UNIT 2
Production of natural drug products

1. Collection (wild)
2. Cultivation (commercial), collection, harvesting, drying, garbling, packaging, storage and preservation
3. Fermentation (Recombinant DNA technology or Genetically engineered drugs)
4. Cell-culture techniques
5. Microbial transformation
6. Biologics (prepared from the blood of animals)

Preparation of crude drugs

1. Collecting of medicinal plants

Suitable time for collection

- The amount of a constituent is usually not constant throughout the life of a plant.
- The stage at which a plant is collected or harvested is, therefore, very important for maximizing the yield of the desired constituent.
- The differences are sometimes not only quantitative but also qualitative.

Rules for collection

The following general rules are based on assuming that the material is best collected when the organ in question has reached its optimal state of development:

- **Roots and rhizomes** are collected at the end of the vegetation period, i.e. usually in the autumn. In most cases they must be washed free of adhering soil and sand.
- **Bark** is collected in the spring.
- **Leaves** and **herbs** are collected at the flowering stage.
- **Flowers** are usually gathered when fully developed.
- **Fruits** and **seeds** are collected when fully ripe.

Methods of collection

- Medicinal plants must be largely collected by hand. This is especially true in the case of wild plants.
- With cultivation on a large scale, it may be possible to use modern agricultural harvesters, but in many cases, e.g. barks, manual collection is unavoidable. Thus, the cost of drug production is largely the cost of the labor involved.
2. Preservation of plant material

- The plant material must first be preserved so that the active compounds will remain unchanged during transport and storage.
- The cells of living plants contain not only low molecular-weight compounds and enzymes, but they also have many kinds of barriers that keep these constituents apart. When the plant dies, the barriers are quickly broken down and the enzymes then get the opportunity to promote various chemical changes in the other cell constituents, e.g. by oxidation or hydrolysis.

Preservation aims at limiting these processes as far as possible:

1. Drying

- The most common method for preserving plant material is drying.
- Enzymic processes take place in aqueous solution. Rapid removal of the water from the cell will, therefore, largely prevent degradation of the cell constituents.
- Drying also decreases the risk of external attack, e.g. by moulds.
- Living plant material has a high water content: leaves may contain 60-90% water, roots and rhizomes 70-85%, and wood 40-50%. The lowest percentage, often no more than 5-10%, is found in seeds.
- To stop the enzymic processes, the water content must be brought down to about 10%.
- Drying must be done quickly, in other words at raised temperatures and with rapid and efficient removal of the water vapor.
- The most efficient drying is achieved in large driers of the tunnel type. The plant material is spread out on shallow trays, which are placed on mobile racks and passed into a tunnel where they meet a stream of warm air.
- The air temperature is kept at 20-40 °C for thin materials such as leaves, but is often raised to 60-70 °C for plant parts that are harder to dry, e.g. roots and barks.
- When the crude drug has been collected under primitive conditions, without access to a drier, it must be dried in the open. Even then, the material should be spread out in shallow layers with good ventilation to facilitate the drying. The choice of sunshine or shade is determined by the sensitivity to light of the constituents.
- In a dried drug the enzymes are not destroyed but only rendered inactive due to the low water content. As soon as water is added, they become active again. Hence, dried drugs must be protected from moisture during storage.
2. Freeze-drying

- Freeze-drying (lyophilization) is a very mild method.
- Frozen material is placed in an evacuated apparatus which has a cold surface maintained at -60 to -80 °C. Water vapor from the frozen material then passes rapidly to the cold surface.
- *The method requires a relatively complicated apparatus and is much more expensive than hot-air drying. For this reason, it is not used as a routine method, but it is very important for drying heat-sensitive substances, e.g. antibiotics and proteins.*

3. Stabilization

- On long storage, enzymatic reactions will slowly destroy the constituents, because the last traces of water can never be removed.
- In order to avoid this degradation, the enzymes should be destroyed before drying, a process usually called stabilization.
- The most common method being brief exposure (a few minutes only) of the plant material to ethanol vapor under pressure (0.5 atm).
- Stabilization may be of value for the isolation of compounds that are very susceptible to enzymatic degradation.

