Int J Ayu Pharm Chem

RESEARCH ARTICLE

www.ijapc.com

e-ISSN 2350-0204

Pharmacognostical Study of Aloe barbadensis Miller

Dhiman Sonia[1]*, Kumar Ajay[2], Dhiman Monika[3], Chawla Kumar Satbir[4] and Priyanka[5]

[1]Dravyaguna department, CDL college of Ayurveda, Jagadhari Haryana, India

[2]Ayush Haryana, India

[3]Fortis Hospital, Kangra, Himachal, India

[4]Agad Tantra Department, Glocal College of Ayurvedic Medical Sciences and Research Center Haryana, India

[5]Stri Rog and Prasuti Tantra Department, CDL College of Ayurveda, Jagadhari Haryana, India

Abstract

Kumari (*Aloe barbadensis* Miller) is non controversial plant, but three types of (*Musabbar*) aloe are sold in market i.e. Barbados aloe, Socotrine aloe and Cape aloe which are prepared from *Aloe barbadensis* Miller, *Aloe perryi* Baker and *Aloe ferox* Miller respectively and other inferior aloe are also sold like Natal aloes by the name of *Musabbar*. These species of aloe are difficult to identify hence pharmacignostical study of *Aloe barbadensis* Miller was done. It is coarse looking, perennial succulent plant, grown close to the ground in a typical rosette shape, Strong, shallow and fibrous roots have arbuscular mycorrhiza. Leaves are crowded on the top of the stem with spiral orientation. Dark green leaves have glaucous spots, wider at the base tapering gradually to the pointed tip, concave above, convex beneath, serrated margins. Rind, sap, mucilage gel, inner gel is layers of leaves. Flowers are born in terminal racemes on the scape.Stalked, numerous having yellow tubular corolla with six stamens. Fruits are oblong, ovoid. The micrograph of the adaxial leaf epidermal layer exhibited the characteristic plasmolyzed cytoplasm, sunken stomata complex having two guard cells, tetracytic, hexagonal epidermal cells and some cells were small square shape. In contrast, the abaxial leaf epidermal layer was less plasmolyzed and cell walls were less thick. Epidermal tissue, chlorenchyma, aquiferous tissue, vascular bundle are four type of tissues present in leaves. The *Tikta Rasa* was observed as the predominant *Rasa* of *Kumari Swarasa* (70%). According to 50% volunteers both *Katu* and *Tikta Rasa* were present.

Keywords *Aloe barbadensis*, *Musabbar*, *Kumari*

Greentree Group

Received 15/10/16 Accepted 01/11/16 Published 10/11/16

INTRODUCTION

Acharya Charaka has mentioned that full knowledge of drug can be gained by knowledge of the name as well as identification[1]. He also stressed on proper identification of drug and told that unidentified drug can kill the person like poison, heat etc[2]. He has mentioned examination of the drug in all respects[3]. As per *Samhita kala* the knowledge of identification of drugs was free from confusion and plants were known by their names and form, not only to physicians, but also to goat headers, sheep headers and cow headers etc[4]. Knowledge was handed over from generation to generation by *Guru-Shishya* method. After extinction of this method; identification of the drugs and their utility was the main base for writing the *Nighantus* and in this period much attention was paid toward the proper identification of plants by organoleptic methods. Commentators of *Samhitas* and authors of *Nighantus*, while on one hand removed many doubts by giving the popular names and synonyms of drugs, on the other hand created complexity in identification of many drugs.

With the advent of modern botany and pharmacognosy, this gap was largely narrowed but could not be filled up till date. Many times we get entirely a different drug from the one we intend to use and consequently results are not obtained that have been mentioned in ayurvedic texts. Such practices mislead and discouraged the practitioner and so create obstacles in progress of *Ayurveda.* Thus it is obvious that there is a vast scope and need for research in this direction.

The versatility of flora of nature always fascinated the man. Each and every species of nature has its own identity. Moreover today, in the age of globalization, raw drugs collection is done by unskilled persons causes doubt in the genuineness and possible adulteration. Unlike the traditional methods the participation of traders in the chain of procurement of drugs, adulteration is increasing day by day when the original genuine material is not available in sufficient quantity and in such instances efforts should be made for a systematic identification by pharmacognostical methods.

