**Biyani Girls College**

**B.Sc. II year Biotechnology**

**I Internal Examination, 2019-20**

**Plant Secondary Metabolites**

**Set-A**

**Time: 1.30 Hours** **Maximum Mark: 30**

**All Questions are Compulsory**

**1. Answer the following questions in very short** 1x8

(i)What do understand by secondary metabolites.

(i)Secondary metabolites are organic compounds produced by bacteria, fungi, or plants which are not directly involved in the normal growth, development, or reproduction of the organism.

(ii)Name 2 steroids.

(ii)Glucocorticoids: cortisone. , Androgens: testosterone

(iii)What do you understand by cryopreservation.

(iii)Cryopreservation is the use of very low temperatures to preserve structurally intact living cells and tissues. Unprotected freezing is normally lethal and this chapter seeks to analyze some of the mechanisms involved and to show how cooling can be used to produce stable conditions that reserve life.

(iv)What are antitumor compounds.

(iv)A great number of antitumor compounds are natural products or their derivatives, mainly produced by microorganisms. In particular, actinomycetes are the producers of a large number of natural products with different biological activities, including antitumor properties. They can reduce the initiation and growth of tumors.

(v)Differentiate primary metabolites from secondary metabolites with examples.

(v)Primary metabolites are produced during the growth phase of cell while secondary metabolites are produced during the non- growth phase of the cell. Secondary metabolites are accumulated by plant cells in very small quantities than primary metabolites.

Examples of primary metabolites are vitamins, carbohydrates, proteins, and lipids while secondary

metabolites examples are phenolics, steroids, essential oils, alkaloids, steroids are few examples

(vi)Which secondary metabolites do we get from *Ginkgo biloba*.

(vi)Ginkgo is rich in secondary metabolites (SMs), mainly including flavonoids, lactones, and ginkgolic acid.

(vii)What is optical density.

(vii)It is the ratio of incident to transmitted radiant power through a material, and spectral absorbance or spectral decadic absorbance is the common logarithm of the ratio of incident to transmitted spectral radiant power through a material

(viii)What is cell immobilization.

(viii)The immobilized whole cell system is an alternative to enzyme immobilization. Unlike enzyme immobilization, where the enzyme is attached to a solid support, in immobilized whole cell systems, the target cell is immobilized.

**2. Write short note on**4x2

(i)Terepenes (isoprenoid compounds)

(i)Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, and by some insects. They often have a strong odor and may protect the plants that produce them by deterring herbivores and by attracting predators and parasites of herbivores.

Monoterpenes: 2 isoprene units, 10 carbon atoms.

Sesquiterepenes: 3 isoprene units, 15 carbon atoms.

Diterepenes:4 isoprene units, 20 carbon atoms.

Triterepenes6 isoprene units, 30 carbon atoms.

Tetraterpenes: 8 isoprene units, 40 carbon atoms

Terpene are the largest single class of compounds found in essential oils, also called Isoprenoids and are made up of isoprene molecules. Each isoprene molecule (sometimes called isoprene unit) contains five carbon atoms with double bonds. The simplest terpenes are monoterpenes that contain two isoprene molecules. Sesquiterepeneshave three isoprene molecules and Diterepeneshave four. Because each isoprene molecule has five carbon atoms, it is easy to calculate the number of carbon atoms per molecule. Terpenes can be subdivided into groups *acyclic* or *cyclic* which indicate their structure. Acyclic terpenes are linear, like the monoterpene. Cyclic terpenes form a ring, like the monoterpene *p*-cymene.

Monocyclic, bicyclic, and tricyclic monoterpenes (meaning one, two, or three nonaromatic rings) occur in essential oils.

(ii)Alkaloids

Alkaloids are a class of naturally occurring organic compounds that mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Alkaloidsare a large and complex group of cyclic compounds that contain N.

About 2,000 different alkaloids have been isolated, some of which are of pharmacological interest. Important alkaloids include morphine, styechine, atropine, colchicine, ephedrine, quinine, and nicotine. They are most common in herbaceous plants, but some occur in woody plants, chiefly tropical species.

Alkaloids commonly are concentrated in particular organs such as the leaves, bark, or roots. For example, although nicotine is synthesized in the roots, 85% of that in a tobacco plant occurs in the leaves, and the cinchona alkaloids are obtained from the bark. Alkaloids also sometimes occur in wood, and the wood of some species of the families Anacardiaceae, Apocynaceae, Euphorbiaceae, the legume families, Rutaceae, and Rubiaceae contains so much alkaloid that it produces dermatitis. Among alkaloids derived from trees, the cinchona alkaloids are best known because of their use in treatment of malaria. They occur in the Andean genera *Cinchona* and *Remijia* of the family Rubiaceae.