4. Fermentation

- Enzymatic transformation of the original plant constituents is sometimes desirable.
- The fresh material is then placed in thick layers, sometimes covered and often exposed to raised temperatures (30-40 °C) and humidity, so as to accelerate the enzymatic processes. (This treatment is usually called fermentation).
- The fermented product must, of course, be dried afterwards to prevent attack by microorganisms, e.g. moulds.
- Fermentation is mostly used to remove bitter or unpleasant-tasting substances or to promote the formation of aromatic compounds with a pleasant smell or taste.
- *It is mainly applied to drugs used as spices or stimulants, e.g. vanilla, tea and cacao.*
3. Storage of crude drugs

- There are great differences in the stability of crude drugs because of slow enzymic changes in the constituents.
- Drugs containing glycosides and esters are usually less stable than those containing alkaloids.
- Drugs with essential oils deteriorate rather quickly through evaporation, oxidation and polymerization of the substances constituting the essential oil.
- Tannins on the other hand, have an almost unlimited durability.

**In order to keep crude drugs as long as possible:**
1. It is essential to store them in a dry condition in carefully closed containers.
2. It is also advisable to exclude light, because - even if it does not affect the active constituents - it almost always causes changes in the appearance of the drug, especially loss of color.
3. It is also necessary to protect the drug against insect attack.

4. Grinding of crude drugs

- Regardless of whether the crude drug is to be used for isolation of a pure compound or for manufacture of a simple preparation, the first operation that must be performed is grinding of the plant material to a powder of suitable particle size.
- It is important that the particles are of as uniform a size as possible.
- Excessive dust can clog percolators and result in a turbid extract which is hard to clarify.
- Large particles take a longer time for complete extraction than small ones and large differences in particle size thus slow down the extraction process.
- Several types of machines are available for grinding crude drugs:
  1. **Hammer mill**: a common type for grinding crude drugs.
  2. **Knife mill**: is useful for production of low-dust powders of leaves, barks and roots for subsequent percolation or maceration.
  3. **Tooth mill**: is used for production of very fine powders.
- Grinding produces a certain amount of heat which must be observed when grinding crude drugs containing heat-sensitive compounds.
- Mills cooled with liquid nitrogen are available for such purposes.
- Cold grinding is also preferable for crude drugs containing volatile oils.
- Following grinding, the material must be sifted to ensure the proper particle size.
Sifting can be performed according to two different principles: **sieving** and **blast sifting**.

- **Sieving**
  
  In sieving the material is passed through a sieve of suitable mesh size giving two fractions. The fraction passing the sieve consists of particles with a size smaller than or corresponding to the mesh size. The remaining fraction consists of coarser particles which are returned to the mill for continued grinding.

- **Blast sifting**
  
  In blast sifting the material to be classified is blown with compressed air into an apparatus which allows the particles to sediment according to their weight. Coarse, heavy particles settle fast whereas small, light particles stay for a long time in the air stream.

5. Extracts

- **Extracts** can be defined as preparations of crude drugs which contain all the constituents which are soluble in the solvent used in making the extract.
- In **dry** extracts all solvent has been removed.
- **Soft** extracts and **fluid** extracts are prepared with mixtures of water and ethanol as solvent.
- **Tinctures** are prepared by extraction of the crude drug with five to ten parts of ethanol of varying concentration, without concentration of the final product.
- For both extracts and tinctures the ratio drug/solvent should always be stated.
- Several factors influence the extraction process.
- Plant constituents are usually contained inside the cells. Therefore, The solvent used for extraction must diffuse into the cell to dissolve the desired compounds whereupon the solution must pass the cell wall in the opposite direction and mix with the surrounding liquid.
- An equilibrium is established between the solute inside the cells and the solvent surrounding the fragmented plant tissues.
- The speed with which this equilibrium is established depends on:
  1. Temperature
  2. pH
  3. Particle size
  4. The movement of the solvent
**Choice of solvent**

The ideal solvent for a certain pharmacologically active constituent should:

1. Be highly selective for the compound to be extracted.
2. Have a high capacity for extraction in terms of coefficient of saturation of the compound in the medium.
3. Not react with the extracted compound or with other compounds in the plant material.
4. Have a low price.
5. Be harmless to man and to the environment.

