UNIT – IV

5. Quality Control of crude Drugs

Adulteration of crude drugs and their detection by organoleptic, microscopic, physical, chemical and biological methods of evaluation including Quantitative microscopy. WHO guidelines for standardization of medicinal plants.

QUALITY CONTROL OF CRUDE DRUGS

The chemistry of plants is as divergent as the great variety of forms in which plants occur. The therapeutically important constituents are usually found to be associated with many inert substances such as colouring matters, cellulose, lignin, cutin, suberin, etc. The active principles are extracted from plant drugs and purified for therapeutic utility for therapeutic utility for their selective and regulated activity. The quality control of herbal crude drugs and their bioconstituents is of paramount importance in justifying their acceptability in modern system of medicine. One of the major problems faced by user industry is nonacceptability in modern system of medicine. One of the major problems faced by user industry is non- availability of rigid quality control profiles for herbal raw materials and their formulations. With the advent of new analytical tools and sophisticated instrumental technology, it is possible to suggest a practicable quality assurance profile for a crude drug or its bioactive constituent.

Owing to the medicinal properties attributed to a crude drug, it is necessary to maintain its quality and purity in commercial market. It is, however, observed that the drugs in commerce are frequently adulterated and do not comply with the standards prescribed for authentic drug.

[A] DRUG ADULTERATION

Adulteration is a practice of substituting original crude drug partially or wholly with other similar looking substances but the later is either free from or inferior in chemical and therapeutic properties. Adulteration in simple terms, is debasement of an article. The motives for intentional adulteration are normally commercial one and are originated mainly with the intention of enhancement of profits. Some of the reasons that can be cited here are

scarcity of drug and its high price prevailing in market. The adulteration is done deliberately, but it may occur accidentally in some cases. It is also very common with the contraband drugs. Adulteration in volves different condition such as deterioration, admixture, sophistication, substitution, inferiority and spoilage. **Deterioration** is impairment in the quality of drug, while admixture is addition of one article to another due to ignorance or carelessness or by accident. **Sophistication** is the intentional or deliberate type of adulteration. **Substitution** occurs when some totally different substance is added in place of original drug. **Inferiority** refers to any substandard drug, and **spoilage** is due to the attack of microorganisms.

A veriety of adulterants are found in natural drugs by which the methods employed for such practices can be enumerated. During the routine quality control, various tests are applied for their detection. The present chapter deals with different

techniques used in adulterating crude drugs and laboratory methods for their detection.

Types of Adulterants

Generally, the drugs are adulterated by substitution with substandard commercial varieties, inferior drugs or artificially manufactured commodities. The different types of adulterants found in market are given here

(i) Substitution with substandard commercial varieties : The adulterants used here may resemble original crude drug by morphological, chemical or therapeutic characters, but are substandard in nature and hence cheaper in cost. This is rather a most common practice of adulteration. The examples are presence of Strychnous nux-blanda or S. potatorum in place of S. Nux- vomica; Capsicum minimum replaced by C. annuum; Indian seena substituted with Arabian senna and dog senna; gentian substituted by kutki; medicinal ginger replaced by its inferior varieties, viz. African, Japanese and Cochin ginger.

(ii) Substitution with superficially similar inferior drugs : These inferior drugs used may or may not be having any chemical or therapeutic value as that of original natural drug. Due to their morphological resemblance to authentic drug, they are marketed as adulterants Balladonna leaves are substituted with Ailanthus leaves; saffron is admixed with

dried flowers of Carthamus tinctorius; scented bdellium is used for myrrh; mother cloves and clove stalks are mixed with clove; and beeswax is subsitituted by Japan wax.

(iii) Substitution with artificially manufactured substances : It has been also observed that substances artificially prepared to resemble original drug are used as substitutes. Generally, this practice is followed for much costlier drugs. Compkressed chicory in place of coffee; paraffin wax made yellow coloured and substituted for beswax, properly cut and shaped baswood for nutmeg are some of the examples representing this type of adulteration.

(iv) Substitution with exhausted drugs : In this type, the same drug is admixed but is devoid of any medicinally active constituents as they are already extracted out. This practice is more common in case of volatile oil containing drugs like fennel, cove, coriander, caraway etc. Sometimes, natural characters of exhausted drugs like colour and taste are manipulated by adding other additives and then it is substituted, e.g. exhausted gentian made bitter with aloes, artificial colouring of exhausted saffron, etc.

