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Extraction, purification and identification of aloe gel from *Aloe vera* (*Aloe barbadensis* Miller)

Mandar Mulik*, Mitesh Phale

Divakar Chemicals ltd., 203-204, Sant Bhavan, Sharma Industrial Estate, Walbhat Road, Goregaon – East, Mumbai - 400 063, (INDIA)

E-mail : mandarmulik@yahoo.com

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ABSTRACT

The current study was conducted to obtained aloe gel from *Aloe vera* leaves and to study various methods of extraction. To achieve crude gel, leaves were subjected to filleting and grinding process and purified using hot treatment method and enzymatic treatment method. Procedure was developed for the extraction, purification and identification of *Aloe vera* gel to ensure the organoleptic stability, and the quality of the final product. The study also reveals that filtration with activated charcoal and Hyflo supercel[R], gives clear appearance to the gel. Gel was analyzed by colorimetric method for estimation of polysaccharide using Congo red as complexing agent. The addition of pectolytic enzyme 'Pectinase' yields *Aloe vera* gel with 45% more polysaccharide content than gel obtained by simple heat treatment.

KEYWORDS

Aloe vera;
Clear gel juice;
Pectolytic enzyme;
Polysaccharides.

INTRODUCTION

Aloe vera, a member of the Liliaceous family with a cactus-like appearance is a popular houseplant with a long history as a multipurpose folk remedy. The herbal practitioners and healers use aloe for promoting and restoring healthy skin due to its inherent healing properties. There are over 240 different species of aloe, growing mainly in the dry regions of Africa, Asia, Europe and America.[1,2]

The aloe leaves are lance shaped with jagged edges and sharp points. The leaf is made of three layers named rind, sap, mucilage. The leaves contain over 75 nutrients and 200 active compounds, mono-saccharides and complex long-chain sugars which are thought to give *Aloe vera* its unique healing and immuno-stimulating properties. The *Aloe vera* gel concentrate shows the presence of glucose, malic acid, and the major polysaccharide acemannan. The polysaccharide acemannan is a β-(1, 4) linked mannan partially acetylated in positions 2, 3, or 6. and forms the major natural component of aloe vera gel.[3]

Aloe vera gel mainly has antifungal[4] anti inflammatory[5] hepatoprotective potential[6] due to which it is in major demand in the herbal area of research. Apart from medicinal use aloe has a wide application in nutraceutical and functional food products. Many producers of aloe vera commercially grow and process the aloe vera plant and supply the gel to manufacturers of cosmetics and food products in bulk. Unfortunately the aloe industry is facing the problems of dilution; addition of cheap non-aloe solids and lack of standardized processing techniques for the processing of *Aloe vera* leaves.[1] There is a need to standardize the processing parameters for the *Aloe vera* gel, and to establish quality control parameters for the *Aloe vera*. It

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therefore becomes essential that a simple but efficient processing technique needs to be developed, to improve product quality, to preserve and maintain almost all of the bioactive chemical entities naturally present in the plant during processing. In order to achieve the above objective the process of extraction, purification and identification of *Aloe vera* gel was studied in the research work. All possible organoleptic and qualitative analysis was done.[7]

EXPERIMENTAL

Chemicals and Reagents

The pectolytic enzyme Pectinase (1000 units/ml) was procured from Lumis Biotech Pvt. Ltd, Thane with certificate of analysis. Calcium hypochlorite Activated charcoal, Hyflo supercel®, Congo red, Sodium hydroxide were obtained from S. D. Fine-Chem Limited (India).

Plant material

The leaves of aloe were procured from local market of Mumbai and were authenticated as *Aloe barbadensis* leaves.

Equipment

pH of the extracts was checked on a pH/ion analyzer (Lab India PHAN, India). The UV system employed in the identification was Jasco V-550. And the Abbeys refractometer was used for measurement of refractive index.

Preparation of Standard Extract

A standard sample of aloe vera gel was extracted by simple drain procedure, where 2-4 leaves of aloe were cut at about ½ inch from the base so as to drain out all the yellow sap material. The sap flows freely with the pressure of the epidermal cells and all other cellular structures above it. Then those leaves were further pressurized with hand till it oozed out clear gel. This procedure was very crude and time consuming as well, but gel obtained by this method was more stable and less degraded than other extraction method. Taking into consideration stability of the gel obtained by this method, the sample thus extracted was used as a standard for further study. The clear gel obtained was labeled as S1.

