Evaluation of Herbs and Processed herbs

Pharmacopoeia specifies and describes in individual monographs tests for identification of herbs and processed herbs. These tests involve morphological, microscopic, organoleptic and chromatography profile as applicable to individual monograph entity. These tests primarily are based on pharmacognostic characterization and are aimed to assist in botanical identification of the herb. Wherever possible the monograph also provides tests to differentiate from potential adulterants or substitutes or closely related herbs. Pharmacopoeia has also provided testing for DNA barcode as a confirmatory test of identity in case of difficulty or dispute for correct identity basis tests mentioned above. Currently DNA barcode test is not mandatory if identity is possible on the basis of data of the other tests mentioned above. Monographs on herbs cover different parts of the plants namely barks, roots, flowers and their parts, whole plants, fruits and their parts, underground stems, leaves, woods, seeds and unorganized herbs and any other specific part of the plant which becomes the ingredient for use in different formats for external or internal consumption for animals and humans. These may also be the starting material for obtaining processed herbs, phytopharmaceuticals or purified chemical compounds. Botanical identity confirmation of such part or plants forms a mandatory requirement in the monograph and is a requirement for quality of products made from them. Monographs in Indian Pharmacopoeia cover either organized herb or unorganized herbs. Organized herbs contain tissues of the plant or part of the plant and powdered herbs. Unorganized herbs are produce of plants such as gums, mucilage, resins, juices and latex. Concentrated extracts, fractionated extracts and distilled or expressed oils are also unorganized herbs. Such unorganized herbs have a fairly uniform composition and are devoid of any cellular structure.

Pharmacopoeia suggests comparison of the test substance with samples of herbs whose authenticity has been confirmed kept as reference material or to a herbarium wherever possible. Indian

Pharmacopoeia also provides for use of pharmacopoeial Botanical Reference Substance (BRS) as part of monograph for comparison while undertaking chromatographic profile test.

Tests for identity by macroscopy is conducted through tests for colour, odour, taste, size, form, fracture, marking and other pharmacognostic features of the underground material received and collected as a sample for evaluation. Except for ready ground material herbs are received in their whole, broken to small sizes or in crumpled form for further use. Where prescribed tests are to be conducted as described below which provide broad methods.

Organoleptic Evaluation

Color**:** Examine the color of the sample in diffused day light or artificial light, similar to day-light. For herbs like barks and leaves, colors on the outer and inner side should be noted separately. The colors are expressed as primary or secondary colors. In case of mixed colors i.e. yellow and brown, if, brown is predominant the color is expressed as 'yellowish brown' and if yellow is predominant express the color as 'brownish yellow'.

Odour***:** If a volatile component is present in the herb, it gives an odour otherwise the herb is odourless. If the material is expected to be innocuous, place a small portion of the sample in the palm of the hand or in a beaker of suitable size, and slowly and repeatedly inhale the air over the material. If no distinct odour is perceptible, crush the sample between the thumb and index finger or between the palms of the hands using gentle pressure. If the material is known to be dangerous, crush by mechanical means and then pour a small quantity of boiling water onto the crushed sample in a beaker. First, determine the strength of the odour (none, weak, distinct, strong) and then the odour sensation (aromatic, fruity, musty, mouldy, rancid, etc.). A direct comparison of the odour with a defined substance is advisable (e.g.

peppermint should have an odour similar to menthol, cloves should have an odour similar to eugenol).

Taste***:** It is known that the odour also contributes to the taste. 'Taste' is used as criteria for evaluation of some herbs containing bitter or pungent compounds, to determine the total bitterness as 'bitterness value' official in British Pharmacopoeia for Gentian. The threshold of bitter concentration of the extract of the herb is compared with a dilute solution of Quinine Hydrochloride. The bitterness value is expressed in unit's equivalent to the bitterness of solution containing 1 g of quinine hydrochloride (R) in 2000ml. Similarly for herbs with pungent taste it is possible to grade them based on heat value which is compared to the pungent and hot effect given by a standard solution of capsaicin. Indian Standard Specification provides details of heat value determination to not only qualitatively determine the taste but also quantitatively estimate the same. Taste is specified by using terms such as sweet, salty, sour, pungent, slimy, bitter, acrid, warm and astringent.

