AS-2284

M.Sc. (Third Semester), Examination - 2013

Rural Technology

(Drug formulation and Evaluation)

MODEL - ANSWER

SECTION - 'A'

1. (i) Pflazenkalien means:
   (b) Alkaloid

(ii) Picrolonic acid is :-
   (c) Hager's reagent.

(iii) ..... are the natural substances without specific chemical properties.
   (c) Resens

(iv) Kattha is used as -
   (d) All above

(v) Aqueous solutions contain sugar are called:
   (c) Syrup

(vi) Which one is no more official in IP:
   (b) Aromatic water

(vii) Substituting original crude drug with other similar looking substance is called as:
   (c) Drug adulteration

(viii) Study of form of crude drug is called:
   (a) Morphology

(ix) Ulcer can be induced by:
   (d) All

(x) Estimation of gastric acid helps in testing:
   (a) Ulcer

SECTION - B

2. Phenolic compounds -

Phenols probably constitute the largest group of plant secondary metabolites. Widespread in nature, and to be found in most classes of natural compounds having aromatic moieties, they range from simple structures with one aromatic ring to highly complex polymeric substances such as tannins and lignins. Phenols are important constituents of some medicinal plants and in the food industry they are utilized as colouring agents, flavourings, aromatizers and antioxidants. The phenolic classes of pharmaceutical interest, namely :- (1) Simple phenolic compounds (2) Tannins (3) Coumarins and their glycosides (4) Anthraquinones and their glycosides (5) Naphthoquinones (6) Flavone and related flavonoid glycosides (7) Anthocyanidins (8) lignans and liguin.

Phenols may also have aromatic rings derived by acetate condensation.

Simple phenolic compounds - The phenolic compounds in this group often also possess alcoholic aldehydic and carboxylic acid groups; the include eugenol, vanillin and various phenolic acids.

Tannins - The term tannin was first applied by Seguin in 1796 to denote substances present in plant extracts which were able to combine with protein of animal hides, prevent their putrefaction and convert them into leather.

Coumarins and their Glycosides - Derivatives of benzo-α-pyrone such as coumarin, aesculetin, umbelliferone and scopoletin are common in plants both in the free state and as glycosides.

Anthraquinones and Glycosides → Rhubarb, aloes, senna and cascara were recognized as forming a natural

group of purgative drugs. Substances of the anthraquinone type were the first to be recognized both in free state and as glycosides. The derivatives of anthraquinone present in purgative drugs may be dihydroxy phenols such as chrysophanol, trihydroxy phenols such as emodin. other groups are often present.

Naphthoquinones :- They are produced by higher plants fungi and actinomycetes and exhibits a broad range of biological actions including fungicidal, antibacterial, phytotoxic etc. In plants they commonly occur in the reduced and glycosidic forms.

Flavone and Related Flavonoid Glycosides → The flavonoids which occur both in free state and as glycosides are the largest group of naturally occurring phenols. They are formed from three acetate units and a phenyl propane unit as has already been outlined. The occur in cell sap. They have been used as chemotaxonomic markers.

Anthocyanidins and Glycosides → Anthocyanidins are flavonoids structurally related to the flavones. Their glycosides are known as anthocyanins. They are sap pigments and the actual colour of the plant organ is determined by the pH of the sap. eg. blue colour of the cornflower.

Lignans and Lignin → Lignans are dimeric compounds formed essentially by the union of two molecules of a phenylpropene derivative. At one time it was thought that these compounds were early intermediates in the formation of lignin but it is now recognized that they are offshoots of the principal lignin biosynthetic pathway.

③ Chemical tests and Storage of Volatile oil –

Presence of volatile oil in the natural drugs can be detected by the following tests :-

1. To the thin section of the drug add alcoholic solution of Sudan III. Red colour obtained by globules indicate the presence of volatile oil.
2. To the thin section of the drug add a drop of tincture alkane. Red colour indicates the presence of volatile oil.

