INDUSTRIAL PRODUCTION AND UTILISATION OF PHYTOCONSTITUENTS

SENNOSIDES

Indian senna

Cassia angustifolia

TINNELVELLY SENNA

Alexandrian senna

Cassia acutifolia

CASSIA SENNA

LEGUMINOSAE

- Senna pods are the dried or nearly ripe pods of the both above species.

1.2-2.5% of anthraquinone 2.5-4.5% Glycosides- sennoside A and B

INDIA- Tamil nadu cultivated more in sudan

- Maharastra, Gujarat, Rajasthan (MGR)

- (EP-European pharmacopeia)

Cultivation in India

- Senna is an annual crop
- Cultivated mainly in Tinnevely, Madurai and Ramanthapuram dist of Tamil nadu and on small scale in other states of Maharashtra,Gujarath and Rajasthan.
- Senna plant grown wild in Kutch a dist in Gujarath.
- By seed propagation (march-April) – (nov-dec).
- Mostly cultivated as a pure crop but on small scale as a mixed crop along with chillies and coriander.

- Cultivated on red soil as well as on black soil.
- Fertilizers required are nitrogen-80 kg, k2-20kg/hectare.
- It needs sufficient rainfall.
- The crop remains in the field for 8-9 months (survive 2-3yrs).
- Senna plant is a small shrub of 1-1.5 m in ht.
- Propagation is done by seeds which are rubbed with coarse sand and sown by broadcasting method or in rows 30cm apart.
- Seed germination on the 3rd day.
- The crop becomes ready after 2 months. first plucking is done after 3 months of sowing – leaves appears thick, mature, bluish green in colour.
- Second plucking is done after a month followed by subsequent pluckings after 4-6 weeks.
- After plucking the plant is up rooted

Production

- Total area under production in India is approximately 25,000 hectar
- Total production of senna leaves -22,500 tons
- Total production of senna pods-7500 tons.

Preparation for the market

- **Drying**: harvested leaves are spread in thin layers on a floor under the shade (avoid overlapping) stir the material frequently for uniform drying for a week or 10 days then leaves become yellowish green in colour then remove the sticks, stems, sand, stones etc by sieving.
- **Drying in sun:** poor colour and quality inferior.

METHOD-A

- Extract the senna leaves in powder form with 70% methanol filtered concentrated under vaccum acidified with HCL at PH 3 filtered

- Extract the aqueous solution with chloroform to remove soluble a glycone if any neutralised with ammonia and centrifuged to separate SENNOSIDES

- Senna leaf powder is extracted with benzene
- Benzene extract marc is left after the filtration is dried at room temperature or in hot air oven not exceeding 40 degree.
- Dried marc is extracted with 70%methanol on electric shakers for 4 to 6 hours at room temperature
- Mix both the extracts . Concentrate to 1/8^{th} volume with reduced pressure.
- Adjust the PH 3 with HCL and keep it aside for 3 hours and filter. To the filtrate add calcium chloride with vigorous shaking.
- Adjust the PH to 8 by adding ammonia solution, keep it for 2 hours, and filter the solution. Dry the precipitate over phosphorous pentoxide.

Manufacturers of calcium sennosides

1. Galxo labs Ltd, worli, Mumbai.
2. Sandoz India Ltd, Mumbai.
3. Chemical industrial pharmaceutical laboratories Ltd, Mumbai.
4. CIPLA, alembic chemical works, Baroda.
5. KOTHARI phytochemical international, Madurai.
6. Bengal immunity co, Ltd, Calcutta.

Utilization (uses)

- The senna leaves and pods (fruits) are mainly used as an cathartic.
- Sennosides the main constituent of senna are useful purgative for either habitual constipation or for occasional use.
- Sennoside preparations are most important pharmaceutical laxatives among the number of synthetic preparations.

CARDIAC GLYCOSIDES

DIGITALIS

PURPLE FOX GLOVE LEAVES

DIGITALIS PURPUREA
DIGITALIS LANATA

SCROPHULARIACEAE

- Perennial herb, 45-105cm
- Height with alternate leaves

glycosides digoxin, lanatoside C.

- Leaves-10cm long
- 4-15cm wide

perennial or biennial herb (1m in height). Shape- ovate, lanceolate, leaves-alternate

- Color- greyish green.
- Margin-serrate or dentate.
- Flowers-pinkish purple with purplish
- Deep purple spots.

45cm width, margin-entire, apex – Acuminate, flower-yellowish white with or without network

Indigenous to UK and grown
As an ornamental plant.
Widely distributed and cultivated
In Egypt, Japan, India,
North America, commercially
Produced in Holland.

Indigenous to Europe, cultivated
In Holland, USA, Asian
countries. In India Digitalis
plants are used medicinally
and as ornamental plant.
Cultivated in Dehradun, Nilgiris,
Chakrata, Jammu, WB, HP,
Hilly Places in Tamil nadu.

OTHER SPECIES CONTAINING CARDIAC GLYCOSIDES

Digitalis dubia, D.grandiflora, D.lutea, D.ferruginea, D.nervosa and D.thapsi. (Not much used commercially)

CULTIVATION

By seed propagation.

