Research Article

Obtaining of hydroalcoholic extract of *Aloe vera* using fresh plant material Scaling process

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Abstract

Objective: *Aloe vera* (Liliaceae family) is a widely used plant for its medicinal properties. The objective of this work was to obtain hydroalcoholic extract using fresh plant material and its subsequent industrial scale. **Material and methods**: An obtaining methodology was designed for laboratory scale, being studied for different variants. The best variant was elaborated to bank, pilot and industrial scale. Stability during 24 months was studied. **Results:** The results were compared with obtained extracts by means of the traditional procedure**.** A technological process for the obtaining of hydroalcoholic extract of *Aloe* using fresh plant material was developed. The technological process was introduced satisfactorily at industrial level. **Conclusion:** The process is homogeneous and reproducible to the studied scale up.

Key word: *Aloe vera*, hydroalcoholic extraction, stability, scale up

Introduction

Aloe vera (Liliaceae family) is a widely used plant for its medicinal properties. Native to the Mediterranean region, particularly from North Africa, it was introduced to America where it is grown abundantly in the Caribbean Basin (Caceres, 1996). Its chemical composition in general is characterized by the presence of phenolic constituents (chromones and anthraquinones), polysaccharides, glycoproteins and amino acids present in greater proportion in the colorless and tasteless gel of the parenchymal cells of the leaf of this plant (Rivero et al., 2002; Yagi et al., 1997; Okamura et al., 1996; Larionova et al., 1989; Fajardo et al., 1988; Esteves, 1972). Gel has been the main component in the development of many medicines and cosmetics internationally. The biological activity of this gel is attributed, fundamentally, to the polysaccharides, present in the same (It represents approximately 20% of the total solids in the leaves of this plant). Polysaccharides are associated with a group of proteins, being responsible for the anti-inflammatory, antiviral and immunomodulatory activities attributed to this plant (Garcia et al., 2001; Rodriguez et al., 2000).

In Cuba, the industrial process of obtaining the hydroalcoholic extract is performed by maceration of dry Aloe drug in 50% hydroalcoholic solution. This process has yields of approximately 1000 L extract per 50 kg of dry plant material and lasts 48 hours. The extract is used as a pharmaceutical active ingredient in the manufacture of Aloe Syrup, a drug industrially manufactured for use in the treatment of catarrh and it is especially helpful for who suffers from chronic bronchitis due to its pharmacological action as a mucolytic (Portilla et al., 2012; Rodriguez et al., 2004; Takao, 1985).

The main limitations of this technology are the drying process of the plant material and the maceration time. The drying process takes about 15 days, due to the high moisture content of this material (greater than 95%), which means that a high amount of it is required. On the other hand, the maceration process lasts 48 hours. The objective of this work was to obtain hydroalcoholic extract using fresh plant material and its subsequent industrial scale.

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Materials and methods

Plant collection

The plant materials were collected in the Experimental Station of Medicinal Plants Dr Juan Tomas Roig, Artemisa province, Cuba. Its quality parameters were evaluated by the NRSP standard 309 (NRSP, 1992). Collected material was washed with abundant water, disinfected with 2% sodium hypochlorite solution and stored at a temperature between 5 and 8 ° C until the moment of its use. Before use, the plant material was milled, collecting the liquid and the ground mass into a tared container. A sample of the species (ROIG 4591) was deposited in the herbarium of the experimental station.

Preparation of hydroalcoholic extract at laboratory scale

Calculations were made to ensure that the hydroalcoholic concentration of the extract was similar to the hydroalcoholic concentration of the extract traditionally made by the industry. The amount of water presented by the fresh material was considered and two extraction variants were elaborated. A batch prepared by the traditional method was used as a control to compare the results. The variables evaluated are shown in table 1.

Table 1. Variants studied at laboratory scale

Parameters	A	B	Control
Extraction method	Maceration	Maceration	Maceration
Extraction time (h)	24	24	24
Agitation	No	No	No
Fresh Aloe: Ethanol ratio (w/v)	1:1	1:1.1	–
Dry Aloe: Ethanol ratio (w/v)	1:37.5	1:41	1:12.6

On the best study variant the influence of agitation and temperature was evaluated. Two methodologies were studied. The first was performed at room temperature and agitation, while in the second variant temperature (100°C, reflux) and stirring was applied. Six experiments of each variant were carried out with the following reaction times: 30, 60, 90, 120, 180 and 240 min. The results were compared with the analytical results of the control sample.

In this scale a glass reactor of 1 liter capacity equipped with a stirrer marine propeller and a heating system were used. Filtration was performed under vacuum at constant pressure (9.99×10^4 kg/ $m.s^2$), using a Buchner porcelain funnel with a filtration area of 1.06×10^{-2} m^2 and cotton canvas filter as filter medium (cotton XX, 2 mm) (Filtronic, Brazil).

