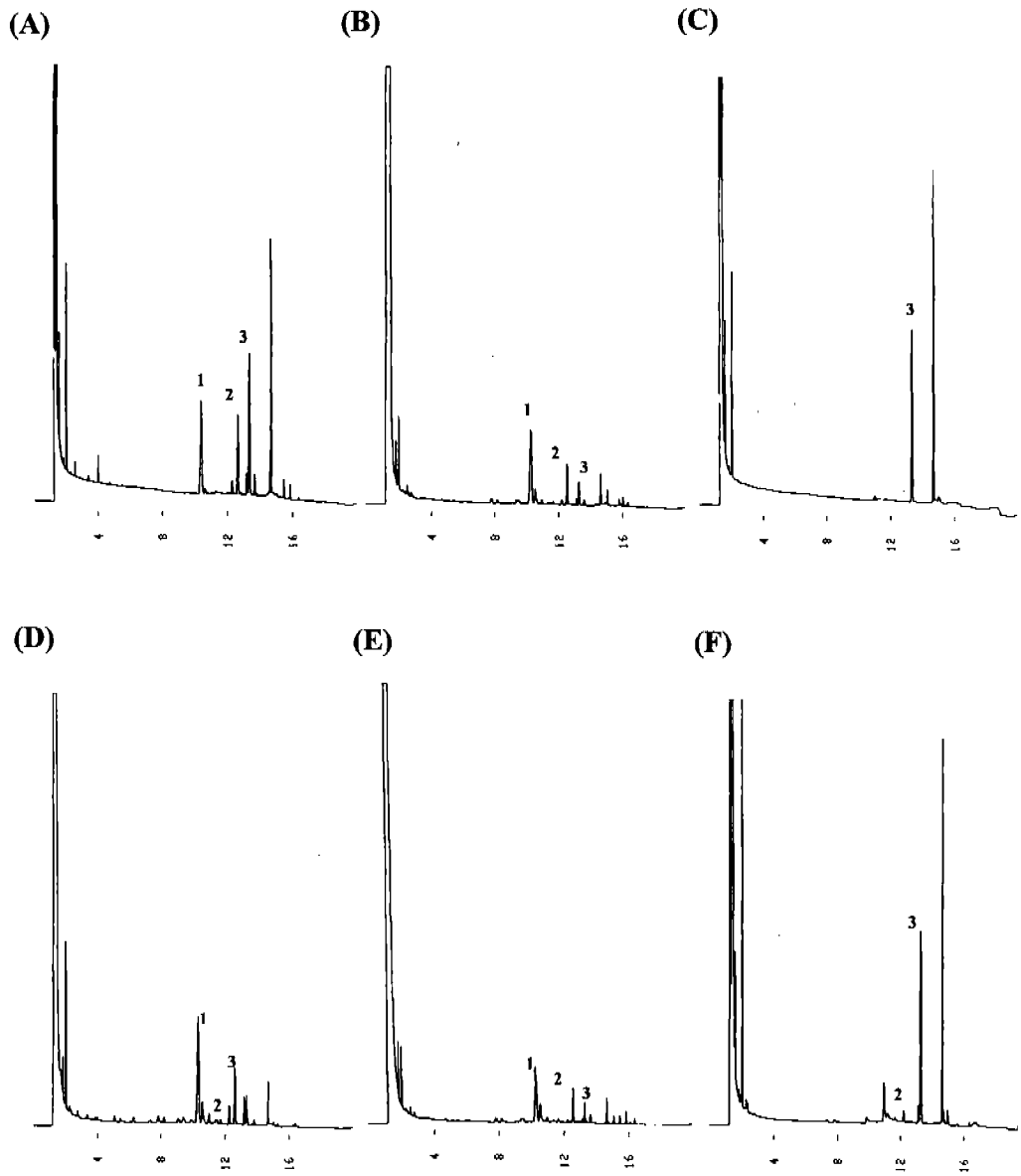


**Fig. 1.** GC chromatograms of (A) standard sample A, (B) standard sample B, (C) maltodextrin, (D) A-9, (E) K-1 and (F) G-2. Mannose, galactose and glucose were determined by Gas Chromatography after trimethylsilylation with trimethylchlorsilane/hexamethyldisilazane. 1, 2 and 3 indicate peak of mannose, galactose and glucose. Condition: column; J&W DB-1 (0.25 μm, 0.25 mm×30 m), column temperature; 160→250°C, carrier gas; He 2.84 ml/min, and detection; flame ionization detector.

of product G1~G3, T2~T4, D1 and P1, the results showed that amount of glucose was very high while amount of mannose was very low. Sugar composition of these products showed typical dextran pattern, so these products could be adulterated with commercial maltodextrin. Typical GC chromatogram was shown in Fig. 1.