Preparation of Sample Extract

The sample extracts were prepared by two different extraction procedures on the aloe leaves separately. These sample extracts thus obtained were compared with the standard extract (S1) prepared to compare the content of polysaccharide acemannan. The process followed is shown in Figure 1

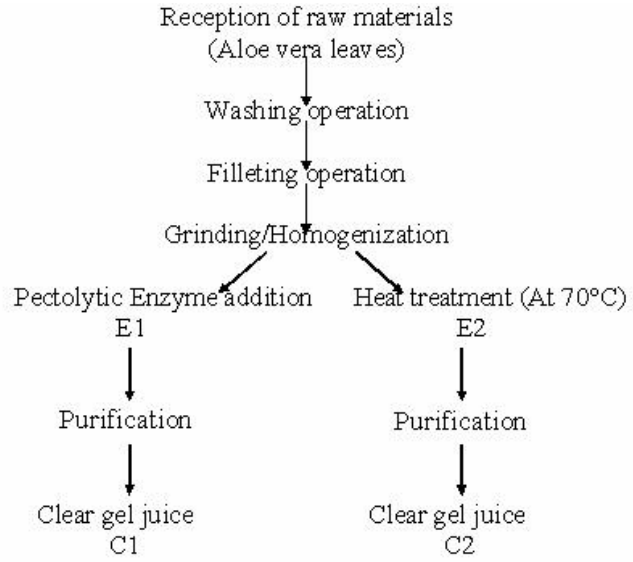

Figure 1 : Extraction Methods for Aloe gel from *Aloe vera* leaves Cleaning and disinfection of the leaves

All the leaves before processing were soaked in a solution of calcium hypochlorite (0.1 % w/v), for disinfection and also to remove any adhered dust or soil material. The leaves were then thoroughly washed with water, till no residue of calcium hypochlorite remains. All the leaves were carefully observed and any infected or damaged leaves were immediately discarded at this stage. Also any immature leaves or leaves containing very little amount of mucilaginous gel were sorted out and discarded.

Filleting and Homogenization

The leaves were then cut at about ½ inch from the base so as to drain out all the yellow sap material which flows free with the pressure of the epidermal cells and all other cellular structures above it. Then the leaves were again cut at about 1½ inch from the apex. The leaves were then cut in to two or more parts longitudinally, which were easy to handle. The spiny margins from the leaves were stripped off using a clean knife. The upper epidermis of the leaves was then removed

with the help of a clean spatula, so as to expose the inner mucilaginous gel from the leaf. The inner gel was then scraped out with the help of a clean spatula carefully avoiding any green leafy part structures to obtain the chunks/fillets of leaf. All the chunks/fillets were carefully observed, as they should not contain any green colour part associated with them. The chunks were allowed to drain naturally at low temperature, which gives the aloe juice. The remaining was subjected to grinder which gives juice with some cellular particles and other solid content, which were cleared off by in following processes.

Clarification of the juice

The clarification of *Aloe vera* gel was the critical step of the entire process. Two different approaches were carried out to obtain clear juice.

Pectolytic enzyme addition[8]

In this process crude *Aloe vera* gel juice was treated with pectolytic enzyme. The time of enzyme reaction did not exceed 20 min for the conditions: 0.06% (w/v) Pectinase in aloe gel mash, temperature of reaction was maintained to 50°C and 70°C. The juice obtained by pectolytic enzyme addition was labeled as E1.

Heat treatment

The crude *Aloe vera* gel juice so obtained was heated in hot water bath at 70°C for 30 min. Heat treatment destroys the enzymes that causes darkening of the juice. The juice obtained by heat treatment was labeled as E2.

Purification

Gel juice (E1 and E2) were further subjected to filtration by adding activated charcoal (0.1%) and passing through G3 sintered glass filter separately. The juices separately were further passed through G4 sintered glass filter with Hyflo supercel^{R} as the filter media to a clear transparent aloe juice.

