PHARMACY INDIA

MODEL PAPER

B.PHARMA

5th SEMESTER

PHARMACOGNOSY & PHYTOCHEMISTRY-II

(BP-504T)

PHARMACOLOGY-II MODEL PAPER

SECTION A

VERY SHORT ANSWERS TYPE QUESTIONS (10 × 2 = 20)

1. Differentiate between primary and secondary metabolites with suitable example.

Answer

Primary Metabolites

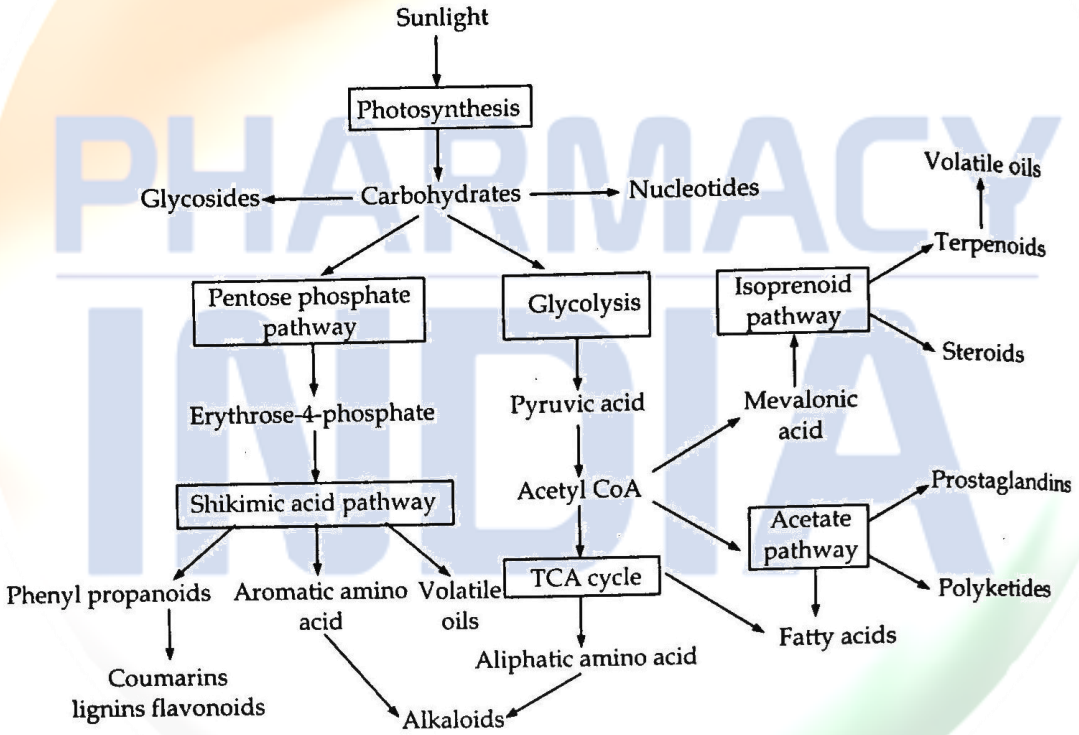
- They are involved in normal growth, development and reproduction. Example – protein, carbohydrate and lipids.

Secondary Metabolites

- They are not involved in normal growth, development and reproduction. Example – Alkaloids, Tannins, Glycosides etc.

2. Give biosynthetic flow of production of various primary and secondary metabolite.

Answer



3. Discuss chemical constituents and uses of asafoetida.

Answer

Asafoetida

Chemical Constituents

- Asafoetida contains resin (40 to 65%), gum (20 to 25%) and volatile oil (4 to 20%).

Uses

- It is used as carminative, nervine stimulant or in intestinal flatulence.
- It is also used as flavoring agent for curries, sauces and pickles.
- It is used in veterinary medicine.

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4. What is the therapeutic significance of ginger?

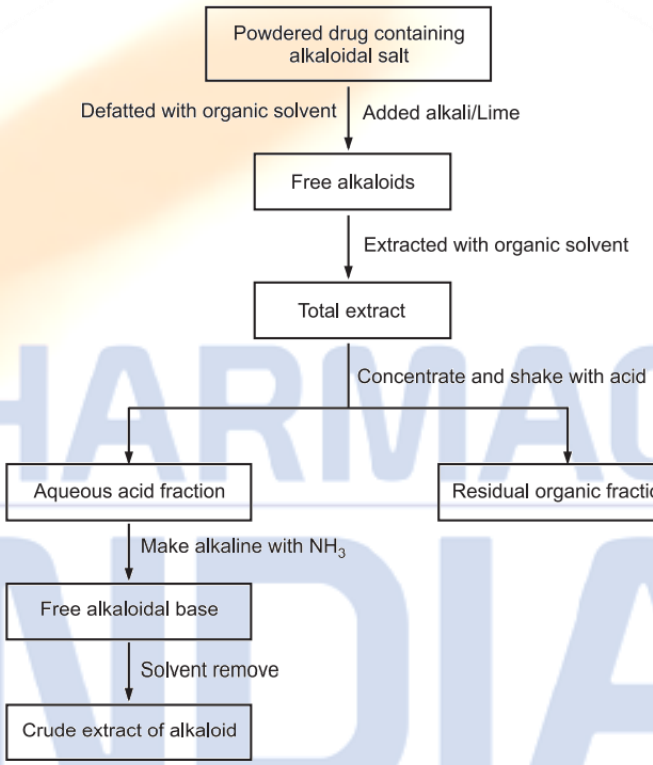
Answer

Therapeutic Significance of Ginger

- Ginger is used as a stomachic, an aromatic, a carminative, stimulant and flavouring agent.
- Ginger oil is used in mouth washes, ginger beverages and liquors.
- Ginger powder has been reported to be effective in motion sickness.

5. Explain stat-otto method for extraction.

Answer



6. Discuss the physiochemical properties of resins.

Answer

Physiochemical Properties of Resins

- Resin is soluble in various liquids but insoluble in water.
- They become soft on heating.
- When ignited, they burn with a smoky flame.
- Resins are hard and amorphous solids; they are heavier than water.

7. What is the biological source and uses of artemisinin?

Answer

Artemisinin

Biological Source

- Artemisnin is isolated from the leaves or aerial part of the plant Artemisia annua (FamilyCompositae).

Uses

- Artemisnin or their derivatives are very useful for their antimalarial activity.

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- It can be dispense in oily suspension (IM) or in tablet or suppositories form.

8. Give chemical identification test of digoxin.

Answer

Chemical Identification test of digoxin

- Dissolve and dilute test sample in hot methanol → The aliquot of the solution is evaporated to dryness → Acid ferric chloride TS is added to the residue → A green colour develops that slowly changes to a deep green blue colour.

9. Explain decoction process of extraction.

Answer

- The process is more suitable for the vegetables drugs which are heat stable and water soluble because in this method the drug is boiled for about 15 min.

Take drug in a vessel add 1000 ml water and cover it
↓
Boil for this for around 15 minutes
↓
Cool it to room temperature
↓
Strain the liquids and press the marc

Flow chart of decoction method

10. What do you mean by theoretical plates in Chromatography?

Answer

Theoretical Plates in Chromatography

- Theoretical plate number (N) is an index that indicates column efficiency. It describes the number of plates as defined according to plate theory, and can be used to determine column efficiency based on calculation in which the larger the theoretical plate number the sharper the peaks.

