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Analytical study of different samples of Aloe Vera Juice

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ABSTRACT

Aloe vera juice (*Kumari Sawarasa*) is a herbal formulation used extensively in *Ayurvedic* system of Indian Medicine, as Anti-ageing, Prevent skin aging, Anti-microbial, Anti-oxidant and Healing agent. In last few years, there has been a significant increase in the number of doctors, dentists, vets, other health professionals and therapists who have come regard using of *Aloe vera* abundance of products available in market of different agencies and people are enhancing the use of the *Aloe vera* in different ways i.e., Juices, Gel in Cosmetics and Beauty products. But on the till date the parameters of active principles have been devoid of search.

Key words: *Anti-Ageing, Cosmetics uses, Indian Medicine.*

INTRODUCTION

Aloe vera juice is the colourless mucilaginous pulp obtained from the parenchymatous cells in the fresh leaves of *Aloe vera* (L) Burm. f. (Liliceae). Health is a dynamic state of complete physical, mental social and spiritual well being and not only absence of disease. Natural substances are useful to treat the diseases as well as to give strength to the body. These natural substances are present in different parts of the plant. 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. Herbal medicine has been enjoying renaissance among the customers throughout the world, however one of the impediments in the acceptance of the Ayurvedic formulations is the lack of standard quality control profile.

The quality of herbal medicine, that is the profile of the constituents in the final product has implication in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of the plant-based drugs, it is difficult to establish quality control parameters and modern analytical techniques are accepted to help in circumventing this problem. Aloe vera juice (*Kumari Swarasa*) is a herbal formulation used extensively in Ayurvedic system of Indian Medicine, as Anti-ageing, Prevent skin aging, Antimicrobial, Anti-oxidant, Healing agent.

AIMS AND OBJECTIVES

1. To standardize *Aloe vera juice* based upon chromatographic and spectral studies. Various extracts of *Aloe vera juice* have been prepared and evaluated.
2. Estimation of aloin which is reported as one of the main constituents of *Aloe.*
3. To analyze heat stability of Aloin.

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Synonyms

Aloe barbadensis Mill., *Aloe chinensis* Bak., *A. elongata* Murray, *A. indica* Royle, *A. officinalis* Forsk., *A. perfoliata* L., *A. rubescens* DC, *A. vera* L. var. littoralis König ex Bak., *A. vera* L. var. *chinensis* Berger, *A. vulgaris* Lam. Most formularies and reference books regard *Aloe barbadensis* Mill. as the correct species name, and *Aloe vera* (L.) Burm. f. as a synonym. However, according to the International Rules of Botanical Nomenclature, *Aloe vera* (L.) Burm. f. [1] is the legitimate name for this species. The genus Aloe has also been placed taxonomically in a family called Aloeaceae.

Description

Succulent, almost sessile perennial herb; leaves 30-50 cm long and 10cm broad at the base; colour pea-green (when young spotted with white); bright yellow tubular flowers 25-35 mm in length arranged in a slender loose spike; stamens frequently project beyond the perianth tube.[2]

General Appearance

The juice is a viscous, original amber nectar slightly yellow colour, transparent liquid.

Organoleptic Properties:

Viscous, colourless, odourless, taste slightly bitter.

Microscopic Characteristics: Not applicable.

Geographical Distribution

Probably native to north Africa along the upper Nile in the Sudan, and subsequently introduced and naturalized in the Mediterranean region, most of the tropics and warmer areas of the world, including Asia, the Bahamas, Central America, Mexico, the southern United States of America, south-east Asia, and the West Indies.

General Identity Tests: To be established in accordance with national requirements.

Microbiology

The test for *Salmonella* spp. in *Aloe vera* juice should be negative. Acceptable maximum limits of other microorganisms are as follows.

For Internal Use: Aloe juice used as a health tonic.

Moisture: Contains 98.5% water.

Pesticide Residues

To be established in accordance with national requirements. For guidance, see WHO guidelines on quality control methods for medicinal plants and guidelines on predicting dietary intake of pesticide residues.

Heavy Metals

Recommended lead and cadmium levels are not more than 10 and 0.3mg/kg, respectively, in the final dosage form.

Radioactive Residues

For analysis of strontium-90, iodine-131, caesium-134, caesium-137, and plutonium-239, see WHO guidelines on quality control methods for medicinal plants.