(v) Besides these common practices, sometimes other methods are employed like use of synthetic chemicals to enhance the natural character as in case of addition of benzyl benzoate to balsam of Peru.

(vi) Presence of vegetative matter from same plant : Sometimes, the other miniature plants growing alongwith medicinal plant are mixed with drug due to their resembling colour, odour and in some cases constituents. The lower plants like moss, liver worts and epiphytes growing on bark portion are mixed with cascara or cinchona. The stem portions are mixed alongwith leaf drugs like stramonium, lobelia and senna.

(vii) Harmful adulterants : Several times, the wastes from market are collected and admixed with authentic drugs. This is particularly noticed for liquids or unorganised drugs The examples like pieces of amber coloured glass in colophony, limestones in asafoetiada, lead shot in opium, white oil in coconut oils, cocoa butter mixed with stearin or paraffin indicate this type of adulteration practice. The addition of rodent faecal matter to cardamom seed is a very harmful adulterant.

(viii) Adulteration of powders : Besides the entire drugs, the powdered forms are frequently found to be adulterated. Some examples which can be cited here are dextrin in ipecacuanha, powdered liquorice or gentian admixed with powdered olive stones, exhausted ginger powder in powdered colocynth or ginger, red sanders wood in capsicum, etc. The powdered bark is frequently found to be adulterated with brick powder.

EVALUATION OF DRUGS

Evaluation of drugs deals with the correct identification of the plant and determination of quality and purity of the crude drugs. Actual collection of the drug is done from the identified plant or animal for this purpose research gardens have been maintained. The characters of an unknown sample are compared with the autentic monographs written in the pharmacopoeia. The high quality of the drug is maintained by collection of the drug from the correct natural source at proper time; preparation of samples of the collected drugs by proper cleaning, drying and to free from dirt, and proper preservation of the cleaned, dried and pure drug.

The evaluation of a drug is done by studying its organoleptic, microscopic, biological, chemical, and physical properties.

ORGANOLEPTIC EVALUATION

Organoleptic evaluation means study of a drug with the help of organs of sense which includes its external morphology, colour, odour, taste, sound to its fracture, etc.

Morphological Characters : To study morphology of a drug, its shape and size, colour and external markings, fracture and internal colour, odour and taste are examined. The organized drugs are classified into :

1. Barks : Which are tissues in a woody stem outside the inner fascicular cambium, e.g. Cinnamon, Cinchona, Quillaia, Ashoka and Kurchi.

2. Underground Structures : Whjich may be rhizomes, roots bulbs, corm, and tubers; they often swollen e.g., roots (Podophyllum, Liquorice, Jatamansi, Rauwolfia), rhizomes and stolons which are underground stems and have buds, scale leaves and scars, (Ginger, Turmet\ric, Dioscorea).

3. Leaves : These are photosynthetic organs arising from a node on a stem. The shape, margin, base, apex and venation of leaves help in the identification of the drugs. Senna, Tulsi, Vasaka and Digitalis leaves can be easily identified.

4. Flowers : These are reproductive organs of a plant and possess different shapes, size, and colour, e.g., Saffron, Banafsha, Pyrethrum.

5. Fruits : Fruits arise from the ovary and contain seeds, e.g. Cardamom, Colocynth, Almond, Vidang, Bahera, Amla and Bael.

6. Seeds : Seeds are developed from the ovules in carpels of the flowers and characterized by the hilum, micropyle and sometimes re\aphe. The seed drugs are Ispaghula, Linseed, Nux-vomica, Psoralia.

7. Herbs : The whole aerial part is sometimes used as a drug, e.g. Brahmi, Chirata, Kalmegh, Pudina, Shankhpushpi, etc.

The shape of a drug may be cylindrical (Sarsaparilla), sub-cylindrical (Podophyllum), conical (Aconite); fusiform, ovoid or pyriform (Jalap), and terete or disk-shaped (Nux-vomica): The drug may be simple, branched, curved or twisted. The length, breadth and diameter are measured in millimeters or centimeters. In case of conical drugs the size of both parts is mentioned.

