Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 2 Issue 8 August 2018

Research Article

Quality Control Study in Various Nutraceutical Aloe vera Formulations

Sushruta Mulay* and Sunita Ogale

VIVA Institute of Pharmacy, Maharashtra, India

***Corresponding Author:** Sushruta Mulay, VIVA Institute of Pharmacy, Maharashtra, India.

Received: May 24, 2018; **Published:** July 02, 2018

Abstract

Many *aloe vera* formulations as nutraceutical supplements are available in Indian market. However they differ in the quality control parameters. Hence it is of prime importance in order to get uniformity in the products. The lack of available information on the quality and composition of *aloe vera* has been a major obstacle to scientific studies. In present research we tried to establish quality control parameters of formulations in market and compared for their therapeutic and safe use as per IASC (International Aloe science Council).

Keywords: Acemannan; Anthraquinones; Maltodextrins.

Introduction

Standardization of herbal formulations is an important step of a consistent biological activity establishment, chemical profiling or simply a quality assurance programme [1]. WHO specifies guidelines for the assessment of safety, efficacy and quality of herbal medicines as a prerequisite for global harmonization.

International Aloe Science Council (IASC) has developed a set of identity, purity and quality standards for *aloe vera* juice products. Products certified by IASC are specifically prepared in a manner that limits the total amount of anthraquinones in raw materials and finished products, ensures presence of polysaccharides at or above a minimum level and includes assays to ensure the absence of specific adulterants.

Materials and Methods

STD barbaloin was a benevolent gift from ICT Mumbai, Reagents used were of IP grade.

Seven marketed products of *aloe vera* were labelled as A1, A2, A3, A4, A5 A6 and A7. A comparative study was performed on organoleptic characters, physical nature, chemical profiling and presence of adulterants if any in the product as per IASC guidelines.

Experimental

Compound	Certification Requirement
Acetylated mannan	≥ 5% by dry weight
Glucose	Present
Aloin	≤ 10 ppm
Maltodextrins	Must be listed on label and analysis must meet label claims
Solids	≥ 1.0%

Table 1: IASC Certification Requirement for *Aloe Vera* Inner Leaf Juice [2].

Organoleptic Parameters	Certification Requirement
Colour	Colourless to caramel coloured
Taste	Tasteless to slightly bitter
Aroma	Odourless to mildly vegetative

Table 2: Organoleptic Characterization as per IASC.

Note: The organoleptic characteristics of products of aloe vera leaf juice can vary considerably depending upon the processing techniques and additives used.

Formulation	Composition	Dosage	Indications
A1	*Aloe vera* Extract 99%	30 ml twice a day	Multipurpose
A2	*Aloe vera* Fibrous Juice	20 - 30 ml dilute with equal volume of water	Dietary supplement
A3	*Aloe vera* Juice	30 ml once a day	Useful in intestinal problems, Constipation and gastric disorders
A4	*Aloe vera* Juice	15 - 25 ml twice a day	Dietary supplement
A5	98% Pure *Aloe vera* Juice	30 ml once a day	Nutritional storehouse, antioxidant and immune booster
A6	*Aloe vera* Extract 100%	5 caps	Cures internally intestinal problems
A7	98% Pure *Aloe vera* Juice	30 ml once a day	Dietary supplement

Table 3: Label Claims of Marketed Formulations.

Organoleptic Evaluation

As *aloe vera* health drink being a beverage consumed regularly, appealing colour, taste and odour form a very important part of the finished product. Added ingredients may serve to modify the original taste of product. It helps to predict the quality and stability by understanding the changes in organoleptic characteristics such as appearance, colour and odour/flavour and taste of formulations. For this purpose six volunteers were selected and judgement of each volunteer on colour, taste odour and flavour was recorded.

Sample	Colour	General Appearance	Taste	Odour/ flavour
A1	Off white	Clear and viscous	Obnoxious	Obnoxious
A2	Pale yellow	Thick and fibrous	Sour and pungent	Pleasant
A3	Dull yellow	Thick and fibrous	Sour and astringent	Pleasant
A4	Faintly yellow	Clear thin	Pungent, slightly bitter acceptable	Pleasant
A5	Dull yellow	Thick and fibrous	Acceptable, Slightly sour	Very pleasant
A6	Faintly yellow	Clear, dilute thin consistency	Oily, slightly sour taste ,watery	Oily
A7	Pale yellow	Clear, dilute thin consistency	Acceptable, Slightly sour	Pleasant

Table 4: Organoleptic Evaluation of Marketed Formulations.