Other Tests

Chemical tests for *Aloe vera* pulp and tests for total ash, acid-insoluble ash, alcohol-soluble residue, foreign organic matter, and water-soluble extracts to be established in accordance with national requirements.

Chemical Assays

Carbohydrates (0.3%), water (98.5%) Polysaccharide composition analysis by gas-liquid chromatography.

Major Chemical Constituents

Aloe vera juice consists primarily of water and polysaccharides (pectins, hemicelluloses, glucomannan, acemannan, mucilage and mannose derivatives). Mannose 6-phosphate is a major sugar component. Aloin present in a small amount. It also contains amino acids (essential & non-essential), lipids, sterols (lupeol, campesterol, and β-sitosterol), tannins, and enzymes (Amylase, Cellulase, lipase). Different minerals have been found in *Aloe vera,* the most important are Calcium, Iron, Magnesium and Zinc.

Dosage Forms

The clear mucilaginous gel. At present no commercial preparation has been proved to be stable. Because many of the active ingredients in the gel appear to

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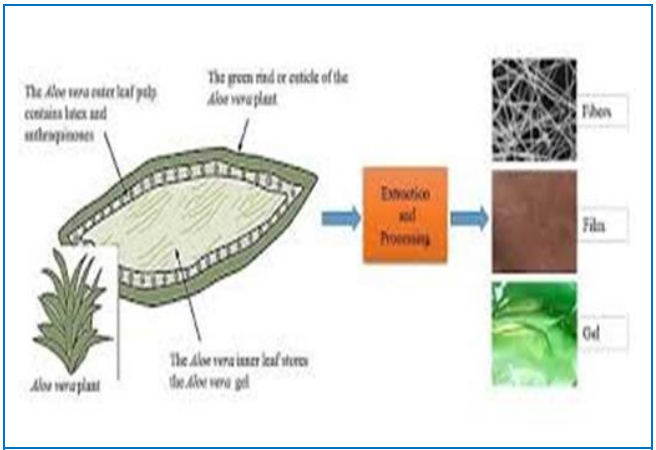
deteriorate on storage, the use of fresh gel is recommended. In Ayurvedic text there is extract mentioned as Alua / Kumari Sara.



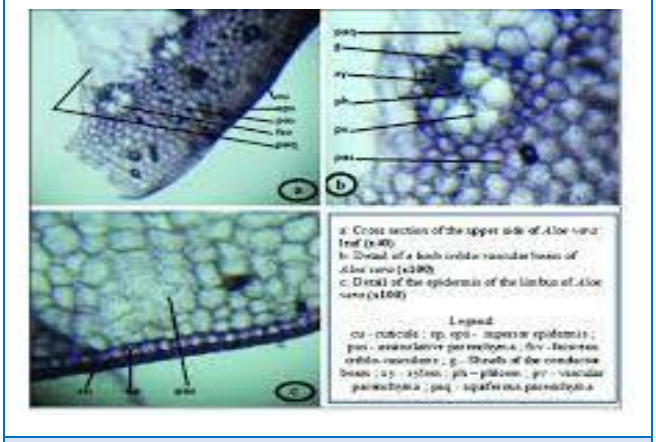


Fig. 1 & 2: Cross sectional study of Aloe vera.

Analytical Study

The leaf of *A. vera* consists of two parts, the inner clear pulp and the outer green rind. Many of the beneficial effects of this plant have been attributed to the pulp, including both immune-stimulation and anti-inflammation. It has been widely believed for several years that many of the beneficial effects of aloe leaf extracts lie in their carbohydrates. The thick fleshy leaves contain both cell wall carbohydrates, such as celluloses and hemicelluloses, as well as storage carbohydrates. The acetylated mannan is the primary polysaccharide in the pulp (inner clear portion of the leaf) and has been most widely studied. It has been claimed to possess many therapeutic properties, including immune stimulation. The leaf *Aloe vera* consists of two parts, the inner clear pulp and the outer green rind. Many of beneficial effects of this plant have been attributed to the pulp, including both immune-stimulation and anti-inflammation.

