Indole Alkaloids

Harmala Alkaloids

Peganum harmala of the family Zygophyllaceae.

It is a woody, perennial, succulent shrub native to arid regions. The leaves are bright green, finely divided and about 1 cm long. Both the roots and seeds contain significant quantities of Beta-carbolines (indole) alkaloids, which are absent in the rest of the plant.

- ***The Traditional and Medical Uses:***
- The traditional uses including as ***the dye "turkey red",*** and as ***incense*** from ancient times.
- **Peganum harmala** was claimed to be an important medical plant. Its seeds were known to possess hypothermic and essentially hallucinogenic properties since it is MAO inhibitor agent .
- Various authors have under taken studies on the antibacterial, anti fungal and antiviral effects of **Peganum harmala** seeds. In Moroccan traditional medicine , seed powder is sometimes used on skin and subcutaneous tumors.

- This work was designed to investigate some aspects of the anti neoplastic properties of **Peganum harmala** in that the active principle at a dose of 50 mg / kg given orally to mice for 40 days was found to have significant anti tumor activity. **Peganum harmala** alkaloids thus posses significant anti tumor potential, which could prove useful as novel anticancer therapy. The pharmacologically active compounds of **Peganum harmala** are several alkaloids ,which are found especially in the seeds (2-7% total) and the roots.
- These include beta-carbolines such as: **harmaine** , **harmaline** and **Harman**.

Introduction to Pharmacognosy

By Lecturer Zena Qaragholi

Pharmacognosy

Introduction

Pharmacognosy is the study of those natural substances principally plants that find use in medicine.

The word pharmacognosy is derived from the Greek Pharmacon means a drug & gnosis which means to acquire knowledge of pharmacognosy forms an important link between **pharmacology** and **medicinal chemistry** on one hand and between **pharmaceutics** and **clinical pharmacy** on the other hand.

- Pharmacognosy is closely related to both botany & plant chemistry & indeed both originated from earlier scientific studies on medicinal plants.
- Pharmacognosy played an important role in the development of other sciences ex: descriptive botany, plant taxonomy, & phytochemistry, chemical plant taxonomy, tissue culture etc.

- The use of modern isolation technique & pharmacological testing procedures means that new plant drugs find their way into medicine as purified substances rather than in the form of galenical preparation.

Pharmacognosy played an important role in the development of other sciences ex: descriptive botany, plant taxonomy, & phytochemistry, chemical plant taxonomy, tissue culture etc…

Terminology

1. **Alphabetical**: Either latin or vernacular names may be used. This arrangement is employed for dictionaries, pharmacopoeias etc…
2. **Taxonomic**: Drugs are arranged according to the plants from which they are obtained , in classes, orders, families, genera, & species.

3. **Morphological**: Drugs are divided into groups such as leaves, flowers, fruits, seeds, herbs etc… These groupings have some advantages for practical study of crude drugs & identification of powdered drugs.
4. **Pharmacological or therapeutic:** This classification involves the grouping of drugs according to the pharmacological action of their most important constituents or their therapeutic use.

5. **Chemical or biogenetic:** The important constituents ex: alkaloids, glycosides, volatile oils etc… or their biosynthetic pathways, form the basis of classification of the drugs.
6. **Phytochemistry:** A branch of chemistry dealing with the chemical processes associated with plant life &the chemical compounds produced by plants i.e the chemistry of the plant, plant processes ,&plant products.OR the scientific study &classification of the chemical constituents of plants.

7-Taxonomy:(from greek taxis meaning arrangement or division &nomos meaning law)is the science of classification according to a predetermined system i.e classification of organisms or others into groups based on similarities of structure or origin

8-Crude drug: Natural products which are not pure compounds i.e. plants or parts of plants extract or exudes.

9-Wild plants:plants that are just that,they have grown in wild with very little help from humans.it grows on its own without human interference.

10Primary metabolites:A metabolite excreted during the growth phase .they mainly contain carbon,nitrogen& phosphorus ex.sugars,amino acids,and nucleotides.they give rise to secondary metabolites.

11-Secondary metabolites: Are compoundes belonging to extremely varied chemical groups , such as organic acids aromatic compounds,terpenoides,alkaloids etc..their function in plants for growth regulation , lignification,coloring of plant parts,protection.

12-Alkaloids: Any of various compounds normally with basic chemical properties & usually containing at least one nitrogen atom in a heterocyclic ring , occurring chiefly in many vascular plants & fungi.

13-Glycoside:Are compound that yield upon hydrolysis, one or more sugar molecules with an organic hydroxide and non sugar part (aglycone).most glycoside are found in plants&exhibit different pharmacological activities.

14-Oils: An unctuous, combustible substance that is liquid ,or easily liquefied on worming . they are soluble in ether but insoluble in water ,such substances depending on their origin , are classified as animal mineral or vegetable oils.

15-Volatile oils: A rapidly evaporating oil of plant derivation(volatilize at ordinary temperature) ,also called essential oil(have odor), that is capable of distillation &that does not have a stain also called ethereal oil.

16-Fixed oil: A nonvolatile fatty oil of vegetable origin consisting mainly of glycerides.

17-Tannin: Any complex phenolic substances of plant origin used in tanning &in medicine. Tannins can precipitate proteins , alkaloids & convert hide into leather including tannic acid some are present in coffee & tea.

18-Extraction: Methods of obtaining the active constituents found in plants .extraction removes only those substances that can be dissolved in liquid or liquid mixture which is referred to as the **Solvent or** more specifically **called menstruum**

19-Marc: The undissolved portion of the substance that remains after the extraction process is completed.

20-Extract: solvent used after extraction process is completed.

Plant nomenclature & taxonomy

Botanical nomenclature:

- In the past the plants were known by a double Latin tittle but Linnaeus (1707-1778) who is a Swedish biologist was the first to describe the present binomial system in which the first name denotes the species
- All species names may be written with small initial letters while the genus name starts with a capital letters

- . Botanical names are followed by the names of a person or their accepted abbreviations
- Ex: Mentha piperita Linnaeus or Mentha piperita L.
- This name (Linnaeus) refers to the botanist who first described the species or variety. This name is useful where there is different names for the same plant.

- **Chemical plant taxonomy**
- The concept that plants can be classified on the bases of their **chemical constituents** is not new but in the last 75years modern techniques of isolation & charecterization have led to the chemical screening of thousands of plants.

- ***Biological sources of drugs***
-
- An examination of the list of drugs derived from natural sources , reveals the followings:
- **Plant** : The majority of plants are derived from **Spermatophyta** (the dominant seed bearing plants). Within the Spermatophyta the number of species & the number of useful medicinal plants are divided unevenly between the phyla Gymnospermae , which yields some useful oils, resins & the alkaloid ephidrine , & the Angiospermae , which is divided into monocotyledons & dicotyledons (both of these provide many useful drugs but especially the dicotyledons).

- **Fungi** : The fungi provide a number of useful drugs especially antibiotics, & are important in pharmacy in a number of other drugs.
- **Algae** : These are source of limited number of drugs ex: agar & alginic acid.
- **Lichens & mosses** : This group contribute little to medicine.
- **Ferns & lycopodium**

- **Land animals** : It provides traditional pharmaceutical materials ex: gelatin , wool fat , beeswax & are a source of hormones and vitamins .
- **Bacteria** : Bacteriophyta is a source for the production of antibiotics , substrates & their employments in genetic engineering ex: in the production of human insulin.
-

Methods of using plants:

- Plants may be used as **isolated parts** e.g. dried leaves of plant as digitalis which contain glycosides as dioxin which is used for the treatment of heart diseases & congestive heart failure.
- **Whole plant** e.g. Catharanthus roseus & its active constituents vincristine & vinblastine which are used as anticancer

Extract of active constituents e.g. extract of unripe fruit of plant as Papaver somniferum which contains morphine which is used as narcotic. The resultant extract is called **extractive** which refers to the principle constituents found in natural substances & are separated or isolated from the natural substances by different means of extraction , these principles are responsible for the medicinal activities of the natural substances & these are found either single or mixtures.

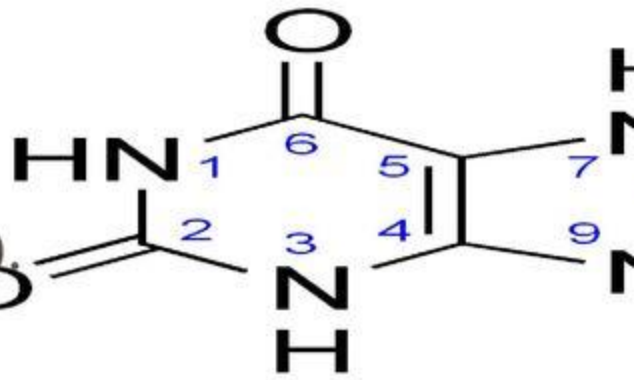
PURINE ALKALOIDS

Purine alkaloids

The purines are consisting of a six-membered pyrimidine rin fused to a five-membered imidazole ring.

three well-known examples are :

- Caffeine (1,3,7-trimethylxanthine),
- Theophylline (1,3-dimethylxanthine)
- Theobromine (3,7-dimethylxanthine).

