CHAPTER – 6

Organoleptic, Morphological & Microscopical Study

ORGANOLEPTIC AND MORPHOLOGICAL CHARACTERS STUDY

Organoleptic study refers to the evaluation of a drug by sensory macroscopic characteristics viz. colour, odour, size, shape, taste and special features including touch, texture etc. Since the majority of the information on the identity, purity and quality of the drug can be drawn from these observations, they are of primary importance before any further testing can be carried out. Organoleptic evaluation can be done by means of organs of sense which include above parameters and thereby define some specific characteristic (s) of the specimen which can be considered as a first step towards establishment of identity and degree of purity. For this purpose authentic specimen of the plant under study and samples of the Pharmacopoeial Quality should be available to serve as a reference. This evaluation procedure provides the simplest & quickest means to establish the identity & purity and thereby ensure quality of a particular sample. If it is found to be devoid of or significantly different from the specified sensory character, it is considered as not fulfilling the requirements. [7-9]

Morphology is the study of the form of an object whilst morpography is the description of that form. Morphological study is commonly applied to "crude drugs", where the material is known to occur in a particular form. It can be studied for the whole drug i.e. macro-morpography or gross morphology, and for cell characteristics i.e. cytomorphology or study of morphological character at a particular level. Majority of natural products used as drugs are derived from plants or from parts of plants. Interpretations of the morphological characteristics by different parameters, for all the plant parts give first hand tool to know the features of whole or powdered drugs and adulterants of commercial significance. It is most useful for only a part of the plant to

be used, either because the active constituent is only found in a particular part or because of economic consideration, which dictate the collection of certain parts of the whole plant. Based upon the anatomical structure of the plant, the different parts of the plant commonly used for therapeutic categories are leaves, bark, flowers, fruits& seeds, wood, herb or aerial parts etc. [6-11]

Organoleptic and morphological characteristics of *Ammania baccifera* whole plant and *Bergenia ciliata* leaves were observed and evaluated botanically.

STUDY OF MICROSCOPICAL CHARACTERS

Microscopical characteristics of plant species are of great importance to establish their identity. The study is specially applied to those species which are morphologically closely related. Microscopical study involves careful examination of microscopic cells form and their arrangement in a plant drug. The plant drugs are generally used in powdered form where the macromorphology is destroyed hence, evaluation of the microscopical cell characters becomes essential. Consideration must therefore, be given to the types of cell & cell inclusion and the manner in which they are distributed in different organs and drugs. The plant drugs contain some basic cell types e.g. parenchyma, collenchymas, sclerenchyma, epidermis, xylem & phloem etc. along with some cell inclusion characteristics i.e. presence of ergastic substances like starch, calcium oxalate crystals, aleurone grains etc. Microscopical study of plant drugs based on the distribution of these various cell types within different organs is of important to ensure the identity and quality of herbal drugs.

The basis of the analysis by evaluating cytomorphological characters is that there are always sufficient differences in the same type or different types of plants as far as the cell characteristics are concerned. Such differences may be very prominent e.g. the presence or absence of a particular specialized cell (such as sclereid) or it may be so marginal that they need to be established by optical micromeritics.

Microscopical techniques provide detailed information about the crude drugs by virtue of its two main analytical uses. Firstly, its property to magnify permits the fine structures of minute objects to be visualized and thereby confirm the structural details of plant drugs under evaluations. Secondly, these techniques can be used in the

determination of optical as well as micro-chemical properties of the crude drug specimen under study. Microscopical inspection of plant crude drugs is essential for their identity, and study often yields information that cannot be obtained by any other method. Though microscopy alone cannot provide complete evaluation profile of a herbal drug, still it can provide supporting evidence, which when combined with other analytical parameters can be used to obtain the full evidence for standardization and evaluation of herbal drugs. [12-14]

Bergenia ciliata leaf was subjected to following microscopical evaluation parameters to establish its identity data.

a) Study of histological characters of transverse section of *Bergenia ciliata* leaf

b) Powder microscopy of dried and grounded *Bergenia ciliata* leaf

c) Determination of linear leaf constants

Transverse section of the leaf

Microscopical study of a section of the crude drug provides information on how various cells / tissues have been arranged in an organized crude drug and which are all the tissues / cells have taken up the applied stain to appear in different in colour. In case of a leaf drug, the important aspects to study are a section through the midrib taken perpendicular to the midrib and observation of a surface preparation. The histological characters (at times diagnostic features) which cannot be observed in the powder drug may be seen in the intact section of a leaf and *vice-versa* is also true. Hence, microscopical study needs to be carried out for both section and powder of a crude drug. [14]