Kumari (*Aloe barbadensis* Miller) is one of non controversial plant, but three types of (*Musabbar*) aloe are sold in market i.e. Barbados aloe, Socotrine aloe and Cape aloe which are prepared from *Aloe barbadensis*

Miller, *Aloe perryi* Baker and *Aloe ferox* Miller respectively and other inferior aloe are also sold like Natal aloes by the name of *Musabbar*. These species of aloe are difficult to identify.

MATERIALS AND METHODS-

Collection of sample-

The authenticity of the plant's species was identified by studying its various characters and comparing them with the characters mentioned in the various Floras. Further identification was also done with the help of botanists of herbal garden and P.G. Department Dravyaguna of Rajiv Gandhi Government Postgraduate Ayurvedic College Paprola (H.P.). The leaves of 1 to 2 feet length, which were changing color from green to brown, were cut without causing damage to the plant from the glass house of herbal garden Jogindernagar (H.P.) and washed properly.

Pharmacognostical study-

Macroscopic Study

The macroscopic characteristics of the root, leaf, stem and flowers were studied systematically as per the standard text books of botany, pharmacognosy as well as with the help of floras and databases.

Organoleptic Study

The fresh sample of leaf and powdered sample of Musabbar were evaluated for their organoleptic characters including taste, odour, colour and touch.

Microscopic Study

Preparation of slide- Finely cut very thin transverse section, upper epidermis and lower epidermis of the fresh leaf was put on the glass slide, heated with the drop of chloral hydrate and then added the drop of diluted safaranine stain and glycerine on it and covered with glass cover slip. It was then watched under dissecting microscope.

a) Microscopic characters of upper epidermis (adaxial surface), lower epidermis (abaxial surface) and transverse section of leaf were studied with the help of Carl Zeiss trinocular microscope; photomicrographs of free hand sections were taken by Cannon digital camera attached to it and figures were drawn with the help of camera lucida attached with dissecting microscope.

b) Powder microscopy: Powder of the aloe was studied microscopically in glycerine with the help of dissecting microscope.

OBSERVATIONS AND RESULTS

A) Macroscopic Study

General features- Aloe vera is coarse looking, perennial succulent plant, grown close to the ground in a typical rosette shape, spreading by offsets and root sprouts. (Figure-1)

Roots are Strong, shallow and fibrous. They have arbuscular mycorrhiza within roots. (Figure-2, 3)

Stem is short, about 50 cm high, 10 inches in circumference**.**

Arrangement of Leaves- Leaves are crowded on the top of the stem with spiral orientation.

Leaves-(Figure-4) Colour of the mature leaves is brownish green whereas fresh leaves are dark green, having glaucous spots, wider at the base tapering gradually to the pointed tip, thick and fleshy, showing white flecks on the upper and lower stem surfaces, concave above, convex beneath, the margins of the leaf are serrated and have small white blunt teeth. Yellow juice is exuded from fibro vascular bundles present just inside the outer skin when the leaf is cut.

The Aloe leaf structure is made up of these layers:

- Rind - It is the outer protective layer.
- Sap - It is a layer of bitter fluid which protects the plant from animals.
- Mucilage gel – It is the movable layer between inner solid gel and outer rind.
- Inner gel- It is innermost part of leaf which is filleted out to make gel.

Flowering- (Figure5, 6, 7) Flowers are born in erect, stout, cylindrical terminal racemes on flower stalks 50 to 100 cm high from centre of leaf tuft. Flowers are stalked, numerous, erect in the bud, pendulous, bracts exceeding the pedicles, membranous, triangular, acute, veined and persistent. Calyx is not present, yellow tubular corolla with six stamens which are little longer than perianth and style is about equal to the stamen and stigma is terminal.

Fruit-(Figure-8) Oblong ovoid in shape, very blunt capsule, about 25mm, long blunty trigonous, 3 celled, thin pericarp, leathery, greenish brown, smooth, dehiscing loculicidall .

Seeds- Seeds are numerous, compressed, testa membranous, lax forming a wide scarious wings.

B) Organoleptic Study-

Kumari Swarasa showed slimy liquid texture, transparent, yellowish tint, bitter in taste and characteristic odour (table-1).

Musabbar showed opaque, slightly vitreous texture, blackish color, and nauseous, pungent and bitter taste with characteristic odour (table-2).