SECTION B

LONG ANSWERS TYPE QUESTIONS (2 × 10 = 20)

1. Discuss shikimic acid pathway with its significance in biogenesis.

Answer

Shikimic Acid Pathway

- Erythrose-4-phosphate and phosphoenol pyruvate interact each other and synthesize the 2-keto-3-deoxy-7-phosphoglucoheptonic acid and enzyme which mediate this reaction is DAPH synthase.
- DHQ (dehyroquinate) synthase catalyse the further reaction and form the 3-dehydroquinate from the 2- keto-3-deoxy-7-phosphoglucoheptonic acid.
- NADC (Nicotinamide adenine dinucleotide) used as a cofactor in this reaction.
- In the presence of another enzyme 3-dehydroquinate dehydratase the DHQ remove the water and form 3-dhydroshikimic acid.
- ATP base phosphorylation occurs of the product dehydroshikimic acid and form the shikimate- 3- phosphate in the presence of enzyme shikimate kinase. Phosphoenol pyruvate reacts with this shikimate- 7- phosphate to form 5-enol pyruvyl shikimate-3-phosphate with the help of enzyme 5-enol pyruvyl shikimate-3-phosphate (EPSP)

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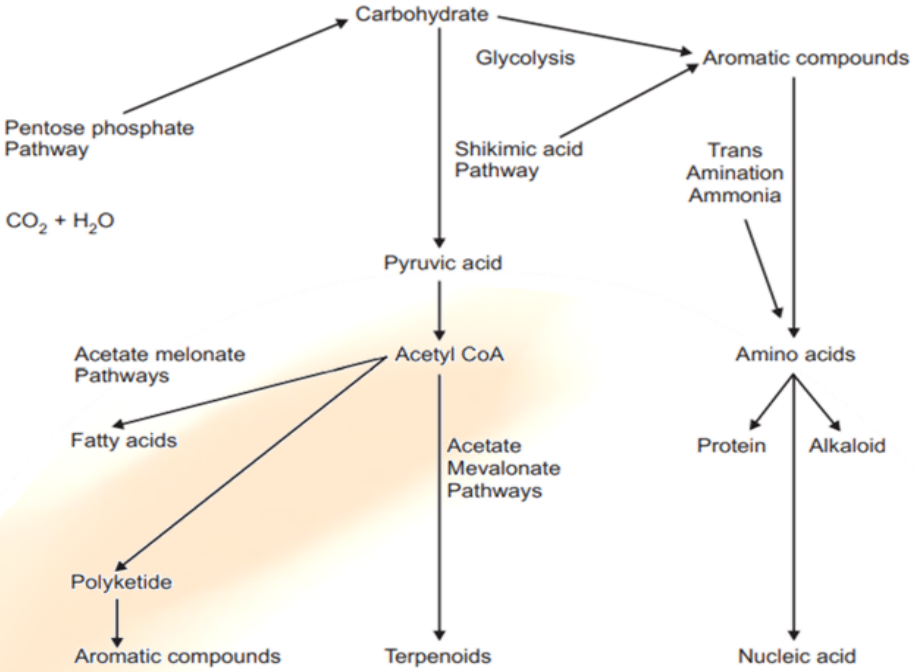
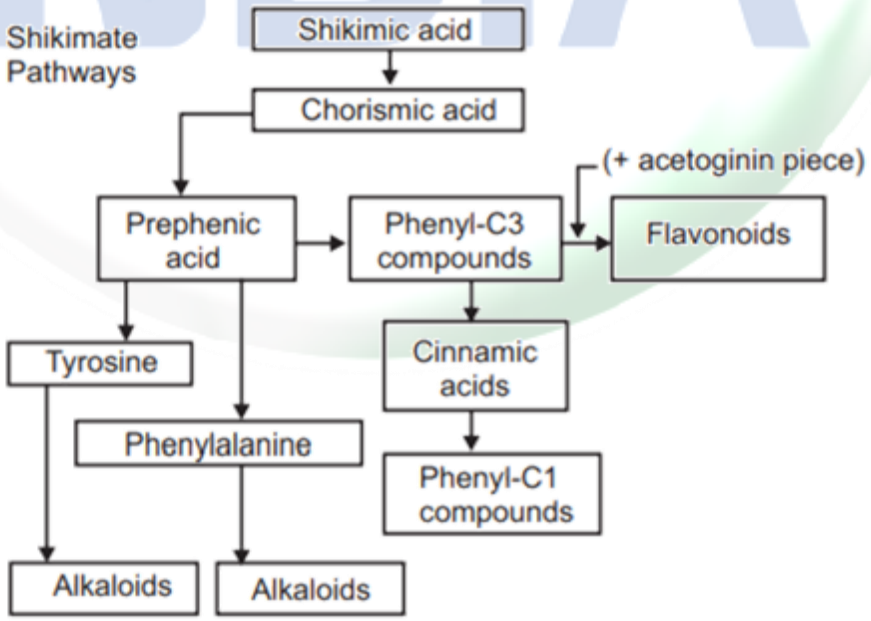
synthase.



Production of primary and secondary metabolites in plants

- The next product chorismate is formed from the product 5-enol pyruvyl shikimate-3 phosphate via the enzyme chorismate synthase. • Clasien rearrangement reaction occurs with chorismate in the presence of enzymes chorismate mutase and prephenic acid was formed.
- From chorismic acid various intermediate product like Anthranillic acid, phosphoribosyl anthranillic acid were formed which will last produce tryptophan.
- While prephenic acid converted into phenyl pyruvic acid or 4-hydroxy phenyl pyruvic acid which in last produce the amino acid phenyl alanine or tyrosine.



