



Pharmacognostical and Phytochemical study of Ghrit Kumari (Aleo-vera)

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The term 'pharmacognosy' is derived from two Greek words 'Pharmacon' means drug and 'gignosco' or 'gnosis' to acquire knowledge means knowledge of drugs. It may be defined as an applied science which deals with the biological, biochemical and economical features of natural drugs and their constituents. Any plant which is used medicinally requires detail study prior to its use because the therapeutic efficacy is absolutely depends on the quality of the plant drug used. The detailed Pharmacognostical study of plant help us to differentiate between closely related species of the same genus or related genera of the same family. It is also the first step to standardize a drug which is the need of the day. If the plant drugs are adulterated, then the quality of preparation cannot give the desirable results. Macroscopic and microscopic pharmacognostical studies will be done in R.R.I (C.C.R.A.S) unit of Lucknow. Thin layer chromatological studies and fluorescence analysis have also been done. These observations will have recored in this chapter.

Apart from this some literature produced from Database and other books related to pharmacognosy and photochemistry of Ghritkumari (Aloe-vera). Apart from this, by the help of the Pharmacognostical study we can suggest substitutes to some rare species and save them from extinction. So before using a drug it is very much essential to carry out it's detailed Pharmacognostical study as it is not only helpful for correct identification but also to get a clue for it's phytochemical, pharmacological and medicinal properties.

Taxonomy

Kingdom-Plantae, Division-Spermatophyta, Sub-division-Angiospermae, Class-Monocotyledoneae, Family-Liliaceae, Genus-Aloe, Species-Aloe-vera Tourn. Ex.Linn; Syn., AloeBarbadensis Mill., Kumarisara (Musabbar) - Aloe barbadensis Mill., Kumarisara consists of dried juice of leaves of Aloe barbadensis Mill. Syn.,Aloe vera Tourn.ex Linn, Aloe indica Royle. (Fam. Liliaceae), shrub

planted in many Indian gardens and found growing throughout India.

Properties and Action

Rasa : Tikta, Madhura

Guna : Laghu, Tikshna, Sukshma

Virya : Ushna

Vipaka : Katu

Karma : Bhedana, Pittanirhara, Rajahpravartaka, Jvaranut

Botanical Description

(a) Macroscopic

(A) Family

Kumari belongs to Liliaceae family. A widely distributed family of about 250 genera and 3,700 species. Habitat-These are herbs and climbers and rarely shrubs or trees with a bulb or rhizome, or

with fibrous roots. Leaves-These are simple, radical or cauline or both. Inflorescence-These may be spike, raceme, panicle or umbel, often on scalp. Flowers--The flowers are regular, bisexual (rarely unisexual) dioecious, as in smilax. They are trimerous, scarious (thin, dry, membranous). Perianth-The tepals are petaloid. There are usually six in two whorls. They may be 3+3 and free (polyphyllous) or 3+3 and united (gamophyllous). Androecium-There are 6 stamens in two whorls, 3+3, rarely 3, free or united with the perianth (epiphyllous) at the base. The anthers are often dorsifixed. Gynoecium-There are 3 carpels (syncarpous). The ovary is superior and 3 celled. There are usually ovules in two rows in each loculus. The placentation is axile. There are 3 styles. Fruit---This may be a berry or capsule. Seeds---The seeds are albuminous



(b) Microscopic

Leaf blade – Not very numerous, sessile, densely crowded on the short stem with wide dilated bases, spreading below, then ascending, one to one and half foot long, tapering to a blunt point, very thick and fleshy, flat or rather concave above, convex beneath, the margin set with hard, distant, somewhat hooked prickles, surface quite smooth and shining, dark glaucous green, sometimes mottled. About 15 inches long, 4 inches broad and ¾ inch thick. Large fleshy green leaves with sharp spines and white specks at the bases of the leaves. Stem - Three feet high, oblique at base, erect, stout, smooth, cylindrical from the centre of the leaf- tuft.