Table 1 Kumari Swarasa (Organoleptic study)

Texture	Colour	Taste	Odour
Slimy liquid	Transparent, yellowish tint.	Bitter	Characteristic

Table 2 Musabbar (Organoleptic study)

Texture	Opaque, slightly vitreous
Colour	Blackish
Taste	Nauseous, pungent and bitter
Odour	Characteristic

Table 3 Perception of Rasa of Kumari Swarasa

Sr. No.	Rasa	Percentage
1.	Tikta	70
2.	Madhura	20
3.	No taste	10

Table 4 Perception of Rasa of Musabbar

Sr. No.	Rasa	Percentage
1.	Katu	20
2.	Tikta	20
3.	Both Katu, Tikta	50
4.	Kashaya	10

C) Microscopic Study-

Leaf is most important part of Aloe vera which is used medicinally hence leaf has been studied microscopically.

Adaxial surface/ upper epidermis and abaxial surface/ lower epidermis- (Figure 9, 10, 11)

The micrograph of the adaxial leaf epidermal layer in 10x exhibited the characteristic cytoplasm which appeared plasmolyzed, its cell wall constituted a thick ridge-like polymer of cellulose. Sunken stomata complex having two guard cells, tetracytic with hexagonal epidermal cells and some cells appeared as small square shape which is characteristic feature of xerophytes.

In contrast, the abaxial leaf epidermal layer was less plasmolyzed and cell walls were appeared to be less thick. Other features were same as that of adaxial surface Observations are supported by previous researches[5].

Figure 1 Showing rosette shape, spreading offsets and root sprouts

Figure 2 Showing roots, stem, leaves arrangement

Figure 3 Showing arbuscular mycorrhiza

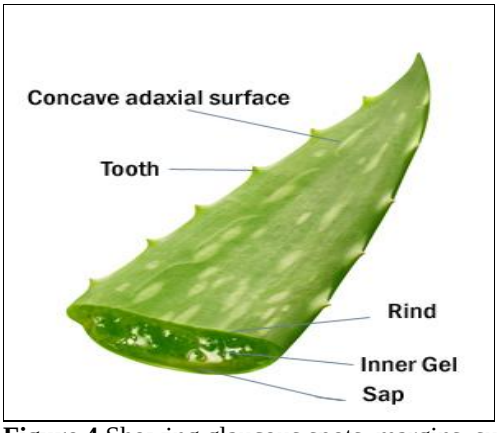

Figure 4 Showing glaucous spots, margins, surfaces, layers

Figure-5, 6, 7 Showing inflorescence, shape, colour and parts of flowers

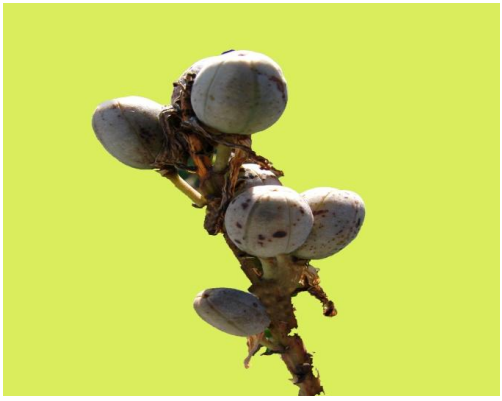

Figure 8 Showing Fruits

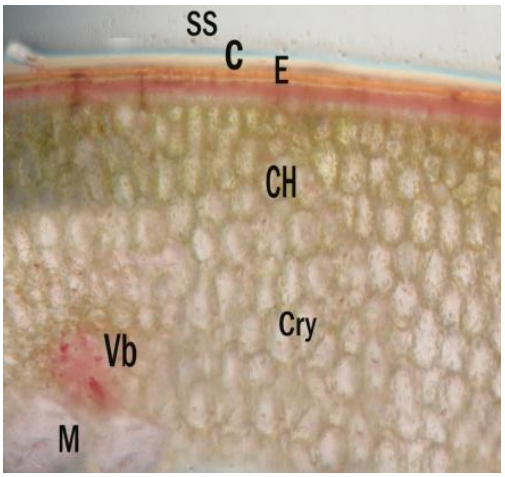

Figure 9 Transeverse section of adaxial surface 10x C- Thick cuticle with waxy appearence, E- epidermis, CH- Chlorenchyma, Vb- vascular bundle, M- mucilagenous layer, SS- sunken stomata.Cry- crystals