*Keeping in mind safety and potential risk tests for odour and taste are being mentioned in monographs only for information purpose and not as tests for quality as part of monograph. These tests to be undertaken by trained personnel with adequate safety precautions when prescribed as a part of the monograph.

Size**:**Measure the length, width and thickness of crude herbal material and report values in millimeter or centimeter.

a. For large samples: Take measurements directly. Place the sample on the scale measure the length, width and thickness.

b. For very small samples: Like small seeds and fruits, arrange about 10 samples in a straight-line lengthwise measure and take the average as length. For width, arrange10 samples width wise in a straight-line measure and take the average.

c. Herbs in shriveled or matted condition: These herbs need re-hydration eg: thin leaves (*Digitalis purpurea*), flowering tops (*Datura*

metel), flowers (pyrethrum) etc., in this condition the size and shape cannot be studied. Care should be taken not to break the mass while rehydrating the dried samples for evaluation

Take the sample of drug that is to be re-hydrated. Soak the herb mass in water for a few minutes. Remove the excess water using blotting paper. Expand the leaves with the fingers on a blotting paper and carefully press and remove the excess water.

OR

Pour some water at the bottom of a dessicator, keep the herb on a blotting paper in the middle. Close the desiccator and leave it over night. Next day take the herb; spread them on a blotting paper. Press and preserve it for morphological and microscopical studies.

OR

Keep the dry herb between wet blotting papers for few hours and carefully spread them out when they are soft and study.

Form**:** Depending on the different morphological group, the form may be whole, separated, broken, cut, peeled and unpeeled.

Surface characteristics (markings), texture and fracture characteristics**:** Examine the untreated sample. A magnifying lens (6x to 10x) may be used. Wetting with water/reagents as required, may be necessary to observe the characteristics of a wet surface. Touch the material to determine if it is soft with hand; bend and rupture it to obtain information on brittleness and appearance of the fracture-fibrous, smooth and granular.

Document the overall form of the sample under examination through photography. Adopt SOP for photography already specified in the pharmacopoeia.

The following section provides broad information on pharmacognostic description to aid in analysis of samples.

Woods

Wood normally is the central portion of the stem or the root of the perennial trees. Commercial supplies of wood may be in large and dense (Sandal wood) pieces either horizontally or transversely cut pieces or as shavings, raspings and chips (Quassia wood) to aid drying.

Wood is differentiated as heartwood or duramen and the sapwood or alburnum consisting of the conducting tissues. Heartwood is the dead tissue darker in colour and adds only to the mechanical strength. Tannins and resins are deposited in this tissue. Sapwood forms the drug. Terms that describe different characteristics of fracture are ring, porous and diffused. Markings are described with terms like annual rings, straight, grained or interlocked grains. Woods may be either heavy or light in nature.

Roots and under-ground modifications of stem

Herbs both roots and under-ground modifications of stem include rhizomes, corm, stolon, tubers and annular roots.
Broad guidance is provided related to roots or modified underground stems.

Table 1: Herbs from roots, stems and their modifications

Part	Examples
Roots only	*Aconitum napellus* (tuberous) *Atropa belladonna*, *Rauvolfia serpentina*, *Carapichea ipecacuanha* (annular roots)
Stem modifications	*Colchicum autumnale* (corm), *Dioscorea alata* (tuber), *Zingiber officinale* (rhizome), *Curcuma longa* (rhizome), *Rheum* (rhizome)
Herbs containing root	Gentian, *Picrorhiza kurroa*,

and rhizome	*Podophyllum peltatum, Valeriana officinalis*
Herbs containing root and stolon	*Glycyrrhiza glabra*

Table 2: Morphological difference between stem and root

Character	Stem & Under-ground stem modifications	Root
Position	aerial	Underground
Growth	vertical, horizontal, or oblique	Vertical
Form	straight	Tortuous
Leaves, scale leaves or scars	present	Absent
Node and inter nodes	present	Absent
Terminal or axillary buds	present	Absent
Centre (dicotyledons)	soft (pith)	hard

Seeds

The Seed is represented by embryo (plumule, radical and one or two cotyledons), endosperm (storage tissue containing protein and fat), nucellus (perisperm containing starch), seed coat, coverings of the ovule (testa and tegmen), pericarp (separate or fuse with the seed coat). There are two types of seeds: Endospermous or Albuminous and Non- endospermous or Exalbuminous.