Certain volatile oils are liable to deteriorate on keeping. The deterioration is accompanied by change in colour, or increase in viscosity of the oil, or change in ~~odd~~ odour of the oil. Therefore volatile oils should be preserved properly in well closed, well filled containers away from light and in cool place. They should be kept in dark bottles at about 4°C temperature in fridge. In presence of light they may denatured. They evaporate when exposed to air at ordinary temperature. they are also called as essential oils.

(4.) Classification of Resin –

Resins are amorphous products of complex chemical nature. These are amorphous mixtures of essential oils, oxygenated products of terpene and carboxylic acids found as exudations from the trunk of various trees. Depending upon the type of the constituents of the resin they further classified as : (1) Acid resin (2) Ester resin (3) Resin alcohol.

1. Acid Resins → Following are few examples of this type of resins alongwith their acids ; Colophony (abietic acid), sandrac (sandracolic acid), copaiba (Copaivic and oxycopaivic acids), Myrrh (Commiphoric acid) and Shellac (Alleuritic acid).

2. Ester Resins → This group contains esters as the chief constituents of the resins, e.g. benzoin and storax, benzoin contains coniferyl benzoate and Storax contains cinnamyl cinnamate.

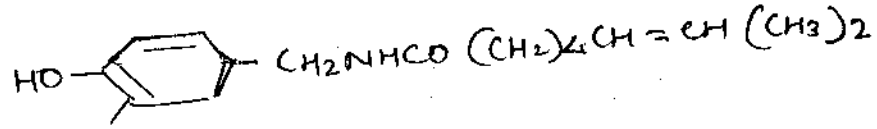
3. Resin Alcohols → The contents are the complex alcohols of high molecular weight. They are either found in free state or as esters. The examples are balsam of peru with peruresinotannol, gurjun balsam with gurjuresinol and and guaiacum resin with guaic-resinol.

Resins and oil in homogenous mixtures are called as oleoresins, e.g. - copaiba, Canada balsam, Capsicum etc. Oleo-gum resins are the homogenous mixtures of volatile oil, gum and resin, e.g. Myrrh, guggul and asafoetida. Glycoresins are made up of resins and sugars and are present in jalap and ipomoea. If the resin contains benzoic acid and/or cinnamic acid, it is called as a balsam, e.g. balsam of tolu, storax, balsam of peru, etc.

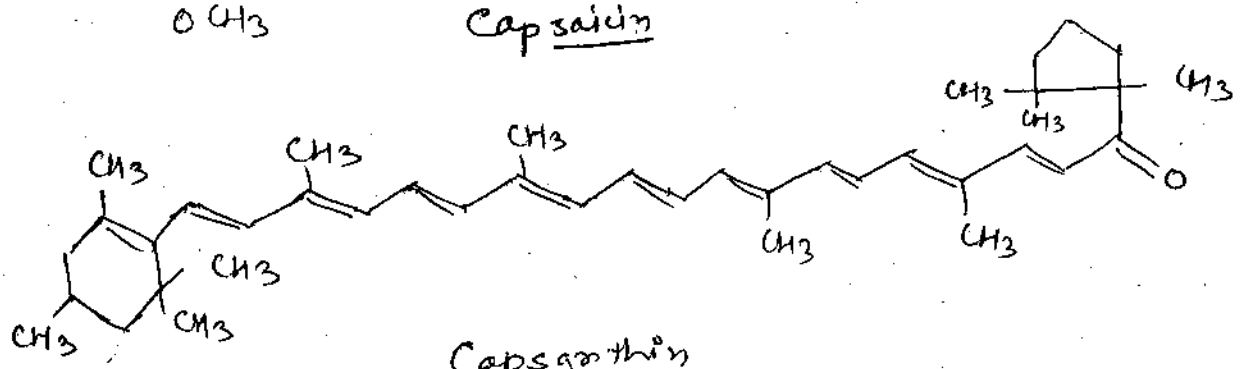
Resenes - These are the complex natural substances without any specific chemical properties. They are inert chemically. They neither form any salt nor they get hydrolysed. Examples of the drug containing resenes are the gum copal, gutta purcha, asofoetida colophony and dammar.