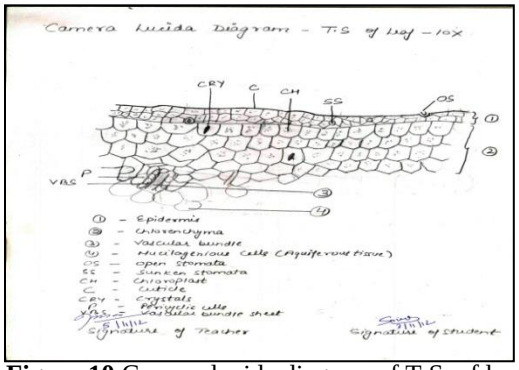

Figure 10 Camera lucida diagram of T.S. of leaf

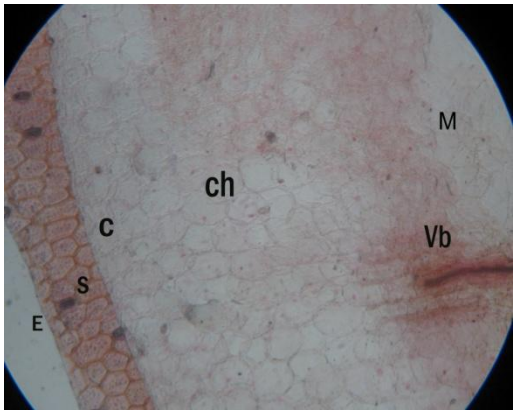

Figure 11 Transverse section of abaxial surface 45x E- epidermis, CH- Chlorenchyma, Vb- vascular bundle, S- sunken stomata,c- chloroplast

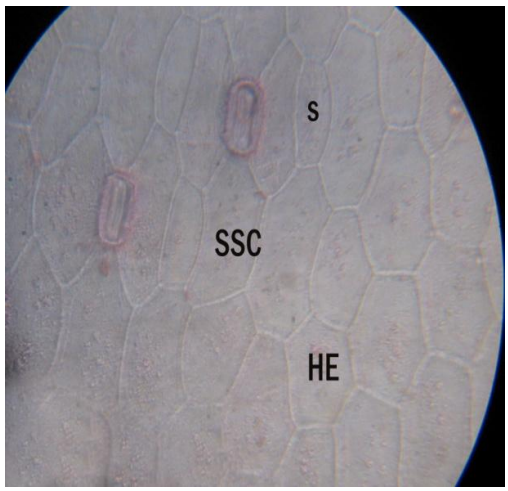

Figure 12 Lower epidermis- 45x S-sunken stomata , Tetracytic. HE- hexagonal epidermal cells, SSC- short square cell

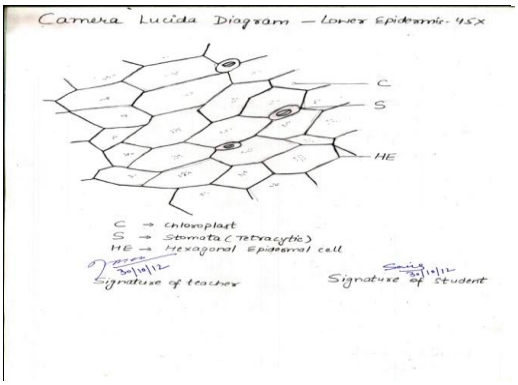

Figure 13 Camera lucida diagram of lower epidermis

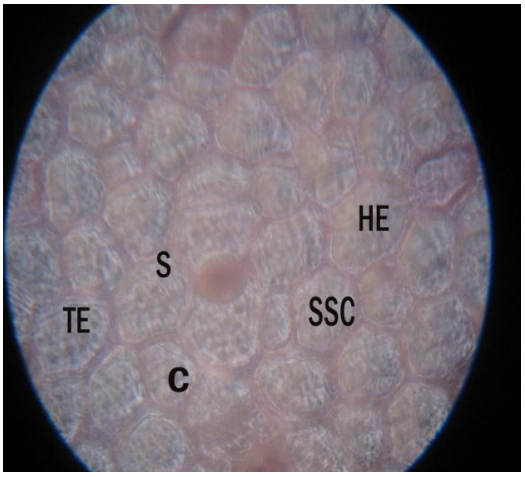

Figure14 Upper epidermis 45x S- stomata, TE- thick walled epidermal cell, C-chloroplast, SSC- Short square cell, HE- hexagonal epidermal cells.