Preparation of hydroalcoholic extract at bench scale

With the best technological variant three batches of 5 L were prepared. In this scale a glass reactor of 10 liter capacity equipped with a stirrer marine propeller and a heating system was used. Filtration was performed under vacuum at constant pressure (9.99×10^4 kg/$m.s^2$), using a Buchner porcelain funnel with a filtration area of 1.06×10^{-2} m^2 and cotton canvas filter as filter medium (cotton XX, 2 mm) (Filtronic, Brazil).

Preparation of hydroalcoholic extract on a pilot scale

Three batch of 100 L were prepared. A 250 L stainless steel reactor (stirred tank type), equipped with a marine propellant stirrer and a heating and extraction system was used. Filtration was performed through a vertical vacuum filter of 200 L of capacity (Sparkle, Italia) with a filtration area of 0.2642 m^2 and cotton canvas filter as filter medium (cotton XX, 2 mm) (Filtronic, Brazil).

Preparation of hydroalcoholic extract on an industrial scale

Three batches of 300 L were prepared. A 500 L stainless steel reactor (stirred tank type), equipped with a marine propellant stirrer and a heating and extraction system was used. Filtration was performed through a vertical vacuum filter of 200 L of capacity (Sparkle, Italia) with a filtration area of 0.2642 m^2 and cotton canvas filter as filter medium (cotton XX, 2 mm) (Filtronic, Brazil).

Assay methods

Chemical and microbiological analysis of all extracts obtained in each scales studied were performed according to the established procedure for quality control in the industry. Analysis procedures were validated according to current requirements (USP, 2008; Mendez, 2001).

Stability study

Stability study of batches of extracts obtained on an industrial scale was conducted. Shelf life stability method was applied. Evaluations at 0, 12 and 24 months were performed in accordance with established regulations (CECMED, 2000).

Statistical analysis

The batch comparison was performed using analysis of variance (ANOVA). The results were considered significant at $p < 0.05$.

Results

Table 2 shows the results obtained from the variants studied. Both variants comply with the established quality parameters, but the total solids contents were lower than the total solids content of the control simple (approximately 44%). On the other hand, the alcohol content in variant B was higher than the content of variant A and similar to the

control. While yields of both variants were less than 70%.

The results were analytically satisfactory, but technologically to increase the extraction of the components of interest and to reduce the extraction time a more dynamic process was necessary. It was decided to continue the studies with the B variant.

Table 2. Results of the study of laboratory-scale variants

Parameters	A	B	Control
Rendimiento de Filtrado (%)	65.2	66.5	83.3
pH	6.0	6.2	5.9
Total solids	0.40	0.36	0.83
Alcohol content	40	50	50
Relative density	0.945	0.928	0.930
Polysaccharides	0.15	0.32	0.35

Note: pH (Between 5.2 and 6.2); Total solids (Between 0.3 and 1.0%); Alcohol content (Between 38 and 52%); Relative density (Between 5.2 and 6.2 g/mL) and Polysaccharides (Between 0.10 and 0.50 g/100 mL). The values are the mean of three replicates.

Figure 1 shows the behavioral results of the total solids content, total polysaccharides and alcohol content for the two variants studied. The contents of total solids were lower in the variant in which heat was applied. Similarly in this variant the alcohol content of the sample decreased over time. This situation may be due to losses occurring during the reflow process. Yields were similar in both variants (between 75 and 80%) suitable for scale studied. However, although differences were observed among the processes, the extracts complied with the quality parameters established for this product.

These results allowed the establishment of an extraction process with the following parameters: Ambient temperature and extraction time of 90 minutes.

Table 3 shows analytical results of batches prepared in bench, pilot and industrial scale. The quality of the same is adequate according to the method of quality control of this product. Technologically the yields were higher than 75%, 90% and 94% for bench, pilot and industrial scale, respectively. In all cases, a slightly opalescent liquid extract amber reddish and characteristic odor was obtained. Statistical analysis of ANOVA showed no significant differences in total polyphenol content between batches in the scales studied ($p = 0.1277$, $p = 0.1005$ and $p = 0.1375$ for bench, pilot and industrial scale respectively) for a level of 0.05. These results indicate that the batches produced are reproducible and homogeneous at any scales.