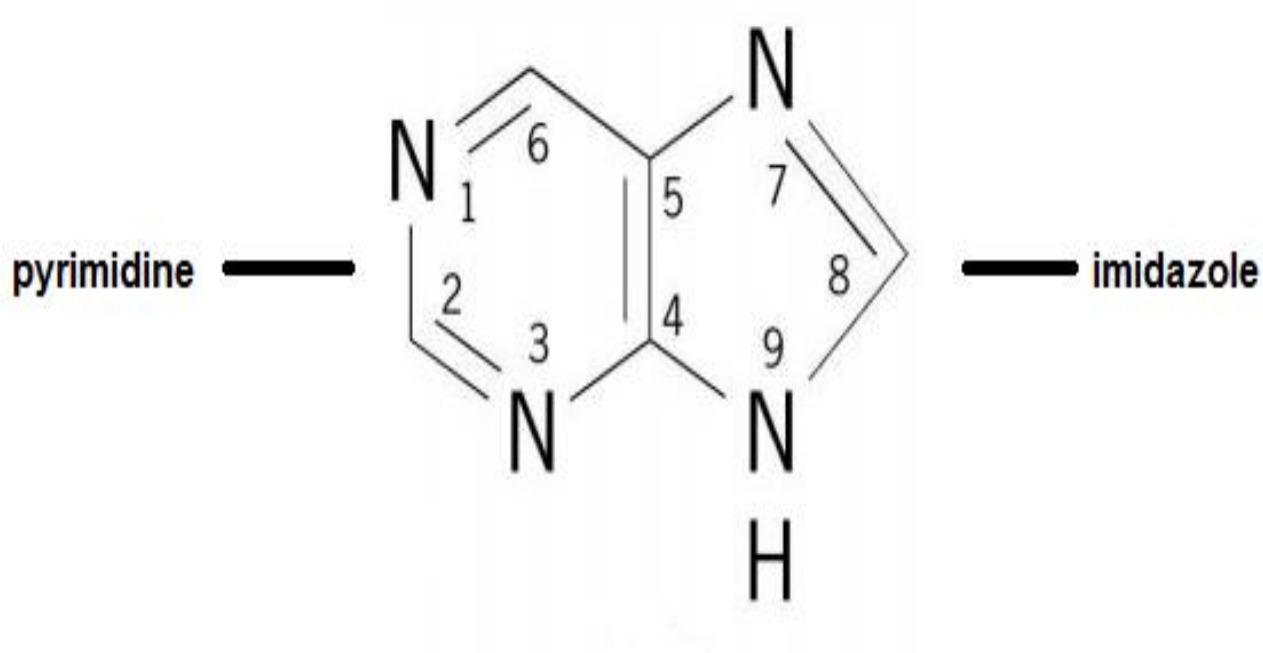


xanthine

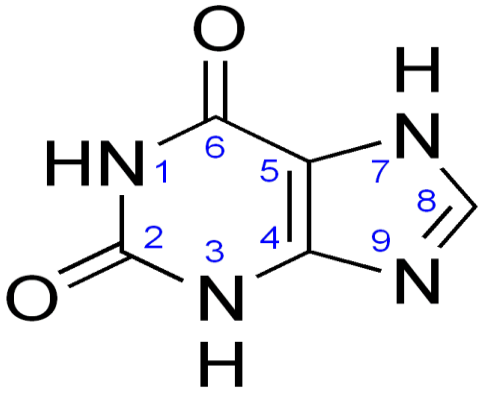
Botanical origin:

- *Coffea arabica*
- *Cola nitida*
- Theobroma Cocoa seeds
- Tae leaves (*camilla sinensis)*

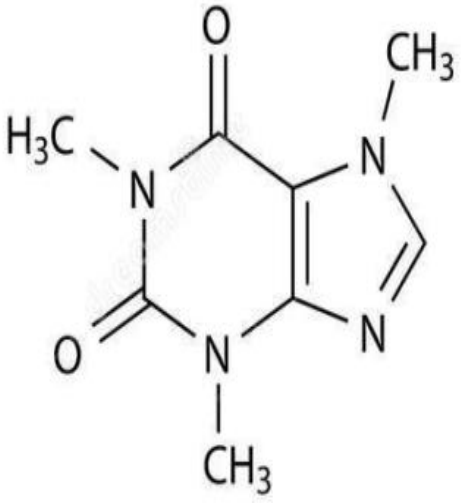
Purine bases



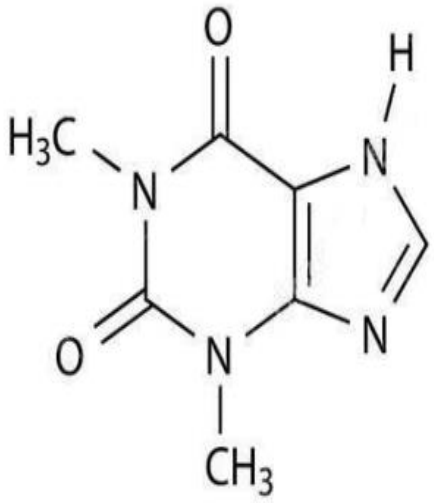
- The purines are derivatives of a heterocyclic nucleus consisting of the 6-membered pyrimidine ring fused to the 5-membered imidazole ring.
- Purine itself does not occur in nature, but numerous derivatives are biologically significant.
- The pharmaceutically important bases of this group are the methylated derivatives of 2,6-dioxypurine (xanthine) i.e. xanthine is diketo purine.



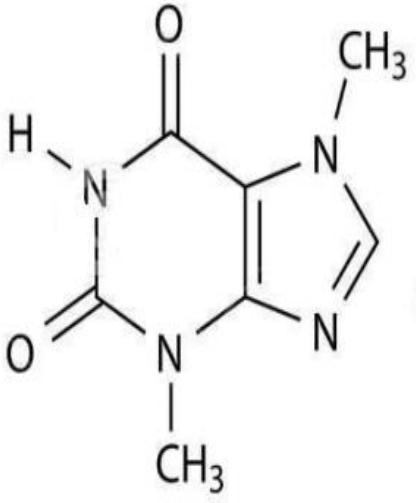
- **Caffeine** is 1,3,7-trimethyl xanthine
- **Theophylline** is 1,3-dimethyl xanthine
- **Theobromine** is 3,7-dimethyl xanthine



Caffeine

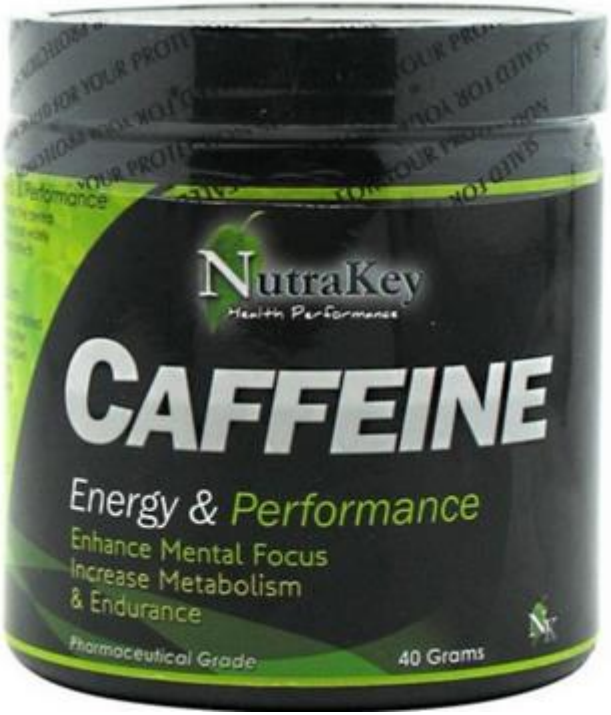


Theophylline



Theobromine

Caffeine


NutraKey
Health Performance
CAFFEINE
Energy & Performance
Enhance Mental Focus
Increase Metabolism
& Endurance
Pharmaceutical Grade
40 Grams

Theophylline

Theobromine

- *Generally the pharmacological activities of these methylated compounds are:*
- Stimulation of the CNS.
- Diuretic effects.
- Increase gastric acid secretion.
- Relaxation of the bronchial smooth muscle (theophylline).
- Positive inotropic and chronotropic effect on the heart.

- *The most important plants in this group are :*
- ***Coffee*** (Coffea arabica of the family Rubiaceae).
- Contain about 1-2 % of caffeine.
- ***Tea*** (Camellia sinensis of the family Theaceae).
- Contain about 1-4 % of caffeine.
- ***Cola*** (Cola nitida of the family Sterculiaceae).
- Contain about 3.5 % of caffeine.

MORINATION
AGRICULTURAL
PRODUCTS

PREMIUM ORGANIC COFFEE BEANS


Pure Health
Naturally
800 mg
Per Serving†
Over
45%
Chlorogenic
Acids‡
Green Coffee Bean
Supports Weight Management*
Dietary Supplement
90 Vegetarian Capsules
Pure Health
Naturally
Green Coffee Bean
Supports Weight Management*
Dietary Supplement


mcaffeine
naked & raw
coffee
body
scrub
coffee with coconut
Normal to Oily Skin
Net Quantity: 100 g | 3.5 oz.
mcaffeine
naked & raw
coffee
face
scrub
coffee with vitamin e
Normal to Oily Skin
Net Quantity: 100 g | 3.5 oz.

Kola


Heritage Store
Old Fashioned
Cola Syrup


KAWAII PHARM
KOLA NUT
(Cola Acuminata)


HERBS OF THE GODS
KOLA NUT

Place ***10 gm*** of the powdered tea leaf in ***50 ml*** of ***water***

↓ Boil for ***(15 mins)***

(Constant stirring)

Strain the resulting hot extract through muslin, express well

↓

Wash the mass remains on the muslin with ***10 ml*** of ***boiling water*** and express again

↓ Add (carefully)

5drops of lead sub acetate

(Heat the mixture to boiling)

↓ Centrifuge (***5 mins.***)

Decant and take the supernatant (upper layer)

↓ Cool & transfer to separatory funnel

[Extract with ***15 ml*** of ***methylene chloride OR chloroform***] two times

↓ (Shake & stand)

Take the lower layer and put it in the conical flask

↓

Tissue Culture 2

Lecturer Zena Qaragholi

A plant tissue culture laboratory

A laboratory devoted to in vitro procedures with plant tissue must have a adequate space for the performance of several functions. It must provide facilities for:

1-Media preparation, sterilization, cleaning and storage of supplies.

2-Aseptic manipulation of plant material.

3-Growth of the cultures under controlled environmental conditions.

4-Examination and evaluation of the cultures.

5-Assembling and filing of record.

- **<u>Design of a PTC lab</u>**
- Lab design must be simple and convenient to work inside it In general, laboratory of PTC, consist of three rooms:-

1-Preparation room:

- This room is essentially a kitchen for three functions:

1-Cleaning and sterilization of glass wares.

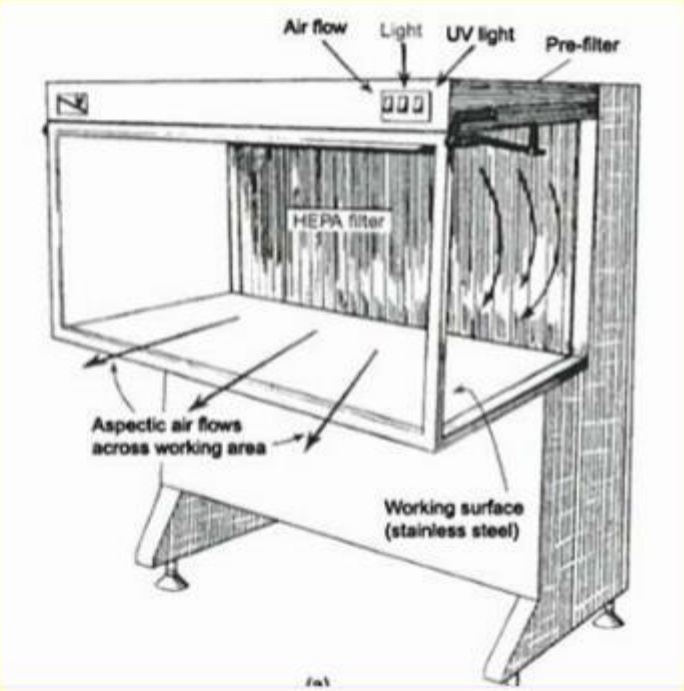
2-Preparation and sterilization of media.

3-Storage of glass wares.