Production of secondary metabolites from shikimic acid pathway in Plants

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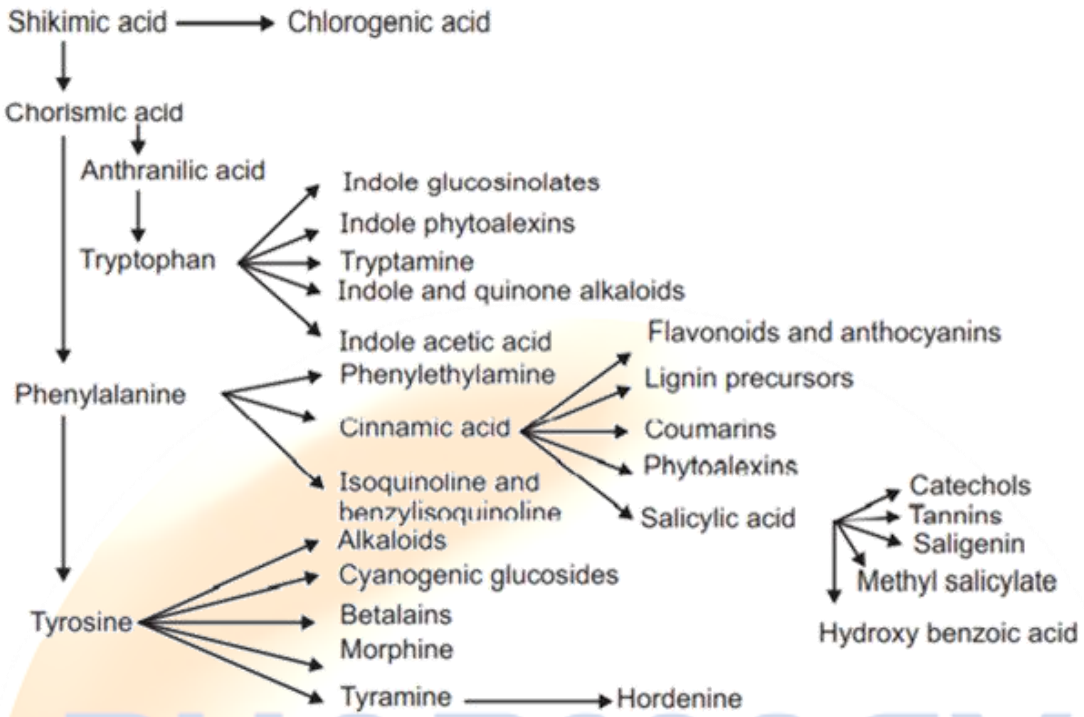
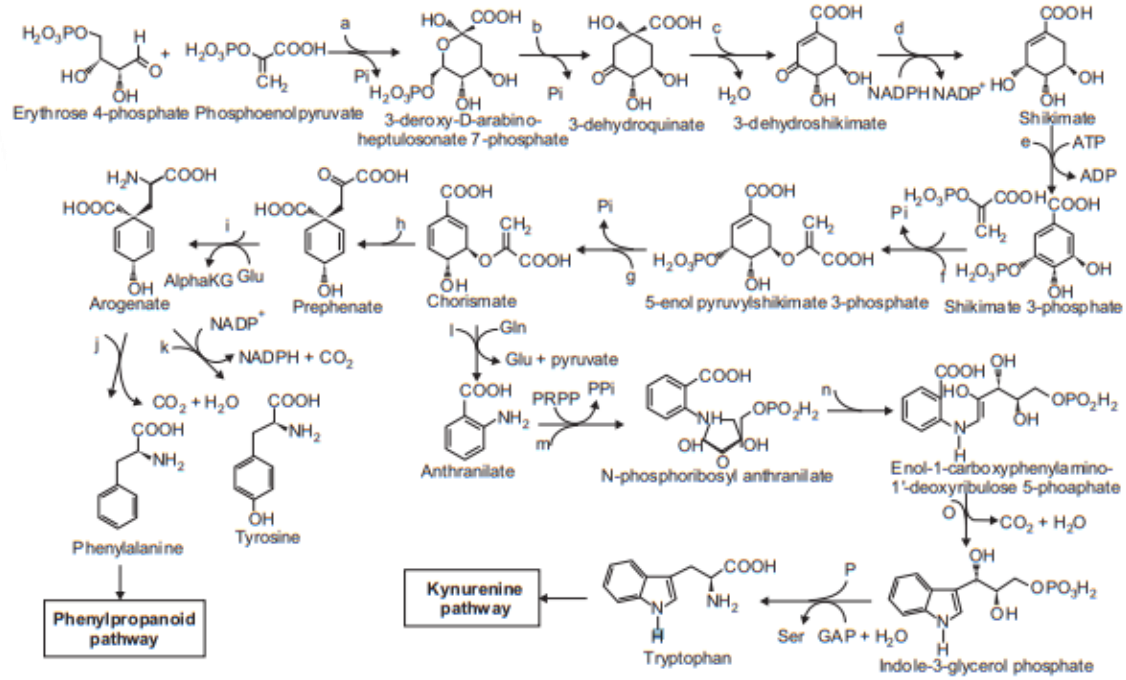


Shikimic acid pathway leading to the biosynthesis of diverse bioactive principles



Production of amino acids by Shikimic acid pathway

Significance

- Phenyl propanoids (Phenyl alanine and Tyrosine) is the main precursor for the synthesis

of coumarin, lignin, tannins and flavonoids.

- Gallic acid is also biosynthesized from shikimic acid pathway (rearrangement of 3-5 didehydroshikimate in the presence of enzyme shikimate dehydrogenase).
- Aromatic metabolite and alkaloids are also formed by this cycle.
- Indole, Indole derivatives are also formed from this pathway.

2. Discuss complete pharmacognosy of opium and digitalis.

Answer

Pharmacognosy of Opium

Synonyms: - Crude Opium; Raw Opium; Gum Opium; Afim.

Biological Source: -

- Air dried milky latex obtained by incision from the unripe capsules of
  - *Papaver somniferum album* (Indian)
  - *Papaver somniferum glabrum* (turkey)
  - *Papaver somniferum nigrum* (European)
- Opium is required to contain not less than 10% of morphine and not less than 2.0% of codeine.
- The thebaine content is limited to 3%.

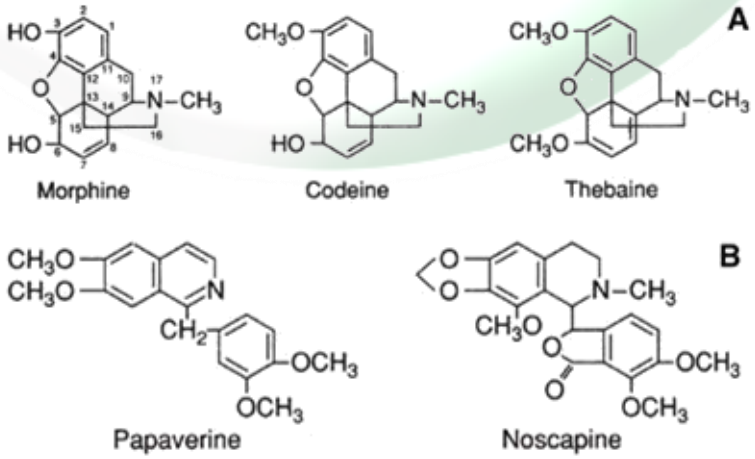
Family: - Papaveraceae

Microscopy: -

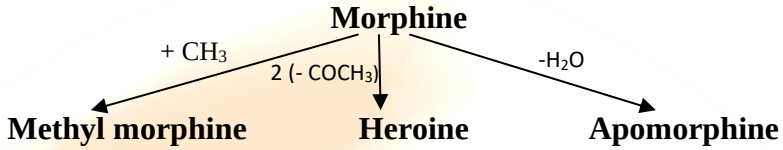
- Agglomerated latex granules in irregular mass
- Stomata – anomocytic type
- Pointed Trichomes; starch granules

Chemical Constituents: -

- Opium contains **morphine (10–16%)** is the most important base.
- The alkaloids are **combined** with **meconic acid**.
- **Phenanthrene nucleus** containg drugs → **Morphine**
  →Codeine
  →Thebaine
- Morphine is **monoacidic** phenolic alkaloid
- Morphine is levorotatory in nature



- Opium alkaloids are present as **salts of meconic acid**
- Opium also contains → sugar, wax, mucilage, and salts of calcium, magnesium and potassium
- Codeine is **strong monoacidic base** and levorotatory
- **Benzylisoquinoline nucleus** containing drugs →**Noscapine (narcotine)**
  →**Narceine**
  →**Papaverine** (optically in active)
- Codeine → **ether of morphine** (methyl-morphine)
- **Cryptopine Type:** Protopine, cryptopine



Chemical Tests: -

1. Aqueous extract of Opium + FeCl3 solution → gives deep reddish purple colour → which persists on addition of HCl. It indicates the presence of meconic acid
2. **Marquise test :**
   Morphine + conc. H2SO4 + formaldehyde → give dark violet colour
3. Morphine + slilcon dioxide → blue colour
4. Morphine + nitric acid → orange red colour

Uses: -

- Narcotic, analgesic and sedative action
- Used to relieve pain, diarrhoea dysentery and cough.
- Poppy capsules are astringent
- Noscapine is not narcotic and has cough suppressant action acting as a central antitussive drug.
- Papaverine has smooth muscle relaxant action and is used to cure muscle spasms.
- Apomorphine → emetic

Pharmacognosy of Digitalis

Synonyms: - Digitalis, purple foxglove, finger flower, lady's glove, Foxglove Leaves, Folia Digitalis.