Evaluation of Anthraquinones [3]

Aloe latex-a bitter yellow exudate from the pericyclic tubules in the outer skin of the leaves may contaminate inner gel of *aloe vera* during extraction process. These are polyphenolic compounds-anthraquinones may impart a bitter taste and orange/reddish brown colour to the product. Being laxative in nature, it helps to cure digestion problems. However health drink being taken regularly, there are consequences of a laxative abuse106. Concerns regarding these compounds are potential carcinogens have recently been raised and restrictions are placed on *aloe vera* products in some countries. Products certified by IASC are specifically prepared in a manner that limits the total amount of anthraquinones in a single strength raw materials and finished products to 10 ppm or less (Table 1).

Identification test for anthraquinones- Modified Brontragers test

Principle: Modified Brontrager's test is used for identification of C anthraquinone glycosides. In this test anthraquinone glycosides are treated with ferric chloride solution in presence of hydrochloric acid to release hydrophobic aglycone which is collected in organic solvent. Liberated C glycosides are detected by alkalinising with ammonia solution.

Procedure

5 ml of juice was treated with 2 ml of ferric chloride solution and 2 ml of dilute hydrochloric acid. It was heated for 5 - 10 minutes. Equal volume of carbon tetrachloride was added in a test tube. The organic layer was separated and shaken with liquor ammonia. The ammoniacal layer was rose pink to cherry in red colour indicating presence of C glycoside in the extracted juice.

Thin Layer Chromatography (TLC) of Barbaloin: The presence of anthraquinones in aloe vera juice was confirmed by TLC. The mobile phase used was ethyl acetate:methanol:water (100:13.5:10). Std Barbaloin was dissolved in methanol to get concentration of 1 µg/ml. A spot of juice was applied on TLC plate. Detection was done by spraying with 5% alcoholic potassium hydroxide solution. Presence of anthraquinone is indicated by fluorescent spot.

Quantification of Anthraquinones [4]

This assay is based on principle of modified Brontrager test in which barbaloin is quantified UV spectrophotometrically.

Procedure

10 ml of juice was transferred to a flask containing 1 ml of 60% w/v solution of ferric chloride hexa hydrate and 6ml of hydrochloric acid. This was heated in a water bath under a reflux condenser for 4 hrs so that the water level was always above that of the liquid in the flask. The solution was transferred after cooling to a separating funnel. The flask was rinsed successively with 4 ml of water and the rinsings were added to the contents of the separating funnel. This was extracted with three quantities each of 20ml carbon tetra chloride. The carbon tetrachloride layers were combined and washed with two quantities each of 100ml of water. The washings were discarded. The organic phase was diluted to 100ml with carbon tetrachloride. 20 ml of the carbon tetrachloride extract was carefully evaporated to dryness on a water bath and the residue was dissolved in 10 ml of 1M NaOH. The absorbance of the resulting solution was measured at 500 nm.

Anthraquinones content in the juice was measured as-
Absorbance = A (1%1 cm) x b x c
Where
A: STD absorbance
b: Path length
c: Concentration of substance

Formulation	Modified Brontragers test	Anthraquinone Content in ppm
A1	Positive	10.2
A2	Positive	11.0
A3	Positive	10.5
A4	Positive	13.0
A5	Positive	12.0
A6	Positive	19.0
A7	Positive	10.5

Table 5: Evaluation of Anthraquinones in Marketed Formulations.

Evaluation of polysaccharide

A Evaluation of Bioactive Polysaccharide-Acemannan [5]

In the production of aloe vera leaf juice special care must be taken to preserve the content of acetylated polysaccharides which can readily degrade due to prolonged storage bacterial fermentation and elevated temperature. The quality of commercial aloe vera juice is strongly dependent on processing and storage conditions. Enzymatic and thermal degradation and bacterial fermentation may affect the quality and decrease the value of final product. IASC ensures presence of acetylated polysaccharides at or above a minimum level (≥ 5% dry weight) (Table 7).