Generally, mass which was retained upon dialysis showed linear relationship with content of total hexose. As in Fig. 4, there was a 96.5% correlation between polysaccharide defined by alcohol precipitable hexose and the mass of alcohol precipitable, non-dialysable material. This analysis also confirm that the data fall into several groups based on polysaccharide content. However, standard sample B, products K1, F1 and M1 was not applied to dialysis because they were sparingly soluble in water.

Qualitative TLC analysis were performed, and adulteration with maltodextrin in some products could be confirmed. $R_f$ value of standard sample A treated with cellulase, was 0.53 and single spot was observed and $R_f$ value of standard sample B untreated with cellulase, was almost 0. Products A-1~A-9 were supposed to be treated with cellulase. $R_f$ values of maltodextrin was,

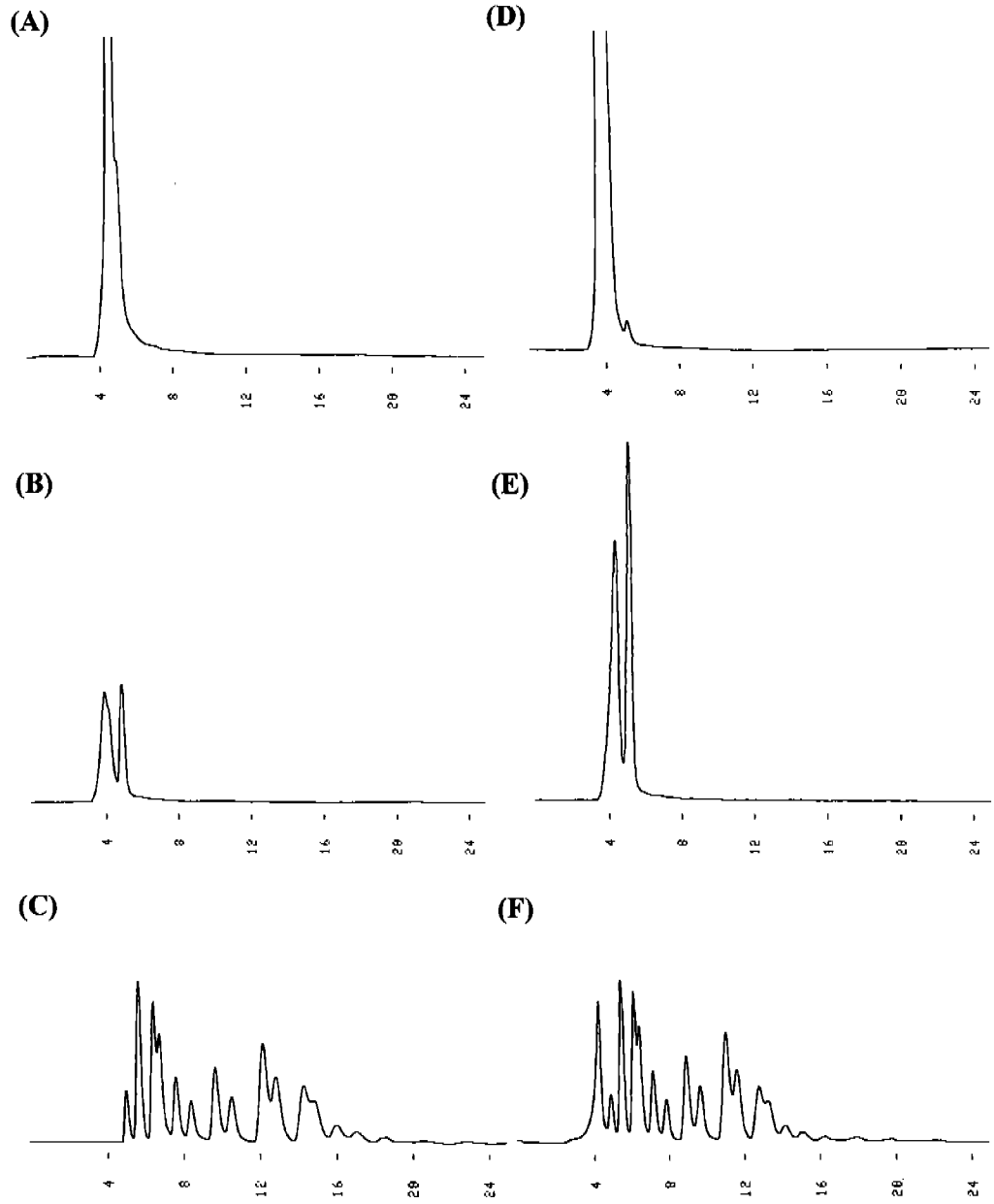


**Fig. 2.** HPLC chromatograms of (A) standard sample A, (B) standard sample B, (C) maltodextrin, (D) A-9, (E) K-1 and (F) G-2. Each aloe gel powder was simply dissolved into DI water, followed by injection directly onto HPLC. Column; GL Science Inertsil ODS-2 (5 μm, 4.6 mm × 300 mm), mobile phases; 100% DI water, detector; refractive index detector.

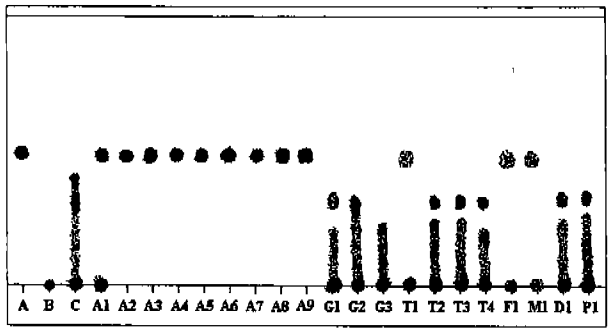


**Fig. 3.** TLC patterns of various aloe gel powders. Stationary phase; Merck Kieselgel 60 F254 precoated TLC plate, mobile phase; acetonitrile/ethyl acetate/iso-propyl alcohol/DI water= 85/20/50/50.

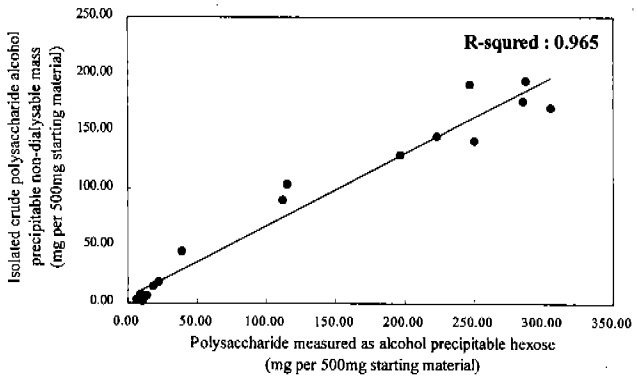


