Journal of Ayurveda & Holistic Medicine
www.jahm.co.in
eISSN-2321-1563
ORA-EXPT STUDY OPEN ACCESS

HISTOLOGICAL COMPARATIVE STUDY OF DIFFERENT SAMPLES OF ORCHIDACEAE WITH REFERENCE TO MUNJAKATA

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Submitted on- 27-05-24	Revised on- 30-05-24	Accepted on-28-06-24

ABSTRACT:

Background: The histological study has been carried out on three different market samples of *Munjataka*. The organoleptic character includes the taste, texture, color, and odor, while, the microscopic study consists of macro, microscopic structure, and powder microscopy along with evaluation of *rasa*. Also, the physical evaluation comprises of determination of foreign matter, moisture content, total ash, acid insoluble ash, alcohol soluble extractive values, pH, and swelling factor tests, etc. The microscopical findings are presented for all the three groups of samples. **Objectives:** For accomplishing the histological study on the three market samples of *Munjataka* is carried out using macroscopical, microscopical, powder analysis, chemical study, respectively. **Materials and Methods:** The samples were collected from the market and were categorized into three groups (i.e., Group I, Group II, Group III). Then after following the essential procedure, the samples were converted into powder form for carrying out the further testing on them. **Results:** It can be interpreted from the result that present situation is the identity of plant/ vegetable drugs through investigation and research study such that it leads to the proper standardization of the drug.

Keywords: Macroscopic, Microscopic, *Rasa*, Samples, Tuber.

INTRODUCTION:

The WHO assembly in several resolutions emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards [1,2]. Many plants have been described in Materia Medica, irrespective of their habitat, quality, despite the texts prescribing based on material for medicaments, some drugs were incorporated from the rest of Greater Indian continent [3,4]. The drugs from other than India are included as *Anukta Dravya's* that are to be perceived by morphological and linguistics way for conversion and utility [5]. *Charaka* while describing *Munjataka* specified it is a habitat of U*ttarapaduika Swalpa Kand Vishesha* [6,7,8] i.e., Kashmir area known as Mumjawan. *Salampanja* and *Salabmisri* are such other drugs from Iran, Afghanistan and Persia considered as *Munjataka* as *Pushpa Shaka Bheda* [9] of plant/ vegetable by many authors but its proper identity is not established. Drugs are very important tool to the physician for prevention of disease and management of health. Ayurvedic Materia medica consists of crude drugs mainly of plant/ vegetable origin. The unstandardized drugs may produce undesirable effects, moreover,**I.** they may not be of any value for the purpose for which they are being given.

Standardization plays important role as Ayurvedic formulations consisting of drugs which are used in the form of whole plant, part of the plant or plant extract. The scientific standards must be developed for checking the uniformity of the product and to have some sort of control on the manufacture of the drugs [10]. This can be achieved by pharmacognostic identification, physical, chemical, biochemical estimation, and stability studies of standardized drugs for clinical use. It is not only important for India**II.** alone but for global acceptance as well. Hence, the histological study has been carried out on the three market samples namely, *Salampanja* (Group I), *Salammisri* (Group II), and *Salabkatta* (Group III) of *Munjataka* (sold by different names) in form of macroscopical, microscopical, powder analysis and the chemical study, respectively.

OBJECTIVES:

The main purpose of this study was comparing the different samples of *Munjataka*, sold under different names in the Indian market i.e., *Salampanja*, *Salammisri*, and *Salabkatta* by carrying out the histological study of the same. The aim was to focus for the standardization of the ayurvedic drug.

MATERIALS AND METHODS:

The histological study of *Munjataka* includes the collection of different market samples sold under the name of *Salampanja*, *Salammisri*, and *Salabkatta*. The collected market samples were cleaned free from sand, stones, and other extraneous material. Tubers were boiled in water and then dried in sun until quite hard. And then cut into small pieces made into fine powder and then powder being allowed to sieve separately to become the fine powder for the use.