(5) Chemical constituents and Uses of Capsicum →

Capsicum contains about 0.5 to 0.9% colourless, crystalline and pungent principle known as capsaicin which is volatile above 65°C, Capsicum also contains fixed oil (4 to 16%), proteins and pigments, viz. capsanthin and carotene. Pigments are responsible for the red colour. Thiamine and ascorbic acid are the other contents of the drug.



Capsaicin



Capsanthin

Unlike ginger, the pungency of capsicum is not destroyed by boiling it with 2% solution of sodium hydroxide. But, it is destroyed by oxidizing agents like potassium permanganate.

Uses :- It is used as a carminative, an appetizer and a stomachic. Externally, it is used as a counter irritant in the treatment of rheumatism lumbago and neuralgia. It is also used in Spices.

SECTION - C

(8) ALKALOID -

Alkaloids are a chemically heterogenous group of natural substances and comprise more than 6000 basic nitrogen containing organic compounds which occur more than 150 different plant families.

The term alkaloid or 'PflanzenKalien' was coined by Meissner, a German pharmacist in 1819. The mankind has been using alkaloids for various purposes like poision, medicines, poultices etc. The French chemist, Derosne in 1803, isolated narcotine. In the same year, morphine from opium was isolated by Sertuerner.

In view of their chemical and physiological diversity there is no comprehensive definition of alkaloids. The term is derived from the word 'alkali-like' and hence, they resemble some of the characters of naturally occuring comples amines. The term alkaloid also covers proto alkaloids and pseudo alkaloids. In view of such variations, the only definition that brings all such compounds under one title is as follows. These are the organic products of natural or synthetic origin which are basic in nature and contain one or more nitrogen atoms, normally of heterocyclic nature, and possess specific physiological action on human or animal body. The true alkaloids are toxic in nature, contain heterocyclic nitrogen which is derived from amino acids and always basic in nature.

Properties –

(A) Physical Properties – With few exceptions, all the alkaloids are colourless, crystaline solids with a sharp melting point or decomposition range. Some alkaloids are amorphous gums, while other like coniine, sparteine, nicotine etc. are liquid and volatile in nature. Some alkaloids are coloured in nature e.g. betanidin is red, berberine is yellow.

The free bases of alkaloids are soluble in organic nonpolar, immiscible solvents. The salts of most alkaloids are soluble in water.

(B) Chemical Properties – Most of the alkaloids are basic in reaction, due to the availability of lone pair of electrons on nitrogen. The basic character of the alkaloidal compound is enhanced if the adjacent functional groups are electron releasing. The alkaloid turns to be neutral or acidic when the adjacent functional groups are electron withdrawing like amide group which reduces the availability of the lone pair of electrons.

Chemical Tests for Alkaloids – The qualitative chemical tests used for detection of alkaloids are dependent on the characters of alkaloids to give precipitates as salts of organic acids or with compounds of heavy metals like mercury, gold, platinum etc.

The different reagents used are Mayer's reagent (Potassium mercuric iodide solution) giving cream coloured precipitate; Dragendorff's reagent giving reddish brown and Wagner's reagent yielding reddish brown precipitate.

Isolation and Extraction of Alkaloid -

The extraction of alkaloids is based on their basic characters and solubility pattern. The normal procedures followed are to treat the moistened drug with alkali so as to set free the base as it exists in salt ~~form~~ form and than to seperate free base with organic solvent. This is known as Stas Otto process. First the plant is defatted with petroleum ether, especially in ~~for small scale~~ case of seed and leaf form of drugs. In the second stage the drug may be extracted with polar solvents like water, ethanol, methanol, aqueous alcohol mixtures or with acidified aqueous solutions. By this treatment the alkaloidal salts are transferred to polar solvent. It also helps in removing pigments, sugars and other organic secondary constituents. The further purification of crude extract of alkaloids is done by following ways -

1. Direct crystallisation from solvent
2. By steam distillation.
3. By chromatographic technique.

The chromatographic method proved to be ideal for separation of vast number of plant alkaloids. The different techniques of chromatography are used for seperation of individual alkaloids, from complex mixture.