**Fig. 4.** Relationship between content of polysaccharide measured as alcohol precipitable hexose and yield of crude polysaccharide isolated by alcohol precipitation and dialysis (500 mg Aloe Powder). Standard sample B, K-1, F-1 and M-1 is excluded because they were non-dialysable owing to their low-solubility in DI water.

however, distributed in the range of 0~0.47 and existence of maltodextrin in adulterated aloe gel powder, therefore, could be detected as is illustrated in Fig. 3. Contents of maltodextrin was determined by RP-HPLC using refractive index detector. In cases of standard sample A and B, no peaks were observed after 5 min as shown in Fig. 2A and 2B. But, peaks of maltodextrin were observed in the range of 5~20 min and chromatogram showed specific elution pattern as shown in Fig. 2C. Therefore, identification and determination of maltodextrin could be performed. The standard curve for maltodextrin gave a linear response with correlation coefficient of 0.999. Contents of maltodextrin was shown in Table I.

Consequently, in the cases of products G1~G3, T2~T4, D1 and P1, the presence of dextran consistent with maltodextrin was definitive, and polysaccharides of products K1, A1~A9, T1, F1 and M1 was most consistent with Aloe vera. In conclusion, adulteration with commercial polysaccharides could be detected by sugar analysis, determination of ash contents, and especially maltodextrin could be detected by TLC and determined by HPLC.

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