Flowers- Stalked, numerous, erect in the bud, afterwards pendulous, arranged the pedicles,

membranous, triangular, acute, reddish, veined, persistent. Perianth - Gamophyllous, tubular, about 36mm. Long, rather fleshy, reddish yellow and green, cut into six oblong, bluntish segments, the three outer a little shorter than the inner, which they closely cover, never spreading persistent. Stamens- Six hypogynous, a little longer than the perianth, anthers small, oblong ovoid, bluntly triangular, 3-celled, with a double row of ovules in each cell. Style-About equally the stamens, simple. Stigma-Terminal

Fruit-(not seen) An oblong-ovoid, very blunt capsule, about 25mm. Long bluntly trigonous, 3-celled, pericarp thin, leathery, greenish brown, dehiscing loculicidally. Seeds-Very numerous, compressed, testa fine and membranous, lax, forming a wide scarious wing.

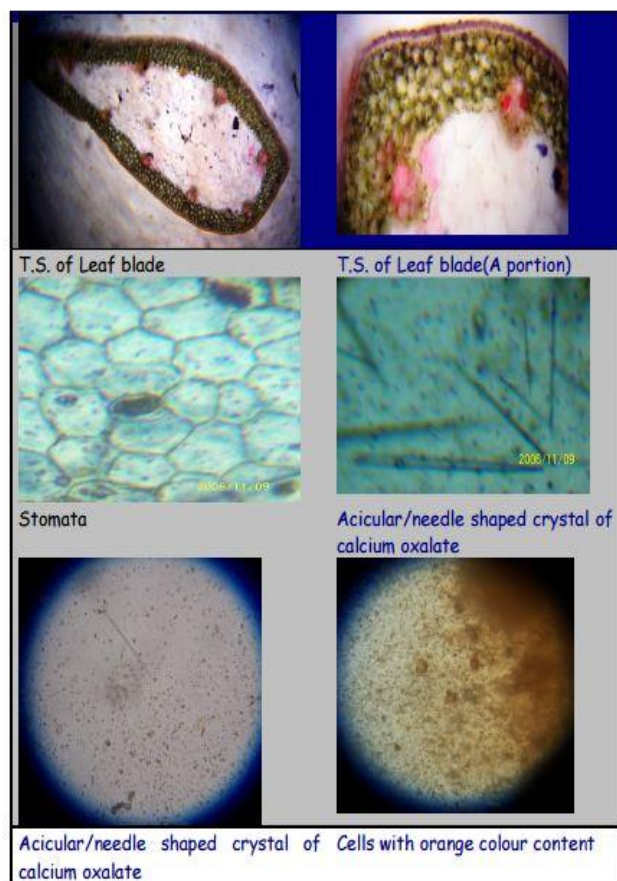
Embryo- In the axis of the endosperm.

Flowering time--winter spring seasons

Formulations and Preparations

Kumari is used in the following formulations:

- Kumaryasava- Kumarika vati- Kumaripaka- Kumari taila- Rajahpravartani vati etc.



T.S. of Aloe-vera

Preliminary screening of Phytochemicals

The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug. 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test.

General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them (Trease and Evan, 1983).

1. Alkaloids

Dragendorff's test: Dissolve a few mg of alcoholic or aqueous extract of the drug in 5 ml of distilled water, add 2 M hydrochloric acid until an acid reaction occurs, then add 1 ml of

Dragendorff's reagent, an orange or orange-red precipitate is produced immediately.

Hager's test: To 1 ml of alcoholic extract of the drug taken in a test tube, add a few drops of Hager's reagent. Formation of yellow precipitate confirms the presence of alkaloids.

Wagner's test: Acidify 1 ml of alcoholic extract of the drug with 1.5% v/v of hydrochloric acid and add a few drops of Wagner's reagent. A yellow or brown precipitate is formed.

Mayer's test: Add a few drops of Mayer's reagent to 1 ml of acidic aqueous extract of the drug. White or pale yellow precipitate is formed.