4. Gradient pH Technique. - Though alkaloids are basic in nature, there are variations in the extent of basicity of various alkaloids of the same plant. Depending on this character, the crude alkaloidal mixture is dissolved in 2% tartaric acid solution and extracted with benzene.

Classification of Alkaloids -

1. Pharmacological classification - Depending on the physiological response, the alkaloids are classified under various pharmacological categories, like central nervous stimulants or depressants, Sympathomimetics, analgesics, purgatives etc.
2. Taxonomic classification - This method classifies the vast number of alkaloids based on their distribution in various plant families, like Solanaceous alkaloids.
3. Biosynthetic classification - This method gives significance to the precursor from which the alkaloids are biosynthesized in the plant. e.g. all indole alkaloids from typtophan are grouped together.
4. Chemical classification - This is the most accepted way of classification of alkaloids. The main criterion for chemical classification is the type of fundamental ring structure present in alkaloid -
   (a) True alkaloids (heterocyclic alkaloids) having heterocyclic ring
   (b) Pseudoalkaloids or biological amines.

Role of alkaloids in plants -

1. They are the reserve substances with an ability to supply nitrogen.
2. They might be the defensive mechanisms for plants.
3. They might have a possible role as growth regulatory factors in the plant.
4. They could act as carriers for transportation of acids.

(9) Cultivation, collection, microscopic characters and chemical Constituents of Ginger →

Synonyms – Zingiber, Zingiberis, Sunthi

Biological Source - Ginger consists of rhizomes of Zingiber officinale, belong to family Zingiberaceae, scrapped to remove the outer skin and dried in the sun. It is known as Jamaica ginger in the market.

Geographical Source — It is said to be native of South East Asia, but is cultivated in Caribbean islands; Africa, Australia, Jamaica, Taiwan and India.

Cultivation and Collection → Approximately, 25,000 hectares of land is under cultivation in India for the production of about 25,000 tonnes of dry ginger annually. In almost all states of India, ginger is cultivated, especially in Kerala, Assam, Himachal Pradesh, Orissa, Karnataka. Ginger needs warm climate with humidity and is cultivated in areas with heavy rainfall. Best at an altitude of 1000 to 1500 m. and even at sea level. If no sufficient rainfall is available proper arrangements for irrigation are necessary. Sandy or clay or red loamy soils are suitable for ginger. It is cultivated by sowing rhizomes in the month of June. Ginger is soil exhausting crop and being a rhizome, needs to be supplemented with good quality of manures and fertilizers. Superphosphate and ammonium sulphate and potash are the common fertilizers used for ginger. It is ready for harvesting in about six months when its leaves become yellow. Harvesting done by digging the rhizomes.

Rhizomes washed properly and then dried to improve the colour and to prevent its further growth. The rhizomes are scrapped, dried and coated with inert material like calcium sulphate.

Macroscopic Characters :-

Colour - Externally, it is buff coloured

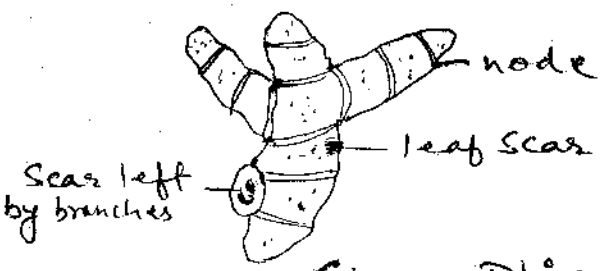
Odour - Agreeable and aromatic

Taste - Agreeable and Pungent

Size - Rhizomes are about 5 to 15 x 1.5 to 6.5 cm.

Shape - The rhizomes are laterally compressed bearing short flat, ovate and oblique branches on the upperside with bud at the apex.

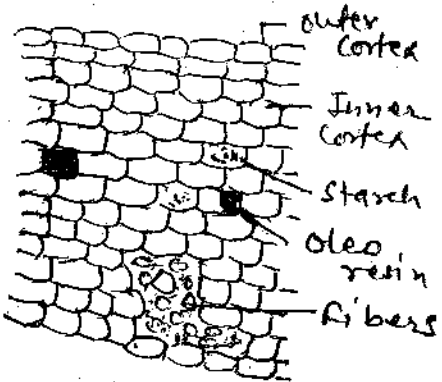
Fractures - Short and fibrous.