- It requires well drained sandy, mild acidic soil for D.purpurea and alkaline soil for D.lanata both the plants require semi-shady places.
- The growth and glycosidal content are maximum between 20-30 degree C. At lower temperature both growth and biogenesis of glycoside are effected.
- The seeds are soaked in the water at 30˚C for 2 days for germination.
- After the germination, the seeds are directly sown into the field in the month of October-November and weeding in the month of April-may following spacing approximately 30*45 cm apart.
- First and second year the leaves are collected in the month of June-August early afternoon for the maximum glycoside content.
- Entire plant 10-15cm above the ground is cutted –D.purpurea, rosette leaves are cutted 2-3cm above the soil – D.lanata gives max leafage and cardenolide content.

ISOLATION OF CARDIAC GLYCOSIES (AS PER BP)

Digitalis leaf powderExtracted with 50% ethanol

At low temperature Lead acetate sol is added

Ppt removed by centrifugation

Supernatant liquid Extracted with chloroform

Chloroform extract is evaporated

Residue of cardiac glycoside (purified by chromatography)

PRODUCTION OF CARDENOLIDE GLYCOSIDE

They are produced by cell and organ culture technique.

Green hairy roots produced by the light exposure gives a max increase in cardenolide accumulation over the roots cultivated in dark.

UTILISATION

"*DIGITALIS LANATA*" is more potent than "*DIGITALIS PURPUREA*"

Both plants contain cardiac glycosides and "DIGOXIN" as main constituent." DIGITALIS LANATA" leaves are used for the manufacture of DIGOXIN and other glycosides.

- The digitalis glycosides used to increase the force of myocardial contraction.
- Used in cardiac arrhythmias such as atrial premature beats, atrial fibrillation and supra ventricular tachycardia.
- Digitalis leaves are used in the manufacture of DIGOXIN and DIGITOXIN.DIGITOXIN is more potent glycoside.
- Digoxin is valuable for rapid digitalization and in the treatment of atrial fibrillation and in the congestive heart failure.
- Digoxin is excreted more rapidly than the other glycosides of digitalis.Digoxin are also used in the treatment of animal heart failure.

VINCA

CATHARANTHUS ROSEUS

Vinca rosea, periwinkle, lochnera rosea

- Sadaphul, nayantara, sadabahar.

BIOLOGICAL SOURCE

- Vinca is the dried whole plant of "*Catharanthus roseus*"

FAMILY:APOCYNACEAE

MACROSCOPICAL CHARACTERS

It is an ever blooming herb or sub-shrub, woody at the base 40-80cm in ht.Flowers-violet, pink, white (used as an ornamental plant)Leaf-shining brilliantly dark green in colour, oblong, acute base and rounded apex.

CHEMICAL CONSTITUENTS

The total alkaloid content in leaves 0.60-0.6%

- In stem o.26-0.31% In roots 0.51-0.75% In root bark 4.5-9.0%
- The plant contains more than 100 alkaloids of indole group out of which about 25 are dimeric in nature.
- Two dimeric alkaloids vinblastine and vincristine are used in the treatment of human neo-plasm. (Present in the leaves)
- Monomeric ajmalicine (raubasine) has been used in the relief of obstruction of normal cerebral blood flow.
- In combination with rauwolfia alkaloids it has been reported to lower high BP (hypotensive alkaloid- ajmalicine-present in root).

CULTIVATION AND HARVESTING OF VINCA

- Seed propagation or vegetative propagation –annual plant.Plant do not grow in highly alkaline or water logged soil it prefers light well drained, sandy loamy soil with a rainfall of 100cm per annum is required.
- The fresh seeds are sown in late june in rows 10-15cm apart in small beds. The seedlings are transplanted at a distance of 30-45cm (75000 plants per hectare).The plants are manured with 20 kg N/hectare
- After 150 days of sowing, the roots penetrate the soil up to 15-20cm then develop lateral roots.
- Roots collection is done after 1yr.The harvested leaves are dried in shade and stored in gunny bags.

PRODUCTION OF VINCA ALKALOIDS BY PLANT TISSUE CULTURE

- Vincristine and vinblastine are produced by shoot suspension culture.
- Ajmalcine, catharanthidine, serpentine, vindaline and yohimbine alkaloids have been produced by hairy root culture obtained by infection with *Arobacterium rhizogenes.*
- Serpentine and ajmalicine have been produced by cell suspension culture of *C.roseus*.

UTILISATION

Used for diabetes as folk remedy.

- The roots are toxic and used as a stomachic.
- Sedative, for hypertension-Vinca alkaloids.
- Vinblastine, vincristine, vindoline, vinleurosine and vincosidine used effectively in the treatment of neoplastic disease in animals and in man (leukemia).
- Vinblastine sulphate used mainly in Hodgkin's disease(lymphoid carcinoma)
- Vincristine sulphate is used in tumor of brain, breast and lungs.
- Root alkaloids: serpentine and ajmalcine used in high BP.
- Madagascar Vinca is used in diabetes, high BP, asthma, constipation, menstrual problems, leukemia, various cancers.
- In Jamaica, "tea of Vinca" was used to treat diabetes.

