AS-2283

M.Sc. (III Semester) Examination - 2013
Rural Technology
RT- 901 M
- Medicinal plant production techniques -

Section 'A'
Multiple Choice questions

Answers

(i) (d) Cymbopogon martinii

(ii) (d) All of them

(iii) (c) Concrete

(iv) (b) Washed sandal wood oil

(v) (d) All of them

(vi) (a) Marc

(vii) (b) NMR

(viii) (c) HPLC

(ix) (c) Pancreas

(x) (a) Renin

Section - B
Short answers

2. Aromatic plants synthesize and preserve a variety of biochemical products, many of which are extractable and useful as chemical feed stocks or as raw materials for various scientific investigations. Many secondary metabolites of plants are commercially important and find use in a number of perfumery, flavouring and pharmaceutical compounds. The characteristic

property of the plant is due to variety of complex chemical compounds and hence aromatic plants are generally referred to as 'natural bio-chemical factories' or 'chemical goldmines'. In today's world of consumer boom, role of essential oils increased many folds. Essential oils and aroma chemicals constitute a major group of industrial products. These oils form indispensable ingredients of necessities in many spheres of human activity. They are adjuncts of cosmetics, soaps, pharmaceuticals, perfumery, confectionery, ice-creams, aerated waters, disinfectants, tobacco, agarbathis etc.

Demand and price of herbal products and essential oils are increasing consistently in national and international market. In the world of fragrance and flavour industry, essential oils contribute to about 17%. Extent of usage of essential oil is 55-60% for flavour in food industry, 15-21% for fragrances in perfumery/cosmetic industry, 10-20% as starting material for isolation of components.

It is estimated that annual turnover of perfumery, cosmetic and flavour industry exceeds US $ 6 billion comprising more than 100 essential oils the world over. World production is likely to touch US $ 5 trillion by 2050.

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3. Enfleurage: Enfleurage is the process of extraction of fragrance by absorbing it from flowers in contact with cold fats.

This process is adopted for fragrante flowers of jasmine and tuberose, which continue to manifest their characteristic fragrance even in plucked condition. Solvents lak this virtue of arresting manifested fragrance.

Method: In this method refined lard or beef suet are preferred. Fat should be saturated and odourless to prevent entrance of fat odours. Fat is thinly layered on both sides of a glass plate supported on a rectangular wooden frame or chassis. Fresh fragrant flowers are lightly layered on fat coated chassis. Several chassis are placed one above the other sandwiching the flowers between two layers of fat. Spent flower are removed, this process called defleurage and fresh charge is made. Reversing of glass slab is called patage Patage is done several times to obtain maximum perfume absorption. Furrows are created with combs to increase absorption surface. The process of defleurage, fresh charging and patage is continued to obtain fat of desired perfume strength.

This method gives a much greater yield of flower oil than other method except extraction with voltile solvents.

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4.

Answer: Factors affecting choice of an Extraction Process.

1. Character of the drugs: A knowledge of pharmacognosy of the drug to be extracted is essential for the selection of the extraction process that will give best results. Thus percolation is recommended in case of hard and tough drug, whereas maceration is suitable in case of hard and tough drugs whereas maceration is suitable in case of soft and parenchymatous drug. Similarly maceration is suitable for drugs e.g. squill which cannot be easily powdered and unorganized drug.

2. Therapeutic value of the drug: Maceration is considered suitable if the drug has little therapeutic value e.g. flavours, bitters but if the drug has considerable therapeutic value and maximum extraction is required, percolation should be used. e.g. Belladonna.

3. Stability of the drug: Continuous extraction has to be avoided if the active constituents of the drug are heat-labile.

4. Cost of drug: In case of costly drugs e.g. Ginger, percolation is economical but for cheaper drugs maceration is good enough. Due consideration has to be given to the cost of the drug and the cost involved in comminution for best possible extraction of the constituents.

5. Solvent: Reserved percolation can be used avoiding continuous extraction if

the desired constituents require a solvent other than a bare boiling solvent or an azeotrope.

6. Concentration of the product: Depending on the previous factors, dilute products such as tinctures can be prepared by maceration & percolation. Concentrated preparations such as liquid extracts or dry extracts are prepared exclusively by percolation. However, continuous extraction can be used if the solvent is suitable and the constituents are heat-stable.

5. Hot continuous extraction method:

This method is also known as soxhelation. This is modified method of simple percolation wherein percolation is effected by the use of a soxhlet extractor whereby a small volume of hot liquid is made to percolate through a column of the drug again and again by evaporation and subsequent condensation.