External markings are mentioned as :

1. Furrows, ridges, etc.,

2. Wrinkles,

3. annulations,

4. fissures,

5. nodules,

6. projections,

7. scars of leaf, stem-base, root, bud, bud-scale, etc.

The fractures may be complete, incomplete, short, fibrous, splintery (breaking irregularly), brittle (easily broken), tough and weak.

Sensory Character : Colour. texture, odour and taste are useful in the evaluation of drugs. This method is especially applicable to drugs containing volatile oils or pungent principles (e.g. Capsicum). and to the detection of the effects of inadequate drying or damp storage. The external colour varies from white to yellowish grey. brown, orange or brownish black. The colour of some drugs changes if they are dried in sunlight in place of shade.

The odour of a drug may be either distinct (characterisic) or indistinact. The terms used to define odour are aromatic, balsamic, spicy, alliaceous (garlic-like), camphoraceous (camphor-like), terebinthinate (turpentine-like) and others.Leaves of different species of Mentha can be distinguished by smell. Clove and exhausted clove are differentiated by odour. Deteriorated Cantharides have ammonical smell while spoiled Ergot has rancid and ammonical smell.

Taste is a particular sensation production by certain substances when these come into contact with taste buds present in epithelial layer of the mouth. The taste may be present in epithelial layer of the mouth. The taste may be sour (acidic), salty (saline), sweet (saccharine), bitter, alkaline and metallic. Substances possessing no taste are mentioned as tasteless. The tastes due to a characteristic odour are grouped as aromatic, balsamic, spicy, alliaceous, grouped as aromatic, balsamic, spicy alliaceous, camphoraceous and terebinthinate. The taste produced by distinctive sensations to the tongue are classified as mucilaginous, oily, astringent (producing a contraction of the tissues of the mouth), pungent (warm biting sensation), acrid (unpleasant, irritating sensation) and nauseous (causing vomiting).

The drugs like Ginger and Capsicum have pungent taste; Gentian, Chirata and Kalmegh have bitter taste; Glycyrrhiza and Honey are sweet in taste. Linseed and Isphagula are mucilaginous; Podophyllum, Kaladana, Jalap and Ipomoea are acrid; while Ipecac, Acorus, and Tylophora indica contain nauseous taste.

Glycyrrhiza has hard and fibrous fracture due to the presence of fibrous and woody tissues. Aconite has a horny fracture due to gelatinization of starch.

Colour of drugs are standardized and determined by the Inter-Society Colour Council-National Bureau of Standard Inter-Society Colour Council-National Bureau of Standard method For example, reserpine is described as a "white or pale buff to slightly yellowish,odourless crystalline powder".

MICROSCOPIC OR ANATOMICAL EVALUATION

Schleiden (1847) used microscope for the examination of drugs. Microscopic examination of section and powder drugs, aided by stains, helps in distinction of anatomy in adulterants. Further, microscopical examination of epidermal trichomes and calcium oxalate crystals is extremely valuable, especially in powdered drugs. In the powdered drugs the cells are mostly broken, except lignified cells. The cell contents such as starch, calcium oxalate crystals, aleurone, etc. are scattered in the powder. Some fragments are specific for each powder which may consist of parts of cells or groups of cells.

Plant parts are made up of specific arranged tissues, spores (Lycopodium) or hairs (Lupulin). Histological characters are studied from very thin transverse, or longitudinal sections properly mounted in suitable stains, reagents or mounting media.

The size, shape and relative positions of the different cells and tissues, chemical nature of the cell walls and of the cell contents are determined. The basic arrangement of tissues in each drug is fairly constant. Fibres, sclereids, tracheids, vessels and cork are least affected by drying. Starch, calcium oxalate, epidermal trichomes and lignin are examinaed carefully.

Microscope is also used for a quantitative evaluation of drugs and adulterated powders. This is done by counting aspecific histological feature such as stomatal index, veinislets and vein termination numbers, palisade ratio, etc. These features are compared with the standard samples.

Plaisade Ratio : The average number of palisade cells beneath each epidermal cell is called as palisade ratio. it is determined from powdered drugs with the help of camera lucida.

Stomatal Number : The average number of stomata per square millimeter of the epidermis is known as stomatal number. The range and average value for each surface are recorded.