Ginger Rhizome

Microscopic characters -

Cork consists of irregularly arranged cells, followed by cortex. Cortex is made up of thin walled parenchymatous tissue. Well marked endodermis distinguished stele and the cortex. Cortical tissue encloses several closed collateral fibro vascular bundles. Vascular bundle just inside the endodermis are free to fibres. Oleo-resinous cells and starch grains are found throughout the ground tissue.



T.S. of Rhizome

Chemical Constituents → Ginger consists of volatile oil (1-4%), starch (40-60%), fat (10%), fibre (5%) inorganic material (6%) residual moisture (10%) and acrid resinous matter (5-8%). Ginger oil is constituted of monoterpene, hydrocarbons, Sesquiterpene hydrocarbons, Oxygenated mono and Sesquiterpenes and ~~phen~~ phenyl-propanoid.

Sesquiterpene hydrocarbon contents of all types of ginger oil from different areas is found to same and include α-Zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene and α-curcumene.

Uses → 1) It is used as a stomachic
2) It is used as aromatic, a carminative, stimulant and flavouring agent.
3) Ginger oil is used in mouth washes, ginger beverages and liquors.
4) Ginger powder is effective in motion sickness.
5) It has been suggested that adsorbent, aromatic and carminative properties of ginger on G.I. tract cause adsorption of toxins and acid enhanced gastric motility. These may have probably blocking effects of G.I. reaction and nausea.

(10) Monophasic Liquid - Liquid dosage forms commonly encountered in pharmaceutical practice are either monophasic or biphasic. Monophasic systems are characterized by the presence of a single homogenous phase e.g. solutions, waters, tinctures etc., whereas biphasic liquid dosage forms consist of two distinct phases

eg. emulsions and suspensions.

A solution may be defined as a monophasic system of two or more substances. The components of a solution are so intimately mixed with each other that a physically homogenous system results. Many solutions are classified separately. ~~Thus~~, ~~Syrup and~~

Syrup – Syrups are aqueous solutions containing sugar. Medicated syrups contain a therapeutic or ~~medicinal~~ medicinal agent. Syrups containing flavouring agents but no medicinal substances are called flavoured syrups.

Elixirs – Elixirs are sweetened hydroalcoholic solutions they are clear intended for oral use. They provide a palatable means of administering potent or nauseous drugs. Elixirs are less sweet and less viscous than syrup.

Spirits → Spirits are solutions of aromatic materials in alcohol and consist of alcoholic or hydro-alcoholic solutions of volatile substances. Brandy and whisky were the first spirits.

Aromatic water – Aromatic waters are clear, saturated aqueous solutions of volatile oils or other aromatic or volatile substances. Their odour and taste are similar to those of the drugs or volatile substances from which they are derived.

Tinctures – According to the pharmacopoeia of India, tinctures are defined as alcoholic or hydroalcoholic solutions usually containing, in comparatively dilute proportions, the active principles of

vegetable or animal drugs.

Injections – Sterile and pyrogen free solutions for parenteral administration are classified as injections.

Solutions may be solid, liquid or gaseous but liquid solutions constitute the most important category from pharmaceutical viewpoint. A solution consisting of only two substances is referred to as a binary solution and the components are known as the solvent and the solute. The component present in the greater amount in a solution is designated as solvent and the component present in the lesser amount is the solute.

In a true solution e.g. a syrup, the particles of the solute dispersed in the solvent are so small that they consist of molecules as ions. Thus a true solution is regarded as a molecular dispersion. A colloidal solution or dispersion e.g. Silver protein in water or methylcellulose in water consists of the particles ranging in size from 0.1 μm to 1 μm and may be detected with the help of an ultra microscope.

When an excess of a solid (solute) is brought into contact with a liquid (solvent) for a period of time, molecules of the former are removed from its surface until an equilibrium is established between the molecules leaving the solid and those

returning to it. The resulting solution is a saturated solution at a given temperature and the extent to which the solute dissolves is referred to as its solubility. It is often posible to correlate some physical properties and solubility characteristics of compounds.