2. Carbohydrates: -

Anthrone test: To 2 ml of anthrone test solution, add 0.5 ml of aqueous extract of the drug. A green or blue colour indicates the presence of carbohydrates.

Benedict's test: To 0.5 ml of aqueous extract of the drug add 5 ml of Benedict's solution and boil for 5 min. Formation of a brick red coloured precipitate is due to the presence of carbohydrates.

Fehling's test: To 2 ml of aqueous extract of the drug add 1 ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' and boil the contents of the test tube for few min. A red or brick red precipitate is formed.

Molisch's test: In a test tube containing 2 ml of aqueous extract of the drug add 2 drops of a freshly prepared 20% alcoholic solution of beta-naphthol and mix, pour 2 ml conc. sulphuric acid so as to form a layer below the mixture. Carbohydrates, if present, produce a red-violet ring, which disappears on the addition of an excess of alkali solution.

3. Flavonoids

Shinoda's test: In a test tube containing 0.5 ml of alcoholic extract of the drug, add 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown colour is produced.

4. Triterpenoids

Liebermann-Burchard's test: Add 2 ml of acetic anhydride solution to 1 ml of petroleum ether

extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A violet colour coloured ring is formed indicating the presence of triterpenoids.

5. Proteins

Biuret's test: To 1 ml of hot aq. extract of the drug add 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution. A red or violet colour is obtained.

Millon's test: Dissolve a small quantity of aqueous extract of the drug in 1 ml of distilled water and add 5-6 drops of Millon's reagent. A white precipitate is formed which turns red on heating.

6. Resins- Biuret's test: To 1 ml of hot aq. extract of the drug add 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution. A red or violet colour is obtained.

Millon's test: Dissolve a small quantity of aqueous extract of the drug in 1 ml of distilled water and add 5-6 drops of Millon's reagent. A white precipitate is formed which turns red on heating.

7. Saponins -In a test tube containing about 5 ml of an aqueous extract of the drug add a drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 mints. Honeycomb like forth is formed

8. Steroids -Liebermann-Burchard's test: Add 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A greenish colour is developed which turns to blue.

Salkowski Reaction: Add 1 ml of conc. sulphuric acid to 2 ml of chloroform extract of the drug carefully, from the side of the test tube. A red colour is produced in the chloroform layer.

9. Tannins- To 1 – 2 ml of plant extract, add a few drops of 5% FeCl₃ solution were added. A green colour indicates the presence of gallotannins while brown colour tannins.

10. Starch- Dissolve 0.015g of Iodine and 0.075g of Potassium Iodide in 5 ml of distilled water and add 2 – 3 ml of an aqueous extract of drug. A blue colour is produced.

11. Glycosides- Detection of glycoside on paper spray solution No. 1 (0.5 % aqueous sol. of Sodium metaperiodate) & wait for 10 minutes after then spray solution No. 2 [0.5 % Benzidine (w/v) in solution of Ethanol – acetic Acid (4:1)], white spot with blue back ground shows presence of glycoside.

Identity, Purity and Strength

Loss on drying at 105° C/Moisture content

Place about 10 gm of drug sample after accurately weighing it in a tared evaporating dish. After placing the above said amount of sample in a tared evaporating dish dry at 105° C for 5 hours and weigh. After drying tared evaporating dish cool in desiccator for 30 minutes and then weight.

$$\text{The \% of Loss on drying} = \frac{\text{Difference in weight after heating} \times 100}{\text{Weight of sample taken}}$$

Determination of Ash-

Determination of Total Ash

About 2.0g of powdered drug was incinerated in a tared silica dish at a temperature not exceeding 450°C until free carbon was left, cooled and final weight was taken. The percentage of ash calculated with reference to the air-dried drug (PASf, 1987).

Determination of Acid Insoluble Ash

The ash obtained as above method was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and collected the insoluble matter on an ash- less filter paper, washed with hot water and ignited to constant weight.