Menthol (oleum mentho piperita)

- Menthol is an alkaloid obtained from volatile oils of the plants of "*Mentha piperita*" and other species of *mentha.*
- The mentha oil (V.oil) is obtained by STEAM DISTILLATION of the fresh flowering tops of *"Mentha piperita*" and other species of mentha. (Family-Labiatae). Mentha oil contains not less than 50% of total menthol.

PRODUCTION

Menthol is produced by the following process.

- Preparation of mentha oil (V.oil)
- Preparation of menthol from mentha oil.

PREPARATION OF MENTHA OIL (V.oil)

The mentha plants are air dried (drying in sunlight will cause loss of volatile oil) and powdered.

- Air dried powders are placed in mild steamer. The steamer outlet is connected to a condenser.
- Steam under pressure from a boiler is charged into the still. Distillation is allowed to take place for 2-3hrs.
- 80% of the oil is distilled during the first half of distillation. Latter distillation brings out the medicinally and commercially important menthol. (process must be carried out carefully in the latter distillation)
- The distillate leaving the condenser is collected separately. The mentha oil is insoluble and lighter than water and floats.

The mentha oil is decanted and filtered

PREPARATION OF MENTHOL FROM MENTHA OIL

- Mentha oil is freezed (-22˚C). 50% of menthol crystallizes out and is recrystallised from alcohol.
- The separated liquid portion still contains about 40-55% of menthol and 5-17% of esters of menthol.
- By boiling a solution of NaOH the esters are decomposed to form menthol.
- On further freezing the saponified oil gives a second crop of menthol crystals.

The following species contain high % of Menthol and cultivated in different countries:

- *Mentha piperita*
- *Mentha arvensis, Mentha piperascens*
- *Mentha viridis (Mentha spicata)* SPEARMINT (contains carvone. In India it is used as PUDINA)
- *Mentha pulegium*- contains pulegone
- *Mentha piperita* oil- it is peppermint oil obtained by steam distillation from fresh aerial parts of "*Mentha piperita*" (family:Labiatae)

- There are about 3000 mentha species of “Labiatae” family.
- The plants are herbs or under shrubs.
- Leaves are dorsiventral and have characteristic glandular trichomes which contain volatile oil.
- Glandular trichome consists of a 2,4 or 8 cells with a raised cuticle forming a bladder like cuticular sac containing volatile oil.

GEOGRAPHICAL SOURCE:

- It grows wild in Europe and it is also cultivated in Japan, England, France, Italy, USA, Bulgaria and USSR.In India it is cultivated in Jammu and UP.

DESCRIPTION OF MENTHA OIL OR PEPPERMINT OIL

Colour – colourless to yellow

Odour – characteristic and pleasant

Taste – pungent followed by cooling sensation

Nature – it is a clear, transparent liquid

Solubility – soluble in 70% alcohol, ether, chloroform. Insoluble in water.

Standard – it is neutral to litmus paper.

CHEMICAL CONSTITUENTS

- The chief constituent of menthol is volatile oil, it also contains 6-12% of tannins.
- The volatile oil (peppermint oil) contains chiefly menthol to the extent of 70% in free form and in the form of esters.
- It also contains menthone, menthofuran, jasmine, menthyl isovalerate, methyl acetate and other terpene derivatives -limonene, cineole, pinene, camphene, etc.
- Jasmone & esters are responsible for pleasant flavour.
- Menthofuran causes resinification (unpleasant smell)

- **USES OF PEPPERMINT OIL**

Used as a carminative, stimulant, aromatic, counter irritant,flavouring agent, antiseptic, in the preparations of toothpaste, toothpowders, shaving creams, tobacco, betal nut, chewing gums, jellies, perfumes,etc. Also used as an ANTIPRURITIC and as a CARDIAC DEPRESSANT.

- **STORAGE OF PEPPERMINT OIL**

Peppermint oil should be stored in well filled and air tight containers protected from light, in cool place. Peppermint oil becomes dark and viscous on storage. On cooling, the menthol crystals will occur

IDENTIFICATION TESTS FOR MENTHOL

- 10mg of menthol is dissolved in 1ml of sulphuric acid. Add 1ml solution of vanillin in sulphuric acid – it gives orange yellow colour which changes to violet colour on the addition of 1ml of water (Distinction from Thymol).
- Few crystals of menthol is dissolved in 1ml of glacial acetic acid to this 3 drops of sulphuric acid and 1 drop of nitric acid is added-No green colour.(Distinction from Thymol)
- On trituration with an equal weight of camphor or chloral hydrate or phenol-the mixture liquifies

SUBSTITUTES AND ADULTERANTS

American oil of peppermint – contains 80% of menthol.

- Japanese peppermint oil – contains 85% of menthol.
- Menthene (De mentholised oil of peppermint).

CHARACTERS OF MENTHOL

Occurs as colourless, hexagonal crystals. Usually needle shaped, as fused masses or as a crystalline powder.Odour – pleasant peppermint Odour.

Taste – pungent and aromatic.

- Natural menthol is LAEVO ROTATORY.
- Synthetic menthol is RACEMIC. Synthetic menthol is prepared by Hydrogenation of thymol and pinene.