Table 3: Types of seeds

Endospermous or Albuminous	Non-endospermous or

	Exalbuminous
Food storage outside embryo	Food storage in the embryo
The embryo is small and endosperm remains as a permanent food storage tissue of the seed.	The growing embryo completely uses up the endosperm resulting in the embryo occupying the complete seed cavity. A thin layer may be present some times as in *Prunus amygdalus* (almond) seeds
i. Herbs as only endosperm example *Strychnos nux-vomica* and *Plantago* (isaphgol) ii. Herbs as endosperm and perisperm example *Myristica fragrans* (nutmeg), *Elettaria cardamomum* (cardamom) and *Areca catechu*	Herbs as oily cotyledons: example *Amygdalus communis* (bitter almond), *Arachis hypogaea* (groundnut) and *Brassica nigra* (mustard) seed

Kernel forms the herb in many seeds. It is embryo with cotyledons covered with a thin testa. The markings of the seeds can be seen on the kernel as depressions or grooves indicating the positions on the seed. Different parts of ovule are seen on the seed: seed coat**,** the outer and inner integuments of the ovule form the testa and tegmen of the seed. The characteristics of the seed coat help in the identification of the seed at species, genus or family level.

Hilum**,** the point of attachment of the stalk or funicle; micropyle**,** the small opening at the apex of the integuments; chalaza**,** the point not visible but can be seen as a depression in some seeds; Raphe**,** the

extension of funicle growing along side of the ovule, in some seeds there are outgrowths of tissues from different parts; Aril, covering arising from the funicle end, example mace in *Myristica fragrans*; Arillode**,** covering arising from the micropyle end as in *Elettaria cardamomum*; Caruncle**,** fleshy growth from the micropyle end as in *Ricinus communis* (Castor) seed; Strophiole**,** along the line of raphe in *Colchicum autumnale* seed; Wing**,** the extension of testa as a fold in *Strophanthus kombe*; Awn, elongated feathery extension as in *Strophanthus kombe*.

Table 4: Type of ovule and presence/absence of Raphe

Type of ovule	Raphe	Example
Orthotropous ovule	Raphe is absent	*Strychnos nux-vomica* and *Plantago*
Amphitropous ovule	Raphe is short; half the length of seed	*Strophanthus kombe* and *Cascabela thevetia*
Anatropous ovule	Raphe is as long as seed	*Prunus amygdalus*, *Linum usitatissimum*, *Myristica fragrans* and *Elettaria cardamomum*
Campylotropous ovule	Raphe is absent	Solanaceous seeds

Barks

Barks are the outermost covering of stems and roots in the dicot plants. 'botanical bark' consists of the cork and cork cambium only. The 'pharmacognostica**l'** bark consists of all the tissues present after the secondary growth, starting from the cork outside to the cambium inside.

If all the tissues are present, bark is known as the **'**whole bark'. The cork and the cork cambium forms 'outer bark'. The secondary cortex

and the primary cortex and endodermis together form the ‘middle bark.’ The pericycle, primary and secondary phloem forms the inner bark along with the medullary rays. In some barks, the outer and the inner barks are scrapped off as these tissues do not contain any therapeutically active constituent’s example *Cinnamomum zeylanicum* and *Quillaja saponaria*.

1. Shape -The shape of the pieces depend upon the type of incision made at the time of its removal from the tree trunk and also upon the nature of shrinkage during drying.

i. Flat - Large pieces of bark often dried under pressure becomes flat in appearance example *Tecomella undulata* and *Boswellia serrata.*

ii. Curved - The bark is slightly concave towards the inside example *Stereospermum suaveolens*.

iii. Re-curved - The concavity is on the outside example *Holarrhena antidysenterica* and *Wrightia tinctoria*.

iv. Channeled - The curvature on the inside is deep enough to form a deep trough or channel example *Myrica esculenta.*

v. Quill – The curvature is present and one edge overlaps the other example *Spondias pinnata*.

vi. Double quill -Each edge is rolled independently into a quill example Cinnamomum *zeylanicum*.

vii. Compound Quill - When quills are packed one inside the other.