Biological Sources: - Digitalis consists of dried leaves of *Digitalis purpurea* Linn.

Family: - Scrophulariaceae.

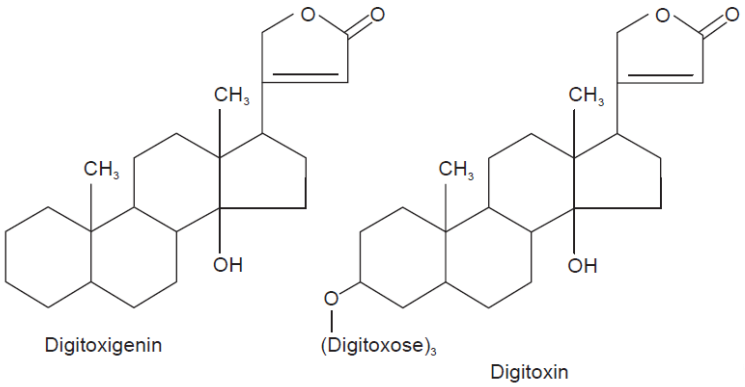
Microscopy: -

- Dorsiventral leaf
- Anomocytic or ranunculaceous type of stomata
- Collapsed cell covering trichome is an important characteristic of digitalis.
- The pericycle is parenchymatous above and collenchymatous below.
- Calcium oxalate crystals and sclerenchyma is absent.

Chemical Constituents: -

- Digitalis leaves contains 0.2–0.45% of both primary and secondary glycosides.
- Primary glycoside- genin + digitoxose + glucose.

- Secondary glycoside- genin + digitoxose.



Purpurea glycoside A → Digitoxigenin + 3 digitoxose + 1 glucose → Digitoxin

Purpurea glycoside B → Gitoxigenin + 3 digitoxose + 1 glucose → Gitoxin

- Additionally it contains 2saponin glycosides – digitonin and gitonin.
- In *Digitalis lanata* other glycosides present are-
  - Lanatoside A (acetylated purpurea glycoside A) - **D**igitoxigenin + 1acetyl digitoxose + 1glucose
  - Lanatoside B - **G**itoxin + 1acetyl digitoxose + 1glucose
  - Lanatoside C - **D**igoxin + 1acetyl digitoxose + 1glucose
  - Lanatoside D - **D**iginatin + 1acetyl digitoxose + 1glucose
  - Lanatoside E - **G**italoxin + 1acetyl digitoxose + 1glucose

Chemical Tests: -

S. No.	Test Name	Procedure	Observation
1.	**Baljet Test**	Thick section of leaf + sodium picrate reagent	Yellow to orange colour indicates the presence of glycoside
2.	**Legal Test**	Glycoside + pyridine + sodium nitroprusside solution→Made alkaline	Pink to red colour is produced
3.	**Keller–Killiani Test**	1gm drug + 10ml 70% alcohol→Boil for 2 to 3 min→Filter→Filtrate + 0.5ml lead acetate (strong)→Shake well an dseparate the filtrate→ Clear filtrate is treated with equal vol. Of chloroform and evaporated to yield extract→Extract + glacial acetic acid→After cooling→Add 2drops of ferric chloride solution→Solution is	Reddish brown layer changes to bluish green colour after standing

		transferred to the test tube containing 2ml conc. H_2SO_4	

Uses: -

- Increases the force of systolic contraction
- Cardiac stimulant and tonic
- Frequency of heart is normalized
- In congestive heart failure
- As diuretics
- Arterial flutter
- Arterial fibrillation
- Also employed in the treatment of internal haemorrhage, in inflammatory diseases, in delirium tremens, in epilepsy, in acute mania.

Adulterants: -

- *Verbascum thapsus* also known as Mullelin leaves.
- *Primula vulgaris* (Primrose leaves)
- *Symphytum officinale* (Comfrey leaves)
- *Inula conyza* (Ploughman's Spikenard)

3. Elaborate various chromatographic techniques with their significance. What is herbal fingerprinting?

Answer

Column Chromatography

- Generally, in all the types of chromatography there is one stationary phase and one mobile phase.
- In a column chromatography we use packed column as a stationary phase.
- The column may be packed with solid or liquid.
- If the packing occurs with the solid the principle of separation is adsorption while if packing will be done by liquid, then the principle of separation will be partition.
- Generally, in the column chromatography the most separation is based on the adsorption principle.
- The adsorption principle is used in the separation of the mixture from column chromatography.
- The extract which is to be analyzed should be dissolve in the mobile phase and add into the column.
- The individual constituent in the extract will move with different rates which depend upon their affinities towards the stationary phase or adsorbent.
- The compounds which have more affinity towards stationary phase will be separated in the last while those have less affinity towards stationary phase will move fast and separated first.
- The stationary phase which is used to fill the column should have uniform spherical size with high mechanical stability, inert with solute or other components and mobile phase have free flow, freely available, inexpensive and able to separate the wide variety of compounds.
- Example of weak adsorbent is sucrose, starch, inuline, talc while medium adsorbent is calcium carbonate, magnesium oxide, calcium hydroxide and magnesium carbonate and the example of strong adsorbent is activated alumina,

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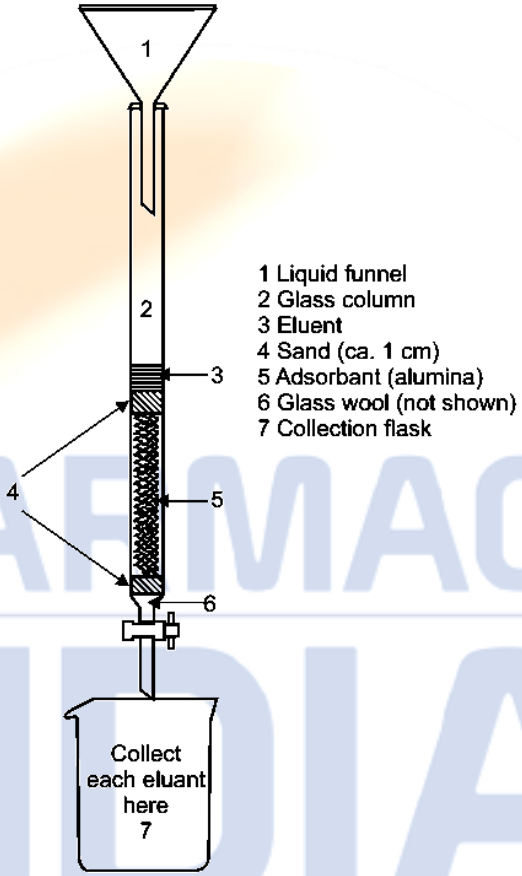
activated charcoal, fuller's earth etc.