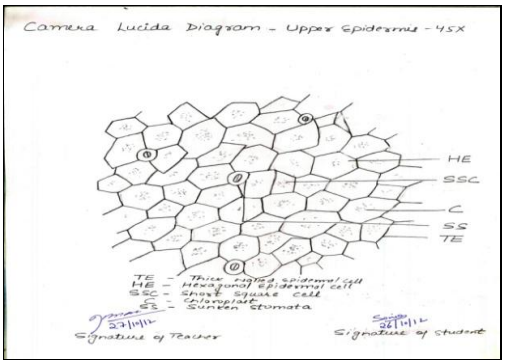

Figure 15 Camera lucida diagram of upper eppidermis

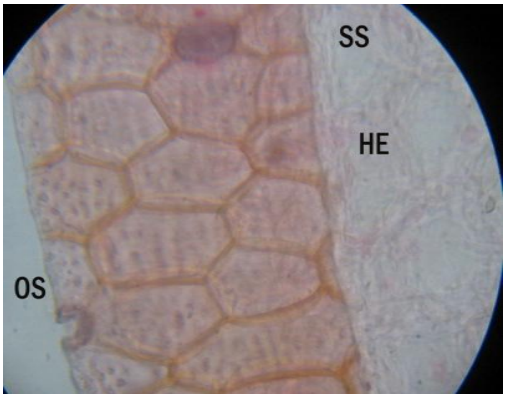

Image- 16- T.S. Epidermis (high megnification)
OS- open stomata, HE- hexagonal epidermal cells, S- sunken stomata

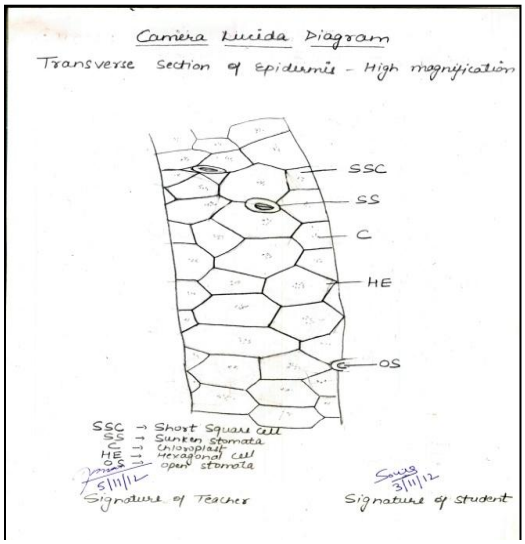

Figure 17 Camera lucida diagram of T.S. epidermis

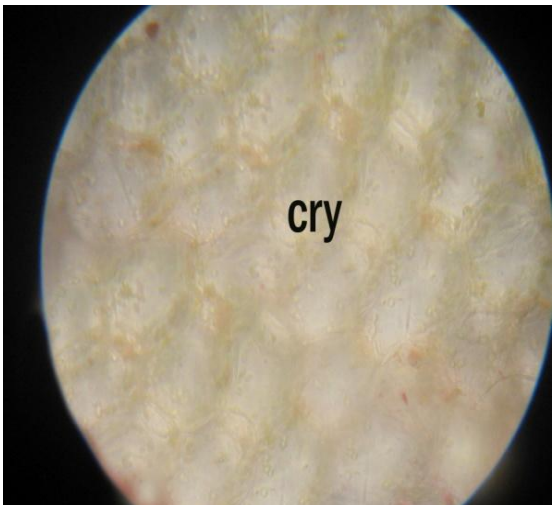

Figure- 18- T.S 45x Chllorenchyma isodiametric cells
Cry- ca oxalate and mg lactate crystals

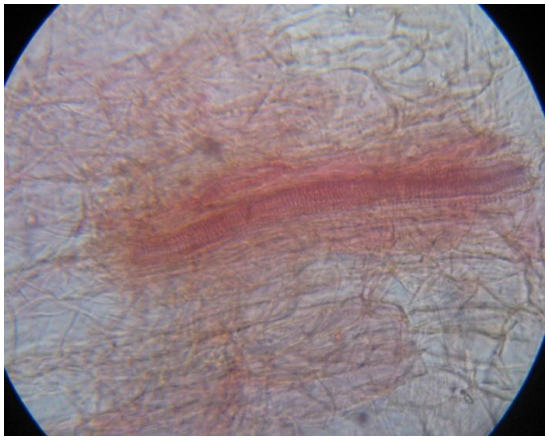

Figure- 19-High magnification of vascular bundle

Four types of tissues are present *in Aloe barbadensis* Miller leaves[6].