Analytical study is the application of a process or a series of processes in order to identify the chemical constituents and also about standards of the preparation. Hence, qualitative and quantitative study of a particular formulation should be carried out by the use of various parameters which helps in standardization and authentication of the drug, using the modern techniques and instruments. For maintaining the quality of drug (formulation) it is essential that the raw materials which are used are of required quality and the processes involved are followed correctly; so that the product manufactured will be a quality product. Hence, it is required to analyze the raw materials, and the finished product. For this study samples were collected from market and also prepared lab samples and named given as follows:

Sample A	Market Sample
Sample B	Market Sample
Sample C	Lab Prepared (Fresh)
Sample D	Lab Prepared (Heated)

To find out the quality of seed drugs and to distinguish the closely related species of the same genus, they are subjected to the following parameters.

1. Physico-chemical parameters
2. Qualitative chemical tests.
3. TLC study.
4. HPTLC study.

Physico-chemical Parameters

According to the procedure mentioned in "Quality control methods for medicinal plant material by WHO" the following physico-chemical constants are performed.

(A) Determination of Foreign matter

Plant drug should be free from moulds, insects, animal faecal matter and other contaminants such as earth,

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stones and extraneous materials. Foreign matter is material consisting of any one or all of the following:

i. Parts of the medicinal plant material or materials other than those named with limits specified for the plant material concerned.

ii. Any organism - part or product other than that the name in the specification and description of the plant material concerned. The amount of foreign matter present should not be more than the percentage prescribed in the monograph. Here the leaves are collected directly from herbal garden for *A.vera* juice and stored separately. Other samples of prepared gels are taken from market, so they are devoid of admixture of foreign or extraneous materials. Hence this test is not performed.

(B) Loss on drying

Presence of moisture in the crude drugs will enhance the growth of microbes, fungi and insects. This leads to deterioration and affects the preservation quality of the drug. Loss on drying is determined by weighing 10gm of the juice of each sample in dried Petri dish and dried in an oven at 110°C till two consecutive weights are arrived, which do not differ by more than 5 mg. The weight after drying is noted and loss on drying is calculated. The percentage is calculated with reference to air- dried sample.

Determination of Extractive values

(C) Water soluble extractives

This parameter is done to have an idea about the amount of water soluble constituents present in a particular drug such as sugar, mucilage, glycosides, tannins etc. Water soluble extractive was obtained by following the same procedure as described for methanol soluble extractive.

(D) Methanol soluble extractive

10 gm of liquid sample was macerated with 500 ml of methanol in a closed flask for 24 hours. The flask was shaken frequently during first 6 hours and allowed to stand still for 18 hours. The extract was filtered rapidly taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a dried, previously weighed flat bottomed shallow dish and dried at 105°C till constant weight. From the weight of the residue obtained, the percentage of alcohol soluble extractive was calculated with reference to the air dried drug.

(E) pH value

pH is defined as the negative logarithm of hydrogen ion concentration. It represents the acidity or alkalinity of an aqueous solution. The pH of the filtrate of a particular concentration of an aqueous solution of the sample is often used as one of the parameters. 5 gm of the sample was taken and extracted with 100 ml of distilled water by keeping it aside for a period of 2 hours and by shaking it intermittently. Then it was filtered and the pH of filtrate was noted with the help of Elico's digital pH meter with combined glass electrode.

Table: 1.0

pH	≤ 5.500
Specific gravity	≤ 1.003
Total solid content	≤ 1.732
Loss of drying % w/v	≤ 0.380
Alcohol soluble Extractive	≤ 3.800

Preliminary phytochemical investigations

The chemical profile of the seed samples are known by performing some preliminary phytochemical tests.[3]

Qualitative Tests

The crude extract of the samples can be qualitatively tested to confirm the presence of different type of constituents. Qualitative tests are used to detect the presence of functional groups, which play very important role in the expression of biological activity. Qualitative tests were carried out by using the methanol or water soluble extract of the samples. These tests indicate the type of phytochemical constituents present in the sample.

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Test for carbohydrates (glycoside / sugars)

Molisch's test (General test)

To 2-3 ml of aqueous extract, add few drops of alpha-naphthol solution in alcohol. Shake and add concentrated H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids, confirming the presence of carbohydrates.

Test for Reducing sugar

Fehling's test

To 1 ml Fehling's A and 1 ml of Fehling's B solutions, 1 ml of test solution was added and heated over boiling water bath for 5 - 10 minutes. Brick red ppt. indicates the presence of reducing sugar.