USES

- As an antiprurutic (Relieves itching).
- On skin, mucous membrane as a counter irritant, antiseptic and stimulant.
- Menthol has depressant effect on heart (cardiac depressant).

QUININE

Quinine is obtained from the dried stem and root bark of Cinchona. Cinchona is indigenous to Columbia, Peru and Bolivia. The different species of cinchona are

- *Cinchona succirubra*
- *Cinchona calisaya*
- *Cinchona ledgeriana* ***Family-*** *Rubiaceae*
- *Cinchona officinalis* and their hybrids

Synonyms: Jesuits bark, cortex cinchonae, Peruvian bark.

GEOGRAPHICAL SOURCE

- India and Indonesia are important producers of cinchona.
- High percentage of cinchona is collected from Tanzania, Kenya, Gutemala and Bolivia.
- The annual world production of quinine and its alkaloids are estimated about 1080 tones. India produces 20-30 tones annually.
- In India cinchona is cultivated in Nilgiris and Annamalai hills of Tamilnadu and in Darjeeling (west Bengal).

CULTIVATION AND COLLECTION

Collected from wild as well as cultivated source. It needs a acid soil, rainfall and altitude are important factors in the production of good quality cinchona – high percentage of alkaloids.

- Propagated by vegetative and seeds.
  - **Grafting technique:** Young "*Cinchona ledgeriana"* scion are grafted on "*Cinchona succirubra*" root stock- given a tree with the production of bark rich in the quinidine alkaloid.
  - **Seed propagation:** Healthy seeds without microbial attack.
  - **Vegetative propagation:** Practiced in west Bengal.

Vegetative and seed propagation methods in Tamilnadu.

- Cinchona plants grown well in moist and warm climate in the temperature ranges from 12-20˚C. (forest land is ideal)Seeds germinate within 3 weeksThe seedlings of about 5 cm height are planted out in nurseries under shade.
- The seedlings reaches 20-25cm height they are transplanted in holes a meter apart during rainy season.After 6 yrs the plants are used for collecting the barks.The whole tree is cut down by “coppicing method” close to the ground.

- In Indonesia plants are grown for 12 yrs and then uprooted. The bark is collected during rainy season for easy separation of bark from the trunks.
- The bark is dried in sunlight shade (not direct sunlight) and by artificial heat (not more than 65˚C).
- Care should be taken to avoid mould formation during drying.
- During drying the pale yellow colour of the inner bark of cinchona changes to dark brown or red due to the action of air on phlobatannins present in the bark.
- The barks with 12-15% of moisture are used for the extraction of quinine alkaloids.

PRODUCTION OF CINCHONA ALKALOIDS BY TISSUE CULTURE

SUSPENSION CULTURE: Production of cinchona alkaloids by *in vitro* culture of *C.ledgeriana*. Quinine and quinidine proved to be the major alkaloids in leaf and root organ suspension culture.

METHOD- II (EXTRACTION OF QUININE)

- Cinchona bark is powdered and extracted with benzene or toluene in presence of alkali.
- The alkaloids are extracted with dil.H2SO4.
- By means of neutralizing the acid extract Quinine sulphate separates which is sparingly soluble in water.

CHARACTERS OF QUININE

Colour – white, Odour – odourless, Taste – bitter, Nature – fine needle shaped crystals (on exposing to light it becomes brown in colour).

- Solubility – soluble in ether, alcohol, chloroform and hot benzene. Sparingly soluble in water.
- Properties – Quinine has fluorescent properties.
    - It shows a strong blue fluorescence in U.V.light.
    - Quinine forms salts with different acids.

CHEMICAL CONSTITUENTS

- Cinchona bark mainly contains quinoline group of alkaloids not less than 6.5%.
- The major alkaloids are quinine, quinidine, cinchonine, and cinchonidine.
- The minor alkaloids are quinicine, cinchonicine, hydro quinine, hydro cinchonidine, quinamine, cinchotin, and homocinchonidine

- Most of alkaloids in cinchona bark are found as a salt in combination with quinic acid and cinchotannic acid.
- The bark also contains quinic acid 5-8% and phlobatannin cinchotannic acid in the form of its decomposition product “cinchona red”.
- A bitter amorphous glycoside quinovin 2% is present.

PROPORTION OF ALKALOID IN VARIOUS SPECIES OF CINCHONA ARE

Source	**total alkaloid (%)**	**quinine (%)**
C.succirubra	6.5	0.5-1.5
C.calisaya	6-7	3- 4
C.ledgeriana	5-10(up to 19%)	4-5

TEST FOR QUININE ALKALOIDS (thalleoquine test)

Residue of the bark extract is mixed with 1 drop of dil.H_2SO_4 and 1ml of water. to this mixture bromine water is added drop wise till a permanent yellow colour is obtained and then add 1ml of dil.NH_3 solution.→ an emerald green colour forms.