1. Epidermal tissue } Rind
2. Chlorenchyma
3. Aquiferous tissue
4. Vascular bundle

Epidermal tissue-(Figure 12, 13, 14, 15, 16, 17) epidermal features are expressed by

thickened cuticle, thickened cell wall and surface roughness increasing in line with the ability of the species to withstand dry climatic conditions.[7]

Thickened waxy cuticle layer was observed which was interrupted by opened stomata, below this layer were some hexagonal cells with sunken stomata embedded in it and immediately below it there were chlorenchymatous rind cells.

Chlorenchyma-(Figure-18) it was located beneath the epidermis; having 8-10 layers of isodiametric pillar shaped thin walled cells. Layers were not differentiated into palisade cells and spongy cells. Cells were rich in chloroplast and some of these cells contained crystals of calcium oxalate and magnesium lactate and termed as idoblasts. This rind tissue was interconnected with the parenchymatous tissues which are assumed to produce and store the leaf exudates and other constituents which are assumed to be associated with the plant defensive strategy. These leaf exudates oozed immediately out of the parenchymatous cells once the leaf had been cut. 2Observations are supported by previous researches[8].

Aquiferous tissue- Central part of leaf was occupied by Aquiferous tissue; just beneath the rind there was presence of thick and slimy lacunar mesophyll. This provides the movable layer between more solid inner gel fillet and the outer rind. This liquid layer is termed as mucilage. The innermost and major portion of the leaf is the spongy constituting the gel fillet. The cells were devoid of chloroplast. These observations are similar to previous researches[9].

Vascular bundle- (Figure-19) the ring of vascular bundles was located within the boundary of Chlorenchyma and Aquiferous tissue. The outer support to the vascular bundle was provided by the vascular sheath cells. Inside the vascular bundle there are three types of tubular structures: the xylem, the phloem and the large pericyclic tubules (containing sap which is rich in anthraquinones). The anthraquinones absorb U.V. rays of the sun and prevent over heating of the central portion of the aloe leaf. The pericyclic portion was adherent to the rind, while the remainder of the vascular bundles was protruded into the lacunar mesophyll (mucilage).

Powder microscopy- In the 10x powder microscopy of Musabbar; fragment of very large number of needles was seen which resembles the textual references of Curacao aloe[10]**.** Under high power; vascular bundles along with Aquiferous tissue were observed.

Evaluation of Threshold

Acharya Charaka has mentioned **'Raso Nipate Dravyanam'**[11]i.e. the taste of a substance is felt when comes in contact with Rasanendriya. The minimum quantity of a substance required to stimulate the taste bud is known as the taste threshold for that drug. This term was used for the first time by Dr. Wescby of Germany. To evaluate taste threshold the method prescribed by Dr. S. C. Dhyani has been adopted. It was carried out in three phases.

(1) ***Phase-I Cold water method*:**

5 gms of Musabbar was dissolved in 100 ml of distilled water in a conical flask and was stirred continuously for 30 minutes, later it was allowed to settle the sediments and then it was filtered with filter paper.

1 ml of this stock solution was diluted gradually with distilled water in different dilutions such as 1:5, 1:10 and 1:15 and so on. Each time the test solution was given to the volunteers for testing. The volunteers were requested to wash their mouth thoroughly with distilled water for each test trial. The dilution on which the least Rasa was perceived was multiplied with 20 (dilution factor of stock solution) and then was noted as the taste threshold of the drug. Same method was adopted in case of Kumari Swarasa.

(2) Phase-II Hot water method:

Some drugs contain certain volatile oils or any such contents, which may subscribe to taste, may be more soluble in hot water. Therefore, the Taste threshold of drug was ascertained in hot water.

5 gms of Musabbar was added to 100ml of distilled water in a conical flask and stirred continuously for 30 minutes for proper mixing. Then the liquid mixture was boiled till it reduces to half. i.e. 50 ml (The water lost i.e. 50 ml distil water, thus making the volume 100 ml) and filtered and then 1 ml of this prepared solution was taken and to it 5 ml of distilled water was added and the drug was tasted. Each time 5 ml of distilled water was added keeping the initial 1 ml. solution constant. Volunteer method was used for taste determinations. In the same way taste threshold of Kumari Swarasa was determined.

(3) Phase-III Method of Taste threshold in 6 hours after boiling:

Some contents of the drug may require time for being fully dissolved in the solution. Therefore, this method was adopted. The method is the same as described previously except that after the volume is reduced to

half after boiling, it was allowed to cool down for 6 hours and then filtered. 1 ml of the stock solution was taken for further experiment.