Polarity of a solute is another property that can be related to its solubility. Organic compounds containing polar groups capable of forming hydrogen bonds with water are generally soluble in water. Polar groups like OH, CHO, COH, CHOH, CH_2OH, COOH, NH_2 and SO_3H tend to increase the solubility of an organic compound in water.

Section – B

Ans 6 – **Organoleptic evaluation of drugs :-**

It is also known as morphological evaluation of drugs. It is the evaluation of drugs by color, odor, taste, size, shape and special features like touch, textuse etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profile of whole drugs. Organoleptic evaluation means conclusions drawn from studies resulted due to impressions on organs of senses. The study of form of a crude drug is "Morphology" while description of the form is called Morphography. The fractured surfaces in cinchona, quillaia and cascara barks and quassia wood are important characteristics. Aromatic odor of umbelliferous fruits and sweet taste of liquorice are the examples of this type of evaluation. Ovoid tears of gum acacia, disc shaped structure of nux-vomica, conical shape of aconite are important diagnostic characters. General appearance of many crude drugs often indicates whether it is likely to comply with prescribed standards such as stalk in cloves. Overdrying makes leaf drugs and flowers brittle and cause them to break in transit making the task of morphological evaluation difficult.

The wavy shape of rauwolfia, pungent taste of Capsicum and ginger, brown color of cinnamon, odor and taste of spice drugs like asafoetida, black pepper, cumin etc. are imp. diagnostic organoleptic characteristics.

Section – B

Ans 7- Biological evaluation of drugs :-

When estimation of potency of crude drug or its preparation is carried out by means of its effect on living organisms like bacteria, fungal growth or animal tissue or entire animal, it is known as bioassay. This method is generally called for, when standardization is not adequately done by chemical or physical means and also for conformity of therapeutic activity of raw material and finished product. In other words, biological evaluation of drugs is the measure of sample being tested capable of producing biological effect as that of standard preparation. Such activity is represented in units known as 'international unit (I.U.). Biological evaluation methods are mainly of three types - (i) toxic (ii) Symptomatic and (iii) tissue methods. In toxic and symptomatic techniques, the animals are used, whereas in tissue methods, the effect of a drug is observed on isolated organs or tissue

Section – C

Ans 11 – Procedure of evaluation of hepatoprotective drug:-

For evaluation of hepatoprotective activity of a drug, both in vitro and in vivo methods may be applied. In in vitro evaluation, primary hepatocyte culture of rats/mice, rabbits or guinea pigs may be used or established hepatic cell lines may be used. On the other hand, in vivo method involves the use of rats/mice, rabbits or guinea pigs. A number of chemicals, drugs, industrial pollutants, ethyl alcohols, or hepatitis virus are known to cause hepatitis.

Liver damaging agents – Most commonly used liver damaging agents are CCl_4 (1.5ml/kg), paracetamol (>2g/kg), rifampicin, ethyl alcohol may be used, which cause subacute or chronic toxicity. The parameters used to assess liver toxicity are –

(a) Physical parameters
  1. Hexobarbital hypnosis
  2. Total body weight
  3. Organ weight index

(b) Biochemical parameters
  1. Serum biochemistry
  1. AST
  2. ALT
  3. Serum protein
  4. " albumin
  5. LDH
  6. serum alkaline phosphatase
  7. Total bilirubin
  8. Cholesterol

⑨ Total tri glycerides
⑩ Serum glucose

(ii) Tissue biochemistry

① Total protein
② " Glycogen
③ Acid phosphatase
④ Alkaline phosphatase
⑤ Triglycerides
⑥ cholesterol
⑦ Lipid peroxidation
⑧ Superoxide dismutase
⑨ Catalase
⑩ Adenosine triphosphatase.

(iii) Histopathology - necrosis, fatty degeneration fibrosis etc.

After ensuring the induction of toxicity, drug treatment is given for 10-15 days and recovery from damage/toxicity is verified by testing the above mentioned variables.