UTILIZATION

- Cinchona bark and its alkaloids are used as analgesic, anaesthetic, antiarrythmic, antibacterial, antimicrobial, antiparasitic, antipyretic, antiseptic, antispasmodic, antiviral, astringent, bactericide, nerve tonic, stomachic, appetizer, and in hemorrhoids
- Quinine is very effective in the treatment of malaria. It kills malarial parasites in the blood. (Plasmodium falsiparum).
- Natural quinine is more potent than the synthetic quinine

- In Venezuela cinchona is recommended for cancer treatment.
- Quinine is also used as a common cold, cough, influenza, and headache. Amoebic dysentery, for strengthening uterine contraction, in labour pain.
- Quinine sulphate is one of the ingredient of unani medicine for fever due to elephantiasis

CINCHONISM:

Over doses of cinchona causes loss of hearing and sight impairment. Cinchonism causes ringing in the ears (due to continuous intake of cinchona).

- **COSMETICS:**

  Cinchona extracts used in hair tonic preparations.

- **FOOD:** Cinchona extracts are used in frozen dairy desserts baked goods and in condiments.

CITRIC ACID

- Citric acid is a weak organic acid found in citrus fruits. Citric acid is mostly present in most concentrated form in Lemon and Lime.

A pale yellow thick skinned oval citrus fruit with acidic juice is called **lemon - citrus limon**

A rounded citrus fruit like a lemon but greener, smaller and more acidic in taste is called **lime** - **citrus auruntifolia**

Family - Rutaceae

HISTORY

- Citric acid was first isolated in 1784 by the Swedish chemist “Carl Wilhelm Scheele” (crystallised citric acid from lemon juice)
- Industrial scale production was started in 1860, based on the Italian citrus fruit industry.
- In 1893 C.Wehmer discovered penicillin mould could produce citric acid from sugar.
- In 1917 the American food chemist James Currie discovered strains of the mould “*Aspergillus niger*” efficient citricacid

- Pfizer – started industrial level production of citric acid by using the mould "*A. niger*" for the first time.
- Citric acid was originally produced from calcium citrate obtained from cut lemon.
- 90% of citric acid supply is from "*A. niger*" fermentation production methods for citric acid from fungus metabolism initiated in 1923 in US.

PRODUCTION

- Cultures of *"Aspergillus niger"* are fed on sucrose to produce citric acid.
- After the mould is filtered out the resulting solution citric acid is isolated by precipitating it with lime (CaOH) to yield calcium citrate salt $\rightarrow$ treated with $H_2SO_4 \rightarrow$ citric acid

USES

- Citric acid is rapidly and almost completely metabolised in the human body and has a wide pharmaceutical uses.
- Lemon fruit is good natural preservative and used to add an acidic (sour) taste of food and soft drinks.
- Used as a cleaning agent and acts as an anti-oxidant.
- Used as an astringent in lotions to adjust the pH of hair rinses and hair setting preparations, in leather tanning.
- Commercially it is used as a flavouring agent, preservative in food, soft drinks, jams, jellies, wines etc

LABORATORY METHOD: (PRODUCTION OF CITRIC ACID)

- It is obtained from the citrus fruits of "*Citrus limon"* and "*Citrus auritifolia"* $\rightarrow$ Rutaceae.
- MATERIALS REQUIRED: Lemon, 20% KOH or NaOH solution, 10-15% $CaCl_2$, dilute H_2SO_4, funnel.

PROCEDURE

- Squeeze out 50-100ml of lemon juice from the fruits and add 20% KOH or NaOH solution with stirring in order to make the solution slightly alkaline.
- Filter the solution to remove the all fruit parts like pulp, seeds etc by using Buchner funnel.
- To the clear filtrate add 10-15% $CaCl_2$ and stir well. The pale yellow precipate formed can be separated and washed with boiling water

- The formed precipitate calcium citrate is then re-dissolved in boiling water and 10-15ml of dilute H_2SO_4 is added till precipitation is complete.
- Discard the residue and take the filtrate, concentrate the filtrate to $1/3^{rd}$ of its volume and keep over night.
- The citric acid crystals formed in the solution is collected and %yield of citric acid formed is calculated.

INDUSTRIAL METHOD

FERMENTATION TECHINQUE

- It is a process in which chemical changes occur in organic substrate through the action of enzyme liberated by micro organism on their optimum condition.

ORGANISM

- *Pencillium leutium*
- *Pencillium cetrinium*
- *Aspergillus niger*
- *Aspergillus clavans*

SURFACE CULTURE MEDIUM

Factors used in this method

1. →Organism
2. →Medium preparation
3. →pH
4. →Ratio of surface area to the volume of solution fermented
5. →Oxygen supply
6. →Temperature

- **Organism used:** *Pencillium leutium, Pencilluium cetrinium, Aspergillus niger, Aspergillus clavans.*
- **Composition of medium:**
- Consists of sucrose, NH_4NO_3, $MgSO_4$ and HCl acid.
- Sucrose, NH_4NO_3, K_2HPO_4, $MgSO_4$.
- Sugars 14-20% is required.
- Maximum yield is obtained from sucrose and fructose.
- Incubation period is 9-12 days.
- Inorganic salts like Na, K, S, Mg, traces of Fe, Zn, Al, Mn etc.
- Restricted supply of N_2 and minimum amount of inorganic salts produce a higher yield of citric acid
- Cu and Ca inhibit the citric acid production.