Test for Starch (non reducing polysaccharides)

3 ml of aqueous extract is treated with few drops of dilute Iodine solution. Appearance of blue colour indicates the presence of starch.

Tests for Mucilage

Powdered seed drug swells in water or aqueous KOH indicating the presence of mucilage. Powdered drug material shows red colour with ruthenium red.

Tests for Amino acids Ninhydrin test (General test)

To 3ml test solution, 3 drops of 5% Ninhydrin solution was added and heated over boiling water bath for 10 minutes. Purple colour indicates the presence of amino acid.

Test for Tannin

To 2-3 ml of aqueous extract few drops of 5% FeCl3 solution was added. Appearance of deep blue black colour shows the presence of tannin.

Test for Resin

Separately 5 gm of the seed powders were successively extracted twice with alcohol (95%) each time. The pooled alcoholic extracts were concentrated and poured in beaker containing distilled water. A white precipitate was observed indicating the presence of resin.

Test for Saponin (glycosides)[4]

0.1 gm of powder was vigorously shaken with 5 ml of distilled water in test tube for 30 seconds. The test tube is left undisturbed for 20 minutes. Formation of persistent froth indicates the presence of saponin.

Test for Anthraquinone glycosides Modified Borntrager's test

To 5 ml of extract 5 ml of 5% FeCl3 and 5 ml dil. HCl was added and heated over boiling water bath. The mixture was cooled and benzene was added to it. After shaking well the organic layer was separated and to that equal volume of dilute ammonia was added. Ammonia layer showing pinkish red colour indicates the presence of Anthraquinone.

Test for Aloin

(i) Schoenteten's reaction (Borax reaction)

5ml of solution was taken; 0.2g borax was added after dissolving it pour few drops of solution into a test tube nearly full of water. It shows green fluorescence.

(ii) Modified Borntrager's test

To 5 ml of extract 5 ml of 5% FeCl3 and 5 ml dil. HCl was added and heated over boiling water bath. The mixture was cooled and benzene was added to it. After shaking well the organic layer was separated and to that equal volume of dilute ammonia was added. Ammonia layer showing pinkish red colour indicates the presence of Anthraquinone.

(iii) Nitrous acid test

In sample crystals of sodium nitrate was added along acetic acid in small quantity sharp pink to carmine colour appear.

(iv) Nitric acid test

In sample nitric acid was added deep brownish-red colour appear.

Test for Sterols (Terpenoids) Libermann-Burchard Test

1 ml of the extract was evaporated to dryness. To the residue 1 ml of chloroform was added first and then 1 ml of acetic anhydride followed by gentle addition of few drops of sulphuric acid from the side. Formation of purple coloured ring at the junction of two layers will indicate the presence of triterpenes and sterols.

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Test for Alkaloids[5]

(i) Dragendorff's test

A small amount of methanol extract was taken in a watch glass. The solvent was evaporated and to the residue few drops of dil. HCl is added followed by few drops of Dragendorff's reagent (potassium bismuth iodine solution). Orange brown precipitate indicates the presence of alkaloids.

(ii) Mayer's reagent test

To the residue of methanolic extract few drops of dil. HCl and few drops of Mayer's reagent (Potassium mercuric iodide solution) were added. Formation of creamish white precipitate indicates the presence of Alkaloid.

(iii) Wagner's reagent test

To the residue to methanolic extract few drops of dil. HCl followed by few drops of Wagner's reagent (Iodine in potassium iodide solution) were added. Reddish brown colour flocculent precipitate indicates the presence of alkaloid.

HPTLC Test

High performance thin layer chromatography is a sophisticated and automated form of TLC. It is a valuable tool for quality assessment and evaluation of plant based drugs. The separations obtained through HPTLC have high resolutions. Compact starting spots allow a number of samples which may be applied to the HPTLC plate. It allows for the analysis of broad number of compounds both efficiently and cost effectively. More samples can be run in a single analysis thus reducing the analytical time. The same analysis can be viewed in different wavelengths of light there by providing a complete profile of the plant.

Materials and Methods

Equipment

A Camag HPTLC system equipped with a sample applicator Linomat IV, twin trough plate development chamber, TLC Scanner III, Reprostar and Wincats 4.02, integration software (Switzerland).