Organoleptic methods:

It describes colour, taste, odour, consistency, etc. H.W. Youngkon claims that in some cases organoleptic test is alone sufficed, these are of first important in the preliminary examination of drug since, the general appearance of the sample, its odour and taste will frequently indicate whether it will satisfy certain official standards or not. T.E. Wallis also claims that, it is more especially colour and general texture of the powder, which is more important than external macroscopical resemblance.

Microscopic Study:

Microscopic study includes the macro, microscopic structure, powder microscopy and the evaluation of rasa.

a. Macroscopic study:

All the samples are studied for external features and their colour, shape, texture etc. have been recorded.

b. Microscopic study:

Schledien (1947) used microscope for the first time for identification of drug. The microscopic study required some preparation of material to facilitate observation. As the samples are larger in size, they are cut into thin slices to expose inner regions and to permit light to penetrate through the object.

The samples were cut into thin slice (0.5 to 0.75 cm thick) and are carefully labelled. As the market samples are dry, hard, they are subjected to boiling in a solution of glycerol and water (1:10) for 30 minutes at 35°C in a thermostat. When they are smooth, they are subjected for dehydration by keeping them in the solution with different proportions of methanol and water and finally with absolute alcohol.

As alcohol is not solvent for paraffin these samples are placed in solutions with different proportions of alcohol and xylene and finally placed in 100% xylene. After this process, keep it in 100% xylene. Then melted paraffin is added according to the ratio of 25:25, 50:50, 25:75, 0:100 to the xylene. The xylene is then eliminated by evaporation i.e., by exposing to heat in the oven. Once the samples were kept in 100% paraffin, for proper infiltration of the paraffin into the samples, kept the samples in melted paraffin for about 24hrs at 55°C.

These samples are moulded into blocks with the help of the blocking machine (Leica). These blocks are made into thin sections with the help of a microtone. These sections are placed on the slide after applying the mixture of Egg albumin Sodium citrate for adhering the sections and then these are placed under the slow running warm water to remove the folds of the sections.

Then these sections were placed in xylene solution for 2 to 3hrs to remove the paraffin (de-paraffinization). After removing the paraffin, sections were stained with fast green and aqueous safranin. Once the staining process is over, these samples are dehydrated and placed in xylene and finally mounted with Canada balsam. In this manner the permanent slides were prepared and subjected for observation and photography, in CH-2 Olympus automatic trinocular microscope.

c. Powder Microscopy:

The shade dried rhizomes were powdered and sieved with 80 mm mesh prepared powders for analysis. Uniformly powdered 100 mg was boiled with 2ml water. After that drop of suspension after thorough shaking was placed on a slide and a drop of Safranin is added to it. After washing with alcohol, the elements were mounted in glycerine and were observed under 15x40 mm magnification. Randomly 5 fields were selected and observed for cell elements.

d. Evaluation of Rasa:

The *rasa* (taste) of any substance can be perceived with *Rasanendriya* (Ch. Su. 26:66). It is evident from the above statement that *Guna* (qualities), *Veerya* (potency), *Vipaka* (post-digestive effect) are to be presumed by action of drug in the body. Hence an attempt has been made to evaluate the *Rasa* of *Salampanja*, *Salammisri* and *Salabkatta* root tuber powder experimentally by Nipata method [11].

Procedure: 30 volunteers were selected to taste the powders of three samples. Before starting experiment, all the volunteers were requested to wash thoroughly their mouth with distilled water. All the volunteers were given by equal quantity (one pinch) of the trial drug for taste. They were instructed

to taste it for complete one minute. The same process was repeated for three times with each volunteer. The results were interpreted as the *Rasa* perceived within half minute, considered as *Pradhana Rasa* (primary taste) and the *Rasa* perceived after half-a-minute as *Anurasa* (secondary taste).