- The mobile phase may be used in individual or can be used into the mixture depending upon their polarity and the type of constituent to be separated.
- The column should be made of neutral glass and not affected by acid, alkali, mobile phase or stationary phase.
- The length: diameter ratio may be 10:1 to 30:1.
- Sometimes it may be 100:1 ratio.



Diagrammatic representation of Column Chromatography

Packing of the Column:

- The lower portion of the column should be pack with glass wool or cotton wool to above which the adsorbent should be placed.
- After packing with adsorbent, a paper disc can be placed above the adsorbent layer.
- So that on introduction of sample or mobile phase will not disturb the adsorbent layer.
- There are generally two types of columns packing techniques

1. Dry packing technique.
2. Wet packing technique.

- **In the dry packing technique,** the column is pack with dry adsorbent and then flown the solvent through the column till equilibrium is obtained but the major drawback is that air bubbles are trapped between the solvent and adsorbent which produce trouble in the separation of compounds.
- **In the wet packing technique,** the mobile phase is mixed with adsorbent and

poured into the column. So the air entrapment is comparatively less and uniform packing occur in the column. If the flow of mobile phase will be uniform there will be no development of crack and a good and uniform separation of the extract will occur.

Sample Introduction:

- The sample should be dissolved in the least quantity of mobile phase.
- The complete sample should be place on the top portion of column and get them absorbed.
- Then the sample can be isolated by elution.
- Elution may be of two types:
  1. In Isocratic elution the composition of mobile phase will be same for entire separation.
  2. In gradient elution the composition of mobile phase can be changed slowly to improve the separation. The polarity of mobile phase can be changed by changing the mobile phase combination.

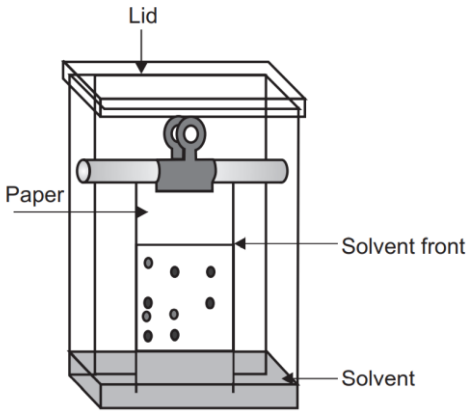
Detection of Components:

- Colored constituents can be detected visually and colored band moving in the column can be collected separately.
- Colourless compound can be detected by UV technique, refractive index detector, monitoring by TLC, by flame ionization detector or such other technique.
- The component can be recovered by different technique some time even by cutting the column (column made of plastic) into several distinct zones but the best method is elution in which the component eluted by the mobile phase and the constituent is separated from the mobile phase.

Paper Chromatography

- Paper chromatography is carried out by specially designed filter paper.
- The principle of separation may be here partition or adsorption. If the filter paper is impregnated with alumina or silica, the adsorption principle will be applied for separation whereas if moisture/water present in the pores of cellulose fibre it works as stationary phase and solvent as mobile phase then the principle of separation will be partition.
- In general paper chromatography refers to the partition principle. Various types of filter paper are used for paper chromatography. It may be Whatmann paper of different grade, acid or base wash filter paper, paper modified with glycol, formamide, methanol, glass fibre type paper, hydrophobic paper (OH group can be acetylated) or paper can be impregnated with alumina, silica or ion exchange resin. The size of the paper should be suitable for the size of the chamber and apply the sample by using capillary or micro pipette.
- The sample should be dissolved in the mobile phase and applied with low concentration with small zone.
- In the mobile phase pure solvent or mixture of solvent or buffer solution can be used. There may be ascending, descending, circular, two dimensional and ascending-descending type different development techniques. Ascending development technique is conventional technique in which the mobile phase

moves against the gravity and spot the sample at bottom portion. While in descending development the mobile phase kept at the top and the solvent flow down the paper.



Diagrammatic representation of paper chromatography

- Here the samples applied at the top and the development is fast due to gravity assisted solvent flow. In circular or radiant development, the sample applied at the centre of the paper and the mobile phase flown through a wick at the centre and spread uniformly in all direction. In two-dimensional development techniques the samples is applied in one corner and develop the paper on one axis then dry the paper.
- After drying turn the paper on ninety-degree angle and develop the paper on another axis. This technique is used for more complex sample. By ascending descending development, the length of separation can be increase. First here ascending takes place later descending development follow.
- After development of the chromatogram the isolated compound can be visualized by detecting agents.
- Detecting agents can be two types:
  (a) Destructive type
  (b) Nondestructive type
- In destructive type the sample cannot be recovered or it will be destroyed due to chemical reaction of spraying reagent with sample.
- While in non-destructive method sample can be recovered. In non-destructive method sample can be detected by UV chamber method, densitometric method or iodine chamber method.
- Paper chromatography can be used for both qualitative and quantitative purpose. For qualitative purpose Rf value can be determined.

$$\text{Rf (Retardation factor)} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

- For quantitative purpose the density of the spot can be measured or the spot can be eluted with the solvent and analysed by conventional techniques like spectrophotometric method or electrochemical methods.

Thin Layer Chromatography (TLC)

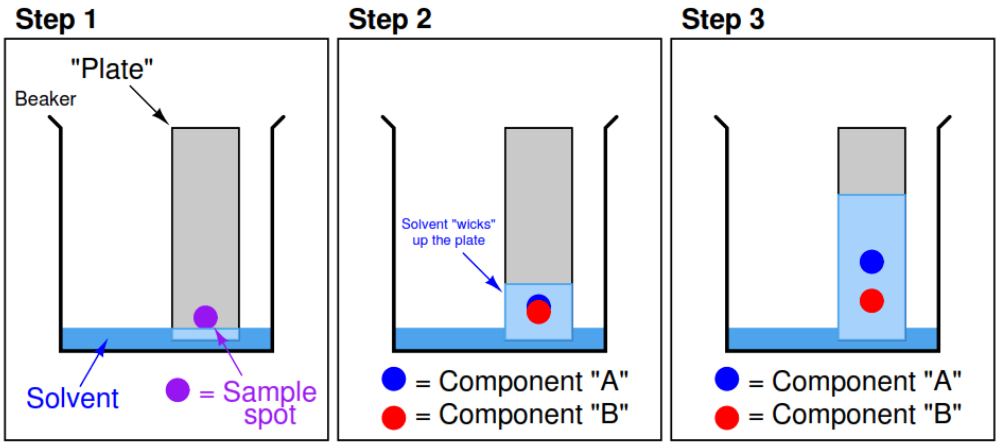
- Stahl in 1958 developed the standard equipment and technique for analysis by thin layer chromatography. Before that in 1938 Izmailou and Shraiber separate the

phytoconstituent using 2 mm thick alumina on glass plate.