Chemicals

Ethyl acetate, methanol and potassium hydroxide obtained from G.A.U quality control lab. Jamnagar, (India). Pure Aloin was obtained from Total Herb Solution labs. Mumbai, (India).

Preparation of standard Aloin solution

Aloin standard (2mg) was accurately weighed and transferred to 2ml of volumetric flask. Crystals were dissolved in methanol and the volume was adjusted up to 10 ml with methanol (0.2mg/ml).

Calibration curve for Aloin

The standard solutions (5-15 per respective spot as track no.1,2,3) were applied in triplicate on TLC plate using CAMAG LINOMAT IV automatic spotter. The plate was developed with mobile phase comprised of Ethyl acetate: Methanol: water (10:1.35:1.0) (v/v). After development the plate was first scanned in UV in scanner III and detection and quantification was performed by densitometry at =359nm.The peak area were recorded. Calibration curve of Aloin was prepared by the plotting peak areas vs. concentrations of Aloin.

Preparation of sample solutions and estimation

Preparation of sample solution for Aloin:

2gm of aloin standard was taken and volume make up to 100ml with methanol. It was prepared in different three concentrations respectively 5, 10, 15µg.

Procedure

Chromatographic Conditions for HPTLC:

Application mode : Camag Linomat V, Hamilton syringe

Development chamber : Camag Twin through Chamber (20 x 10 cm^2)

Plates : Precoated silica plates

Chamber saturation : 45 min

Development distance : 5 cm

Development time : 30 min

Scanner : Camag Scanner II (ver. 3.14)

Detection : Deuterium lamp, Tungsten lamp.

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Photo Documentation : Camag Reprostar

Data System : Cats soft ware (ver. 3.17).

Drying device : Oven

Visualization : Under 254 nm to Under 359 nm

Derivatization : Spray reagent – 10% ethanolic potassium hydroxide and plate is heated at 110°C for 10 minutes.

Different samples were soaked in 20 ml of methanol and refluxed on boiling water bath for 30 minutes. The filtrates were concentrated and made up to 5 ml in a standard flask. 0.01µl was of each sample applied on E Merk Aluminium plate of 0.2 mm thickness precoated with silicagel 60 F254 using Linomat IV applicator. The plate was developed in Ethyl acetate : Methanol : Water (10 : 1.35 : 1.0). Solvent system up to 8 cm, plate was scanned at 254 nm and 359 nm using Deuterium tungsten lamp in a Camag µPhotographs were taken using Camag Photo Documentation System. The Rf value and percentage area of each spot were calculated.

To calculate the concentration of Aloin in each sample following equation was developed:

$$\frac{\text{Vol. made concentration total solubility}}{\text{Wt. of dried extract sample loaded } 1000}$$

Table 1.1: Showing amount of Aloin

SN	Sample for analysis	Amount of aloin found
1.	Sample A	…….
2.	Sample B	…….
3.	Sample C	0.456
4.	Sample D	0.186

The aloin content of aloe vera is not thermo stable so it should be protected from excessive heating.

DISCUSSION

Ayurveda has given the greatest emphasis on comprehensive knowledge of drugs including identification, procurement, processing, and preservation and dispensing of prepared drug under a broad heading known as *Bhaishajya Kalpana*. The basic processing techniques of Bhaishajya Kalpana are elaborately explained in *Samhitas*. They were called the *Panchavidha Kashaya Kalpana*.[6] But due to less shelf life, the need for preparing a formulation with a longer shelf life arose. During the last few decades considerable work in the field of natural products of pharmaceutical significance has been done globally.

The development of science of phytopharmaceuticals and the hopes for remedies in chronic diseases generated a new enthusiasm in the research workers to develop herbal medicines. Approximately 150 pure chemical substances were isolated from some 100 species of higher plants and are used in medicines world wide. Herbal medicine has been enjoying renaissance among the customers throughout the world, however one of the impediments in the acceptance of the Ayurvedic formulations is the lack of standard quality control profile. The quality of herbal medicine, that is the profile of the constituents in the final product has implication in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to establish quality control parameters and modern analytical techniques are accepted to help in circumventing this problem. *Kumari Sawarasa* is a herbal formulation used extensively in Ayurvedic system of Indian Medicine, as Cathartic, Prevent skin aging. Standardization of Ayurvedic formulation is the prime need of the current time. Many of them do not have uniform standards and analytical procedures to justify their quality and purity.