2. Outer Surface- The colour and condition of the outer surface gives a characteristic identification to the bark.

i. Scaly - Layers of dead phloem alternating with bands of cork frequently form a massive external covering of the bark (rhytidoma) and gives a scaly appearance.

ii. Smooth - When the cork is evenly developed.

iii. Lenticels - The smooth surface of bark is frequently marked with lenticels, which are commonly elongated and generally placed transversely to the long axis of the bark.

iv. Cracks and Fissures - Due to the continuous increase in girth of tree and lack of elasticity of dead tissues, cracks and fissures are formed and are often characteristic e.g. with clean cut edges as in *Cinchona succirubra* but thickened, uncurved edges as in *C. officinalis.*

v. Warts - On older barks small circular dusty patches frequently develop in the cork.

vi. Wrinkle - The shrinkage of barks during drying occurs generally transversely because the fibres prevent the shrinkage in longitudinal direction and the greater shrinkage of the softer tissues results in the formation of wrinkles.

vii. Furrows - If troughs between the wrinkles are very wide.

viii. Presence of Epiphytes - The additional character is provided by the presence of lichens or bryophytes, including both liverworts and mosses.

3. Inner Surface - The color and condition of the inner surface of the bark also has diagnostic value.

i. Striations - The shrinkage results in the formation of parallel longitudinal ridges which are sometimes very fine or quite coarse.

ii. Corrugations - The longitudinal shrinkage produces parallel transverse wrinkles and is termed as corrugations.

iii. Network of raised lines - Very unequal shrinkage of portions of differing hardness associated with an anastomosis of the fibre strands example *Gmelina arborea*.

iv. Smooth - When the innermost tissues are uniformly soft, on drying, they form a firm dense layer giving a very hard smooth inner surface, example *Myrica nagi.*

4. Fracture - The appearance and behavior of the bark when broken across transversely, subjected to sufficient pressure is known as fracture.

i. Complete- breaking clean across.
iii. Incomplete- breaking only part of the way across.
iv. Fibrous- breaking that the broken ends have a jagged appearance from projecting fibres or fibro-vascular bundles.
v. Flexible- breaking only by tearing or twisting.
vi. Brittle -crumbling readily into fragments.
vii. Granular-peculiar, grain-like appearance of the broken surface.
viii. Hard- exhibiting a compact surface when broken.
ix. Horny- exhibiting a hard, horn-like surface.
x. Mealy-breaking readily across the drug and exhibiting a starchy, broken surface.
xi. Resinous- exhibiting a smooth, glossy, broken surface.
xii. Conchoidal- resembling the resinous fracture, but the surface is characteristically curved in convex and concave fashion.
xiii. Sharp- breaking straight across.
xiv. Short- breaking quickly.
xv. Smooth -breaking with a smooth surface.
xvi. Splintery -breaking irregularly across into pieces with larger and smaller projecting edges and splinters.
xvii. Tough -breaking only after twisting or tearing.
xviii. Uneven- breaking in such a way as to exhibit irregularly broken surfaces.
xix. Waxy- exhibiting peculiar, dull, granular, broken surfaces.
xx. Weak -breaking quickly with little effort.
xxi. Laminated- the fractured region breaks into stratified layers.

Fruits

Fruits develop from the fertilized ovary. The ovary wall develops as pericarp of the fruit and the ovules as seeds. The crude drugs may be derived from the whole fruit including the seeds, like umbelliferous fruits, *Piper nigrum*, *Cassia angustifolia* (senna) fruits, or the outer part of the fruit 'pericarp', example *Citrus limon* (lemon peel).
The pericarp is divided into outer epicarp, the middle mesocarp, and the inner endocarp. The mesocarp contains the vascular tissue. The endocarp may fuse with the seed coat as in *Foeniculum vulgare* and *Piper nigrum*, or form a hard cover for the seed as in *Prunus amygdalus* and *Myristica fragrans*.

The fruit also shows the number of locules from which it develops. The fruit developing from epigynous (superior) ovary may have some floral parts attached to it, persistent calyx at the base of the fruit, e.g. *Capsicum annuum*, *Datura metel*. In hypogynous (inferior) ovary; calyx and stylopod are seen on top of the fruit as in umbelliferous fruits.