Above three methods for determination of taste-threshold was done in P.G. Department of Dravyaguna R.G.G.P.G.A.C. Paprola. Results obtained are as follows

RESULTS

Kumari Swarasa- The Tikta Rasa was observed as the predominant Rasa (70%). (Table-3)

Musabbar- According to 50% volunteers both Katu and Tikta Rasa were present.(Table-4)

Taste Threshold-

Musabbar

- Taste threshold of Musabbar in cold water - 1:35 X 20 = 1:700 ml.
- Taste threshold of Musabbar in hot water – 1:40 X 20 = 1: 800 ml.
- Taste threshold of Musabbar in 6 hours after boiling – 1: 45 X 20 = 1: 900 ml.

Kumari Swarasa

- Taste threshold of Kumari Swarasa in cold water – 1: 35 X 20 = 1: 700 ml.
- Taste threshold of Kumari Swarasa in hot water – 1: 20 X 20 = 1: 400 ml.
- Taste threshold of Kumari Swarasa in 6 hours after boiling -1: 20 X 20 = 1: 400 ml.

CONCLUSION

- Aloe barbadensis Miller is beautiful plant having miraculous medicinal properties. Macroscopic and microscopic studies like tetracytic sunken stomata with hexagonal epidermal cells and some small square shape cells showed typical xerophytic characters. Aloe leaf structure is made up of outer protective layer called as rind, sap which contains bitter fluid to protect the plant from animals, movable layer between inner solid gel and outer rind is mucilage gel and innermost part of leaf is filleted out to make gel. Epidermal tissue, chlorenchyma, aquiferous tissue, vascular bundle are four type of tissues present in leaves. Fragment of very large number of needles was seen in powder microscopy which resembles the textual references of Curacao aloe hence the drug prepared as Musabbar was a product of Aloe barbadensis Miller called as Curacao aloe.

REFRENCES

1. Shastri Satyanarayana, Shastri Kashinatha, Chaturvedi Gorakhnatha, Charaka sahmita,part1, sutrasthana chapter1, shalok122, vidyotini hindi commentary, Reprint2001,published by chaukhamba Bharti Academy, reprint2001, p48.

2. Shastri Satyanarayana, Shastri Kashinath and Chaturvedi Gorakhnath, Charka Samhita, Part1,Sutra Shana chapter 1, shalok 125, Vidhyotini Hindi Commentary, Reprint 2001, Chaukhambha Bharati Academy Publications, p48.

3. Shastri Satyanarayana, Shastri Kashinath and Chaturvedi Gorakhnath, Charka Samhita, Part1,Vimana sthana chapter 1, shalok 87, Vidhyotini Hindi Commentary, Reprint 2001, Chaukhambha Bharati Academy Publications, p768.

4. Shastri Satyanarayana, Shastri Kashinath and Chaturvedi Gorakhnath, Charka Samhita, Part1,Sutra sthana chapter 1, shalok 121, Vidhyotini Hindi Commentary, Reprint 2001, Chaukhambha Bharati Academy Publications, p47.

5. Sharma Vinay, Pharmacognostical assessment of *Aloe barbadensis* Mill. Leaf, International journal of science innovations and Discoveries 2011, 158-164.

6. Sharma Vinay, Pharmacognostical assessment of *Aloe barbadensis* Mill. Leaf, International journal of science innovations and Discoveries 2011, 158-164.

7. Roy Upton RH DAyu, Standards of identity, Analysis and Quality Control, American Herbal Pharmacopoeia.

8. Sharma Vinay, Pharmacognostical assessment of *Aloe barbadensis* Mill. Leaf, International journal of science innovations and Discoveries 2011, 158-164.

9. Ivan e denhof et al, Aloe leaf handling and constituent variability-www.simplynaturalproducts.com date of browsing-07 -03-2013

10. Kokate C.K., Purohit A.P., Gokhale S.B., Pharmacognosy,Volume1 and 2, edition46th, published by Nirali prakashan,p8.26.

11. Shastri Satyanarayana, Shastri Kashinath and Chaturvedi Gorakhnath, Charka Samhita, Part1,Sutra sthana chapter 26, shalok 66, Vidhyotini Hindi Commentary, Reprint 2001, Chaukhambha Bharati Academy Publications, p513.