The juices (E1 and E2) thus obtained from both the process were analyzed for organoleptic and qualitative analysis. Further analysis was performed by determining the polysaccharide content by Congo red method to study the effect of temperature on juice. The clear gel juice obtained was labeled C1 and C2. The process is summarized in the Figure 1.

Identification and Analysis of the Aloe Gel

The *Aloe vera* gel so obtained by all the extraction procedures was evaluated for following parameters:

Organoleptic Evaluation

Appeal, taste, and odour form a very important part of the products. Added ingredients may modify the original taste of any product. The organoleptic characteristics include appearance; colour, odour/flavor, and taste of the two in-house preparations were evaluated.

Physical Evaluation[9-11]

Determination of pH

The measurement of pH was done with a suitable calibrated potentiometer know as pH meter fitted with two electrodes, one constructed of glass and sensitive to hydrogen-ion and the other is calomel reference electrode. The determination was carried out at a temperature of 30°C (Room temperature).

Determination of Total Solids

Accurately measured 10 ml of samples was placed in a tarred dish, and the contents were evaporated on steam bath until the residue was apparently dry. The residue was further heated on steam bath to dry it to constant weight. The dish was cooled in desiccator and then again weighed. Total solid content is an indication of the aloe solids.

Determination of Methanol Precipitable Solids (MPS)

To 1 ml of each sample 10 ml of methanol was added slowly with continuous shaking and the contents were centrifuged for 10 minutes. The supernatant was discarded and the precipitate was dried, by centrifuging it with diethyl ether for 5 minutes. The precipitate was then dried in vacuum desiccator, weighed and percentage of precipitate was calculated on w/v basis.

Determination of Weight per ml

A thoroughly clean and dry pycnometer was taken. It was calibrated by filling it with recently boiled and cooled water at 25°C. The temperature of the substance to be examined was adjusted to about 25°C. The tare weight of the pycnometer from filled weight of

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pycnometer was subtracted. The weight per milliliter was determined by dividing the weight in air, expressed in grams, of the quantity of liquid which fills the pycnometer at a specified temperature, by capacity expressed in ml, of the pycnometer at the same temperature 30°C.

Determination of Refractive Index

The samples were placed between the two surfaces of prisms of Abbes refractometer. Light was adjusted with the help of mirror for maximum reflection and the knob was rotated to bring the boundary (between bright and dark halves of light) to the centre of the cross wire. Reading was noted which is the refractive index of the sample.

Chemical evaluation

Quantitative Estimation of Polysaccharides by Congo Red Method[7]

The active polysaccharide acemannan can be identified and estimated by the use of Congo red as a complexing agent. In this method Congo red was allowed to react with bioactive polysaccharides i.e. acemannan in an alkaline medium. The formation of a complex between gel forming D-glucose and Congo red has been reported. It was postulated that the complex formation of polysaccharide with Congo red was due to orderly conformation of the molecule.

Standard Curve of Polysaccharides

Preparation of Stock Solution

100 mg of the reference aloe vera standard, made up of lyophilized aloe vera gel was dissolved in 10 ml distilled water. (1% w/v is the arbitrarily assigned 200% aloe vera value). The stock solution was diluted to concentration of 0.1% w/v polysaccharide. This solution was further diluted by taking 0, 0.8, 1.6, 2.4 and 3.2 ml in separate 10 ml test tube and making up the volume with distilled water to 4 ml in each tube. In sample tube 4 ml of aloe juice was taken instead of standard solution. The color was developed by adding 5 ml of 0.2 M NaOH to each sample tube, followed by addition of 1 ml of 2 x 10^{-4}M Congo red. The tubes were vortexed to mix the reaction mixture was left at room temperature for more than 20 min before absorbance was read at 540 nm in UV spectrometer. The R^2 value was 0.997which showed the precision and accuracy of the standard plotted.

RESULTS AND DISCUSSION

The aloe gel (S1, C1 and C2) so prepared was evaluated for parameters like organoleptic, physical, and chemical parameters, which included determination of *pH*, total solids, methanol precipitable solids (MPS), refractive index, weight per ml, qualitative and quantitative estimation of polysaccharides, carbohydrates, amino acids and preservatives. The results are summarized in TABLE 1.