- is room must containing the following equipments & apparatus:-
-

General plant tissue culture laboratory design

1. Glassware washing and storage area
2. Media preparation and sterilization area
   - Refrigerator/freezer
   - Balances
   - Hot plate/stirrer
   - Ph meter
   - Autoclave
3. Growth room
4. Aseptic transfer area
   - Laminar flow hoods



2-Transfer room:

- In this room, the explant or the culture transfer into sterilizes media. This work carried out inside aseptic hood (laminar air flow cabinet). كابينة انسياب الهواء الطبقي
- This cabinet having:
- Vertical or horizontal air flow. The air coming out of the fine filter (0.22-0.30mm) is ultraclean (free fungal or bacterial contaminant).
- A glass or plastic door either sliding or hinged fitted with UV tube (to make area free from any live contamination).
- A spirit burner or gas micro burner for flame sterilization of cabinet.

3- Growing or culture room:

- In this room, the cultures grow under controlled environmental conditions for a period of time, therefore, in this room must be provide the following environmental conditions:
- Temperature, humidity and illumination by using :- (air condition, oil heater, neon lumps) also should provide: thermometer, timer, different roofs or shelves to placed the culture vessels.

- **Aseptic technique in plant tissue culture**
- Aseptic technique is the name given to the procedures used by microbiologists to prevent microbial contamination of themselves, in plant tissue culture, contamination the cultures by different microorganisms is one of the major problems, therefore, aseptic conditions must be established and maintained. Microorganisms tend to have faster growth rates than plant cells and if aseptic conditions are not maintained, the growth media can quickly become contaminated, micro-organisms growing on any medium will quickly alter the chemical environment by not only using the growth media and plant cells as food substrates, but also by excreting metabolites into the media. All these processors will lead to a rapid loss of defined and controlled conditions in the culture vessels.

- Source of contamination may come from:

1-The explant itself 2- culture vessels 3- media 4- working persons
5- from the instruments used in this technique.

- **Sterilization methods:**
- Several techniques are employed for the sterilization of different requirement in PTC. The methods can be classified as follows:

- **Wet heat**
- This type of sterilization is achieve by using autoclave at a temperature 121°C and pressure 1.04kg/cm (i.e this sterilization employs an autoclave operated with steam under pressure). If the laboratory is not equipped with an autoclave, a home pressure cooker can be used. This method is used for sterilization:- media, glassware, metal tools, plastic, cotton and paper materials.

Moist/steam heat sterilization

AUTOCLAVE

- Sterilization with Steam Under Pressure
- Time required at 121^0 C is 15 mins at 15 psi of pressure.

Advantages

1. Time efficient.
2. Good penetration.
3. The results are consistently good and reliable.
4. The instruments can be wrapped prior to sterilization.

Disadvantages

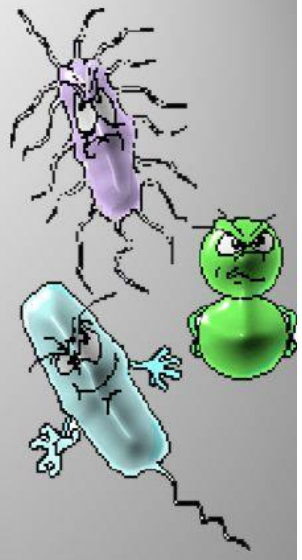
1. Blunting and corrosion of sharp instruments.
2. Damage to rubber goods.

AUTOCLAVE

- The autoclave is a equipment used to remove microorganisms (Virus, Bacteria, fungus etc.) and spores using high pressure and high temperature steam Sterilization

- **Dry heat**
- This method is used for glass ware, metal instruments or other materials that are charred by high temperatures objects containing cotton, paper or plastic and media can not be sterilized by this method. This method is achieve by using the oven at a temperatures between 160-170˚C for 3-4 hours.
-

2. Dry heat sterilization



Conventional dry heat ovens:

- **Achieved at temperature above 160^0 C**

for 2 hours

Advantages of dry heat sterilization

1. No corrosion is seen in carbon-steel instruments and burs
2. Maintains the sharpness of cutting instruments
3. Low cost of equipment

Disadvantages

1. Long cycle is required because of poor heat conduction and poor penetrating capacity
2. High temperature may damage heat sensitive items

such as rubber or plastic goods

3. Generally not suitable for handpieces

- **Ultra filtration**
- Some media components (like vitamins and growth regulators) are unstable at high temperatures and must be sterilized by ultra filtration at room temperature.
- Usually a small volume is sterilized by passage through a membrane filtration unit attached to a graduated syringe. Nuclepore and Millipore are examples for filters this method. The diameter of their openings is 0.22 micron or less to remove all of the microorganisms and get full complete sterilization.

- **Chemical sterilization**
- This method used for sterilization the following:
- Surfaces of working tables 2- the hands 3- explants 4- glass ware
  5- metal tools 6- laminar air flow cabinet.
- By using different chemical materials such as: ethyl alcohol, $HgCl_2$, sodium and calcium hypochloride.
-
- Efficiency of this method depends on:

1-Kind and concentration of chemical materials.

2-Period of sterilization.

3-Degree of contamination.

- **Breeding and improvement of the plants**
- PTC is used to produce plants (hybrid plants) by:
- Cultured sexual cells like: egg, ovule, ovary, anther and microspore to production of haploid plants culture.
- Crosses between distantly related species through protoplast fusion of these species.
- Crosses pollinate between distantly related species.
- Induction of mutagenesis.

- **Genetic transformation**
- This technique is used to:

1-Introduction of foreign DNA to generate novel genetic combinations.

2-Study the function of genes.

- **Production of secondary metabolites by using tissue culture technique**
- PTC was used to produce secondary metabolites since 1950. The importance was given mostly to the production of the medicinal compounds especially those medicinal compounds characterized by the following:

1-It is difficult to produce these compounds by chemical or microbiological methods (until now can't be synthesis).

2-They have a high healing efficiency.

3-No sound chemical compound can match the activity of these medicinal compounds

- Ex: vincristine, vinblastine, digoxin, and digitoxin.

- **Problems of production the secondary metabolites from the farm plants.**
- Despite of the easiness of the production of the secondary products from the farms without sophisticated requirements of the PTC, but there are found some problems in the form plants.
- The presence of the secondary compound in a specific part of the plant like the roots. In this case this plant should be left or bluck out.
- Some plants need a long period of time to produce the required products.
- Productions of high quantities of secondary products require a large amount of the plants and a large area.
- Some plants are grown in places hard to reached.
- Extraction of the secondary metabolites from the farm plants are expensive.

Tissue Culture 3

Lecturer Zena Qaragholi

- **<u>Regulation of medicinal compounds production in cultured cells:</u>**
- Efficient production of secondary metabolites by PTC is largely depends on the following factors:

- **<u>The components of the nutrient media:</u>**
- One of the major advantage of plant cell cultures over animal cell culture is that plant cells can be grown in a simple synthetic medium, the chemical composition of commonly employed media have been primarily devised for the production of secondary metabolites.
-
- Generally the components of the media are divided into various groups:
- Inorganic compounds:
- A-1- Macro elements:
- These are nitrogen compounds as NO_3 and NH_4, phosphorus compounds as PO_4, K, Ca and Mg, all of which are usually present in a study quantity. These elements have both structural and functional roles in protein synthesis (N and S), nucleotide synthesis (P, N, S), cell wall synthesis (Ca), enzyme co factors and membrane integrity (Mg) and addition to other functions.

- A-2- Micro elements:
- These elements are found in study quantities and include Mm, Zn, Br, Cu, CO, Fe, Mo and other elements. Many of these elements have roles in enzyme function as co-factors, in addition to other functions.
- Usually the production of the secondary compounds are affected by the type and concentrations of these elements like the cardiac glycosides production from the digitalis plants that needs a medium rich with salts like Murashige and Skoog medium, whereas this media is a rich source for high quantities of N elements which is necessary to form the great structure formulas for these compounds. Also this medium is a source of Mn and Mg which are necessary for the stimulation of charged enzymes to form these compounds.
-

- B- Organic substances:
- The most commonly organic substances used are:
- B-1- Vitamins:
- As thiamine HCL, Myo-inositol, Nicotinic acid, pantothenate, biotin and pyridoxine HCL, generally these vitamins are important co enzyme for the secondary metabolism.
-
-

- B-2- Amino acid:
- The most frequent used amino acid is the glycine; arginine, asparagine, aspartic acid, alanine, glutamic acid, glutamine, and proline are also used. Amino acids provide a source of reduced nitrogen and like ammonium ions; uptake causes acidification of the medium.
-

- Carbon source:
- Carbohydrates are the source of nutrients in the growth media. Sucrose is used to satisfy the carbohydrate requirements. Glucose has been superior to sucrose. Other carbohydrate can be used but non shown superiority over sucrose and glucose. The carbohydrate components in the media play many roles:
- Preservation of the osmotic pressure.
- They are a source for energy.
- They are a source for glycone part.
-
- All of these are reflected on the production of secondary metabolites. This is clear in the case of Digitalis plant whereas the presence of sugar in the media lead to the increasement of the digitalis compounds as secondary products. This is because that the sugar content is needed in the structural formation of the glycosides as the glucose attachment to the aglycone moiety.

- Other additions:
- Precursors:
- An exogenous supply of a biosynthesis precursor of culture medium may increase the yield of the final product, when the productivity is limited by lack of the precursor. In scopolia and Datura cultures, experiments showed that production of tropane alkaloids could be markedly increased by the addition of tropic acid, the direct precursor.
- Then, the administration of a direct precursor is necessarily effective precursor for increasing the content of the final product.
-

- Gelling agents:
- Tissue culture media are presented to the explant in a liquid or semi-solid state, then the explant may be immersed in a liquid medium or may be positioned above the medium, whereas the forms of media are depended on the type of culture being grown.
- For any culture types that require the plant tissue to be grown on the surface of the medium, it must be solidified (gelled). Agar, produced from seaweed (Gellidium cartilliginum), it is the most common type of gelling agent and is ideal for routine applications. This gelled agar will support the explant and keep it from submerging in the medium yet allow diffusion of the medium ingredients into the plant tissues. The agar quality can vary from supplier to supplier, where a range of purer gelling agents are available, ex: Agar-Agar, and Difco-Bacto-Agar.

- Plant growth regulators (PGR):
- Plant growth regulators are the critical media components in determining the developmental pathway of the plant cell. PGR is used most commonly are plant hormone or their synthetic analogues.
- The most critical PGR of plant propagation media are auxin and cytokinin. These two classes of PGR help maintain differentiation of cell growth, promote cell division and effect secondary metabolism.
-

- Plant growth regulators (PGR):
- Plant growth regulators are the critical media components in determining the developmental pathway of the plant cell. PGR is used most commonly are plant hormone or their synthetic analogues.
- The most critical PGR of plant propagation media are auxin and cytokinin. These two classes of PGR help maintain differentiation of cell growth, promote cell division and effect secondary metabolism.
-

- 3-2- Cytokinins:
- Cytokinins are substances of organic bases. Naturally occurring cytokinine are a large group of structurally related (they are purine derivative compounds). They have many chemical specifications and physiological effects on the growth in small quantities like:
- Stimulation of cell division
- Stimulation of cell enlargement towards the cross axis
- Cytokinine involved into the controlling of most diverse development procedures
- Increasing the growth of lateral shoots by breaking the apical dominance (inhibition of apical dominance).