A review of a limited number of commercial *aloe vera* leaf products suggest that polysaccharide concentrations vary widely with some products that have undergone prolonged pasteurization and/or uncontrolled enzymatic treatment contain as low as 1% dry weight of acetylated polysaccharides.

Procedure

Evaluation of Bioactive Polysaccharide present in *aloe vera* juice.

Principle

This assay is based upon qualitative and quantitative determination of bioactive polysaccharide-acemannan present in inner leaf juice of *aloe vera*. The complexing agent used is Congo red which reacts with bioactive polysaccharide, acemannan to give colour change. Congo red is the sodium salt of 3, 3'-([1, 1'-biphenyl]-4, 4'-diyl) bis (4-aminonaphthalene-1-sulfonic acid). Congo red is also used in quantification of cellulase and amyloids (non-uniform proteins) in tissue. Size Exclusion Chromatography (SEC) can be used to detect acemannan, however it is difficult to regulate commercial products containing aloe vera by this method. Light absorption maximum of Congo red in 1% w/v aqueous solution is approximately 488 nm wavelength. This absorption maximum of Congo red is shifted towards a longer wavelength in presence of a large molecular weight polysaccharide acemannan. The complex formation of this polysaccharide is due to ordered linear confirmation of acemannan.

Procedure-Preparation of *Aloe vera* Reference Standard by freeze drying method

About 150 ml of *aloe vera* juice was frozen at -20^{0}C to -50^{0}C in a suitable container. The frozen sample container was covered with filter paper and transferred into the lyophilizer- Virtis 6K (from Spinco Biotech Pvt Ltd) and freeze-dried at -90^{0}C under vacuum (100-160 mTorr). Approximate time taken for 400ml sample to be made into powder depends on total solid content. Time required for lyophilisation was about 12 - 18 hrs. The solids obtained were 0.9g.

Assay Procedure

90 mg of the reference standard made up of freeze dried *aloe vera* gel was accurately weighed The solid was dissolved in 9 ml of distilled water to give concentration 1%w/v. This aliquot (1% solution) was filtered through whatman filter paper No. 40 and subsequently diluted with distilled water to get concentration levels of 0.1, 0.2, 0.3, o.4, 0.5, 0.6, 0.7, 0.8 and 0.9%. 4 ml of juice was transferred into a vial then added successively 5 ml of 0.2M NaOH was added followed by 1 ml of 2 X 10^{-4}M congo red solution for a total of 10ml solution. The test tube was vortexed to mix and the colour was developed immediately. The reaction mixture was allowed to stand at room temperature for 20 minutes. Absorbance was read at 540 nm wavelength using UV visible spectrophotometer (Make-Systronic).

Percent Concentration	Mean Absorbance
0.1	0.111
0.2	0.158
0.3	0.200
0.4	0.260
0.5	0.344
0.6	0.350
0.7	0.448
0.8	0.554
0.9	0.664
1.0	0.701

Table 6: Absorbance at various concentrations of acemannan.

Figure 1: Standard curve of acemannan (absorbance Vs % concentration of acemannan).

The *aloe vera* bioactive polysaccharide concentration of an aloe beverage was directly estimated from standard calibration plot. Each bottle containing *aloe vera* beverage was shaken vigorously before an aliquot sample was taken for analysis. The aliquot was filtered through Whatman filter paper No. 40. Each product was analysed in triplicate and the mean was used to determine the percentage aloe vera gel bioactive polysaccharide.

Formulation	Mean Absorbance	% Acemannan Content
A1	0.104	15.34
A2	0.034	04.34
A3	0.249	36.12
A4	0.279	40.45
A5	0.125	17.89
A6	0.029	04.12
A7	0.125	17.89

Table 7: Evaluation of bioactive polysaccharide in marketed formulations.

Interference Study of Other Polysaccharide in Marketed Formulations [6]

Other polysaccharides are commonly used to improve organoleptic characters, physical nature as well as stability of the products. Colorimetric assay of various polysaccharides using congo red was performed. The measured absorbance of different dilutions of *aloe vera* gel polysaccharide was compared with other polysaccharides.