TABLE 1 : Oraganoleptic Evaluation of Aloe Gel obtained by Various Extraction Methods

Extract	Color	Taste	Odor	pH at 30°C	Total Solids (%)	MPS (%w/v)	Weight per ml (g/ml)	Refractive index at 30°C
C1	Colorless	Slightly Sour	Odorless	4.05	0.641	0.582	1.0111	1.3425
C2	Colorless	Sweet & sour	Odorless	4.14	1.1580	1.131	1.0072	1.3420
S1	Colorless	Slightly sour	Odorless	4.08	1.1641	1.128	1.0083	1.3420

The standardization of aloe juice by determination of polysaccharides content was done. The major polysaccharide acemannan present in the aloe gel was estimated selectively by Congo red method. A comparison of the major polysaccharide acemannan present in aloe gel extracted and purified by different methods, showed variation in percent content of acemannan. The content obtained by the pectolytic enzyme addition method was 1.1410 % while the extract obtained by heat treatment was 0.6315 %. The standard sample showed 1.1545 % of polysaccharide content. The comparison is shown in the Figure 2.

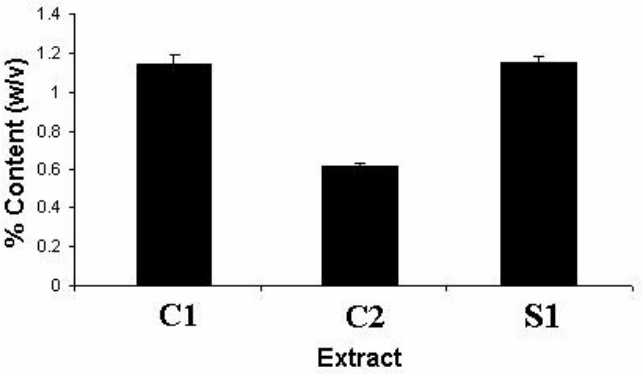

Figure 2 : Polysaccharide Content in Aloe Gel Obtained by Various Methods

The study also reveals that filtration with activated charcoal and Hyflo supercel[R], gave clear appearance to the gel. Gel was analyzed by colorimetric method for estimation of polysaccharide using Congo red as complexing agent. The addition of pectolytic enzyme 'Pectinase' yields *Aloe vera* gel with 45% more polysaccharide content than gel obtained by simple heat treatment.

CONCLUSION

The aloe gel obtained by different methods showed the presence of the major polysaccharide acemannan in all the extracts. The pectolytic enzyme addition can be used widely as it gives45% more polysaccharide content than gel obtained by simple heat treatment. The standardized methods of extraction, purification and isolation methods reported can be successfully used in nutraceutical and functional food products industry.

REFERENCES

[1] D.Grindlay, T.Reynolds; J.Ethnopharmacol., **16**, 117 **(1986)**.
[2] T.Reynolds, A.C.Dweck; J.Ethnopharmacol., **68**, 3 **(1999)**.
[3] A.Bozzi, C.Perrin; Food Chemistry, **103,** 22 **(2007)**.
[4] O.R.Casian, M.Parvu, L.Vlase, M.Tamas; Fitoterapia, **78**, 219 **(2007)**.
[5] B.Vfizquez, G.Avila, D.Segura, L.B.Escalante, J.Ethnopharmacol., **55**, 69 **(1996)**.
[6] B.K.Chandan, A.K.Saxena, S.Shukla, N.Sharma, D.K.Gupta, K.A.Suri, J.Suri, M.Bhadauria, B.Singh; J.Ethnopharmacol., **111**, 560 **(2007)**.
[7] N.R.E.Alxis, H.M.Bill; United States Patent 20060134238, **(1996)**.
[8] H.Qian, C.Liu, K.Eshun, T.Zhang; Food Control, **16**, 95 **(2000)**.
[9] R.P.Felly, Y.T.Wang, T.A.Waller; SOFW Journal, **119**, 255 **(1993)**.
[10] Indian Pharmacopoeia, Government of India **II**, pg. 465, A-125, A-130, A-135, A-137, Ministry of Health and family welfare, Controller of Publication, **(1985)**.
[11] S.Ranganna; Handbook of Analysis and Quality Control for Fruit and Vegetable Products, pg. 874, Tata McGraw Hill Publishing House, **(1992)**.