- TLC is comparatively better separation tool then paper chromatography.
- Though the adsorbent term uses various times in TLC but principle of separation may be any one of four fundamental mechanisms of separation:
  1. Ion exchange
  2. Partition
  3. Adsorption
  4. Molecular exclusion
- Principle of separation totally depend upon the coating substance apply on the plate. If silica or alumina is applied as coating substance adsorption principle will take place. If coating substance is sephadex molecular exclusion will be principle of separation. Ion exchange will be the mechanism of separation if resin will be the coating substance and cellulose like coating substance adopt partition mechanism.
- The extract of plant spotted on the plate and it moves along with mobile phase. The phytoconstituent of extract have different affinity towards the stationary phase and mobile phase. Those constituents have more affinity towards mobile phase separate faster compare to those who have more affinity with coating substances.
- Silica gel G is one of the most common adsorbents uses for the coating of plate. Here G represents the gypsum (CaSO4) which is around 15 percent of the silica gel and act as binder.
- The other adsorbent which are used in the stationary phase is alumina, cellulose, kiesselguhr, polyamide powder and others. Most often the adsorbent are applied on the glass plate.
- The dimension of the glass plate may be varied from 20X20 cm to 20X5 cm. sometime even microscopic plates are also used to examine the progress the chemical reaction by TLC method.
- The glasses are coated with adsorbent with different techniques like:
  1. Pouring,
  2. Dipping,
  3. Spraying,
  4. Spreading techniques.
- In **pouring technique** the prepared slurry is poured onto the glass plate and try to maintain an equal level surface.
- In **dipping technique** two plates are stick together and dip into the slurry and then remove from the slurry. Separate both plates, one surface will be coated and other one will be dried.
- In the **spray technique** adsorbent is sprayed on glass plate using a machine sprayer.
- The best applying technique is **spreading technique** in which the slurry is kept in the TLC spreader box.
- The glass plates of particular size are kept on base plate. The prepared slurry kept in the reservoir of spreader box. Adjust the thickness of slurry by rotating the knob present in the spreader box then spreader box is moved over the glass plate. Allow them for air drying and activate them at 100-120°C for one hour.
- Sample can be applied by micro pipette or capillary tube. It should be spotted at least 2 cm above the base of or on such height that spotted area should not

immersed in mobile phase. The area of the spotted sample should be minimum with sufficient concentration.

- Mobile phase and TLC plate should be kept in the development chamber.
- Chamber should be saturated with mobile phase otherwise edge effect may found in which the solvent front in the middle of plate move faster than the edge.
- The mobile phase selected on the basis of phytoconstituent which have to be separated and the nature of stationary phase.



- It may be single or the mixture of the solvent.
- The polarity of the solvent adjusted such that it easily separated the phytoconstituents.
- The different development techniques used in the TLC are one dimensional, two dimensional, horizontal and multiple developmental techniques.
- In one dimensional or vertical technique, the mobile phase flows against the gravity due to the capillary action.
- Most separations are done by this technique in which one spot is applied at the corner of the plate and the plates are developed in one axis then dried it. Further turn the plate to 90 degree and developed the plate on another axis.
- After development of the chromatogram the isolated compound can be visualized by detecting agents.
- Detecting agents can be two types
  a) Destructive type
  b) Non-destructive type.
- In **destructive type** the sample cannot be recovered or it will be destroyed due to chemical reaction of spraying reagent with sample.
- While in **non-destructive method** sample can be recovered. In non-destructive method sample can be detected by UV chamber method, densitometric method or iodine chamber method.
- This chromatography can be used for both qualitative and quantitative purpose. For qualitative purpose Rf value can be determine.

$$\text{Rf (Retardation factor)} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

- For quantitative purpose the density of the spot can be measured or the spot can be eluted with the solvent and analysed by conventional techniques like spectrophotometric method or electrochemical methods.

Herbal Fingerprinting

- Chromatographic fingerprinting is used for both establishing the identity and quality of the single herbs being added into herbal formulation . Nowadays chromatographic fingerprinting is used at two stages during the manufacture of a herbal formulation.

SECTION C

SHORT ANSWERS TYPE QUESTIONS (5 × 7 = 35)

1. Write a note on the application of radioactivity in the investigation of biogenetic pathway.

Answer

Application of Radioactivity in the investigation of Biogenetic Pathway

- Some research can be done which uses the whole organism with minute disturbances like bacteria, moulds and fungi.
- These can be cultivated and studied biochemically but in animals test material is added into feed and blood and fecal material is analyzed.
- In the case of plants the destruction of plant is compulsory for analysis purpose. Few examples like minces, breis and homogenates are the preparations in which the cell wall and tissues are destroyed but intracellular particles remains intact.
- The components of these mixtures can be separated by centrifugation and biological activities of these fractions are examined.

Radioactive Tracers:

- The radioactive carbon and hydrogen are used mostly in biological investigation.
- Others like sulphur, phosphorus, alkali and alkaline earthy metals are also used but they should be used in specific purposes.
- These compounds enable the compound metabolism in living organisms. In the study of proteins, alkaloids and amino acids the labeled nitrogen atom gives more specific information in comparison to labeled carbon.
- The available nitrogen isotopes are stable and necessitate the utilization of mass spectrometer for their use as tracers.
- The naturally occurring carbon isotopes are stable having mass number 12 and 13. The latter isotope is most abundant.
- Other radioactive isotopes of carbon having mass number 10, 11 and 14 have varied half life (^{10}C half life is 8.8s, ^{11}C half life is 20 min and ^{14}C half life is about more than 5000 years).
- The 10C and 11C has usefulness in biological research. The use of organic compound with prelabeled carbon atoms lead to the compound synthesis from inorganic carbon compounds which is produced in the pile by routes not previously commercially utilized.
- In this synthesis the compound will be more pure because strong radioactive impurity even in less proportion causes serious risk or damage of the experimental result.
- Many compounds which are produced by natural sources like certain amino acid produced by the hydrolysis of proteins are prepared by developing Chlorella in $^{14}CO_2$ rich environment. All the carbon compounds of this organism are prelabelled.
- The tritium ^{3}H labeled compounds are available commercially. Its labeling is effected by catalytic exchange in water or aqueous media or by irradiation of organic compounds by tritium gas and by hydrogenation of unsaturated compounds with tritium gas.

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Tritium is a pure beta emitter of less toxicity having half life 12.43 years and radiation energy is less than ^{14}C.

Detection and Assay of Radioactively Compounds:

- The adequate methods are essential for the detection and estimation of labeled compounds.
- The liquid scintillation counter instrument is preferred for the soft and easily absorbed radiation from ^{3}H and ^{14}C labeled compounds.
- It mainly depends on the conversion of kinetic energy of a particle into a fleeting pulse of light as result of its penetration in a suitable luminescent material.
- Liquid scintillation media consists of solvent where excitation occurs and a fluorescent solute emits the light to actuate the photomultiplier.
- This have been devised the purpose of sample enabling incorporated into the same solute and attains optimum balance between sample and scintillator.
- Today's modern instruments are fully automatic. Nearly 100 samples can be run at a time and measurement of mixed radiations is also possible e.g. ^{3}H and ^{14}C both is beta emitter and possess different energies.
- The instrument containing all counters is connected to a ratemeter which counts in a given time and records the readings. With 14C isotope no decay correction is essential for normal biogenetic experiments because ^{14}C has long half life. So the half life is important in carbon dating of old materials.
- 'Curie' is the unit for radioactivity. It is defined as 'Quantity of any radioactive nuclide which has 3.7X1010 numbers of disintegrations per second.

Autoradiography:

- Autoradiography is a technique used for locating the radioisotope in biological and other material. In autoradiography the sample or specimen is placed in contact with suitable emulsion for example X-ray sensitive film, and after exposure the emulsion media is developed in normal manner.
- The resultant autoradiograph shows the distribution pattern of radioactive material in the sample or specimen. This method can be applied to whole morphological part of plant or its section.
- The resultant negative is observed under microscope. The radioactive compounds can also be detected on paper chromatogram or thin layer chromatogram. The different spots determined by densitometer or by calibrated films.