Modern techniques such as HPTLC, HPLC, GC etc. can be used to develop the methods for the quantification of marker compounds in these types of multicomponent herbal formulations. *Kumari Swarasa* is the juice of *Aloe vera* mill. The standards mentioned in Ayurvedic pharmacopoeia are not adequate enough to ensure the quality of plant drugs or their formulations. Therefore, selected formulations were subjected to HPTLC study.

In the last few years, there has been a significant increase in the number of health professionals and

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therapists who have come regard using of the *Aloe vera* in abundance of its products available in market. Different agencies are enhancing the use of the *Aloe vera* in different ways as Juices, Gel and in Cosmetics and Beauty products.

Aloe vera and products with aloe vera has increased dramatically and number of companies connected to aloe vera industries and most of industries have experience phenomenal growth in a short period, one often finds that demands can outstrip supply and this can lead to proliferation of products of lower quality being introduced to particular markets.

There are some biological markers present in the drug which are responsible for the medicinal value and responsible for therapeutic activity, it can be state that these are the main constituents of drug. In aloe vera Aloin is reported as the biological marker. This is responsible for therapeutic activity along with other chemical constituents. So, in any Aloe formulation it should be present. In market there are abundance of products of Aloe vera are reported. But none of pharmaceutical company and other agencies are describing chemical profile of their product. Though there are some works reported on aloe vera, but no work has found reported on it for there active principle i.e., Aloin.[7]

This is when the need for nationally and internationally accepted standards is paramount. This is an age of globalization and the time has come for companies with products of unquestionable purity, quality and efficacy to take positive action to the activities of those manufacturers and supplier who do not meet the highest quality standards. When assessing the quality of an aloe vera product remember to consider these points.

- The quality and maturity of aloe vera plants used
- The age at which they are harvested
- The harvesting methods used

Inner gel versus whole leaf

According to Ayurvedic principals it mentioned that any plant should be use in as natural state as possible. Excessive filtration and concentration can affect the balance of the naturally occurring nutrients. The companies discard the rind whose products are based on the inner gel. By this process the two activities most likely to damage

- Destroy polysaccharides early in manufacturing process are firstly, exposure to the natural enzyme, cellulose and
- Secondly, the activity of bacteria.

Both are present in aloe vera just under the rind or on the outer green leaf. So, it should be use along green rind part by using the effective filtration or other techniques.

Present study shows clearly that Aloin which is the reported bio-marker of aloe vera effected by heat. Quantitative data received from HPTLC study shows this fact.[8]

The essential component!

Aloe vera must be “biologically alive” and must be in bio-available from if customers around the world are to experience the full scope and range of benefits from this plant. The only way that aloe vera can reach the customer in a fresh state and still be of the highest quality is if the raw inner gel is stabilized immediately. This stabilization process must also preserve and retain all the active ingredients without the use of any damaging chemicals or exposing to excessive heat.

This kind of study correlates the Ayurvedic formulation with analytical studies. Which define the accurate results regarding the presence of bio-marker or other essential compound present in any kind of formulation. Also, this kind of study can be correlate with specific species of medicinal plants. Because plant species varies with their chemical constituents and from where it is collected and the harvesting process also varies the chemical constituents.

This kind of studies confirm the quality of any market samples, which are sold in market as a crude form and they don’t label any chemical profile and plant description. So, by the quality control agencies run by Government should concern about these kind of

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products which are present in plenty in market. Because without chemical profile nobody can't say about the presence of essential chemical constituents which are responsible for therapeutic activity of that drug. Especially for Ayurvedic formulations which are present in market need this kind of study which define the many aspects of the drug formulation like about plant species, harvesting method, processing methods etc. About the chemical changes and region wise plant occurring conditions and wild and cultivated plants (these days people are enhancing the medicinal plant cultivation and those are taken in pharmacy industries) it varies the chemical constituents.

CONCLUSION

So, on basis of this study it is concluded that: *Aloe vera* has used during the *Samhita Kala* for different purposes. The quality assurance of market products is must now a days. It should be sold as along chemical profile. Analytical study shows that following parameter may be present in the products of aloe vera juices.

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