A soxhlet extractor consists of a flask holding the menstruum, the extractor or a cylindrical percolator provided with an attached called the soxhlet extractor and a reflux condenser fitted at the top of the soxhlet.

The material to be extracted is usually placed in a thimble made by filter paper and then inserted into the extractor.

The menstruum is placed in the flask and boiled. The vapours arising from the flask pass by the side tube into the condenser. The vapour is condensed and drips into the body of the extractor as pure menstruum. It percolates through the drug to be extracted dissolving the soluble substance(s). As soon as the level of the menstruum in the main extractor rises above the siphon bend, the extract is drained out flowing through the siphon into the flask. This alternate filling and emptying of the body of the extractor goes on continuously. Thus the same quantity of menstruum is recycled every time and a complete extraction is achieved with a small volume of the menstruum.

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6 Plate preparation method for TLC

The substances most frequently used as coating material are silica gel, alumina and cellulose. The coating material may also contain an inorganic fluorescent indicator (e.g. zinc silicate) which fluoresces when irradiated at a suitable wavelength. Silica gel and alumina are available with different specific surface areas and these grades are identified by a number which indicates the mean pore size in Angstroms.

Method: The size of glass plates for use with commercially available spreaders are usually 20×20, 20×10 or 20×5 cm.

Mix the adsorbent (30g) in a mortar to smooth consistency with the requisite amount of water or solvent specified as per manufacturer's instructions and Transfer the slurry quickly to the spreader. Spread the mixture over glass plate and allow the thin layer to set. Transfer the plates carefully to a suitable holder and after a further 30 min, dry at 100-120°C for 1 h to activate the adsorbent. Cool and store the plates in a desicator over silica gel.

Microscope slides are also used as glass plate for TLC. These slides are coated by a dipping technique in the following way - prepare a slurry of the adsorbent by shaking with chloroform or chloroform - methanol (2:1) and insert two slides (back to back) into the slurry. Withdraw the slides, allow to drain, separate the slides and dry.

7. Collagen:

Source - It is the protein which consists of major portion of white fibres in connective tissues of the animal body specifically from the tendons, skin bones and teeth.

Chemical nature - Glycine and proline are the important amino acids in the central core of the triple helical molecule of collagen. Collagen is characterised by the presence of glycine, proline, hydroxyproline, and hydroxylysine and low tyrosine and sulphur contents. Various types of collagen exist depending upon the amino acid sequence. The five most common types are:

- Collagen - I : skin, tendon, organ, bone
- Collagen - II : Cartilage
- Collagen - III : reticulate and commonly found alongside type-I
- Collagen IV : forms basal lamina, the epithelium - secreted layer of the basement membrane
- Collagen V : Cell surfaces, hair and placenta.

Uses: It is used in the preparation of sutures, as a gel in food casing and photographic emulsions.

- Oral administration of type-II Collagen improves symptoms of rheumatoid arthritis.
- It is widely used in cosmetic surgery.
- It is also used in bone grafting and tissue generation.

Section - C

Long answers

8. Scientific name:

Cymbopogon winterianus

Family: Poaceae

English: Citronella grass

Habitat and distribution: Citronella thrives well under tropical and sub-tropical conditions. It requires abundant moisture and sunshine for good growth. Plant grows under a wide range of soil conditions sandy loam uplands with abundant organic matter. It grows under a wide range of pH (5-8) though 6-7 is ideal.

It is widely distributed throughout tropics comprising Srilanka, Java, Central America, Guatemala, Taiwan, Brazil, India and west Indies etc. In India, it is cultivated in Assam, West Bengal, Uttar Pradesh, Maharashtra, Karnataka, Tamil Nadu, Gujrat, Arunachal Pradesh, Manipur, Mizoram, Meghalaya, Nagaland and Tripura.

Cultivation: Grass is propagated only vegetatively by slips which are obtained by dividing well grown clumps. Clumps are separated in a manner that each slip contains 1-3 tillers. Roots and leaves are trimmed off before planting. Slips should be obtained from at least 6 month old plantation.

slips are planted during June - July at 60 - 90 cm spacing and 10 cm. deep. Care should be taken to avoid waterloggin in field.

Fertilizers: FYM is applied (10 tonnes/ha) before planting during field preparation. A fertilizer dose of 200 kg N, 80 Kg P_2O_5 and 40-80 Kg K_2O/ha/annum is recommended for optimum growth and yield.