Precursor-product Sequence:

- In this a prelabelled precursor is fed into the plant and after a definite period of time the compound is isolated and purified. After purification the radioactivity is determined. If specific atom of the precursor is labeled, there may be the chances of isolated metabolite degradation.
- Radioactivity of the isolated constituent is not sufficient proof that the particular compound fed is direct precursor. The substances enter the metabolic pathway of plant and are distributed throughout the whole plant and its products. For solving this problem of none providing the complete evidence of precursor double or triple labeling experiments are developed.
- The multiple labeling simplifies the procedure for determination of particular label of molecule. It provides tremendous information in comparison to the information

provided by single experiment. In multiple labeling process atleast two or more than two positions are labeled at a time by using same isotope or different isotope.

- If we take an example of ergot alkaloids it is seen that ergot alkaloids are formed by precursor amino acid tryptophan and mevalonic acid. The cis-trans isomerism take place when chanoclavine-I is converted into agroclavine and elymoclavine.

2. Discuss biological source, chemical constituents, and uses of belladonna. Give extraction of atropine.

Answer

Belladonna

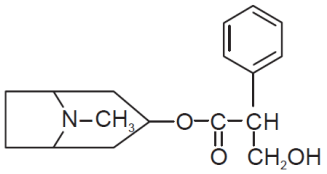
Biological Source: - dried leaves and flowering tops of *Atropa belladonna* (European Belladonna) and *Atropa acuminata*

Family: - Solanaceae

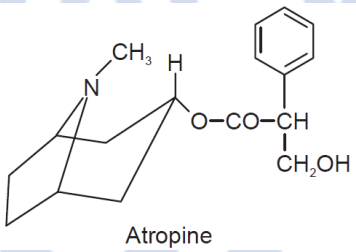
Note: - It contains about 0.35% of total alkaloids calculated as hyoscyamine.

Chemical Constituents: -

- Belladonna contains 0.3–1.0% total alkaloids
- Main alkaloids → **l-hyoscyamine** and **atropine**
- Ammonia + alcoholic solution of scopoletin → shows blue florescence. This test is useful to detect Belladonna poisoning



Hyoscyamine



Atropine

Uses

- Treatment of peptic ulcer
- Functional digestive disorders, including spastic, mucous and ulcerative colitis; diarrhoea, diverticulitis and pancreatitis
- Used to control excess motor activity of the gastrointestinal tract and spasm of the urinary tract
- Belladonna is anticholinergic, narcotic, sedative, diuretic, mydriatic
- Used as anodyne and to check secretion.
- It relieves spasm of gut or respiratory tract.
- Consumption of Belladonna checks excessive perspiration of patients suffering from tuberculosis.
- Belladonna acts as a parasympathetic depressant.

Extraction of Atropine

- The powdered drug is moistened with sodium carbonate aqueous solution and then it is extracted with benzene or ether.
- The residue is then extracted with acidified water. Remove the coloring matter by treating the aqueous extract with solvent ether.
- From this solution add sodium carbonate to precipitate the alkaloid. Wash the

precipitate and dry it.

- Dissolve the dried precipitate in ether or acetone and dehydrate it using anhydrous sodium sulphate.
- Concentrate the filtrate and make it cool.
- After cooling the crystal of hyoscyamine and atropine are separate out.
- Separate out the crystal and dissolve in alcohol. To this solution add the sodium hydroxide.
- Then allow standing this mixture. The crystal of hyoscyamine will be completely racemised in the atropine.
- For the purification the crude atropine, dissolve it into acetone and then recrystallised it.

3. Write a note on application of various spectroscopic techniques in zthe identification of crude drugs.

Answer

Application of various spectroscopic techniques in identification of crude drugs

UV Spectroscopy

- UV spectroscopy plays a very important role for the identification of plant constituents. This may be either in the screening of crude plant extract or observation of the eluents of chromatographic column during separation of plant products.
- Generally, the UV spectroscopy of the phytoconstituents should be measured in dilute solution using an appropriate blank.
- The absorbance of colored compounds can be measured in between 200 to 700nm while the colorless compound can be measured in between 400-700nm.
- The particular wavelength at which the maximum and minimum absorption take place should be recorded.
- A pure phytoconstituent which show the characteristic UV spectra should be purified repeatedly from the mixture until the particular characteristic spectra will obtain with the help of UV visible spectra.

Infrared Spectroscopy

- In the infra-red region the energy involve in the absorption is very small and the spectrum in this region is much more complex.
- The energy associated to absorption is so small that it will not produce electronic transition but enough to produce rotational and vibrational energy changes.
- The spectrum of infrared can be divided into two regions.
- First one is the Fingerprint region (7-11µm) and another one is the functional group region.
- In fingerprint region it is difficult to assign the peak but can assign the peak in functional group region.
- The intensity of the band can be recorded as (S) strong (M) medium (W) or weak.
- By the help of characteristic vibrational frequencies, we can assign the various functional groups in the molecule (mentioned in the table).

- We can compare the natural phytoconstituents to the synthetic compounds by comparing the fingerprint region.
- IR spectra are most commonly use to identify known essential oil component which are separated by gas liquid chromatography (GLC).
- IR spectroscopy is a great tool in the structure elucidation of a new phytoconstituent isolated from the natural origin. The interpretation of the spectrum of new compound is very complex and need very experiences.
- By the help of IR spectroscopy we can also perform the quantitative analysis of crude herbal sample or their formulation.
- In the quantitative analysis the area of the peak of the band are measured and the ratio of area of two sample are compare by the help of which we can assign the percentage of pure drug or the herbal drug present in the formulation.

Mass Spectroscopy

- By the help of mass spectroscopy, we can get the accurate molecular weight of the phytoconstituent by using only microgram quantity of the sample.
- Mass spectroscopy produces the characteristic fragmentation pattern which is helpful to identify the phytoconstituents.
- The microgram amount of phytoconstituents is introduced into the mass spectrometer where the compound become ionized and caused fragmentation of the chemical bonds.
- Mass spectra are the graph in between the relative proportion of a fragmented ion versus the ratio of mass/charge of these ions.
- Mass spectroscopy nowadays is very useful to get the information of about structure of unknown phytoconstituent.
- Nowadays mass spectra are attached to the high-performance liquid chromatography (HPLC) where isolated constituent direct enter into the mass spectra and help the identification of the unknown phytoconstituents.
- Mass spectrometer is very complicated and sophisticated instrument and unlike the UV and IR where the instrument operated by individual phytochemist, it is operated by some technical expert person.
- The mass spectroscopy is also useful to very low molecular weight phytoconstituents.
- In generally the mass spectroscopy is used for the structure elucidation of phytoconstituents but sometime when two mass spectrometers are attached simultaneously it is also useful for the quantitative analysis.