III. Physico-chemical Methods

Physico-chemical standards viz, foreign matter, total ash, acid insoluble ash and determination of swelling factor test of crude as well as finished herbal drugs of products.

a. Determination of foreign matter:

Foreign matter: Drugs should be free from grass, moulds, insects, animal faeces and other contaminations as stones and extraneous material. For this, drug should be carefully observed.

Determination of foreign matter: Weigh 100gm of drug sample to be examined and spread it out in a thin layer. Thin layer matter should be detected by inspection with an unaided eye or by use of a lens (6x). These foreign matters than should be separated and weight is taken.

b. Moisture:

About 10g of the material is weighed into a weighed moisture box and dried in an oven at 110°C and cooled in a desiccator. The process of heating and cooling is repeated till a constant weight is achieved. Now the percentage of moisture with reference to the air-dried drug is calculated.

c. Ash Determination:

When plant/ vegetable drugs are incinerated, they leave some inorganic carbon called ash. 3gms of all the compounds are taken in a silica crucible and heated below 450°C for 6 hours and the weight of ash was calculated.

d. Determination of acids soluble ash:

Obtained ash is boiled for 5min in 25ml of HCl insoluble matter collected in an ash less filter paper washed with hot water and ignite to constant weight.

e. Determination of alcohol/ water soluble extractive value:

The water-soluble and alcohol soluble extractives also help in the quality of the plant material, several pharmacopoeias recommended this test as well as quality control. ***Procedure:*** Accurately weighed 5 grams of coarsely powdered air – a dried material was, placed in glass stoppered conical flask. Macerated with 100 ml of 90% of ethyl alcohol and weighed to obtain the total weighed including the flask. After this it was closed with the lid and allowed to stand for 24 hours with frequent agitation. After filtered using the vacuum filter, 25ml of the filtrate was transferred to a flat-bottomed dish. Then kept in water bath for drying and was dried until no liquid content was left. Thereafter, cooled in desiccators for 30 min. and weighed without delay. The content of extractable matter in mg per gm of air-dried material was calculated.

f. Determination of pH values:

Material required- pH meter, Buffer tablet, and Potassium chloride solution.

The measurement of pH is generally done with a suitable potentiometer known as pH meter fitted with two electrode, one constructed of glass and sensitive to hydrogenation activity and the other a calomel reference electrode. The determination is carried out at a temperature of 25+ or -2°C unless otherwise specified in the individual monograph. ***Procedure:*** The value of solution was determined potentiometrically by means of a glass electrode, a reference electrode and pH meter either of the Potentiometer or the deflection type. First the apparatus was calibrated using buffer solution pH 4 at 20°C. The coarsely powdered drug was added to

the water and stirred for 5 minutes and adjusted at 25°C. The electrode was immersed in the solution to be examined and the pH was measured at the same temperature as for the standard solutions. At the end, a set of measurement and readings were taken (if the difference between the reading and the original value is greater than 0.5, the set of measurements to be repeated). pH can also be measured with the help of pH indicator papers. 1gm of drug was dissolved in 100ml of distilled water, stirred, filtered, and then drop was put on paper. First compared with wide range pH indicated paper, then with acidic or basic paper.

g. Determination of swelling factor:

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin of hemicellulose. ***Procedure:*** Take 1gm of the powder in a 25ml stoppered cylinder, add water up to 20ml marking. Shake occasionally, during 23 hours, keep aside for one hour. Measure the volume occupied by the swollen seeds. Example: Swelling factor of the Isabgol seed is not less than 10ml.

RESULTS AND OBSERVATIONS:

With the help of organoleptic properties, the market samples can be categorized into three groups, i.e., Group I, Group II, and Group III.