- 3-3- Gibberellins:
- Gibberellins are organic acid. They are formed in the plant in more than 21 kinds for example: GA_3 and GA_7. They have roles in some physiological process like:
- Stimulation of cell division
- Stimulation of cell enlargement toward linear axis
- Roots formation
- Callus formation

- **2-Environmental condition:**
- 2-A- light:
- The illumination of plant should be considered in terms of intensity, light period and quality.
- Light is needed to regulate certain morphological processes, as well as important for the formation of shoots, the initiation of roots and formation of secondary compounds.
- Including cardinolides, flavonoids. It has been clearly demonstrated that large increases in the quantities of all enzymes involved in the accumulation of the flavone and flavonal glycosides occur upon illumination of the cultures.
- If the metabolites could be produced in dark, so it will be more economic but most of the activities of the enzymes involved in the biosynthesis of the products are initiated by light illumination.
- It has been found that fluorescent tubes are generally used as light source, with intensities in the range of 1000 to 5000 lux, for a period 16-18 hours.

- 2-B- Temperature:
- The main physical requirement for the growth and maintenance of plant cell tissue cultures is the ability to maintain a constant temperature of 25°C ± 2°C.
-
- **3- pH of the culture media:**
- Optimal growth in PTC usually occurs in media with initial pH values in the range of 5-6. pH of the media is usually remaining constant during the course of the growth.
-
- **Nature of cultured explaants:**
- The production of secondary products by tissue culture depends on the type cultured part. That is to say if cardiac glycosides are needed, it is better to cultivate Digitalis shoot tips (قمم الافرع) rather than other parts of the explant, because the storage of these compounds is occur in this part.
-
- **Genetic factors:**
- Quantity and quality of secondary products depends on certain genetic factors in the genus or the species of the explants. Example: Digitalis lanata contains digoxin that is not present in D. purpurea.

Types of cultures in PTC:

Shoot culture:

Plant regeneration from shoot tips, meristem tips, and nodes has been successful in many medicinal and aromatic plants. Shoot culture, often produces a higher rate of products than callus and suspension cultures. More cardiac glycosides are accumulated in shoot culture, of Digitalis than undifferentiated cultures of this species.

Root culture:

It can be established from explants of the root tip of either primary or lateral roots and can be fairly cultured on simple media.

The establishment of root culture was one of the first achievements in PTC but it is not widely used.

Ex: production atropine alkaloid from the root culture of Atropa belladonna plant.

Callus culture:

Callus cultures are larger aggregates of undifferentiated plant cells usually grown on solidified nutrient media. The state of undifferentiated growth is maintained by the phytohormon balance, mainly auxins and cytokinins, added to the medium.in PTC, this tissue can be used for production different medicinal compounds.

Ex: nicotine and morphine.

Cell suspension culture:

Callus cultures can be transformed into liquid medium to establish suspension cultures, which are placed on a shaker to supply the cells with sufficient oxygen. Suspension cultures are an excellent source of material for enzyme purification and investigation to the molecular regulation of biosynthetic pathways.

Tissue Culture

By Lecturer Zena Qaragholi

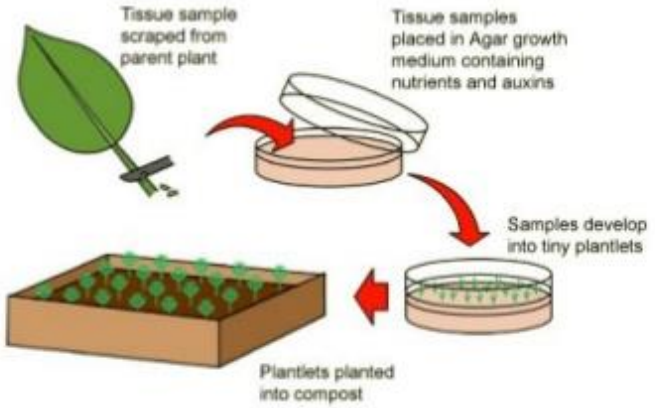
What is Plant tissue culture?

DEFINITION

- **Tissue culture** is *in vitro* cultivation of plant cell or tissue under aseptic and controlled environmental conditions, in liquid or on semisolid well defined nutrient medium for the production of primary and secondary metabolites or to regenerate plant.

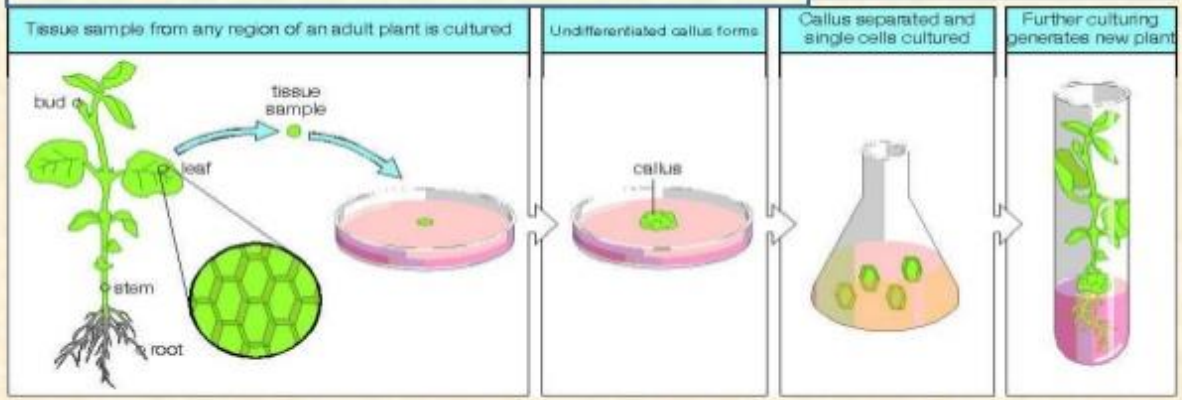
Plant Tissue Culture

Plant tissue culture is the science of growing plant cells, tissues or organs isolated from the Mother plant, on artificial media in vitro under controlled conditions

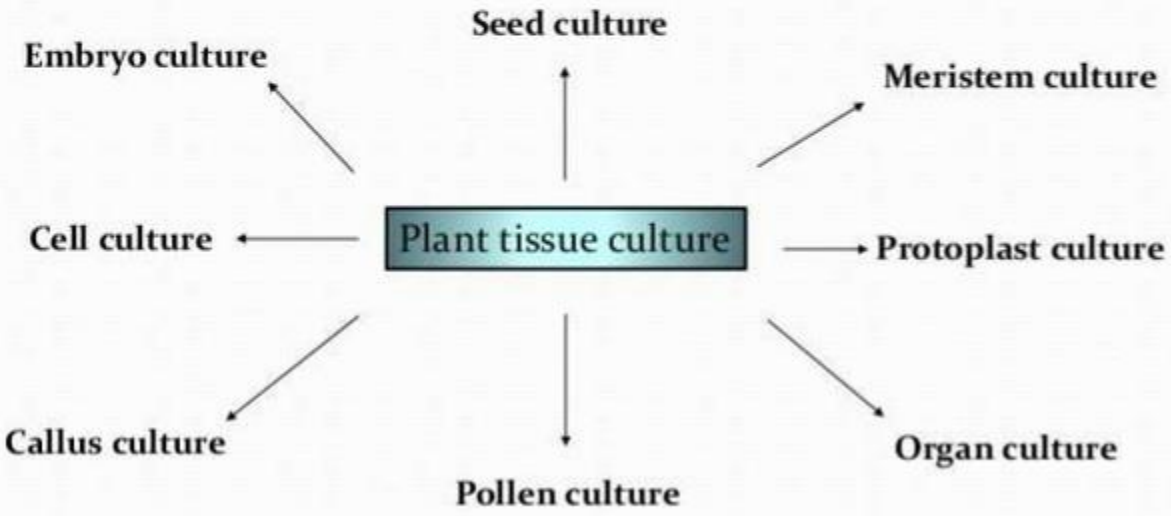


TYPES OF PLANT TISSUE CULTURE

- SEED CULTURE
- EMBRYO CULTURE
- MERISTEM CULTURE
- BUD CULTUR
- CALLUS CULTURE
- CELL SUSPENSION CULTURE
- ANTHER CULTURE/OVARY CULTURE
- PROTOPLAST CULTURE



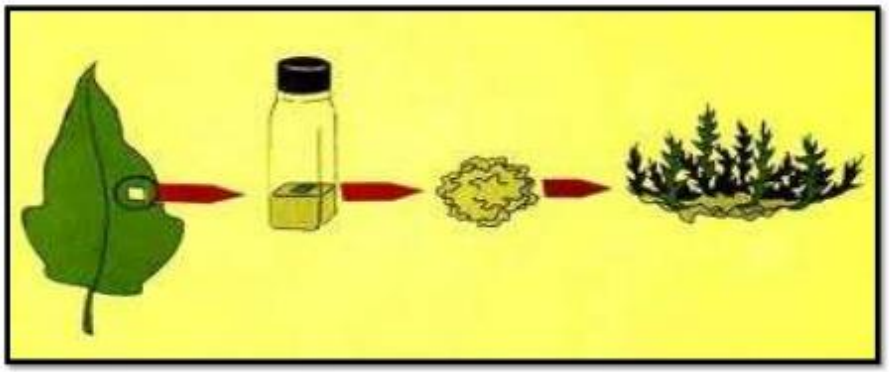
TYPES OF PTC


Plant tissue culture
Seed culture
Embryo culture
Meristem culture
Cell culture
Protoplast culture
Callus culture
Pollen culture
Organ culture