- pH- optimum pH should be 1.6 -2.2.
- Temperature: 8-28˚C (optimum temperature 28˚C)
- Oxygen supply: essential for good yield.
- Yield: about 90% citric acid.
- Recovery: after complete fermentation the solution is precipitated from (calcium citrate precipitate) the neutral solution. The precipitate is treated with concentrated H_2SO_4 to liberate free citric acid

MOULD CULTURE MEDIUM

- Successive transfer of the spore from one medium to another medium of same composition may stimulate the mould production in large amount of citric acid.
- Inoculate the spore of *A. Niger* in a flask containing standard medium containing 145 sucrose and maintain the ph 2.
- The prepared single culture is incubated at 20˚C for 10 days

BY FERMENTATION METHOD

- Sterile solution in a shallow pan is inoculated with mould spores. Incubated at favourite temperature for 2-5 days. The surface of the layer is coverd by the mycelial layer. After 7-10 days the citric acid fermentation is completed and removed immediately.

TROPANE ALKALOIDS

- The major tropane alkaloids of pharmacological importance or therapeutical importance are those occurring in number of species of the plant belonging to the Family – Solanaceae.
- Those occurring in certain *Erythroxylon* species “coca leaf”
- (Family - Erythroxylaceae)

SOLACENEOUS AKLALOIDS

- Major alkaloids present in Quantity&therapeuticallly
- A number of varying species of the genera Atropa, Stramonium, Datura, Hyoscyamus,Duboisia and Scopolia (Family – Solanaceae) produces varying quantities of “Tropane alkaloids”. Hyoscyamine (racemic form is “atropine”)
- “Scopolamine” (also called as “hyoscine”)

- Meteloidine,Belladonine, and several other alkaloids related to these.
- These “Solanaceous alkaloids” also called as “belladonna alkaloids”

BELLADONNA HERB

(Deadly Night Shade Plant)

- Consists of fresh or dried leaves and flowering tops of "*Atropa belladonna* Linn" (European Belladona) or "*Atropa acuminata"* (Indian Belladonna) – "Solanaceae"
- It is indigenous and cultivated in England and other European countries. In India, it is found in Western Himalayas from Shimla to Kashmir and in Tamilnadu.
- Over the years it has been procured only from wild sources

- The plant is a tall perennial herb producing dull purple bell shaped flowers followed by shiny black cherry fruits.
- Propagated from seeds.
- Leaves contain richest in alkaloids. At the end of June or in July.
- Plants about “3 years old” $\rightarrow$ Gives large amount (yield) of leaves.
- The tops of the plant are harvested 2-3 times/year and dried immediately after collection and stored carefully. The plant is a tall perennial herb producing dull purple bell shaped flowers followed by shiny black cherry fruits

- The tops of the plant are harvested 2-3 times/year and dried immediately after collection and stored carefully.
- To obtain good coloured leaves drying is done in thin layers starting with a moderate heat, which is gradually increased to about 60˚C and then gradually decreased.
- Roots from 2-3 year old plants are collected for the isolation of alkaloids- generally it contains small quantity.

STRAMONIUM LEAVES
(Thorn Apple Leaves)

- It consists of dried leaves or flowering tops of "*Datura Stramonium"* (Solanaceae). It should contain not less than 0.25% of alkaloid calculated as Hyoscyamine. "*Datura tatula"* (Solanaceae).
- The generic name datura is derived from "Dhat"- An Indian poison. (Datura metal – has narcotic properties)
- The plant is cultivated in Europe and South America. In india plant is grown widly.
- It contains 0.2-0.45% of total alkaloids mainly – Hyoscyamine and hyoscine.

DATURA LEAF

- Consists of the dried leaves and flowering tops of "*Datura innoxia*" and "*Datua metal*"
- Family – solanaceae
- It is obtained principally from India.
- Leaves contain about 0.5 % alkaloids.

HYOSCYAMUS LEAF
("HENBANE LEAF")

- Hyoscyamus leaf (henbane) consists of dried leaves and flowering tops of *Hyoscyamus niger* (Solanaceae).
- It should contain not less than 0.05% of total alkaloids (Hyoscyamine), 0.45-1.4% of tropane alkaloids as hyosyamine.
- It is an annual or biennal herb.

COCA LEAF

- Derives from the shrubs of the:
- *Erthroxylum truxillensa*
- Peruvian or Truxillo
- *Erythroxylum coca*
- Bolivian or Huanuca

  family - Erythrozylaceae
- Cultivated in peru ,Bolivia, Colombia and Indonesia
- cocaine was isolated in the year 1688
- Used as local anaesthetic due to its toxic and addictive properties cocaine is restricted only for ENT surgery

- “**COCAINE TRADE HAS BEEN BANNED”**
- Cocaine leaves contains tropane alkaloids mainly cocaine.
- Local anaesthetic effects is it has hallucinogenic actions leads to addiction – “Narcotic Drugs and Psychotropic Subatances Act 1985” and by relevant acts by other countries.

CULTIVATION AND COLLECTION

- By seed propagation in nursery beds, seedlings of 15-20 cm transplanted to open fields to 2mts distance in each plant. Over a period of 3 years the leaves are collected at one year interval. The leaves are collected in dry weather and dried in shade or by artificial heat.
- This “COCA PLANT” is considered as “DIVINE PLANT”.