The percentage of acid - insoluble ash with reference to the air dried drug was calculated (PASf, 1987).

Determination of Sulphated Ash

Heat a silica crucible to redness for 10 minutes; allow cooling in a desiccators and weighing. Put

about 2.0g of powdered drug into the crucible; ignite gently at first, until the sample is thoroughly charred. Cool, moisten the residue with 1 ml of sulphuric acid, heat gently until white fumes are no longer evolved and ignite at $800^{\circ} \pm 25^{\circ}$ until all black particles have disappeared. Allow the crucible cool; add few drops of sulphuric acid and heat. Ignite as before, allow to cool and final weight was taken after two successive operation repetitions.

Determination of Extractable Matter in water and alcohol

About 4.0g of coarsely powdered air dried material, was accurately weighed in a glass stoppered conical flask and macerated with 100ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, then allowed to stand for 18 hours. Filtered rapidly, taking care not to lose any solvent. The extracted matter was dried at 105°C for 6 hours, cooled in desiccators for 30 minutes and then weighed. The percentage extractable matter was calculated.

Identity, Purity and Strength

Foreign matter	=Not more than 1.00 %
Moisture content	= 2.25 %
Total ash	=6.63 %
Acid – insoluble ash	=2.05 %
Sulphated ash w/w	=8.56 %
Alcohol – soluble extractive	= 56.33 %
Water – soluble extractive	=43.67 %

Chemical Test

Steroids	-	+ve
Glycosides	-	+ve
Flavonoids	-	+ve
Tannins	-	+ve
Resins	-	+ve

Fluorescence Study

[Chase & Pratt, 1949. Kokaski, et al., 1958) with some modification]

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S.N-Treatment - Under ordinary light- Under UV % Long (366nm)

1. Drug as such	- Yellowish brown	- Light brown
2. Drug+ Nitrocellulose	- Blackish green	- Dark green
3. Drug + Picric acid	- Yellowish green	- Black
4. Drug + HCl conc.	-Black Dark	- brown
5. Drug + H ₂ SO ₄ conc.	-Reddish yellow	- Yellow
6. Drug + HNO ₃ (50%)	-Green	-Black
7. Drug + 1 N Na OH in Me OH	-Black	-Light green
8. Drug + 1 N Na OH in Water	-Blackish green	- Dark brown
9. Drug + NH ₄ O	-Dark green	- Dark blue
10. Drug + FeCl ₃	-Blackish brown	-Light yellow
11. Drug + Acetic acid Glacial	-Light brown	- Dark yellow
12. Drug + Sudan-III	-Dark grey	-Dark yellow

Mix 0.5 g with 50 ml of water, boil until nearly dissolved, cool, add 0.5 g of Kieselguhr and filter, to the filtrate apply the following tests-

(i)Heat 5 ml of filtrate with 0.2 g of Borax until dissolved, add a few drops of this solution to a test-tube nearly filled with Water, a green fluorescence is produced.

(ii) Mix 2 ml of filtrate with 2 ml of a freshly prepared solution of Bromine, a pale yellow precipitate is produced.

Therapeutic Uses -Udararoga, Jvara, Yakritvikar.

DOSE - 125 - 500 mg of the drug in powder form.

Thin Layer Chromatography

The principle underlying the separation of the compounds is their adsorption at the solid-liquid interface. For successful separation the compounds of the mixture should show different degrees of affinity for the solid support (or adsorbent) and the interaction between adsorbent and the component must be reversible. As the adsorbent is washed with the fresh solvent, the various components move down the column and arrange themselves in the order of affinity to the adsorbent. Those with the least affinity move down the column at a faster rate than those with greater affinity.

Materials used

All the TLC plates used for the analysis were prepared with silica gel containing binder. Most frequently used binder in silica gel is calcium sulphate (Silica gel 60 F254, Merck). Pre coated aluminium sheets were also used which is coated with silica gel 60 F254 (Merck).