4. Explore industrial production, estimation and utilization of sennosides.

Answer

Sennosides

- Senna, the popular drug uses for constipation was introduced by Arabian physician and they use their leaves and pods.
- The two varieties of Senna are the Cassia acutifolia Family Leguminosae (popularly known as Alexandrian or Khartoum Senna) and the other variety is Cassia angustifolia (Tinnevelly senna).
- The drug contains important phytoconstituents sennoside responsible for their

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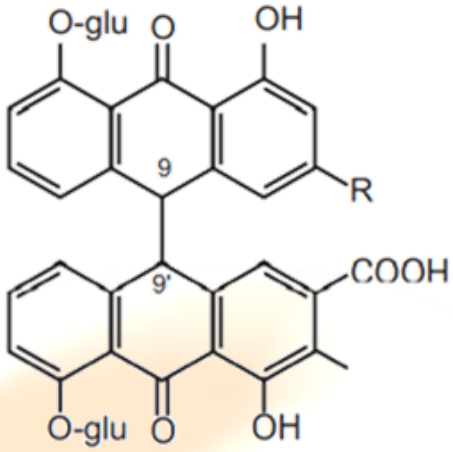
purgative activity.



Structure of Sennoside

- **Production of Sennoside:**

Method A:

- The dried senna leaves extracted with 70 percent methanol.
- Shake the drug with methanol for around 4 hours at room temperature.
- Filter the extract and reduce the volume to 1/8 under vacuum.
- The extract is acidified to pH 3 with the help of Hydrochloric acid.
- After acidification filtration is done and removes any soluble aglycone with chloroform.
- The filtrate is neutralized with liquid ammonia and then centrifuge to isolate the sennoside.

Method B:

- The extraction of the crude drug was performed with the help of benzene for two to three hours with frequent shaking at room temperature.
- Dry the residue (marc) left after the extraction with benzene at oven or room temperature which should be not exceeded to 40°C. The dry drug or marc should be extracted with 70 percent methanol for 4-6 hour on shaker at room temperature. Repeat the same process with fresh methanol for 2 hours.
- Club both the methanol extract and concentrate to 1/8 volume under reduced pressure. The pH is adjusted to 3 with hydrochloric acid and kept on side for 3 hours afterwards filter it. Anhydrous calcium chloride dissolves in denatured spirit and added to the filtrate with vigorous shaking.
- Maintain the pH 8 of the solution by ammonia solution and kept aside for two hours. Filter the solution and dry the precipitate over dessicant like phosphorus pentaoxide in a desiccator.

- **Estimation of Sennoside:**
  - Hot water is useful for the extraction of anthraquinone glycosides.
  - Acidified the aqueous extract and treat with chloroform to make free the aglycone which is largely present in the solution.

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- First neutralize the solution and then centrifuge it afterwards add ferric chloride to the solution.
- Reflux the solution and further acidified it to bring oxidation and hydrolysis.
- The aglycone present should be extracted with ether and re dispersed in the magnesium acetate solution.
- Measure the absorbance of the sennoside and express the concentration in comparison to sennoside B at 515 nm.

- **Utilization:**
  - The main use of sennoside is cathartic.
  - It may be used in habitual constipation or occasional constipation.
  - The sennoside glycoside absorb in the gastro intestinal tract and aglycone portion released in the colon.
  - The released aglycone stimulates and irritates the colon. Thus, movement in the colon portion is increased due to local action.
  - The peristalsis movement is increased which generate soft and bulky stool.

5. Discuss biological source, chemical constituents, uses of rutin and Citral extraction.

Answer

Rutin

Biological source:

- Rutin is a flavonoids glycosides obtained from the powered of dried food grains of Fagopyrum esculentum belongs to family- Polygonaceae. It is also obtained from various citrus fruit.

Chemical Constituents: Rutin

Utilization:

- It is used to treat capillary bleeding along with increased capillary fragility andthereby useful in treatment of retinal haemorrhages. It is also used as Antioxidant.

Citral

Biological source:

- Citral is a monoterpene aldehyde obtained from oils of Cymbopogon flexuosus, C.martini belongs to family- Graminae. It contains not less than 75% of aldehyde calculated a Citral. It also present in Lemon oil (Citrus limonus) and Orange oil (Citrus aurantium),family Rutaceae.

Chemical Constituents - Citral

Utilization:

- It is used as a flavoring agent and perfumery. Commercially citral is act as precursor for the synthesis of β-ionone. β-ionone is used as starting material for the synthesis of Vitamin A.

6. Discuss industrial production, estimation and utilization of podophyllotoxin.

Answer

Podophyllotoxin

- The biological source of podophyllotoxin consists of dried roots and rhizome of Podophyllum emodi or Podophyllum hexandrum (family berberidaceae).
- **Production:**
  - Root and rhizome of podophyllum emodi is the commercial source of podophyllotoxin. Powder the root and rhizome and extract with methanol or ethanol and concentrate the extract under vaccum.
  - Treat the semisolid mass with acidulated water (10 ml HCl in 1000 ml water) at cool to 10°C slowly. Decanted the settled precipitate and wash it with cold water. On drying this precipitate we get amorphous dark brown powder known as podophyllin.
  - Extract podophyllin with chloroform and purify by recrystallisation with benzene or benzene and ethyl alcohol mixture and then wash with hexane/ petroleum ether which gives the commercial podophyllotoxin.
- **Utilization:**
  - The famous anticancer drug teniposide and etoposide are semi synthesized by the podophyllotoxin (natural teralin lignan).
  - The production of podophyllotoxin in total synthetic way is still not economical and production of podophyllotoxin is based on either use of tissue culture technique or the systematic cultivation.
  - Alone with anticancer activity it also shows purgative, bitter tonic and emetic property.
  - In veterinary medicine it is also used as cathartic for dog and cat but nowadays its major use is in anti-cancerous drug synthesis.
- **Estimation:**
  - Weigh the sample and shake it with chloroform for half an hour.
  - Transfer the filtrate in Erlenmeyer flask which contains 50 ml of petroleum ether (40-60°). Collect the precipitate.
  - Rewash the precipitate and conical flask with the petroleum ether.
  - Dry the precipitate at 70°C for one hour and weight the podophyllotoxin.

TLC study

- ✓ **Solvent -** Toluene: Ethyl acetate (5:7)
- ✓ **Stationary phase:** Silica gel G
- ✓ **Detecting reagent:** Sulphuric acid
- ✓ **Sample:** Dissolve in methanol
- ✓ Compare with standard by scanning 280nm densitometric scan.
- ✓ Violet colour spot will be seen with approx Rf value 0.39.

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7. Discuss biological source, chemical constituents, uses of rauwolfia and reserpine extraction.

Answer

Rauwolfia

Biological Source: - dried roots of *Rauwolfia serpentina*

Family: - Apocynaceae

Chemical Constituents: -

- Rauwolfia contains about **0.7–2.4%** total alkaloidal bases from which **more than 80 alkaloids** have been isolated
- The prominent alkaloids isolated from the drug are – **reserpine**
  - **Rescinnamine**
  - **ψ-reserpine**
- The other alkaloidal components are – ajmalinine
  - ajmaline
  - ajmalicine (8-yohimbine)
  - serpentine
  - serpentinine
  - yohambinine

Uses: -

- as hypnotic, sedative and antihypertensive
- It is specific for insanity, reduces blood pressure and cures pain due to affections of the bowels.
- increase uterine contractions
- neuropsychiatric disorders
- Ajmaline treatment of cardiac arrhythmias

Reserpine Extraction

- Plant Material → Macerated with alcohol → Alcoholic extract → Reduce in volume and residue is treated with water → Filter → precipitate insoluble in water → Precipitate contains reserpine

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