Extraction and utilization: Harvested grass is wilted in shade for a short time and steam distilled within 24 hours. Oil yield varies with season, soil fertility and distillation efficiency. On an average 0.8 — 1.2% of oil is recovered from grass.

Citronella oil contained citronellal, geraniol, citronellol, geranyl acetate, citronellyl acetate, limonene, sesquiterpene alcohols, α-elemene, α-cadinene etc.

Citronella oil serves as a starting material for extraction of geraniol and citronellal which can be converted into aroma chemicals. These components are extensively use in soap, perfumery, cosmetic and flavouring industries throughout world.

Spent grass can be used as a source of raw material for cellulose pulp and paper production by using sulphate, sulphite and cald caustic soda.

9. Distillation and expression of essential oil

Distillation: Distillation may be defined as separation of components of a mixture of two or more liquids by virtue of difference in their vapour pressure.

The bulk of essential oils are produced by distillation. There are three systems of distillation - hydro, hydro-steam and steam distillation.

(i) Hydrodistillation: This method is widely practised for essential oil extraction. The plant material is in direct contact with boiling water in a crude metallic distillation unit. The material floats on water or be completely immersed, depending upon its specific gravity and quantity of material handled per charge.

(ii) Hydro-steam distillation: Hydro-steam distillation is employed where perfumery material is vulnerable to direct steam. Consequently, plant material is supported on a perforated grid or screen inserted at some distance above bottom of still. Lower part of still contains water up to a level just below grid. Water may be heated by any of the method. In this method plant material is in contact with steam only and not with boiling water and steam is always fully saturated, wet and never superheated.

(iii) Steam distillation: In this method live steam saturated and superheated, under pressure (up to 7 kg/(cm²)) is injected through steam tubes below the charge and pressure within the distillation vessel is controlled according to nature of the material being distilled. It resembles hydro-steam distillation except that no water is kept in bottom of still.

Maceration: Extraction of essential oil with hot fat. Oil cells of fragrant flowers are ruptured by immersion in a hot fat or oil at 60-70°c which in turn absorbs essential oils. Fat is separated from spent flowers and reused for absorbing fragrance from next batch of fresh flowers. Fat retained by flowers is recovered by hydraulic pressing. Resultant perfumed pomade is frequently marketed as such but is often extracted with strong alcohol to yield extracts.

Enfleurage: Enfleurage is the process of extraction of fragrance by absorbing it from flowers in contact with cold fats. This process is adopted for fragrant flowers of jasmine and Tuberose, which continue to manifest their characteristic fragrance even in plucked condition. Solvents lack this virtue of arresting manifested fragrance. Enfleurage gives a much greater yield of flower oil than other methods.

Super critical fluid extraction: This is emerging as a versatile and important tool to separate components that are susceptible to thermal degradation. It is employed for extraction of flavours, fragrances and perfumes from a wide variety of natural products. this method of extraction is superior and faster than distillation.

Expression: This method is employed when essential oils are thermosensitive. It is used for isolating essential oils from lemon and orange peels. In general, expression involves squeezing any plant material at great pressures to press out oils or other liquids. The process is carried out by hand-operated presses or crushes is isolated or by gigantic mechanical presses in industrial centres.

1a Proteolytic enzyme:

(i) Pepsin: It is proteolytic enzyme and present in the gastric juic of animals.

Source: It is obtained from glandular layer of fresh stomach of hog (Sus scrofa var. domesticus) belonging to family Suidae.

Chemical nature: Pepsin is light buff or white coloured amorphous powder. It also occurs as translucent scales. It has a little acidic or saline taste with slightly meaty odour. It is soluble in water, but insoluble in alcohol, ether and chloroform. If pepsin is heated with alkali or pancreatic enzymes, its biological activity is lost. It shows maximum activity at pH 1.8.

Uses: Pepsin degrades proteins into peptones and proteoses. Pepsin has the capasity to digest 2500 times its weight of coagulated egg albumin. It is also available in other forms which may digest even upto 10,000 times their weight of coagulated egg albumin.

(ii) Tripsin: It is a proteolytic enzyme obtained from mammalian pancreas.

Source: It is obtained from mammalian pancreas like ox (Bos Taurus, family Bovidae) If is obtained by alcoholic or aqueous acid extraction of its precursor trypsinogen and further conversion to crystalline trypsin.