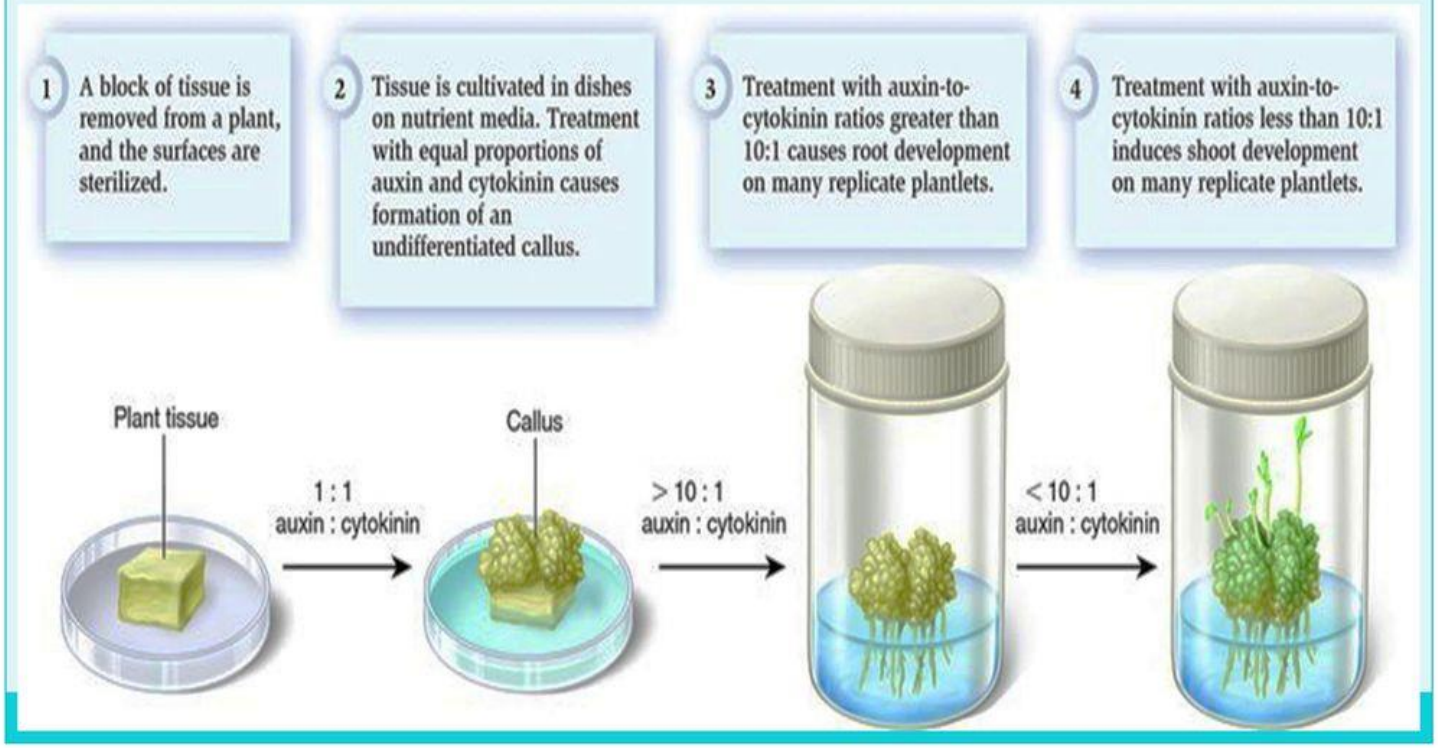
❖ ESTABLISHMENT OF PLANT TISSUE CULTURE

In vitro culturing of plant tissue culture involves the following steps.

- Collecting & sterilization of glassware tools/vessels.
- Preparation of explant.
- Surface sterilization of Explant.
- Production of callus from explant.
- Proliferation of culture.
- Sub culturing of callus.
- Suspension culture

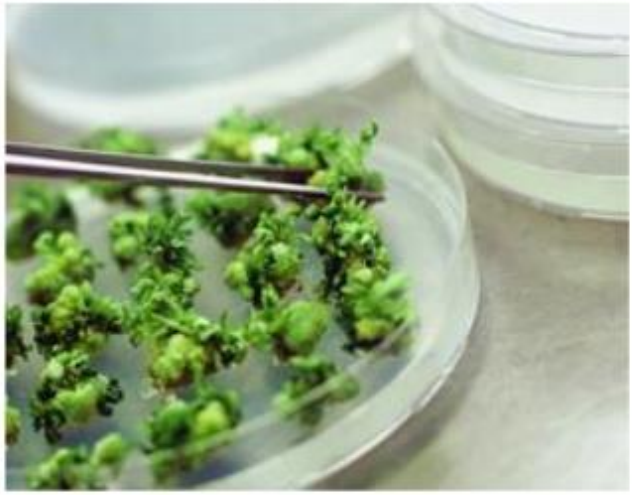


The ratio of auxins and cytokinins play an important role in the morphogenesis of culture systems. When the ratio of auxins to cytokinins is high, embryogenesis, callus initiation, and root initiation occur. For axillary proliferation and shoot proliferation, the ratio of auxins to cytokinins is kept low.

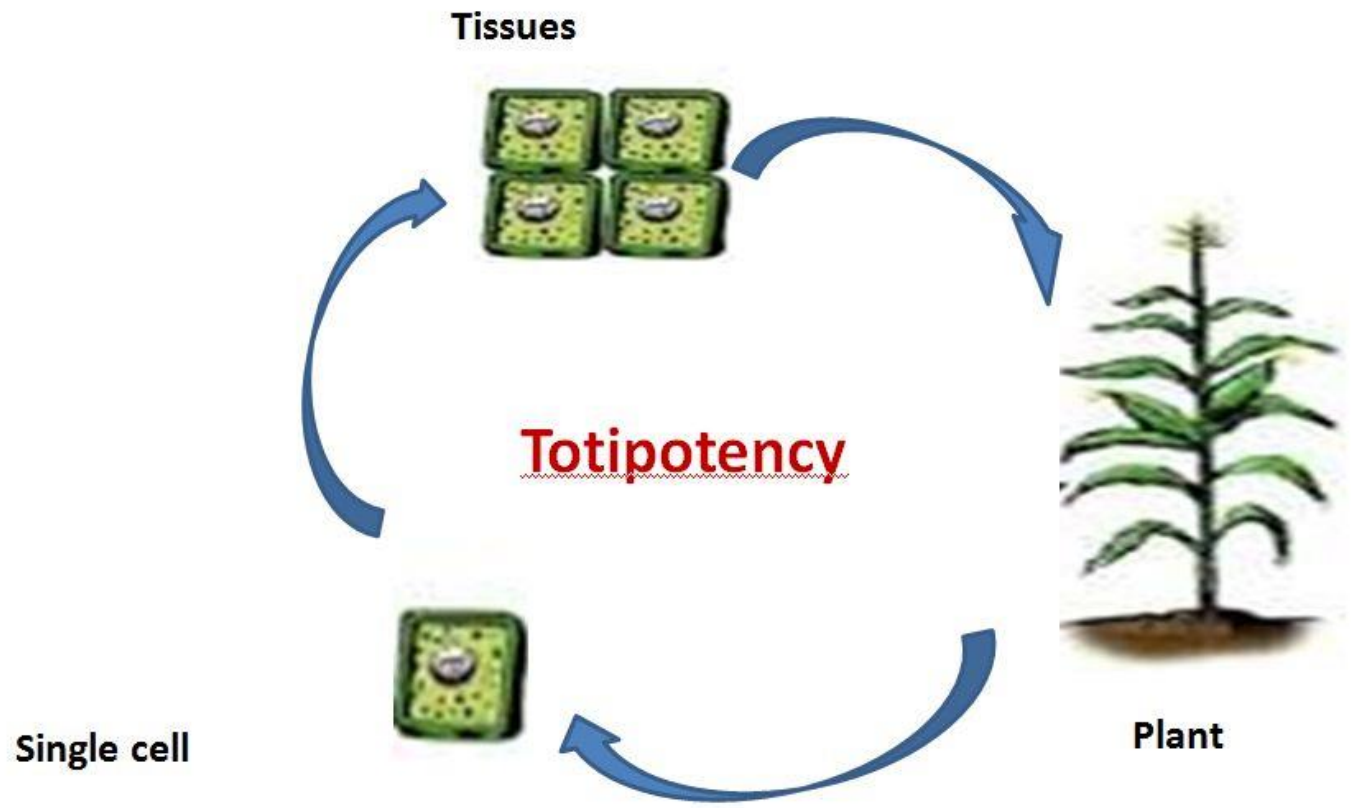


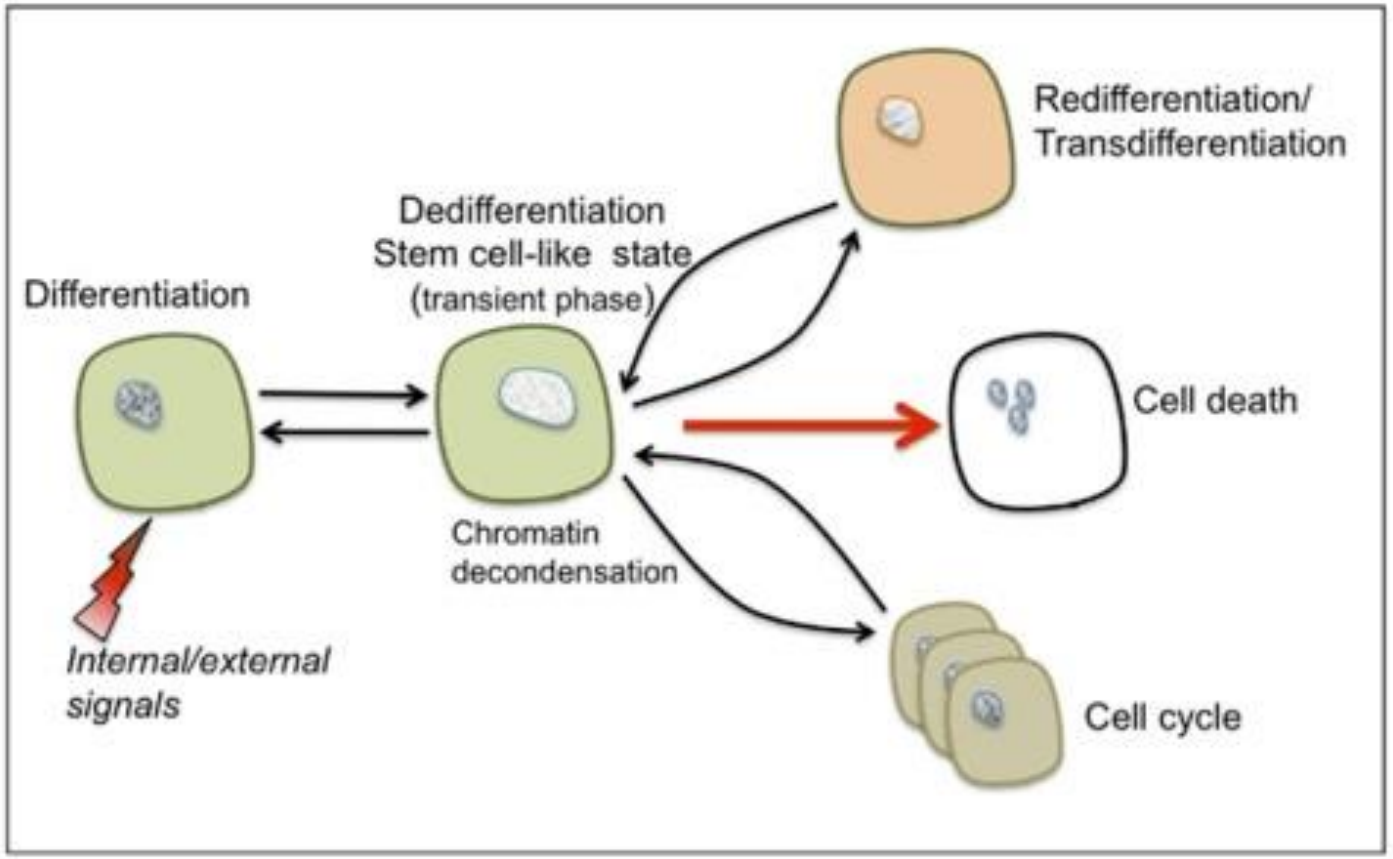
CALLUS PRODUCTION

- **Callus**: a thickened and hardened part of the skin or soft tissue, especially in an area that has been subjected to friction