Stomatal Index : The percentage proportion of the number of stomata form to the total number of epidermal cells of a leaf is termed the stomatal index :

S.I. = S/E + S x 100; where S = number of stomata per unit area, E = number of ordinary epidermal cells in the same unit area.

Stomatal number varies considerably with the age of the leaf but the stomatal index is highly constant for a given species.

Vein-Islet Number : The word 'Vein-islet' is used for the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. Vein-islet number is defined as the number of vein-islets per square mm calculated from four contiguous square mm in the central part of the lamina, midway between the midrib and the margin. The average range of vein-islet numbers for Senna are : Cassia senna (26), C. angustifolia (21); for Coca: Erythroxylum coca (11), E. truxillense (20); for Degitalis. Digitalis purpurea (3.5) D. lanata (2.7); D. lutea (4.4), D. thapsi (1.2).

Veinlet Termination Number : It is defined as the number of veinlet terminations per mm^2 of leaf surface. A vein termination is the ultimate free termination of a veinlet or branch of a veinlet. By this character different Coca leaves and Senna leaflets are differentiated.

LYCOPODIUM SPORE METHOD

Lycopodium (syn. Club-moss spores, Lycopodium seeds; vegetable sulphur) consists of teh spores of the clubmoss, Lycopodium clavatum Linn. (Fam. Lycopodiaceae, Phyllum Pteriodophyta); grows in the North America, Russia, Poland, Indian and Pakistan. The sporangial spikes are cut and dried and the spores are separated by shaking. Lycopodium is a light yellow, extremely mobile and flammable powder without odour or taste. It contains

about 50 % fixed oil, which consists mainly of glycosides of lycopodiumoleic acid; sugars (3%), phytosterin and alkaloids of the annotine type.

Lycopodium spores are exceptionally uniform in size (about 25µm) and 1mg of lycopodiun contains an average of 94.000 spores. The number of spores per milligram is determined by direct counting and by calculation based on specific gravity and dimensions of the spores. It is possible to evaluate many powdered drugs if well-defined particles may be counted as in case of pollen rains or starch grains; or if single layered tissues or cells of the area of which may be traced at a definite magnification and the actual are calculated; or if characteristic particles of a uniform thickness, the length of which can be measured at a definite magnification and the actual length calculated. Mounts containing a definite proportion of the powder and lycopodium are used and the lycopodium spores counted in each of the fields in which the number or area of the particles in the powder is determined.

In this method the moisture content of the powdered material is determined. A mixture of weighed quantity of the powedr and lycopodium spores is suspended in a suitable viscous liquid. A drop of this suspension is mounted and examined with a 4mm objective. The number of lycopodium spores and the number of characteristic particles are counted in 25 various fields. The same experiment is repeated with a second similar suspension. From the mean of these results and a knowledge of the weights of lycopodium and powder in the mixture, the number of characterisatic particles in 1 mg of the powder may be determined.

By employing lycopodium spore method the number of pollen grains in pyrethrum powder (1000-2000/mg), starch granules in wheat powder (400 granules/mg) and starch grain in Ginger (261400 grains/mg) have been determined.

Lycopodium spore method is also used to determine size of a particular type of particle in powders such as epidermal fragmenjts of leaves, single layer of scalerenchyma, or isolated fibres. The procedure is almost the same as used for counting of particles. The particle size counted. The tracings are cut out and weighed and their area calculated by weighing a sheet of known area of the paper used. This area divided by the magnification used $(420)^2$ gives the actual area of the particles in a certain weight of the powdered drug. which is calculated from the number of spores counted and the weight of spores and powder in the the suspension. By this method epidermal area of Indian Senna stalk (100 cm^2),

sclerenchyma layer in Linseed, fibres in the Cinnamon bark and number of beaker cells in testa of Cinnamon seed have been measured.