Procedure- The sections were taken by placing the leaf portion cut along with the midrib in between the two flat surfaces of pith. Pith is usually a piece of potato (about 3x1x1cm) in which the longitudinal slit of 2 cm depth was made, into which leaf placed and sections were taken by slicing. Transferred the sections into watch glass containing water, added chloral hydrate solution (clearing agent), heated for few minutes and the sections were stained with phloroglucinol & hydrochloric acid (1:1); and then mounted in glycerin and observed under low power (10X). The microscopical characters were traced on the black paper using camera lucida. [15-17]

Powder microscopy:

The leaf was dried naturally and made into a fine powder. Sufficient chloral hydrate solution was added, mixed well & heated for few minutes. Then added few ml of phloroglucinol & toludiene (1:1, staining agents) mixed well and allowed to react for few minutes and observed under low (10X) and high power (40 X) for the microscopical features. [18-19]

Linear leaf constants:

These are the values of leaf that remains constant, within a range, irrespective of the age of the leaf. These constants are of diagnostic value in differentiating closely related species.

1) Stomatal number and Stomatal index
2) Vein-islet number and Vein-termination number
3) Palisade ratio.

Stomatal number and stomatal index:

The Stomatal Number (SN) and Stomatal Index (SI) are very specific criteria for identification and characterization of leafy crude drugs. Stomatal number is the average number of stomata present / sq.mm of epidermis on each surface of a leaf. Each stoma consists of two guard cells and the central spore, and is counted as a single unit. Stomatal index is one of the more distinguishing characteristics of herbal leafy drugs. Stomatal index is defined as the percentage which the stomata form to the total number of epidermal cells, each stoma being counted as one cell.

Procedure: Placed fragments of leaves, about 5x5 mm in size, from middle and lamina portion, in a test tube containing about 5 ml of chloral hydrate and heated on water bath for about 15 minutes or until the fragments are transparent. Transferred fragments to the slide, added drop of glycerol and mounted under microscope. Examined under the microscope with a 40X objective. With the help of stage micrometer and prism camera lucida drawn a square on a black drawing sheet and drawn the epidermal cells and stomata. Stomatal Index $SI = S \times 100 \div E + S$

Where, S = Number of stomata in a specific area E = Total number of epidermal cells in the same area of leaf.

Vein-islet Number:

Vein-islet is the term used to indicate the minute areas of the photosynthetic tissue encircled by vascular stands. A vein islet is the smallest unit of the tissue encircled by the ultimate divisions of the conducting strands of the leaves. The number of vein islet present per square mm of leaf surface area, calculated from the lamina midway between the midrib & margin, is termed as **vein-islet number**. This number per unit area of leaf is constant. It can be used as a distinguishing characteristic to differentiate between different species of the same plant or between different plants.

An ultimate free end or termination of a veinlet is called veinlet termination, and the number of vein terminations per square mm of leaf surface midway between the midrib & margin is termed as **Veinlet termination number**.

Procedure: A portion of leaf taken from the midway between the midrib and the margin and cleared with chloral hydrate. Using 5mm objective lens, stage micrometer and prism camera lucida, focused stage micrometer (1mm) and fixed the prism camera lucida. Marked the first and the last line of the stage micrometer (mm scale). Measured these two points, joined them and made a square (1mm square). Removed the stage micrometer and mounted the slide with the leaf specimen and focused the same. Adjusted the square drawn on the paper in such a way that it lies exactly in the middle of the field of vision. Closed the iris diaphragm partially and adjusted the illumination. The image of the leaf piece mounted appears to overlap (superimpose) the square on the drawing sheet. Started from any one side and traced all the vein islets inside the square and also completed those, which are on the boundary of the square. Along with veins-islets, traced the vein terminations also, which are inside the square only. Took 4 readings from continuous squares (or rectangle as the case may be) and traced the vein-islets within it.

Palisade ratio:

Palisade ratio is defined as the average number of palisade cells present beneath each upper epidermal cell. This value does not alter based on geographical variation and difference from species to species so it is a very useful diagnostic feature for characterization and identification of different plant species.

Procedure: Cleared a small portion of leaf with chloral hydrate solution. Mounted and examined using a 5mm objective lens. Arranged a prism camera lucida in such a way that the epidermal cells and the palisade cells lying below them can be traced. First, drawn a number of groups of each of 4 epidermal cells then change the focus to enable the palisade cells to be drawn within the epidermal cells. Counted the palisade cells including those, which are more than half covered by the epidermal cells. [19-22]