- Aliphatic alcohols with up to three carbon atoms, or mixtures of the alcohols with water, are the solvents with the greatest extractive power for almost all natural substances of low molecular weight like alkaloids, saponins and flavonoids.
- According to the pharmacopoeias, *ethyl alcohol* is the solvent of choice for obtaining classic extracts such as tinctures and fluid, soft and dry extracts.
- The ethanol is usually mixed with water *to induce swelling of the plant particles* and *to increase the porosity of the cell walls* which facilitates the diffusion of extracted substances from inside the cells to the surrounding solvent.
- For extraction of *barks, roots, woody parts* and *seeds* the ideal alcohol/water ratio is about 7:3 or 8:2. For *leaves or aerial green parts* the ratio 1:1 is usually preferred in order to avoid extraction of chlorophyll.

**Extraction procedures**

There are many procedures for obtaining extracts like:

- Infusion
- Maceration
- Percolation
- Digestion
- Decoction
- Continuous hot extraction
- Solvent-solvent precipitation
- Liquid-liquid extraction
- Distillation
- Specific procedures
**Infusions** are prepared by simply soaking a drug in water for a specified time. This might be hot or cold, depending on whether decomposition of ingredients could occur at higher temperatures. Infusions would normally be prepared for immediate use, as there is no preservative present. In some cases concentrated infusions might be prepared by boiling to reduce the water then adding a preservative such as alcohol.

**Decoctions** are prepared in a similar way to infusions but with the ingredients and water boiled for a specified period of time or until a certain volume is achieved.

**Maceration** differed from the above two processes in that the drug was left in contact with the menstruum, usually alcohol but sometimes water, for a longer period of time. The usual procedure would be to add the liquid to the drug in a closed vessel for seven days, shaking occasionally, straining, pressing the marc (the remaining drug/liquid mixture), mixing the two solutions and clarifying by filtering or by standing.

**Percolation** differs slightly from maceration. The powdered drug is dampened with the menstruum, left for four hours then packed into a percolator. Sufficient menstruum is added to cover the drug and left for twenty-four hours. The liquid is then allowed to very slowly drain from the bottom of the percolator (about twenty drops per minute). More menstruum is added and the process continued until the volume in the collecting flask reaches about three-quarters of the required volume. The marc is pressed, this liquid added to the flask, more menstruum added to make the specified volume then the whole liquid is clarified.
Good cultivation and for medicinal plants

Good agricultural practices for medicinal plants:

- Selection of medicinal plant for cultivation
- Botanical identity
- Seeds and other propagation material
- Cultivation
  - Site selection
  - Ecological environmental and social impact
  - Climate
  - Soil
  - Irrigation and drainage
  - Plant maintenance and protection
- Harvest
- Personnel

Selection of medicinal plant for cultivation

- Species or botanical variety selected for cultivation should be same as that specified in national Pharmacopoeia or recommended by other authoritative national documents of the end-user’s country.

- In case of newly introduced medicinal plant, the species or botanical variety selected for cultivation should be identified and documented as the source material used or described in traditional medicine or the original country.

Botanical identity

- Scientific name (genus, species, subspecies/variety, cultivar, family) should be verified and recorded. Cultivar name, ecotype, chemotype or phenotype may also be provided, as appropriate
- Name of the material supplier should be recorded
- In case of land races collected, propagated, disseminated and grown in a specific region, records be kept of the locally named lines, including the origin of the source seeds plants or propagation material.
Seeds and other propagation material

- All information relating to identity, quality and performance (as well as breeding history where possible) of the propagation material be obtained from the supplier and recorded.
- Planting material should be free from contamination and disease to promote healthy plant growth
- Take care to exclude extraneous species, variety or strain
- Any genetically modified germplasm should comply with regional and/or national regulations and be appropriately labeled and documented, as required.

Cultivation

- If no scientific published or documented agrotechnology data are available, traditional method of cultivation be followed or agrotechnology be developed through research work
- Principles of good plant husbandry, including appropriate rotation of plants selected according to environment suitability, should be followed
- Conservation agriculture technique (CA*) be followed where appropriate, particularly in the build-up of organic matter and conservation of soil humidity

CA* aims to conserve, improve and make more efficient use of natural resources through integrated management of available soil, water and biological resources combined with external inputs. It contributes to environmental conservation as well as to enhanced and sustained agricultural production.