Procedure

Aloe vera gel solid and each compound (Non-Aloe Polysaccharide) were weighed separately and then dissolved in deionized water to a concentration of 1% w/v solution. Different dilutions of aloe vera gel polysaccharide as well as other polysaccharides were prepared and compared to other polysaccharides which are commonly used to adulterate aloe vera products. Absorbance of aloe gel was compared to non-aloe polysaccharides.

Sample	Mean Absorbance			
	0.125%	0.25%	0.5%	1.0%
Aloe vera gel	0.148	0.178	0.344	0.701
Maltodextrin	0.003	0.004	0.005	0.006
Locust bean gum	0.004	0.005	0.006	0.007
Gum acasia	0.004	0.005	0.006	0.007
HPMC	0.002	0.003	0.004	0.007
Gellan gum	0.005	0.006	0.007	0.008
Xanthan gum	0.005	0.006	0.007	0.007
Guar gum	0.003	0.004	0.005	0.007
Liquid glucose	0.004	0.005	0.006	0.007
Pectin	0.002	0.003	0.004	0.005
Sodium CMC	0.003	0.004	0.005	0.005

Table 8: Interference Study of Non-Aloe Polysaccharides.

Figure 2: Interference study of non-aloe polysaccharides.

Presence of Maltodextrin in Formulations [7]

Adulteration represents a major concern for the *aloe vera* market mostly because of the high cost of raw materials. Historically the most common substance used to adulterate the *aloe vera* products is maltodextrin. The manufacturers try to reform the aloe texture by adding non-aloe polysaccharide such as maltodextrin. It is used artificially to enhance polysaccharide values. Dextran, plant gums, dextrose, glycerine have also been occasionally used in the products. This colorimetric test is used as an initial screening tool for identifying adulterated juice. The assay modified from a commonly known starch detection method. The test is performed with a control that is free of maltodextrin.

Procedure

In 250 ml volumetric flask, 10 g of potassium iodide was dissolved in 25 ml of water. 3.175 g of iodine (I2) was added and was diluted to make the final volume, 250 ml with distilled water. 10 ml of the liquid sample being tested was added in empty vial. 2 Drops of iodine solution were added to the sample being tested. Vials were capped inverted once or swirled. *Aloe vera* juice was used as a control.

Formulation	Colour Change	Maltodextrin
A1	-	Absent
A2	Slightly Brown	Present
A3	-	Absent
A4	-	Absent
A5	-	Absent
A6	Slightly Brown	Present
A7		

Table 9: Detection of maltodextrin.

Determination of Total Solids: Total solids are applied to the residue obtained after the prescribed amount of preparation is dried to constant weight. It is indication of aloe solids..

Procedure: 10 ml of accurately measured sample was placed in a tared dish and contents were evaporated in a steam bath until the residue was apparently dry. The residue was further heated on a steam bath to dry it to constant weight. The dish was cooled in a desiccator and weighed again.

Formulation	Total solids content
A1	4.44
A2	7.40
A3	1.36
A4	1.57
A5	5.15
A6	8.70
A7	2.39

Table 10: Determination of total solids.

Determination of Total Carbohydrates [8]

Total carbohydrates content (in terms of glucose) was determined by phenol sulphuric assay method. The active polysaccharides were precipitated with ethyl alcohol to get rid of alcohol soluble complexes of organic acids and others. The precipitated polysaccharide was measured by Dubois assay. In acidic medium glucose present in the sample is dehydrated to hydroxymethyl furfural. This forms a yellowish orange coloured product with phenol and has a maximum absorption at 490 nm.

Preparation of Standard Curve

Standard curve solution 7 mg/ml stock was prepared by using 700 mg of glucose. Solution was further diluted with distilled water to give a concentration of 700 µg/ml. In six volumetric flasks 1, 2, 4, 6, 8 and 10 ml of 700 µg/ml stock solution was taken volume was made to 10ml with distilled water. 1ml of each so-

lution was added to six different test tubes. To it 1 ml of 5% phenol solution was added and mixed well on a vortex mixer. A blank was also run with water. After 20 minutes colour was read at 490 nm. Standard curve was then plotted for absorbance vs. concentration.