Macroscopic Study:

The macroscopic study of all the three groups is shown in Table 1, as:

Table 1: Macroscopic Study of Samples

S. No.	Group I	Group II	Group III
1.	Fleshy dried tuberous root, palmate in shape and divided into 2-5 finger like lobes, round, thick, and sharp.	Round, ovoid small tuberous root, no lobes.	Obpyriform, bulbous root.
2.	Yellowish in colour.	Creemish brown colour.	Black in colour.
3.	Variable sizes and shapes, 1-2.5cm in length and 0.5-2cm in breadth.	2-4cm in length and 0.5-2cm in width.	Variable sizes, 4-6cm in length, 2-3cm in width.
4.	Fracture is hard, round surface, wrinkled lobes, irregular and pointed.	Fracture is hard, surface often unfolded and finely reticulate with vertical striations.	Fracture is hard, surface blackish with vertical striations, often indented on drying at several places.
5.	Mucilaginous and sweet and salty in taste.	Mucilaginous and somewhat sweet in taste.	Mucilaginous and bitter in taste.
6.	Mucilaginous odour.	Milky in odour.	Disagreeable.

The macroscopic study on Group I sample consists of dried tuberous roots of yellowish-brown colour. It occurs in variable size and shape. The outer surface is smooth, longitudinally wrinkled, or sunken. Fracture is hard and the taste is mucilaginous and starchy with peculiar smell. The dried tuberous

rhizome, oblong, ovate, cylindrical, surface often infolded and finely reticulates with vertical striations, often appearing transparent is the macroscopical findings of Group II sample. For the Group III sample, the rhizome is bulbous, obpyriform, with tapering base of varying sizes, internally blackish on exposure.

Microscopic Study:

Group I Microscopic: T.S. of root tuber: The outer most layer of epidermis is single layered consisting of irregular shaped tangentially elongated cells, radially narrow, thin walled covered by a cuticle, contents scanty. Hypodermis 2-3 layered with tangentially elongated cells with few large mucilaginous cells occur in between the cortex is extensive consisting of parenchymatous cells, which are large, polygonal, thin walled, contents dense, with starches, mucilage and few raphide cells are present. Some large vacuolated cells intersperse the cortex. Vascular bundles are present and distributed in the cortex. Vascular bundle is endarch, bicollateral treachery cells in longitudinal section with helical thickenings few annular.

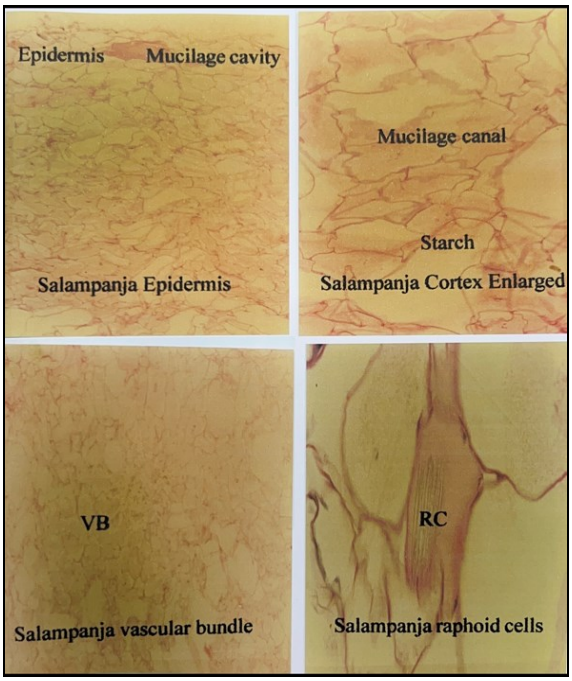

Figure 1: Histology of Salampanja

Group II Microscopic: The outermost layer of epidermis is single layered, often ruptured. The cells of epidermis tangentially elongated, thin walled, radially compressed, contents scanty. The hypodermal cortex is 4-5 layered, made up of barrel shaped to tangentially elongated cells, contents scanty. The inner cortex is extensive made up of polygonal to spherical cells, walls thin, content, dense, with starches, tannins, and raphide sacs, resinous mass, and mucilaginous cells few, dispersed throughout. The cortex is interspersed with many large vacuolated cells. Mucilaginous cells also found towards inner cortex. Vascular patches are present in the cortex.