PRODUCTION OF COCAINE

- The coca plant contains 0.7-1.5% of total alkaloids. The major alkaloids are tropane esters (Ecgonine derivatives)

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- Coca leaf powder extracted by dil H_2SO_4, or petroleum ether or organic solvents.

Crude alkaloid(solid form)

- **USES**: cocaine and its salts were used as local anaesthetic because of their toxic, addictive properties, ENT surgery.
- 0.02% of coca extract (after the removal of cocaine) is used with the extracts of cinnamom, ginger, lime, orange peel as a flavouring agent in cola drinks.
- 0.055% used as ingredient in alcoholic beverages, frozen diary desserts and candy.
- Cocaine is a powerfully addictive stimulant that directly affects the brain —— “HALLUCINATION EFFECTS”.

DUBOSIA LEAVES

- *Dubosia myoporoides, D.leichhardti*
- major world sources of tropane alkaloids
- *D.hopwoodii* contains nicotine
- Contains not less than 2- 4% of total alkaloids. (60%hyoscine, 40% hyoscyamine)
- Duboisia sps is indigenous to Australia. It is also cultivated in Japan.
- Family – “SOLANACEAE”

USES

- Used as a substitute for Hyosyamus and Datura.
- Source for hyoscyamine and hyoscine
- Mydriatic, Antispasmodic, anti cholinergic
- The tincture of Dubosia used in paralysis and in eye infections (homeopathy treatment)

SCOPOLIA

- It is indigenous to Eastern Europe. In shape and size it resembles Belladonna.
- *Scopolia japonica, Scopolia caucasia, Scopolia lurida, Scopolia tangutica, Scopolia carniolica.*
- Family – “SOLANACEAE”
- Useful source of Hyoscyamine
- “Daturamine” Hydroxy Hyoscyamine alkaloid, anisodine is obtained

UTILIZATION OF TROPANE ALKALOIDS

- Tropane alkaloids possess antispasmodic, Mydriatic and anti cholinergic properties. (atropine, hyoscine and hyoscyamine are strongly mydriatic)
- Belladonna Preparations are used as Pain relieving drugs, counter irritants, in rheumatism, neuralgia. Leaf extracts are used in the peptic ulcer, constipation, dysmenorrhoea, bronchial asthma and in whooping cough.
- Belladonna berries are highly poisonous and are used as specific antidote to opium and muscarine poisoning.

- Belladonna is used in the form of plasters and liniments for local applications. Belladonna suppositories used for spasm of anal fistula.
- Belladonna herb and extracts used internally in various diseases like Parkinsonism (paralysis) and encephalitis. The natural drug and total alkaloids are used as sedative, tranquiizers and in labour pain.

- Atropine alkaloids is CNS stimulant and has a depressing action on nerve endings of the secretory glands and plain muscle (hyper secretion). Atropine and hyoscine $\rightarrow$ Dilates the pupil of the eye. Hyoscine has less CNS stimulant effect and used in motion sickness.

SOLANUM KHASIANUM

- The genus “Solanum” comprises of about 2000 species distributed in the warmer region of the world.
- Represented about 100 species including potato.
- Tuberous group
- Non – tuberous group
- Include rest of species and have been reported to contain glyco alkaloid.
- On the basis of presence of tubers the genus is divided in to 2 main groups
- Solasodine” occur in the form of a glycoside.
- *Solanum laciniatum, S.aviculane, and S.khasianum*(dried berri)
- FAMILY – SOLANACEAE

- It is grown in central India, Myanmar and China. It is wildly distributed in khasia, jainitia, Naga Hills, Assam, Bengal, Orissa, and in Nilgiris.
- **CHARACTERS:** It is much branched under shrub varying in height between 0.75- 1.5m with spines on the skin.
- **Leaves:** are oval, lobed triangularly with hairs and prickles on both surfaces.
- **Flowers:** are white, 1-4 flowered raceme
- **Seeds:** are brown to yellowish or greenish, globule in shape and smooth.

ISOLATION OF SOLASODINE

- The berries of *solanum khasianum* are dried, powdered and the oil is removed by defatting.
- The defatted material is extracted with ethanol (50-250g) in a soxhlet extraction for 6 hours.
- Concentrate the extract by distillation off the solvent to syrupy mass and to this add 5ml of boiling water and make alkaline (pH9) with 10% ammonia solution.
- Cool the mixture below 5˚C for 2hrs and filter. To the precipitate, add a few ml of boiling water and make alkaline (pH 9) with 10% ammonia solution

- Boil the mixture under reflux for 2hrs.Cool and filter. The residue obtained is washed, dried and dissolved in chloroform.
- The above mixture is filtered, Solasodine in the form of residue is obtained and dried to remove the solvents

CULTIVATION AND COLLECTION

By seed propagation. Seeds are sown in nursery beds.

Urea, potash and super phosphate are used as fertilisers.

- The seedlings after sufficient growth are transplanted into open fields at a distance of 50*50 cm.
- After 6months, plants are harvested and the berries are collected.
- The berries are immediately dried in shade or artificially at low temperature to reduce large content of moisture.

- The berries are greenish – brownish in colour with compressed smooth brown seeds.
- The berries contain about 3% steroidal glycol alkaloid called “Solasodine”. The berries also contain 8-10% of greenish – yellow fixed oil.