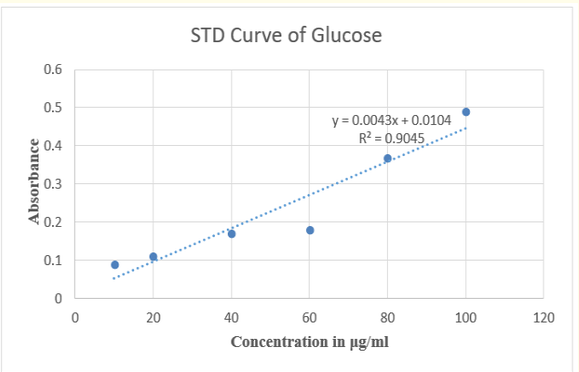

Figure 3: Concentration Vs absorbance.

Formulation	Absorbance	Total Carbohydrates Content g%
A1	0.03	4.56
A2	0.19	42.21
A3	0.02	2.23
A4	0.02	2.23
A5	0.04	6.88
A6	0.21	46.77
A7	0.03	4.58

Table 11: Total carbohydrates content in formulations.

Determination of pH and Titrable Acidity

Determination of pH

The pH value of a solution was determined potentiometrically by means of a glass electrode. A reference electrode and a digital pH meter. pH meter was operated according to manufacturer's instructions. Apparatus was calibrated by using buffers pH 4.0, 7.0 and 9.2. 50 ml of sample was taken in a beaker. The electrodes were immersed in the solution and pH was measured.

Determination of Titrable Acidity

Titrable acidity is indicative of acids present in formulations.

Procedure: 10 ml of sample was mixed thoroughly by shaking and filter through filter paper. Titrated 10 ml of juice sample with 0.1M NaOH using a few drops of 1% phenolphthalein solution as indicator. The titre value was noted. Calculated the result as % anhydrous citric acid.

$$\%\text{Total Acid} = \frac{\text{Titre X Normality of alkali X Equivalent Weight of Acid X 100}}{\text{Volume of sample taken X 1000}}$$

Name of sample	pH	Titrable Acidity
A1	4.89	0.217
A2	4.83	0.322
A3	4.54	0.329
A4	5.35	0.154
A5	4.92	0.273
A6	5.15	0.147
A7	4.91	0.219

Table 12: pH and titrable acidity of formulations.

Determination of Viscosity

Viscosity was determined using Brookfield Viscometer LV-DE Spindle No 62.

Formulation	Viscosity in Centipoise
A1	574
A2	942
A3	676
A4	48
A5	1062
A6	54
A7	876

Table 13: Viscosity of marketed formulations.

NMR Study of formulation M2 [9]

This 1H quantitative NMR (Q NMR) can be used for the direct detection and quantitation of the primary components of interest in aloe vera juice products for compliance with International Aloe Science Council (IASC) certification requirements. Degradation products e.g. lactic acid, succinic acid, fumaric acid, acetic acid, formic acid, ethanol and other atypical adulterants. Fermented aloe vera products and raw materials may also contain high levels of lactic acid and acetic acid due to malolactic bacterial fermentation, hydrolysis or thermal degradation of the material during production and/or storage. To identify degradation products 1H NMR analysis was performed on freeze dried sample 2.0 mg, 5 mm diameter tube, using D2O water at frequency 500.13 MHz on Bruker AV NMR spectrometer.

Figure 4: NMR study of product A2.

Degradation Product	Lactic Acid	Ethanol	Acetic Acid	Pyruvic Acid	Succinic Acid
Chemical shift (ppm)	1.33	1.15	1.96	2.35	2.6

Table 14: Degradation products study.

Acemannan Peak: 2.2 ppm

Results and Discussion

Physical Evaluation

Colour of the formulations was found to be varying from pale yellow to dull yellow. This may be due to oxidation of tetrahydroxyanthraquinone to red compound with subsequent condensation to brown/black polyphenolic material, caramelization of glucose and polysaccharide by heat during processing. The taste of formulations A3, A4 and A5 was very sour due to presence of high acidity. Preparation A5 was very pleasant with slightly sour but acceptable taste. A1 had extremely obnoxious odour A2, A3, A4 and A7 had a pleasant odour. Formulation A6 had oily odour. Overall all the formulations had fermentable odour. Formulations A2, A3 and A5 were fibrous with chunks appeared due to insufficient homogenization (Table 4).