Some synthetic compounds of similar structure may also be termed alkaloids.Alkaloids are widely distributed in species belonging to several families within angiosperms. They are versatile heterocyclic nitrogen compounds with reported antimicrobial activity against fungal or bacterial phytopathogens. Furoquinoline alkaloids, quinolizidine alkaloid extracts, isoquinoline alkaloids (corynoline and acetylcorynoline), pyrrolizidine alkaloids have been mentioned as active compound. The multitarget mechanisms of action are represented by the outer membrane or cytoplasmic membrane disruption, respiratory inhibition, the Z-ring perturbation, and nucleic acid synthesis/cell division inhibition.

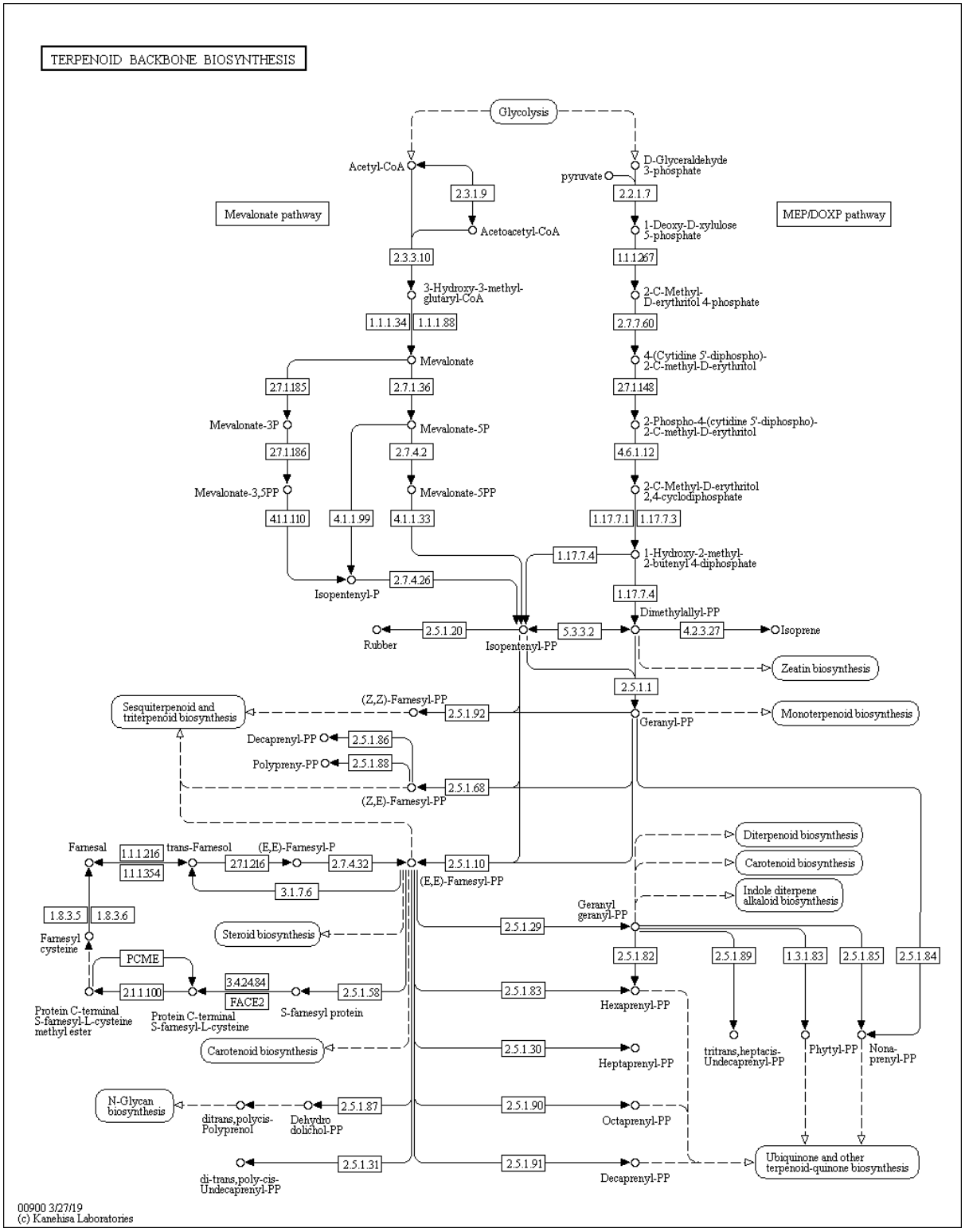
3. Explain different pathways responsible for biosynthesis of terepenoids? 7