Chemical nature: It is crystalline or amorphous, yellowish-white powder without any odour. It is soluble in water, but insoluble in alcohol, glycerine, chloroform and ether. It shows maximum enzymatic activity at pH 7.6 to 8. It is stable in dry air, but the solution should be freshly prepared because of degradation. In the presence of calcium ions, its stability is increased.

Uses: Trypsin is used to enhance the proteolysis of different protein substrates, like blood clot, necrotic tissue, purulent exudates, etc. It does not act on living tissue due to presence of inhibitors.

(iii) Rennin: It is a partially purified milk-curdling proteolytic enzyme

Source: It is obtained from glandular layer of the fourth stomach of the calf (Bos taurus)

Chemical nature: Rennin has peculiar odour and saline taste. It is available as scales or powder. It has hygroscopic nature, yellowish-white or greyish-white colour.

Uses: Rennin is used to prepare junkets and cheese. It is also used to coagulate milk and hence making the milk easily digestable for weak patients.

11.

Chromatography: It originate from Greek word 'Chroma' - colour and 'graphia' - to write. It is the collective term for a set of laboratory techniques for the separation of mixtures.

The mixture is dissolved in a fluid called the 'mobile phase', which carris it through a structure holding onothe material called 'stationary phase'.

The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases.

Types of Chromatography:
- Paper chromatography
- Column chromatography
- Thin layer chromatography
- Gas chromatography
- High performance liquid chromatography

Paper chromatography: Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of chromatography paper a stationary phase and this strip is placed in a jar containing a shallow layer of solvent a mobile phase. The movement of components on the paper depends on the amount and nature of mobility of solute and movement of the mobile phase.

R_F values are of considerable importance in paper chromatography.

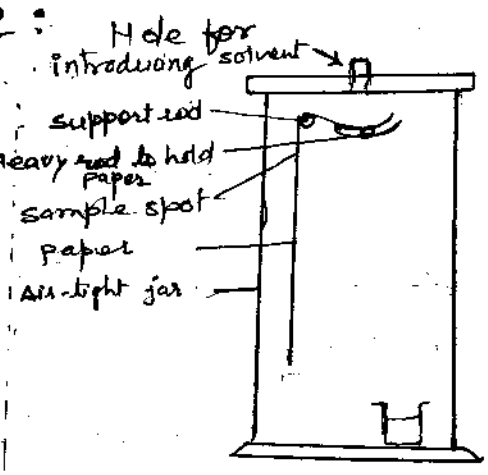
$$R_F = \frac{\text{distance travelled by centre of component}}{\text{distance travelled by solvent front.}}$$

Methods

- Descending chromatography
- Ascending Chromatography
- Two - dimensional chromatography

Descending chromatography:

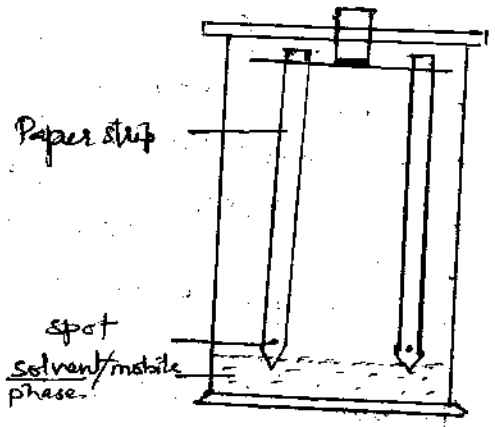
In this type of chromatography mobile phase is added through the stoppered hole situated at the top of the jar. Paper strip is place in air tight jar which is held firmly with heavy rod. spot or loading is done below the support rod and not larger than 5 mm, if a larger number of sample is examined, spot should not closer than 2 cm. Remove the paper after the proper separation and allow paper to dry in a current of air or in an oven and locate the components.



Apparatus for descending chromatography

Ascending chromatography

In method solvent fill in a jar and paper strip is placed in jar. Loading is done at bottom of the paper strip, 2 - 3 cm away from tip. Allow to equilibrate for 2 to 3 h. At the end of this time lower the paper into the mobile phase and allow development to take place until the solvent front has reached a suitable height. Remove the paper, dry and locate the components.



Apparatus for Ascending paper chromatography

Two dimensional Chromatography

After spotting loading (3 cm from its end) develop the chromatogram in one solvent system by the method of ascending chromatography. Dry the paper and refold it into a cylinder at right angles to the first. Develop with a second solvent system, dry and locate the components of the mixture. Identify by treating reference components in the same way.