- Tissue culture relies on three fundamental abilities of plant there are:
  - **Totipotency**: the ability of a single cell to divide and produce all of the differentiated cells in an organism
  - **Dedifferentiation**: a process by which structures or behaviors that were specialized for a specific function lose their specialization and become simplified or generalized.
  - **Competency**: the ability to do something successfully or efficiently


Tissues
Totipotency
Plant
Single cell


Redifferentiation/
Transdifferentiation
Dedifferentiation
Stem cell-like state
(transient phase)
Differentiation
Cell death
Chromatin
decondensation
Internal/external
signals
Cell cycle

Key Concepts

- **Plasticity** – ability of a plant to endure extreme conditions by changing growth and development of plant organs
- **Totipotency** – concept that any part of the plant can give rise to a entire new plant given the right conditions

  Both concepts allow plants to be cloned and generated via cell or tissue culture.
- **Phytotoxic** – Compounds that is toxic or inhibits plant growth

Cell Differentiation

The process by which cells become specialized in form and function. These cells undergo changes that organize them into tissues and organs.

Morphogenesis

As the dividing cells begin to take form, they are undergoing morphogenesis which means the "creation of form."

Morphogenetic events lay out the development very early on development as cell division, cell differentiation and morphogenesis overlap

• **Organogenesis**: The process of initiation and development of a structure that shows natural organ form and/or function.

• **Embryogenesis**: The process of initiation and development of embryos or embryolike structures from somatic cells (Somatic embryogenesis).

Advantages of tissue culture

- To produce many copies of the same plants then which may be used to produce plants with better flowers, odors, fruits or any other properties of the plants that are beneficial to the human beings.
- To produce plants anytime we want although the climates are not appropriate to produce a plant. Moreover, if seed is not available, it is possible to produce a plant with this method.
- If there is plant with partially infected tissue, it is possible to produce a new plant without infection.
- Very helpful in the genetically modified organism studies.

APPLICATIONS OF PLANT TISSUE CULTURE

1.It helps in rapid multiplication of plants.

2. A large number of plantlets are obtained within a short period.

3. Plants are obtained throughout the year under controlled conditions, independent of seasons.

4.. It is an easy, safe and economical method for plant propagation.

5. In case of ornamentals, tissue culture plants give better growth, more flowers and less fall-out.

6. Genetically similar plants are formed by this method.

7. The rare plant and species are multiplied by this method and such plants are saved.

Toxic Plants- Part 1

Lecturer Zena Qaragholi

Toxic Plants: are plants that are allergenic or that cause dermatitis or mechanical injury are annoying, but the victim usually recovers. Plants that are poisonous when eaten, however, may cause serious illness or even death.

Poisonous or toxic plants are those plants that produce toxins as a defense mechanism that deter herbivores from consuming them.

Tannin, for example, is a defensive compound that emerged relatively early in the evolutionary history of plants, while more complex molecules such as polyacetylenes are found in younger groups of plants such as the Asterales. Many of the known plant defense compounds primarily defend against consumption by insects, though other animals, including humans, that consume such plants may also experience negative effects, ranging from mild discomfort to death.

- Some plants can be poisonous if you eat them. Others can hurt you if you get them on your skin. For some plants, all parts of the plant are poisonous. For others, only certain parts of the plant are harmful. The danger can range from mild irritation to severe illness or death.

Plants are divided into 2 parts:

- poisonous plants
- non-poisonous plants

Also mushrooms can be poisonous and need to be taken under consideration.

Poisionus plants

1- Apple (seeds, leaves), *Malus spp* Seeds are mildly poisonous, containing a small amount of amygdalin, a cyanogenic glycoside. The quantity contained is usually not enough to be dangerous to humans, but it is possible to ingest enough seeds to provide a fatal dose.

2-Apricots, cherries, peaches, plums, nectarines (seeds, leaves) *Prunus spp.* Leaves and seeds contain amygdalin, a cyanogenic glycoside.

3-Daffodi, *Narcissus* زهرة النرجس

The bulbs are poisonous and cause nausea, vomiting, and diarrhea; can be fatal. Stems also cause headaches, vomiting, and blurred vision.

4-Atropa belladonna

One of the most toxic plants, all parts of the plant contain tropane alkaloids. The active agents are atropine, hyoscine (scopolamine), and hyoscyamine, which have anticholinergic properties.The symptoms of poisoning include dilated pupils, sensitivity to light, blurred vision, tachycardia, loss of balance, staggering, headache, rash, flushing, dry mouth and throat, slurred speech, urinary retention, constipation, confusion, hallucinations, delirium, and convulsions. The root of the plant is generally the most toxic part, though this can vary from one specimen to another. Ingestion of a single leaf of the plant can be fatal to an adult. Casual contact with the leaves can cause skin pustules. The berries pose the greatest danger to children because they look attractive and have a somewhat sweet taste. The consumption of two to five berries by children and ten to twenty berries by adults can be lethal. In 2009, a case of *A. belladonna* being mistaken for blueberries, with six berries ingested by an adult woman, was documented to result in severe anticholinergic syndrome.The plant's deadly symptoms are caused by atropine's disruption of the parasympathetic nervous system's ability to regulate involuntary activities such as sweating, breathing, and heart rate. The antidote for atropine poisoning is physostigmine or pilocarpine. In humans its anticholinergic properties will cause the disruption of cognitive capacities like memory and learning.

5-Elephant ear, *Colocasia esculenta*

6-Foxglove, *Digitalis purpurea* The leaves, seeds, and flowers are poisonous, containing cardiac or other steroid glycosides. These cause irregular heartbeat, general digestive upset, and confusion; can be fatal.

7-Datura stramonium same toxic ingredients as in Atropia

*8-Nerium oleander*All parts are toxic, the leaves and woody stems in particular. Contains nerioside, oleandroside, saponins and cardiac glycosides. Causes severe digestive upset, heart trouble and contact dermatitis. The smoke of burning oleander can cause reactions in the lungs, and can be fatal

9-Epipremnum aureum اللبلاب

10-Rhubarb leaves, Rheum spp.

The leaf stalks are edible, but the leaves themselves contain notable quantities of oxalic acid, which is a nephrotoxic and corrosive acid present in many plants. Symptoms of poisoning include kidney disorders, convulsions and coma, though it is rarely fatal. The LD_{50} (median lethal dose) for pure oxalic acid in rats is about 375 mg/kg body weight, or about 25 grams for a 65 kg . Cooking the leaves with soda can make them more poisonous by producing soluble oxalates.However, the leaves are believed to also contain an additional, unidentified toxin, which might be an anthraquinone glycoside.

11-Rosary pea, *Abrus precatorius*

Symptoms of poisoning include nausea, vomiting, convulsions, liver failure, and death, usually after several days. Ingesting a single seed can kill an adult human. The seeds have been used as beads in jewelry, which is dangerous; inhaled dust is toxic and can be fatal. The seeds are unfortunately attractive to children.

Non poisonous plants
1- Rose, Rosa

2- Wild strawberry

3-Coleus

- **Mushrooms**
- Eating any amount of any wild mushroom could be very dangerous. Mushrooms may look alike but be very different.

- **Treatment**
- **Mouth**
- Remove any remaining portion of the plant, berry or mushroom.
- Save a piece of the plant or mushroom in a dry container for identification.
- Have the person wash out the mouth with water.
- Check for any irritation, swelling or discoloration.
- **Skin**
- Remove contaminated clothing.
- Wash skin well with soap and water.
- **Eyes**
- Wash hands with soap and water to avoid further irritation to the eye.
- Rinse eye with lukewarm tap water for 10-15 minutes.

- **Prevention**
- Identify and label the plants in your area, yard, and home.
- Wear gloves while gardening.
- Keep plants, seeds, fruits and bulbs stored out of reach of children. A leaf can block an infant's airway.
- Remember Christmas plants may be dangerous.
- Teach children to keep plants out of their mouths and not to suck on flowers or make "tea" from leaves.
- Do not eat wild plants, especially mushrooms.
- Do not make homemade medicines, shampoos, potions or teas from plants.
- Avoid smoke from burning plants.
- Never chew on jewelry made from seeds, beans, or grasses from plants.
- Recognize plants that may cause a rash, such as poison ivy, poison oak, or bull nettle.
- Do not make toys or whistles from unknown flowers or trees

Toxic Plants- Part 1

Lecturer Zena Qaragholi

Toxic Plants: are plants that are allergenic or that cause dermatitis or mechanical injury are annoying, but the victim usually recovers. Plants that are poisonous when eaten, however, may cause serious illness or even death.

Poisonous or toxic plants are those plants that produce toxins as a defense mechanism that deter herbivores from consuming them.

Tannin, for example, is a defensive compound that emerged relatively early in the evolutionary history of plants, while more complex molecules such as polyacetylenes are found in younger groups of plants such as the Asterales. Many of the known plant defense compounds primarily defend against consumption by insects, though other animals, including humans, that consume such plants may also experience negative effects, ranging from mild discomfort to death.

- Some plants can be poisonous if you eat them. Others can hurt you if you get them on your skin. For some plants, all parts of the plant are poisonous. For others, only certain parts of the plant are harmful. The danger can range from mild irritation to severe illness or death.

Plants are divided into 2 parts:

- poisonous plants
- non-poisonous plants

Also mushrooms can be poisonous and need to be taken under consideration.

Poisionus plants

1- Apple (seeds, leaves), *Malus spp* Seeds are mildly poisonous, containing a small amount of amygdalin, a cyanogenic glycoside. The quantity contained is usually not enough to be dangerous to humans, but it is possible to ingest enough seeds to provide a fatal dose.

2-Apricots, cherries, peaches, plums, nectarines (seeds, leaves) *Prunus spp.* Leaves and seeds contain amygdalin, a cyanogenic glycoside.

3-Daffodi, *Narcissus* زهرة النرجس

The bulbs are poisonous and cause nausea, vomiting, and diarrhea; can be fatal. Stems also cause headaches, vomiting, and blurred vision.