3. Terpenoids are a group of largest natural products with important biological functions, and their efficient biosynthesis is of particular importance to both academia and industry. As the building blocks for terpenoid biosynthesis, a suitable supply of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) is extremely crucial for efficient terpenoid biosynthesis. With this focus, we first

introduce biosynthetic pathways of IPP and DMAPP, and then summarize the current strategies adopted for manipulating IPP and DMAPP supply. At last, how to further manage IPP and DMAPP supply to improve terpenoid biosynthesis is also proposed.

Terpenoids, at least those containing an alcohol functional group, often arise by hydrolysis of carbocationic intermediates produced from geranyl pyrophosphate. Analogously hydrolysis of intermediates from farnesyl pyrophosphate gives sesquiterpenoids, and hydrolysis of intermediates from geranylgeranyl

pyrophosphate gives diterpenoids, etc




4. Explain different techniques for separation of secondary metabolites?

4.Techniques for separation of secondary metabolites.

**Liquid Chromatography**: The primary method of compound purification to be used is chromatography. While chromatography comes in many forms, the basics are always the same. In the phytochemical analysis of the target plant liquid chromatography will be used primarily due to its availability, the simplicity of collecting samples from this technique, and the fact that it will likely be effective for all compounds in the extract. Liquid chromatography involves the interaction of two separate components, the mobile phase and the stationary phase. The mobile phase is a solvent or mixture of solvents that will dissolve the sample to be separated. The stationary phase is immiscible in the mobile phase and is chosen for its affinity for the compounds found in the sample. The mobile phase moves through the stationary phase and the interactions of the sample compounds with the stationary phase are what will cause the separation to occur.

**Thin Layer Chromatography (TLC),** a type of planar chromatography, is used to test the effectiveness of a mobile or stationary phase with a small part of the sample to be separated in order to maximize effectiveness on the large scale. TLC is also used to analyze the eluent and eluate collected during traditional column chromatography. The eluent is collected in batches called fractions. These fractions are analyzed by TLC and the fractions that contain the same compound or mixture of compounds are combined.

**High Performance Liquid Chromatography (HPLC)** High performance liquid chromatography is the other type of column chromatography. It is abbreviated HPLC and is sometimes referred to as high pressure liquid chromatography. HPLC has a permanently packed column as opposed to open column chromatography, and the column is generally made out of an unreactive metal. The reason for this is that the mobile phase is pushed though the stationary phase at high pressures. The typical pressures used in HPLC range from 500 to 4000 psi.

**Quantitative HPLC** uses the detector to measure the amount of material eluted. The most common type of detector is a UV-Vis absorption detector. These detectors measure the amount of ultraviolent and visible light absorbed by the analyte components as they elute. While variable wavelength detectors and photodiode arrays are capable of measuring more than one wavelength, a commonly analyzed wavelength is 254nm. Absorption is measured at 254nm because compounds with substituted aromatic chromophores usually absorb around this wavelength.

**Nuclear Magnetic Resonance Spectroscopy (NMR)** The primary method of identifying isolated compounds, as well as a guiding force in prioritizing which fractions should be examined, is nuclear magnetic resonance spectroscopy (NMR). NMR at its very core is based upon the interactions between individual nuclei, their neighboring atoms, and the magnetic field in which NMR takes place. NMR works because the magnetic spin of the individual nuclei will align when placed inside a larger electromagnetic field, which is generated by either a large magnet or superconductor solenoids that generate an electromagnetic field

**Correlation Spectroscopy** In addition to the hydrogen and various carbon one dimensional NMR experiments, several other two dimensional correlation techniques are performed as well. COSY or 1 H1 H correlation spectroscopy can determine the relationship between different nuclei within the sample molecule, and generates a two dimensional spectrum that shows which hydrogen atoms are coupling with each other. COSY is performed by using a series of pulses designed to make the spins of the nuclei interact with each other. The receiver coil records the changes in the magnetic field caused by the interactions. COSY is the form of correlation NMR where the interactions between hydrogen nuclei are recorded. COSY gives important information through these proton couplings that is very useful in structure elucidation.