Preparation of TLC plates

Required quantity of silica gel was mixed in a glass mortar to as smooth consistency with the requisite amount of water and the slurry quickly transferred to the spreader. The mixture has been spreaded over the plates in thickness of 0.2mm and was allowed to set into a thin layer. The plates were transferred carefully to a suitable holder and after 30 minutes, dried and heated at $100-120^{\circ}\text{C}$ for at least one hour. The plates were kept in a

desiccates after cooling, until required for further use. The pre coated plates were also activated by heating them for 30 minutes at 100°C.

Application of sample

A known quantity of sample was dissolved in a known volume of solvent and the sample applied on pre coated TLC plates.

Selection of Solvent Systems

The choices of the solvents depend upon the nature of the substances to be separated and also in the material on which the separation is to be achieved. The solvents system was selected on the basis of trial and error method and by elutropic series. It has been found that combination of two solvents gave better separation than with a single solvent.

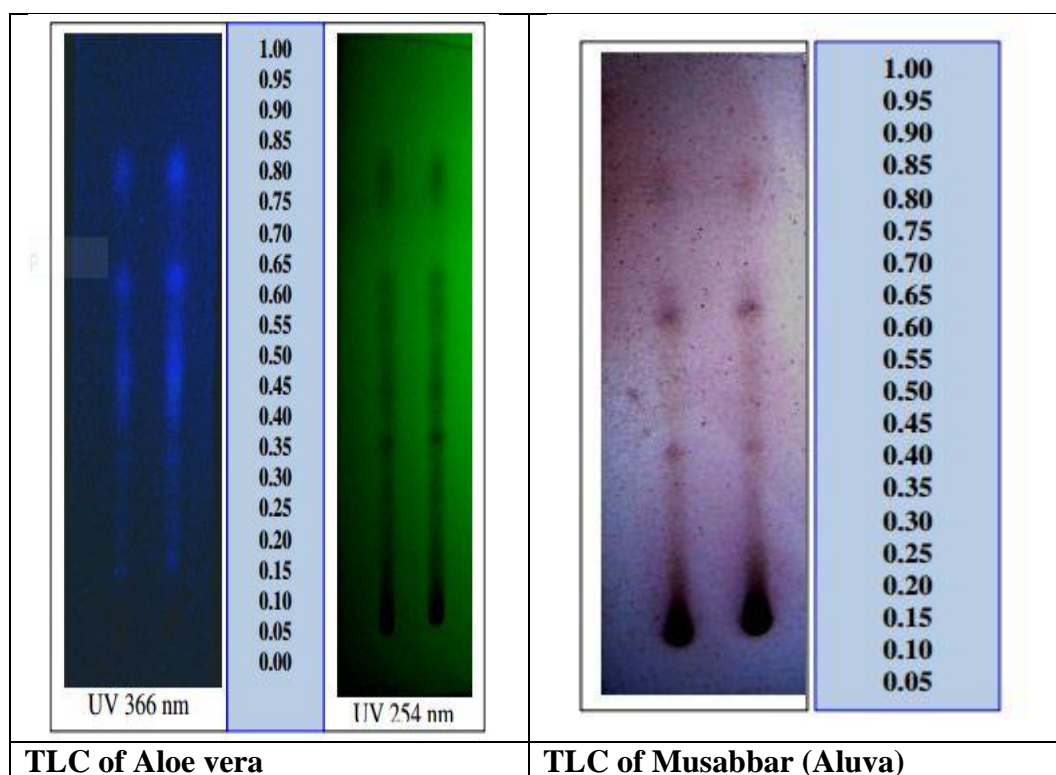
Chromatographic development (separation)

Stationary phase	TLC Aluminium sheet silica gel 60 F 254 plate
Mobile phase	Toluene – Ethyl Acetate – Methanol – Acetic acid (7: 3: 1:0.3)
Rf value of spots Visualized in UV 366 nm	0.10, 0.30, 0.40, 0.60 & 0.75
Rf value of spots Visualized in UV 254 nm	0.24, 0.30, 0.40, 0.50, 0.55 & 0.75
Rf value of spots Visualized after spray of sulphuric acid reagent and heated 110 °C for 5 minutes	0.20, 0.30, 0.55 & 0.75

Development of the chromatogram is effected after the solvent of the applied sample is completely evaporated. Rectangular glass chambers or twin trough chambers are commonly used for TLC development.