Anthraquinones Content

Qualitative test Modified Brontrager's test was positive in all the samples. On further quantitative analysis by UV spectrophotometry in all the formulations Anthraquinone level was higher than the limit prescribed by IASC. In formulations A1 to A5 it was slightly higher than 10 ppm whereas in formulation A6 it was 19 ppm indicating that the adequate care was not taken to remove anthraquinones from the juice (Table 5).

Bioactive Polysaccharide Content

Authentic *aloe vera* gel polysaccharide in a product was determined even at a very low concentration and in presence of large amounts of non-aloe polysaccharides and monosaccharides which may be used to adulterate an *aloe vera* containing product. The colorimetric assay was sensitive to specific polysaccharides found in *aloe vera* while remaining were virtually non-responsive to other non-aloe polysaccharides (Table 8, Figure 2). In the products A2 and A6 acemannan content was lower than the limit prescribed by IASC (Table 6 and 7, Figure 1).

Maltodextrin Analysis

Qualitative iodine test prescribed in *Aloe vera* monograph-American Herbal Pharmacopoeia was performed. For formulations A1, A3, A5 and A6 there was no colour change. Formulations M2 and M6 had shown a slightly brown colouration indicating presence of maltodextrin in the samples (Table 9).

Total Solids

Total solids of juices indicate the total amount of aloe solids present in the formulations. Ideally total solids of fresh *aloe vera* juice without any additives should be in the range of 0.5 to 1%w/v. However if additional substances are present in the formulation then total solid content increases. Formulation A2 and A6 had a solid content 7.40 and 8.71 indicating presence of several additives apart from aloe solids (Table 10).

Total Carbohydrates Content

Total carbohydrates content was found to be higher in formulations M2 and M6. This indicates addition of maltodextrin in the sample (Table 11, Figure 3).

pH and Titrable Acidity

Juices contain food acids like citric acid, malic and tartaric acid. These are indicative of sourness of a product. Titrable acidity was higher in formulations A2 and A3 (0.322 and 0.329) as they were highly acidic (pH 4.83 and 4.54 respectively) (Table 12).

Viscosity

Viscosity of formulations was not uniform as formulations A2 and A6 were of thin consistency with viscosity 48 and 54 centipoise respectively. Formulation M5 was very thick and fibrous with highest viscosity of 1062 centipoise. Viscosity of formulation A2 was 942 centipoise was approximately equal to that of formulation A5. (Table 13).

Degradation Study

In NMR study acemannan peak was very small at 2.0 to 2.2ppm indicating very low amount of acemannan in the samples (Figure 4) Lactic acid peak was found at 1.33 ppm indicating lactobacillus fermentation of juice. Ethanol on further fermentation was converted to acetic acid indicating peaks at 1.15 ppm and 1.96 ppm respectively. Other organic acids found were pyruvic acid and succinic acid (Table 14).

Conclusion

In marketed formulation study, all the marketed formulations differed in physical as well as in chemical parameters but claimed the same dose. Acemannan content and anthraquinone levels were evaluated in these formulations by colourimetric assay method. Almost all the formulations were unpalatable, had slight fermented odour. Some products were adulterated with maltodextrin. In NMR study of the marketed formulations for some of products acemannan peak was absent.

Bibliography

1. Sojitra J., *et al*. "Standardization study of polyherbal formulations Caspa drops". *International Journal of Pharmaceutical Sciences and Drug Research* 5.3 (2013): 113-119.

2. www.IASC.COM

3. Reynolds T and Grindlay D. "The aloe vera phenomenon: a review the properties and modern uses of the leaf parenchyma gel". *Journal of Ethnopharmacology* 16.2-3 (1986): 117-151.

4. IP 1996- Monograph Aloe.

5. U.S. Patent 5512488, Colorimetric assay of bioactive polysaccharide (1956).

6. Austin A., *et al*. "Quality and authenticity of commercial aloe vera gel powders". Research Gate (2006): 1-10.

7. American Herbal Pharmacopoeia- Aloe Vera inner leaf juice (2012): 29.

8. Dubois M., *et al*. "Colourimetric method for determination of sugars and related substances". *Analytical Chemistry* 28.3 (1956): 350-356.

9. American Herbal Pharmacopoeia-Aloe Vera inner leaf juice (2012): 46.

Volume 2 Issue 7 July 2018