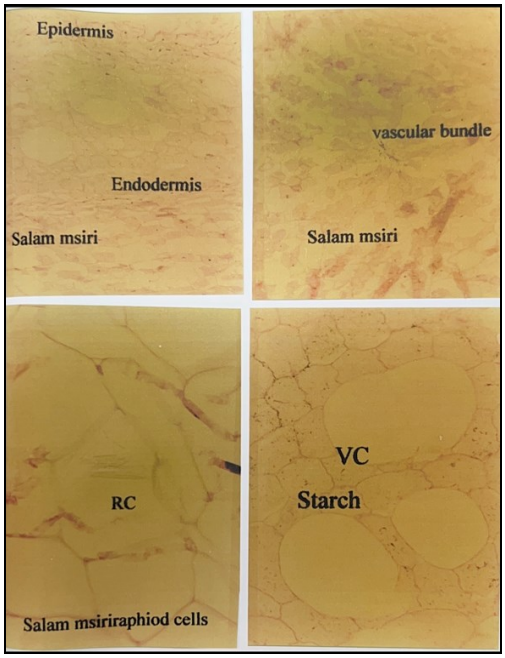

Figure 2: Histology of Salammisri

Group III Microscopic: In T.S, the tuber rhizome shows the single layered epidermis, as the outer most tissue, comprising of tabular cells, which is undulated and often broken, epidermal cells slightly thick walled with a thick cuticle covering. The 6-8 layered hypodermis consists of parenchymatous cells, which tangentially elongated and undulated. Often the cells contain tannins, mucilaginous cells, are frequently encountered in the cortex, which are either solitary or in groups of 2-4 cells. Cell polygonal, slightly thick walled with dense mucilaginous mass. Vascular bundles scattered throughout in the cortex. The vascular bundle consists of a few xylary cells surrounded by phloem with a parenchyma and sieve cells. The cells of the cortex are polygonal to spherical, walls thin, with small intracellular spaces and often filled with starches.

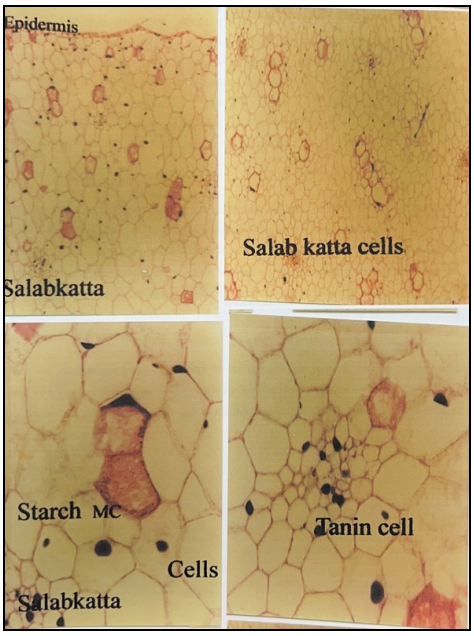

Figure 3: Histology of Salabkatta

Powder Microscopy:

GROUP I

Organoleptic:

Color: Creamish on normal light and light brownish on UV light

Touch: Coarse

Taste: Sweetish

Smell: No characteristic or oily

Microscopic:

a. Large mucilaginous cells, several.

b. Starch grains, compound and single, isolated and in groups of various size and shapes numerous.

c. Isolated cortical parenchyma cells.

d. Cortical cells containing starch grains, few.

e. Pieces of treachery tissue with vessels are tracheid and having helical thickenings.

f. Unidentified cells several.