The arrangement of ovules/seeds on the placenta is known as 'placentation'. Dehiscence of the fruit and its carpels are some of the points to be noted in the identification of fruits.

Table 5: Classification of fruits

Drug	Part	Type of fruit	Characteristics
Black pepper	Mature unripe fruit	Sorosis	From catkin
Capsicum	Ripe fruit	Berry	Persistent calyx
Lemon peel	Epicarp and mespcarp	Outer part of hesperidium	Oil glands
Myrobalan	Epicarp and mespcarp	Drupe	Hard endocarp
Star anise	Follicles	Composite fruit (etaerio of follicles)	Apocarpous ovary
Umbelliferous fruits	Mature fruit	Cremocarps	Sepals, stylopod

Leaves

Leaves are the appendages of the stem. They are green in colour due to chlorophyll and traversed by supporting or conducting tissue, the veins. ‘Lamina’ or ‘blade’ is the expanded portion attached to the stem by a stalk or petiole. It has two surfaces: the one closer to the internode or faces upwards is adaxial surface and the one lower is abaxial. The leaf with petiole is petiolate and without, it is sessile**.** In some leaves, stipules are present at the base of the leaf called as stipulate**,** if the stipules are absent, called as **exs**tipulate**.**

Leaves may be simple or compound. The leaf makes an angle with the stem, ‘axil’. Usually an axillary bud or a branch can be seen in this axil of the leaf.

Compound leaf**:** the lamina is divided into smaller units ‘leaflets’, all lying in the same plane. A leaf with even pairs of leaflets is a paripinnate leaf and a leaf with even pairs of leaflets but with one odd leaflet at the tip is an imparipinnate leaf. The leaflets usually have asymmetric bases.

Venation**:** The conducting tissue from the stem, through the petiole, extends as a midrib (1°) into the lamina and gives rise to secondary (2°), tertiary (3°) and smaller veins going up to (7°). Dicotyledons show network of veins, the reticulate venation and Monocotyledons show parallel venation in which all veins are same and run parallel to each other.

In some leaves with reticulate venation, the secondary veins join the upper veins near the margin forming a continuous line of vein at the margin, known as anastomisation

Important identification features in the study of leaves are the shape, apex, margin, base, texture, surface and the projection of the midrib

towards upper or lower surfaces, the angles at which the secondary veins leaves the midrib and anastomisation.

The terms describing the shape, apex, margin, base, etc., are the same for leaflets also. Texture is described by the terms membraneous, chartaceous, thick, stiff, coriaceous, leathery and tough. Surface by terms glabrous, pubescent and hirsute.

Flowers

Few flowers or their parts are used as herbs. Calyx, corolla, androecium and gynoecium are the four main parts in a flower. These are arranged on top of the vegetative axis, the thalamus or torus in a definite pattern. Pedicel or stalk, bract and bractiole are the other minor parts.

Study the flower as it is if ‘firm’. Rehdydrate the flower if in crumpled form. Look for other parts associated with the flower like pedicels, peduncle, etc. Note the colour, odour, taste and size. Study the types of inflorescence.

Calyx**:** Sepals: Note the number, colour, shape and aestivation.

Corolla: Petals: Note the number, colour, shape and aestivation. Study the aestivation either in the flower or in the bud.

Androecium: Stamen: Note the number, size and colour and their arrangement with respect to the petals, their grouping and attachments. Study the filament, anther lobes, their dehiscence and pollen grains.

Gynoecium: Study the ovary, style and stigma. Note any nectar glands at the base of the ovary. Study the position of ovary whether inferior, superior or half superior and half inferior.

Entire Organisms

These herbs contain the whole aerial parts including stems of particular diameter, leaves, buds, flowers, fruits and roots.

The respective morphological groups may be referred to identify the specified parts of the herb. Few examples of entire organisms are as follows. Cannabis (Cannabis sativa), Ergot (Claviceps purpurea), Ephedra (Ephedra gerardiana), Lobelia (Lobelia nicotiana), Belladonna (Atropa belladonna), Hyoscyamus (Hyoscyamus niger), Stramonium (Datura stramonium), Taxus (Taxus brevifolia).