CHEMICAL EVALUATION

- Chemical evaluation involves the determination of active constituents by a chemical process. Chemical tests are used to identify certain crude drugs to determine purity.
- Chemical tests for alkaloids, carbohydrates, steroids, phenolic compounds, saponins, proteins, amino acids, fixed oils and volatile oils are performed.
- Titrimetric assay, iodine value, saponification value, acid value, acetyl value, ester value, peroxide value, hydroxyl value and ash value are determined.
- Tropane alkaloids in Datura, Belladonna and Stramonium are determined by Vitali-Morin reaction. Potassium chlorate and hydrochloric acid are used to estimate emetine in Ipecac.
- Strychnine in Nux-vomica is detected with ammonium vanadate and sulphuric acid. Borntrager's test is useful for detecting anthraquinone glycosides, present in Senna, Rhubarb, Cascara and Aloe.
- Alkaloid contents can be evaluated by determining total alkaloidal contents by acid base titration.

- Preparation of an extract by an appropriate solvent is sometimes applied to determine the quality of drugs. The solvent may extract a single constituent. e.g. fixed oil form crushed Linseed. Further examples of the use of extractive tests are in cases of Gentian , Colocynth seeds, Indian hemp, Ginger, Calumba, Rhubarb, Glycyrrhiza and Myrrh.

- Drugs containinjg volatile oils are examined for authenticity and quality by determining the percentage of volatile oil yielded by steam distillation in a suitable apparatus. Standards for content of volatile oil in drugs usually allow a somewhat smaller percentage from powdered drugs as compared with the whole drug due to inevitable loss on grinding, volatilization and decomposition.

On ignition of crude drugs a residue of meneral substances or ash remains, derived from the cell wall and cell contents. The ash value is useful in determining authenticity and purity of drugs. For a number of official drugs, a limit is placed on the yield of acid-insoluble ash, i.e. the ash remaining after extraction of the total ash with dilute acid. This residue consists chiefly of Silica, partly derived from the constituents of the cells and their walls and partly from foreign mineral matters, mainly soil. Acid-insoluble ash limits are imposed especially in cases where foreign silica may be present or when the calcium oxalate contents of the drug is high. Pharmacopoeial limits for acid insoluble ash very from 0.5 (Agar) to 12 percent (Hyoscyamus). Glandular trichomes present in Hyoscyamus have a capacity of retaining clay and thus the acid insoluble ash value is higher in such cases. In case of Glycyrrhiza the total ash figure is of importance which indicates the care taken in the preparation of the drug. For the determination of total ash values the carbon must be removed below $450^{O}C$, since alkali chlorides would be lost due to volatile at high temperature. The total ash usually consists of carbonates, phosphates, silicates and silica. In case of Ginger a minimum percentage of water-soluble ash is determined to detect the presence of exhausted ginger.

PHYSICAL EVALUATION

Physical constants such as elasticity in fibres, viscosity of drugs containing gums, swelling factor of mucilage containing materials, froth number of saponin drugs, congealing point of volatile and fixed oils, melting and boiling pionts and water contents (loss on drying at $110^{O}C$) are some important parameters used in the evaluation of drugs. Ultraviolet light is also used for determining the fluorescence of extracts of some drugs (Gambir, Senna) and colours of alkaloids as: aconite (light blue), berberine (yellow), emetine (orange) and quinine (dense fluorescence in dilute sulphuric acid) The florescence of Belladonna leaf and root. Wild Cherry bark and jalap is due to the presence of a coumarin, β-methyl asculetin. Pale Catechu shows fluorescence in alkaline solution due to gambir-fluorescin. Aloe exhibits a green fluorescence in a solution containing borax. Many other drugs show a marked intensity of colour or a characteristic colour under UV light. Rhubarb is differeentiated from Rhapontic, Chinese or Indian Rhubarb is differentiated from Rhapontic, Chinese or Indian Rhubarb by its marked fluorescence in UV light.

Physical constants are extensively applied to the active principles of drugs. such as alkaloids. volatile oils. fixed oils, etc. Solubility expresses number of ml of solvent require ot dissolve one gram of the drug. For example. 1g of codeine sulphate is soluble in 30 ml of water, and in 1300 ml of alcohol, Alkaloids and other nitrogenous compounds are soluble in dilute hydrochloric acid. Melting points are recorded for solid fixed oils(fats) and alkaloids.

Most of the monoterpenes have asymmetric carbon. Therefore, they are optical active. For example, Peppermint oil has optical rotation as -18^{O} to -32^{O}. Specific gravity is important with nutgalls. The galls that will not sink in water are considered to be inferior quality. In Jalap the specific gravity should be higher than water. This constant is also important for volatile oils and lipids. Refractive index is particularly Important in volatile oils and fixed oils. It is in between 1.45-1.46 for Peppermint oil at 20 degree.