Detection of spots

Detection of Rf. Values of spot done by using Iodine exposure and Vanillin Sulphuric Acid Reagent.



Results

TLC results indicate Musabbar (Aluva) Rf value of spots Visualized in UV 366 nm at Rf. 0.10, 0.30, 0.40, 0.60 & 0.75 (5 spots). Rf value of spots Visualized in UV 254 nm at Rf. 0.24, 0.30, 0.40,

0.50, 0.55 & 0.75(6) Rf value of spots Visualized after spray of sulphuric acid reagent and heated 110°C for 5 minuts at Rf. 0.20, 0.30, 0.55, 0.75 (4 spots).

Bibliography for Ghrithkumari (Aloe-vera)**Books**

1. Pandeya G. Dravya Guna Vijnana, Krishnadas Academy, Varanasi.
2. Yagi A. Aloe Vera 1st Ed., DHC, Tokyo, 1997
3. Frawley D. & Lad V., The Yoga and Herbs, 1986
4. Fujita K., Aloe Daihyakka, Shufunotomo, Tokyo, 1996
5. Tanaka K. Medicinal Plant Encyclopedia 1st Ed. Ienohikari5kyokai,

Samhitas

1. Agnivesha, Dridhabal –Charaka Samhita V5VI Part. Shri Gulabkunvarba Ayurvedic Society, Jamnagar 1st Ed. 1949.
2. Ashtaga Hrdaya “Sarvanga Sundara”. “Ayurvedic Rasayana” Tikadvaya Krishnadasa Academy, Varanasi.
3. Bhavamishra, Bhavaprakash Samhita, Vidyotini Commentary, Caukhamba Sanskrit Series, Varanasi, 2nd Ed. 1949.
4. Bhela: Bhela Samhita, Caukhamba Sanskrit Series, Varanasi, 1961.
5. Kashyapa: Kashyapa Samhita Ed. Pandit Hemaraja Sharma & Y.T. Acharya, Nirnaya Sagar Press, Bombay.
6. Sharangadhara: Sharanghadara Samhita Baidyanatha Ayurveda Bhavan, 1975
7. Sushruta Samhita, Ayurveda Tatva Sadipika “Commented by Ambika Dutta Shastri, I5II Part, Caukhamba Sanskrit Bhavan, Varanasi 8th Ed.1993.Singh D.P.
8. Role of certain indigenous drugs on Yuvanapidika in 1982, Jamnagar.Agravat H.P
9. Yuvanapidika and role of Lodhradi lepa there in 1993 from Jamnagar.Gandhi M.
10. Asthentic in medical science and personality damaging disease particular reference to Yuvanapidika in 1995 from Jamnagar.

Web

1. Aloe Vera Company UK, Properties of Aloe Vera Constituents, <http://www.aloevera.co.uk/aloeprop.thm>
2. Aloe Vera Products, http://www.aloeveraproduct.com/about_aloe_vera.htm
3. Herbal Information Center, Aloe Vera, <http://www.kcweb.com/herb/aloevera.htm>
4. Holistic5 online, Herbal Information, AloeVera, http://holisticonline.com/Herbal5Med/_Herbs/h2.htm
5. Properties and Actions of Aloe Vera, <http://www.miyakojima.ne.jp/kyuyou/shoukai.htm>
6. .Aloe Senka, <http://www.wind.ne.jp/hirata/aloesenk.htm>
<http://www.wind.ne.jp/hirata/aloesenk.htm>