4-Atropa belladonna

One of the most toxic plants, all parts of the plant contain tropane alkaloids. The active agents are atropine, hyoscine (scopolamine), and hyoscyamine, which have anticholinergic properties.The symptoms of poisoning include dilated pupils, sensitivity to light, blurred vision, tachycardia, loss of balance, staggering, headache, rash, flushing, dry mouth and throat, slurred speech, urinary retention, constipation, confusion, hallucinations, delirium, and convulsions. The root of the plant is generally the most toxic part, though this can vary from one specimen to another. Ingestion of a single leaf of the plant can be fatal to an adult. Casual contact with the leaves can cause skin pustules. The berries pose the greatest danger to children because they look attractive and have a somewhat sweet taste. The consumption of two to five berries by children and ten to twenty berries by adults can be lethal. In 2009, a case of *A. belladonna* being mistaken for blueberries, with six berries ingested by an adult woman, was documented to result in severe anticholinergic syndrome.The plant's deadly symptoms are caused by atropine's disruption of the parasympathetic nervous system's ability to regulate involuntary activities such as sweating, breathing, and heart rate. The antidote for atropine poisoning is physostigmine or pilocarpine. In humans its anticholinergic properties will cause the disruption of cognitive capacities like memory and learning.

5-Elephant ear, *Colocasia esculenta*

6-Foxglove, *Digitalis purpurea* The leaves, seeds, and flowers are poisonous, containing cardiac or other steroid glycosides. These cause irregular heartbeat, general digestive upset, and confusion; can be fatal.

7-Datura stramonium same toxic ingredients as in Atropia

*8-Nerium oleander*All parts are toxic, the leaves and woody stems in particular. Contains nerioside, oleandroside, saponins and cardiac glycosides. Causes severe digestive upset, heart trouble and contact dermatitis. The smoke of burning oleander can cause reactions in the lungs, and can be fatal

9-Epipremnum aureum اللبلاب

10-Rhubarb leaves, Rheum spp.

The leaf stalks are edible, but the leaves themselves contain notable quantities of oxalic acid, which is a nephrotoxic and corrosive acid present in many plants. Symptoms of poisoning include kidney disorders, convulsions and coma, though it is rarely fatal. The LD_{50} (median lethal dose) for pure oxalic acid in rats is about 375 mg/kg body weight, or about 25 grams for a 65 kg . Cooking the leaves with soda can make them more poisonous by producing soluble oxalates.However, the leaves are believed to also contain an additional, unidentified toxin, which might be an anthraquinone glycoside.

11-Rosary pea, *Abrus precatorius*

Symptoms of poisoning include nausea, vomiting, convulsions, liver failure, and death, usually after several days. Ingesting a single seed can kill an adult human. The seeds have been used as beads in jewelry, which is dangerous; inhaled dust is toxic and can be fatal. The seeds are unfortunately attractive to children.

Non poisonous plants
1- Rose, Rosa

2- Wild strawberry

3-Coleus

- **Mushrooms**
- Eating any amount of any wild mushroom could be very dangerous. Mushrooms may look alike but be very different.

- **Treatment**
- **Mouth**
- Remove any remaining portion of the plant, berry or mushroom.
- Save a piece of the plant or mushroom in a dry container for identification.
- Have the person wash out the mouth with water.
- Check for any irritation, swelling or discoloration.
- **Skin**
- Remove contaminated clothing.
- Wash skin well with soap and water.
- **Eyes**
- Wash hands with soap and water to avoid further irritation to the eye.
- Rinse eye with lukewarm tap water for 10-15 minutes.

- **Prevention**
- Identify and label the plants in your area, yard, and home.
- Wear gloves while gardening.
- Keep plants, seeds, fruits and bulbs stored out of reach of children. A leaf can block an infant's airway.
- Remember Christmas plants may be dangerous.
- Teach children to keep plants out of their mouths and not to suck on flowers or make "tea" from leaves.
- Do not eat wild plants, especially mushrooms.
- Do not make homemade medicines, shampoos, potions or teas from plants.
- Avoid smoke from burning plants.
- Never chew on jewelry made from seeds, beans, or grasses from plants.
- Recognize plants that may cause a rash, such as poison ivy, poison oak, or bull nettle.
- Do not make toys or whistles from unknown flowers or trees

Toxic plants –part 2

Lecturer Zena Qaragholi

CLASSIFICATION, IDENTIFICATION & CHEMICAL CONSTITUENTS OF POISONOUS PLANTS

INTRODUCTION TO PLANT TOXICOLOGY

- Toxic plant may be defined as “one which detrimentally affects the health of man or animal when eaten in such amount as would be taken normally or under special circumstances like restriction of choice of diet or extreme hunger”.
- The toxic (active) principles present in the plants = PHYTOTOXINS.
- The basic framework of protoplasm = Amino acids (20)
- Pathway of metabolism 1-Primary metabolites :glucose, AA. 2-Secondary metabolites :alkaloids, glycosides, terpenes, resins.

SECONDARY PLANT METABOLITES {SPM}

Plant toxins may be referred as SPM. SPM : defense mechanism / survival adaptations. Toxic plants are of 2 types i. Plant containing toxic ingredients & are known to be toxic to animals. ii. Plants which are normally not toxic to animals but becomes so under unfavorable conditions.

CLASSIFICATION OF TOXIC PLANTS

I. Alkaloids

II. Terpenes

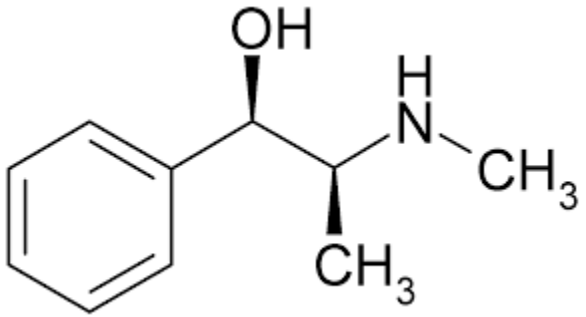
III.Glycosides

IV. Proteinaceous compounds

V. Organic acids

VI. Resins & Resinoids

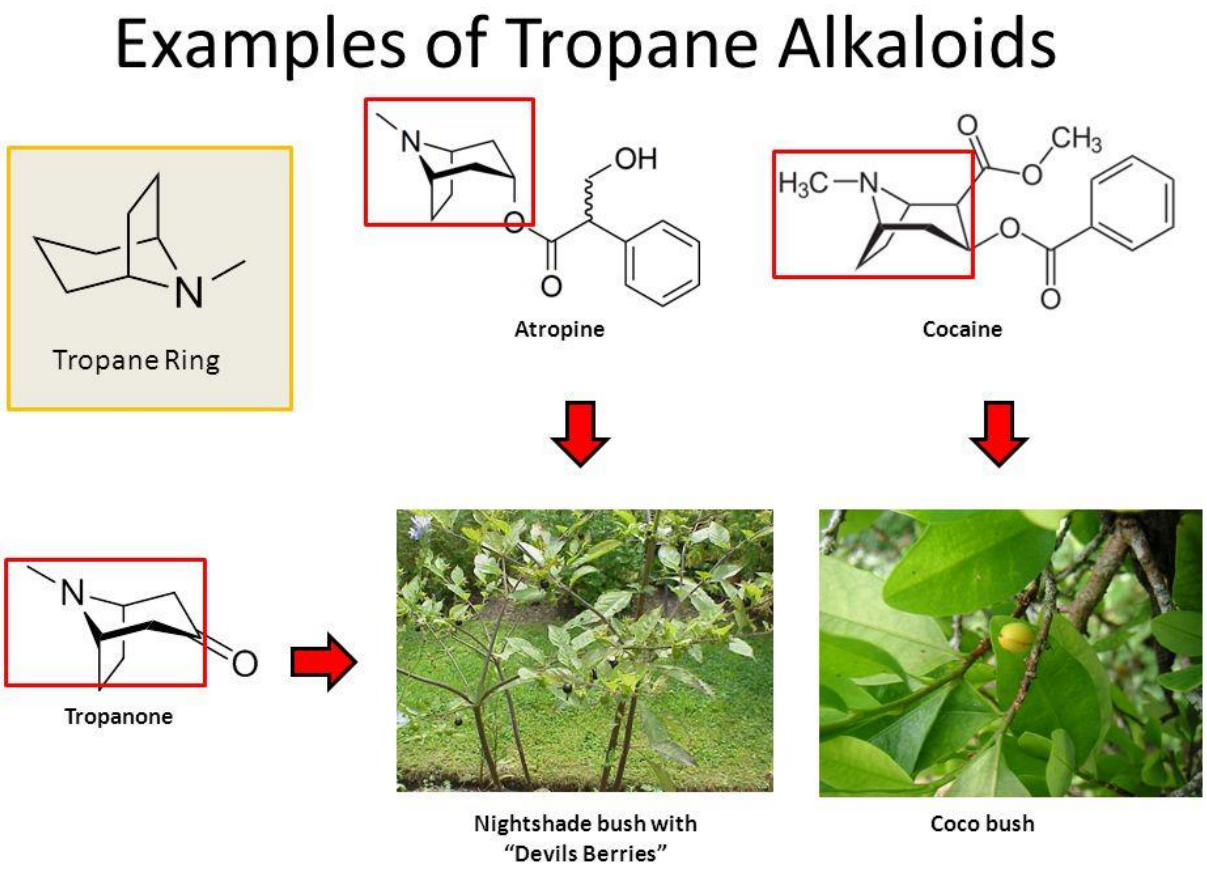
ALKALOIDS



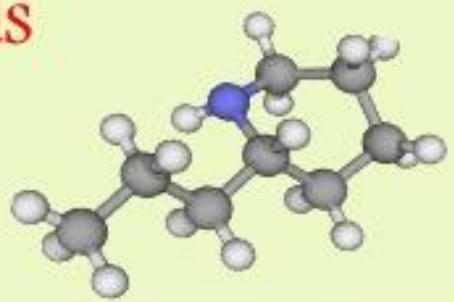
Properties:

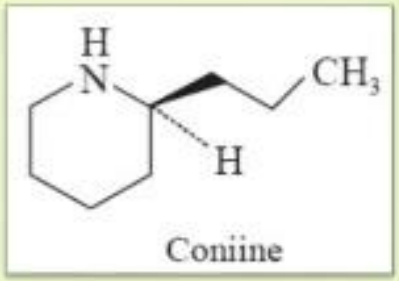
- Complex nitrogen containing organic compounds having one/more heterocyclic rings.
- Alkaline in nature.
- Readily soluble in alcohol, but sparingly soluble in water.
- Both alkaloids + alkaloid salts precipiated by tannic acid & oxidized by potassium permanganate.
- Bitter in taste & often poisonous.
- Name ends with suffix –ine. Eg: atropine, epinephrine, ergotamine, apomorphine.