GROUP II

Organoleptic:

Color: Creamish yellow on normal light and wheat brownish on UV light

Touch: Smooth

Taste: No characteristic

Smell: Milky smell

Microscopic:

a. Fragments of resinous masses appearing translucent.

b. Few sclerified cells of the vascular bundles.

c. Prismatic crystals of calcium oxalate several.

d. Isolated mucilage cells, few.

e. Raphide isolated, randomly distributed.

f. Starches mostly elongated too elliptic and irregularly shaped numerous.

g. Pieces of vascular tissue with helical, scalariform thickenings.

h. Unidentified elements several.

GROUP III

Organoleptic:

Color: Blackish in visible light and snuff brown on UV light

Touch: Finely coarse

Taste: Mucilaginous

Smell: Disagreeable

Microscopic:

a. Mucilaginous cells isolated with dense contents.

b. Fragments of cortical cells in groups, slightly thick walled.

c. Pieces of epidermis with tabular cells and covered by thick cuticle.

d. Pieces of vascular tissue with xylem elements with helical thickenings and attached phloem.

e. Starches isolated few, ovate, oblong, elliptic and spherical slightly large.

f. Unidentified elements several.

Evaluation of *Rasa*:

For evaluating the *rasa* of the samples, 10 volunteers were selected in each group and the result was noted as (Table 2):

Table 2: Evaluation of Rasa

	No. of Volunteers	***Rasa***	***Anurasa***
Group I	10	*Madhura*	-------
Group II	10	*Madhura*	-------
Group III	10	*Katus*	*Tikta*

Maximum number of volunteers i.e., 90% opined that, the *Rasa* of trial drugs, Salampanja, *Salammisri* were *Madhura Rasa* and *rasa* of *Salabkatta*, is *Kattu Tikta*. However, none of the volunteers have given different opinion on rasa in three test trial. Hence, it is concluded that the rasa of *Salampanja*,

Salammisri root tuber powders is *Madhura* and *Salabkatta* is *Kattu Tikta Rasa*.

Physico-Chemical Tests:

For Physico-chemical tests various methods have been adopted for all the three groups, i.e., Group I, Group II, and Group III. The values are tabulated in the Table 3, given as:

Table 3: Physico-Chemical Tests

S. No.	Method Adopted	Group I	Group II	Group III
1.	Foreign matter	1%	0%	2%
2.	Total ash	1.22%	2.73%	3.05%
3.	Acid insoluble ash	2.87%	4.02%	7.01%
4.	Alcohol soluble extractive value	0.96%w/w	1.35%w/w	0.83%w/w
5.	pH value 1%	6	6	6
6.	pH value 5%	6.5	6.5	6.5
7.	Moisture	12.12%	10.38%	10.15%
8.	Swelling factor	7.85ml	6.86ml	6.34ml

CONCLUSION:

The histological study confirms the contents of Group I that contains the sample of *Salampanja* (Dactylorhiza Hatagirea) and Group II consisting *Salammisri* (Eulophia campestris) belongs to the same family of Orchidaciae [12], both market samples having similar characteristics, many vacuolated cells, starch, resinous mass, mucilaginous, raphoid cells and prismatic crystals of calcium oxalates, etc. The market sample of *Salabkatta* with tabular cells, tannins, starch, and dense mucilaginous mass. The pH values shows that all the three samples are in acidic media and swelling factor values shows a content of mucilage 7.85ml, 6.86ml, and 6.34ml for *Salampanja*, *Salammisri* and *Salabkatta* respectively. Also, the taste threshold in human volunteers confirms that the taste of *Salampanja* and *Salammisri* are *Madhura* (sweet) and of *Salabkatta* as *Kattu* (pungent) and *Tikta* (bitter) *rasa*.

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CITE THIS ARTICLE AS

V.V. Rama Rao, Menakshi Pachori. Histological comparative study of different samples of Orchidaceae with reference to Munjakata. *J of Ayurveda and Hol Med (JAHM)*. 2024;12(6):3-12

Conflict of interest: None

Source of support: None