Spectrocopic analysis (UV. IR. NMR, Mass), and radioimmuno assays are applied more frequently to the active individual drugs components. Chromatiographic techniques such as paper, column, thin-layer, gas-liquid (GLC) and high performance liquid chromatography (HPLC) provide information about the chemical constituents present in the drug.

The foreign organic (animal, animal excreta, insects, fungi, bacteria, or mould) and inorganic matters should be in pharmacoepeal limits. They are determined by sedimentation or floatation method. If the drug is not prepared properly the total ash value will be more.

BIOLOGICAL EVALUATION

The drugs, which cannot be assayed satisfactorily by chemical or physical means, are evaluated by biological methods. Tests are carried out on intact animals, animal preparations, isolated living tissues or micro-organisms. Since living organisms are used, the assays are called'biological assays' Biological standardization procedures are gemera;;u ;ess [recose, more time consuming and more expensive to conduct than chemical assays. Therefore, they are generally used if the chemical identity of the active principle has not been fully elucidated: if, no adequate chemical assay has been derived for the active principle as in case of insulin; if the drug is composed of complex mixture and activity, e.g. Digitalis; if the

purification of crude drug is not possible, e.g. separation of vitamin D from irridiated oils; and if the chemical assay is not a valid indication of biological activity.

A biological assay measures the actual biological activity of a given sample. In any on test the animals of only one strain are used. For some assays a specific sex must be used. The male rat has faster growth rate than the female. Therefore, use of both male and female in a growth test should be avoided. Bioassays are conducted by determining the amount of a solution of unknown potency required to produce a definite effect on suitable test animals or organs under standard conditions.To minimize the source of errors resulting from animal variation, standard reference preparation are used in certain bioassay procedures.

Bacteria such as a Salmonella typhi and Staphylococcus aureus are used to determine antiseptic value of certain drugs. In another microbiologic methods the living bacteria, yeast and molds are used for assaying vitamins and to determine the activity of antibiotic drugs. Mice are used to test Rabies vaccine, Diphtheria toxoid and other biologics. The rat line test is utilized for the assay of vitamin D preparation. Guinea pigs are employed to test the toxicity and antigenicity of diagnostic Diphtheria toxin and tetanus toxoid. Oxytocic activity of vasopressin injection is also tested on guinea pigs. Oxytocic injection is assayed on young domestic chickens by injecting into an exposed crural or brachial vein and observing changes in blood pressure. Digitalis glycosides are assayed on pigeons by transfusing the drug through the alar vein into the blood stream and nothing the lethal effects. Cats are utilized in tests for drugs with depressor activity and glucagon injection. Mydriatic drugs such as atropine are evaluated on cat's eye. Curare alkaloids. e.g. tubocurarine chloride, and pyrogens in antibiotic solutions are assayed on rabbits. Ophthalmic preparations are tested on rabbit eyes. Dogs are the test animals to determine pressor activity in drugs and to assay veratrum viride preparations. Anthelmintic drugs(Male fern) are evaluated on earthworms. Evaluation of Ergot is carried out on cock's comb or rabbit intestine or its uterus. Human beings are also used to note the activity of drugs in clinical trial.

There are some disadvantages of bioassays. Quantitative accuracy is usually less than observed with most chemical analyses. Techniques and interpretation involved vary with different operators. The effect measured in the test animals is different form that observed in treating patients.

A simple bioassay utilizing brine shrimp (Artemia salina) is available for determining new biological activities in plant extracts. The eggs of this creature, which serve as food for tropical fish, are allowed to hatch in a brine solution. The shrimp are exposed to different concentrations of the test material and an LC_{50} (median lethan concentration) value in µg/ml is calculated. A broad range of compound show toxic effect to the shrimp. The procedure is rapid, reliable and cheap. Another procedure, called potato-disc assay, involved observation of the inhibition of crown gall tumors induced on potato discs be Agrobacterium tumefaciens by plant extracts or isolated compounds. This method is used for detecting in preliminary fashion anticancer activity.