Site selection

- When cultivated at different sites, same medicinal plant may exhibit differences in quality due to soil, climate and other factors
- These differences may relate to physical appearance or to variations in their constituents
- Risk of contamination as a result of pollution of soil, air or water by hazardous chemicals should be avoided
- The impact of past land uses on cultivation site, including the planting of previous crops and any application of plant protection products, should be evaluated

Ecological environmental and social impact

- The ecological impact of cultivation should be monitored overtime, where practical because:
  - Cultivation of medicinal plants may affect ecological balance and in particular, the genetic diversity of the flora and fauna in surrounding habitats
The quality and growth of medicinal plants can also be affected by other plants, living organisms or by human activities

- Introduction of non-indigenous medicinal plant species into cultivation may have a detrimental impact or biological and ecological balance of the region
- The social impact of cultivation on local communities should ensure that negative impacts on local livelihood are avoided. Small scale cultivators versus large scale cultivation

**Climate**

Climate conditions e.g.

- Length of the day, rainfall (water supply) and field temperature, significantly influence the physical, chemical and biological qualities of medicinal plants.
- Duration of sunlight
- Average rainfall
- Average temperature
- Daytime and night time temperature differences influence the physiological and biochemical activities of plants and prior knowledge should be considered.

**Soil**

- Appropriate amounts of nutrients, organic matter and other elements to ensure optimal medicinal plant growth and quality
- Optimal soil conditions including soil type, drainage, moisture retention, fertility and pH.
- Use of correct type and quantity of fertilizers with minimum risk of leaching
- Human excreta as fertilizer to be avoided due to potential presence of infectious microorganisms and parasites
- Animal manure should be thoroughly composted to meet safe sanitary standards of acceptable microbial limits

**Irrigation**

- Irrigation and drainage should be controlled and carried out in accordance with the needs of the individual medicinal plant species during its various stages of growth
- Water used for irrigation should comply with local, regional and or national quality standards
- For choice of irrigation, as a general rule, the health impact of different types of irrigation (various forms of surface, sub-surface or overhead irrigation), particularly on the risk of increased vector-borne disease transmission, must be taken into account
• **Plant maintenance and protection**
  
  o The growth and development characteristics of medicinal plant as well as part of the plant destined for medicinal use, should guide the field-management practices (e.g. timely topping, bud nipping, pruning and shading) to improve the quality and quantity of medicinal plant material
  
  o Use of any agrochemical for growth promotion and protection should be kept to the minimum
  
  o Integrated pest management should be followed
  
  o When necessary, only approved herbicide/pesticide be applied at minimum effective level as per label instructions
  
  o All applications should be documented
  
  o Growers should comply with maximum pesticide/herbicide residues

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**Harvest**

✓ Time of harvest depends upon the plant part to be used. Consult national Pharmacopoeia, official monographs, published standards

✓ MPs parts be harvested during optimum season or time period to ensure best quality of harvested material

✓ Concentration of bioactives varies with the stage of plant growth and development

✓ Best time for harvest (quality peak season/time of day) should be based on maximum concentration of bioactives

✓ During harvest, no foreign matter, weeds or toxic plants are mixed with the harvest

✓ Best harvesting conditions avoiding dew, rain and humidity

✓ Immediate drying after harvest is advisable

✓ Cutting devices, harvesters and other mechanical devices should be clean and adjusted to reduce damage and contamination from soil and other material

✓ Store under uncontaminated dry place free from insects, rodents, birds & other pests & inaccessible to livestock and domestic animals

✓ Contact with soil & humidity to be avoided to minimize microbial load in harvested material

✓ Clean baskets, dry sacks, trailers, hoppers or other well aerated containers be used for transporting harvested material to central place

✓ Any decomposed material should be discarded
Personnel

✓ Growers & producers should have adequate knowledge of the MAPs including botanical identification, cultivation characteristics and environmental requirements

✓ Should receive instructions on all issues relevant to the protection of the environment, conservation and proper agricultural stewardship for producing quality MAPs material

✓ All personnel (including field workers) involved in propagation, cultivation, harvest and post harvest stages should maintain appropriate personal hygiene and should have received training regarding their hygiene responsibilities and in MAPs cultivation & harvesting