TROPANE/ATROPINE LIKE ALKALOIDS Atropine Datura (jimsonweed) Erythroxylum (coca tree) Hyoscyamus



Pyridine/Piperidine alkaloids



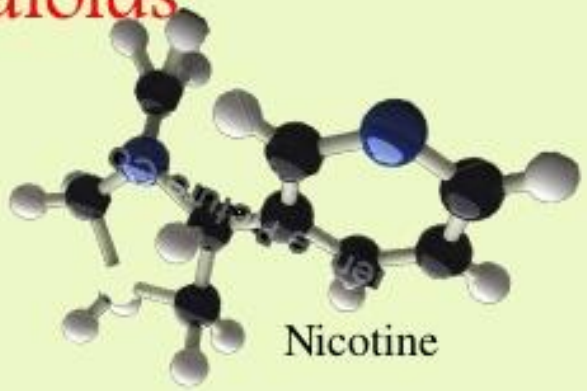


Coniine

Conium (Hemlock)

Lobelia (Indian tobacco)

Pyrrolidine-pyridine alkaloids

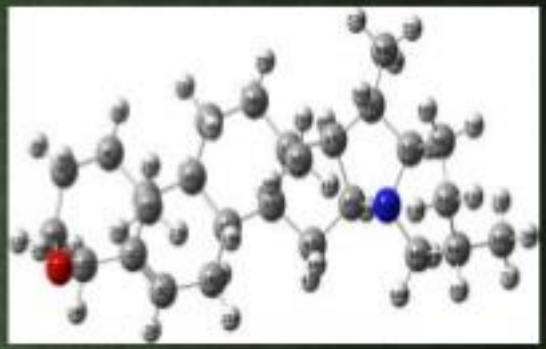


Nicotine

Nicotiana sp. (Tobacco)

Equisetum sp. (Horse tail)

STEROIDAL GLYCOALKALOIDS



Solanidine

Lycopersicum sp. (Tomato)

Solanum sp. (Nightshades)

TERPENES--- Characteristics

1-Biosynthesized by plants .Contains the branched 5-carbon skeleton of isoprene.

2-On the basis of number of isoprene units present in the structure of the molecule, terpenes are categorized as C-10 compounds - monoterpenes C-15 compounds - sesquiterpenes C-20 compounds -diterpenes C-30 compounds -triterpenes

GLYCOSIDES---Charecteristics

1-Complex organic compounds having glycone attached to aglycone/genin moiety by ether linkage

2-Neutral in reaction

3-Soluble in alcohol, less soluble in water & insoluble in ether

4-They don't combine with acids to form salts

5-Names ends with suffix –in. Eg: digitoxin, ouabin, scillarin, glycyrrhizin, senegin.

CYANOGENIC GLYCOSIDES Amygdalin Amygdalin (Almond seed) HCN in Hydrangea, Linum (Linseed) Prunus (Wild cherry) Sorghum vulgare (Jowar) Sorghum sudanese (Sudan grass) Gossypol (cotton seed)

STEROIDAL (CARDIONILIDES/CARDIAC GLYCOSIDES) Digitoxin Digoxin from Digitalis sp. Oubain from Strophanthus Convallarin from Convallaria Ascleipas (Milk weed) Nerium oleander

PROTEINACEOUS COMPOUNDS… Characteristics

1-Plant proteins = harmless + beneficial agents.

2-Plant protein + seed reserve proteins

3-important source of food

4- There are no. of proteins, peptides/amines which are of toxicological importance. Eg: toxalbumins, polypeptides, amines.

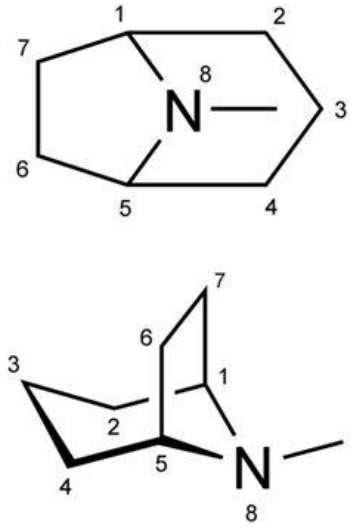
RESINS & RESINOIDS---Characteristics

1-Toxic plant resins = phenolic compounds 2-Important naturally occurring phenolic resin in plants 3-Exists as amorphous & brittle solids 4-Insoluble in water, soluble in organic solvents (alcohol, chloroform & ether) I. Tetrahydrocannabinol II. Hypericin III. Urushiol

Tropane Alkaloids

Lecturer Zena Qaragholi

Tropane Alkaloids

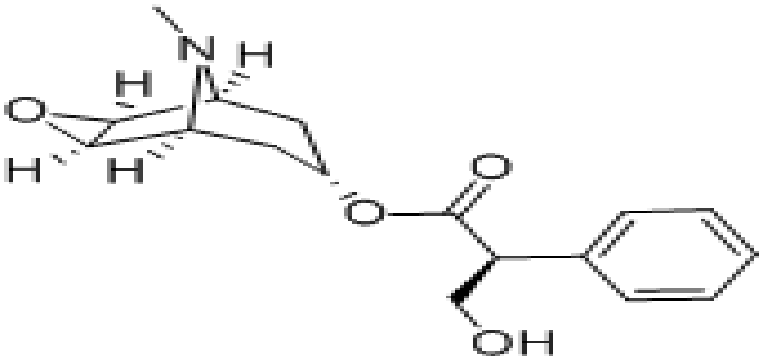


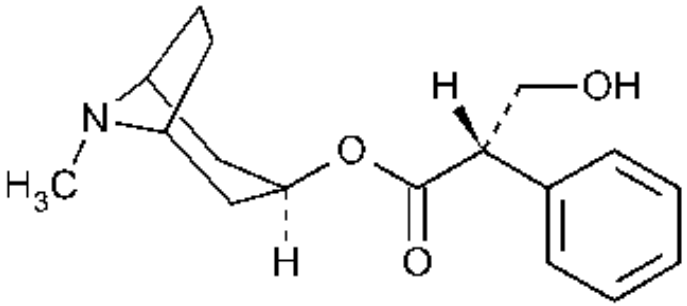
Tropane ring system

- Class of alkaloids that contain a tropane ring system in their chemical structure.
- Naturally produced in plants of the family *Solanaceae*.
- Tropane alkaloids biologically perform one of two functions:
  1. Anticholinergics (blocks the activity of acetylcholine)
  2. Stimulants

- ***]*** **Tropane Alkaloids**
- **Datura stramonium**, known by the common names **Jimson weed** or **datura**, is a plant in the ***Solanaceae*** (nightshade) family. For centuries, datura has been used as a herbal medicine to relieve asthma symptoms and as an analgesic during surgery or bone setting. It is also a powerful hallucinogen and deliriant, which is used spiritually for the intense visions it produces. However, the tropane alkaloids which are responsible for both the medicinal and hallucinogenic properties are fatally toxic in only slightly higher amounts than the medicinal dosage, and careless use often results in hospitalizations and deaths.

- ***Constituents of datura are***:
- ***Hyoscyamine*** and its isomer ***atropine***, which is formed during extraction procedure. Also it contains ***hyoscine*** (scopolamine) alkaloid, which is found in trace amounts.
- The medicinal use is mostly due to the hyocsyamine (atropine), used as mydriatic, antispasmodic, antidote to the toxicity of cholinergic compound, decrease in the secretion (upper and lower respiratory tract) before surgery. While the use of scopolamine mostly in motion sickness. The tropane alkaloids (hyocsyamine and hyoscine) have the following structures:
-
-





- **Hyoscine (scopolamine)** **Hyoscyamine**

Buscopan Tablets

Buscopan is an anticholinergic medicine which relieves the pain of stomach and bowel cramps by helping your digestive system to relax.
Each Buscopan tablet contains 10 mg of Hyoscine Butylbromide.
It is also available as Buscopan Plus which contains Paracetamol 500 mg and Hyoscine Butylbromide 10 mg.


Injectable Solution
100 ml
ATROPIN
PAIN-KILLER
ANTI-SPASM
PREMEDICATION
GMP
Anvet Pharma
GMP-WHO
www.anvetpharma.com
COMPOSITION: 100ml contains
Atropine sulfate.....50 mg
INDICATIONS:
- The treatments for intestinal spasms, severe diarrhea, tracheal and bronchial spasms.
- Pain relief, premedicationin surgery and detoxication for cases poisoned by Dipterex, Pilocarpine
DOSAGE & ADMINISTRATION:
For subcutaneous injection or intramuscular injection
- Dogs, cats: 1ml/10-15 kgs B.W.
- Swine, sheep, goats and bovine: 1ml/ 7-10 kgs B.W.
Withdrawal time: 7 days before slaughter.
Contra - indications: The medicine must not be used in cases with gastroparesis
PRESERVATION: Store it in a cool place and keep out of direct sunlight.
FOR VETERINARY USE ONLY
Lot :
Mfg :
Exp :
Manufactured by: ANVET PHARMA JSC
Address: Plot B2-19 Tan Dong Hiep B Industrial Park, Tan Dong Hiep Ward, Di An, Binh Duong, Vietnam.
Tel: (+84.650) 377 6891 - 377 6892 / 93 / 94 Fax: (+84.650) 377 6895
Email: anvet.jsc@anvetpharma.com * Website:www.anvetpharma.com
ANVET PHARMA

These alkaloids are also present in other plants as **Hyoscyamus niger** of the family Solanaceae, **Atropa belladona** of the same family, and others.

- **Isolation and Identification of the Datura Alkaloids:**
- ***Extraction:***
- ***Aim***: to isolate datura alkaloids.
- ***Equipments:***
- *Reflex apparatus.*
- *Conical flasks.*
- *Stirrer.*
- *Funnel.*
- *Separatory funnel.*
- *Water bath.*
- *Filter paper.*
- *Litmus paepr.*

- ***Reagents:***
- *90% ethanol.???*
- *2% HCl.???*
- *Ammonium hydroxide solution.???*
- *Chloroform.*

- Extract ***50 gm*** of the datura fruits in ***150 ml*** of *90%* ***ethanol*** under Reflex condenser for **1 hrs.**

Filtration

Take ***20 ml*** of alc. Extract in conical flask and concentrate on the water bath to about ***2 ml*** to remove all of ethanol

Pour the concentrated in to ***10 ml*** of ***2% HCl***

Heat gently

(5 min$_{s.}$)

Cool and filter the Acidic extract and place in a separatory funnel

[Wash with ***5 ml*** of ***Chloroform***] two times

Take supernatant (upper layer) and made alkaline by addition of

- ***Ammonium hydroxide*** solution (check by litmus paper)
-
- [Partition with ***5 ml*** of ***Chloroform***] two times
-
- Take the lower layer, dehydrate by adding ***anhydrous sod. Sulphate*** filter (or decant) , evaporate to dryness