In all cases the batches elaborated complied with the microbiological parameters according to the USP (Bacterial

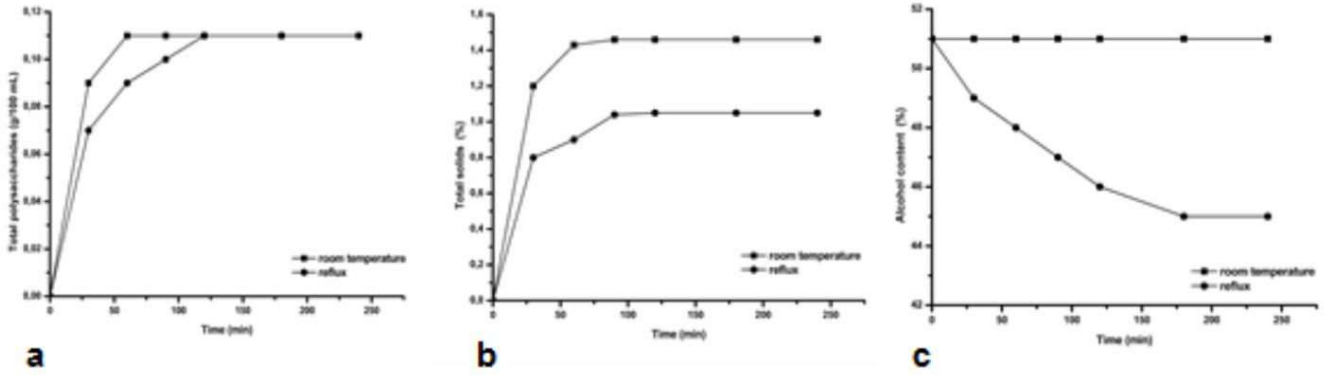

Figure 1. Behavior of total polysaccharides (a), total solids (b) and alcoholic content (c) in the studied variants

Table 3. Analytical results of lots obtained at bench, pilot and industrial scale

Batch type	Bench			Pilot			Industrial		
Batch	0601	0602	0701	1001	1101	1102	2001	2002	2003
pH	5.4	5.8	5.3	5.4	5.8	5.3	5.4	5.2	5.2
Total solids	0.8	0.6	0.5	0.5	0.7	0.6	0.6	0.4	0.5
Alcohol content	51.8	49.2	46.9	51.8	49.2	46.9	45.0	43.0	45.0
Relative density	0.933	0.931	0.928	0.948	0.945	0.941	0.939	0.941	0.936
Polysaccharides	0.34	0.36	0.34	0.44	0.32	0.41	0.25	0.25	0.38

Note: pH (Between 5.2 and 6.2); Total solids (Between 0.3 and 1.0%); Alcohol content (Between 38 and 52%); Relative density (Between 5.2 and 6.2 g/mL) and Polysaccharides (Between 0.10 and 0.50 g/100 mL). The values are the mean of three replicates.

Table 4. Analytical results of the stability study of extracts on an industrial scale

Time	12 months			24 months		
Batch	**2001**	**2002**	**2003**	**2001**	**2002**	**2003**
pH	5.4	5.1	5.1	5.3	5.2	5.1
Alcohol content	40.3	41.4	43.7	39.6	39.9	40.3
Polysaccharides	0.23	0.22	0.36	0.21	0.20	0.32

Note: pH (Between 5.2 and 6.2); Alcohol content (Between 38 and 52%); and Polysaccharides (Between 0.10 and 0.50 g/100 mL). The values are the mean of three replicates.

count < 104 UFC/g; Fungi count < 103 UFC/g and Absence of pathogens) (USP, 2008).

Table 4 shows results of the stability study performed by shelf life to industrial scale batches obtained. It is demonstrated that hydroalcoholic extracts obtained by this proposed technology maintain their chemical and microbiological characteristics 24 months.

Discussion

The results obtained in this work show that the technological process proposed is scalable, demonstrating the reproducibility and homogeneity of the batches obtained in each scale studied. The quality of the batches obtained is good, complying with the established quality control methodologies for this product. On the other hand, they maintain their quality parameters for 24 months which is considered suitable for an extract of natural origin.

Most important of this methodology was the significant savings of time and costs. The Cuban industry produces Aloe hydroalcoholic extract by maceration. This process takes 48 hours and uses dry plant material for extraction.

Aloe leaves have a high water content in their constitution (greater than 95%). The drying of this material takes approximately 15 days according to the established technological process, which causes that the costs of the productive processes are high.

The main advantages of the technology proposed in this study is that it only requires 33% of the vegetal material used in the maceration process and a time of 90 minutes for the elaboration of 1000 L of extract.

This process does not generate wastes that affect the environment, since the vegetal residue, after the extraction process, can be used as organic material in the plant's own cultivation process, which makes the system more ecological and friendly with the medium, which guarantees cleaner and more rational productions.

Conclusion

The results of this study showed that the proposed technology is scalable in the working conditions of the Cuban industry, obtaining yields higher than 90% and extracts that meet the established quality and stability parameters. It is corroborated that the procedure is reproducible and homogeneous. It is an eco-friendly process.

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