UTILISATION

Solasodine is used as a precursor for steroid synthesis. Like diosgenin, it is first converted into 16-dihydro pregneselone acetate. The latter is precursor for steroids like corticosteroids, pregnane androstanes. All the above are used as sex hormones and oral contraceptive.

DIOSCOREA

Synonym – WILD YAM

- Dioscorea is one of the largest plant genera containing 600-800 species.
- They are large starchy tubers commonly known as “YAM”.
- About 15 species are reported to contain Steroidal sapogenin, chiefly “DIOSGENIN”.
- Most of the world production of diosgenin is from Central American species,”*DIOSCOREA FLORIBUNDA* and *D.COMPOSITA”.*
- In India, “*D.DELTOIDEA* and *D.PRAZERI*” occurs wildly in Himalayas. (family – DIOSCOREACEAE)

- “Dioscorea deltoidea” grows wild – North West Himalayas, Jammu and Kashmir, HP, Darjeeling, Sikkim, Bhutan, Nepal, UP.
- “*D.DELTOIDEA*” grows better in the areas where the annual rainfall is 100-200cms.
- Minimum temperature in summer does not exceed 32˚C.
- Shady slopes with well drained conditions.
- Diosgenin % in *D.DELTOIDEA* is 0.6-10.3%.
- It is the best natural source of diosgenin.
- In tubers diosgenin occurs as dioscin and closely related species gracillin or both.

CULTIVATION AND COLLECTION

- Dioscorea is obtained both from wild and cultivated sources.
- Dioscorea tubers can be grown by seed propagation but it takes long time for harvesting. Since it is economical to grow it from tubers.
- Soil should be rich with pH 6.8 – 7.2, free from weeds, virus, fungus and insects for healthy growth of tubers.

- Healthy tubers with 50-70gm in wt are selected and treated with fungicides are sown in nursery beds.
- Tubers take 4-5 weeks to sprout. After 2-3 months of growth tubers are retransplanted into well manured fungicides and insecticide treated soil.
- The tubers are placed at a distance of 30*60cm.
- According to the species the tubers reach maturity in 3-5 yrs and on average yield 1-8% of the total sapogenin.
- "*Dioscorea floribunda"* yields 16-18tons of tubers approximately and 500kg of diosgenin per hectare.

PRODUCTION

Method 1

- Fresh tubers are cut into small pieces and crushed
- Fermentation for 4-10 days
- Fermented mixture
- Hydrolysed with mineral acid
- Filtered and washed
- Dried
- Sapogenin (water insoluble)

Method 2

- Cut the tubers into small pieces dried and powdered treated with Mineral acid liberates diosgenin filtered
- insoluble Fraction is neutralised washed
- Extracted with pet. Ether or toluene
- Diosgenin

Method 3

Tubers cut into small pieces Washed dried

Extracted with hot water or 95% ethanol for several hrs

Alcoholic extract

- Concentrated under vaccum
- Glycoside ppts
- Solvent ether or lead acetate
- Hydrolysis
- Extraction with pet.ether 40-60˚C

DIOSGENIN

UTILISATION

- Dioscorea is the major source for the production of diosgenin.
- Diosgenin is used for the synthesis of corticosteroids, sex hormones and anti fertility compounds.
- It is used as a precursor for 50% of the total steroidal products in the world.
- It is used in the treatment of rheumatic arthritis, for the relaxation of muscles and to promote glandular balance in women.
- “wild yam’ is used as nervine tonic and for the improvement of digestion.

PODOPHYLLOTOXIN

- Podophyllum roots and rhizomes

- Indian Podophyllum American Podophyllum
  Should contain not less than 10% resin

- Syn-*Rhizoma podophylli Indici* May apple

B.S.- dried rhizomes and dried roots of
Podophyllum hexandrum *P.peltatum*
P.emodi

FAMILY – BERIBERIDACEAE

IDENTIFICATION TEST

- Alcoholic extract of podophyllum resin with strong sol of copper actetae developes brown ppt – Indian podophyllum
- Green colour without ppt - American podophyllum

COLLECTION AND PREPARATION

- Podophyllum is a perrinial herb, grows wildly in moist and shady places. Most of the drugs are collected from the wild plant.
- After 2 yrs the rhizomes are dug up, washed with water to remove soil and are cut into smaller pieces.
- The adventitious roots present on the rhizome are removed and dried in sun.

PRODUCTION

- 100gm of powdered drug is extracted with 90% alcohol in soxhlet apparatus for 4 hrs.
- Remove alcohol by distillation and evaporate the extract to a syrupy consistency.
- 200ml of water containing HCl is added to it by continuous stirring, cooled below 5˚C for 2 hrs, filter under vacuum. Wash the residue with 50 ml acidified water, cool below 5˚C
- Dissolve the residue in sufficient hot alcohol 90%, filter and evaporate the alcohol off and dry the residue to a constant wt at 80˚C.

USES

- Used as a purgative in constipation.
- A paint is used in warts treatment.

CAUTION

- Podophyllum is a gastro-intestinal irritant. In large doses it produces inflammation of the stomach and intestines.