

PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 1

Lecture3

Week 1: Lecture 3: Introduction to Primary, Secondary Metabolites and Biosynthetic building blocks

Hello everyone, and welcome to session 3 of the NPTEL course in Pharmacognosy and Phytochemistry. By now, we know what pharmacognosy and phytochemistry are, what the scope of the subject is, and what the different drugs of natural origin are that we saw in the previous session. Now, in this session, we are going to take a step further in understanding what compounds these drugs contain. So, in this session, we are going to learn about plant metabolites and, more predominantly, we are going to classify them as primary and secondary metabolites. And we are going to understand their biosynthetic precursors.

So, what are these plant metabolites, and what are primary metabolites? Now, whenever a plant thrives in an environment, there are numerous compounds that are produced by it, and this process of producing compounds is called metabolism, which is why they are called metabolites. Now, some of this metabolism happens to benefit the plant. The compounds resulting from it are called primary and secondary metabolites. Now, if you look at primary metabolites, they are vital or essential metabolites that are required for the growth, development, and reproduction of plants.

Now, if you consider molecules such as sugars, proteins, lipids, nucleic acids, and vitamins, by now, understanding the biochemistry of plants, we know that they are very vital. So, whenever there is sunlight, the first thing that the plant produces is it takes carbon dioxide and water and, through the process of photosynthesis, gives rise to sugars.

Now, these sugars are the main source of energy for the plant, which will help in its development. Now, these sugars will further undergo numerous pathways to develop other metabolites that are essential for its survival. So, from sugar pathways, we will get our proteins.

We will get our lipids. Now, the plant genetic material is very vital. So we have our nucleic acids and also vitamins, which are cofactors to enzymatic biosynthetic processes. So all these compounds put together are called primary metabolites. Now, there are some compounds or some products of metabolism which are not essential, but they attribute a particular quality to the plant.

This quality can be a defensive quality, or this quality can help them prevent disease. Not only that, in some cases, these principles also help attract beautiful, beautiful insects. Now, these compounds or this set of metabolites are called secondary metabolites. So secondary metabolites are distinct metabolites which are further derived from primary metabolites. And their function is to prevent the plant from disease, prevent the plant from infection, and communicate with other plants.

They help the plant withstand stress, be it environmental conditions, the pH of the soil, drought, rain, or any stressor. Now, these stress metabolites will help the plant thrive in these particular conditions. Now, because these metabolites are produced to repel insects or, in certain cases, to attract insects, they are defensive or protective in function, as compared to primary metabolites. These compounds are found to have more pharmacological effects on animals as well as on us humans. And as a result, when you're searching for bioactive molecules such as drugs, the secondary metabolites are predominantly important to us.

Some of these metabolites render antibacterial properties, essentially the phenols and the tannins. Some of them are cardiotoxic. When we know digitalis, it produces something like digitoxin, which is a radioactive compound. And there are certain substances which plants produce that cause hallucinogenic effects. Now, you know something like cherries or ganja—they are all plant-based metabolites, but they have what is called a CNS-stimulating effect.

So that is also one way for a plant to be defensive and to tell the animal that it should not consume this. Some substances are intensely bitter, and as a result, they deter the animal from consuming them. Some of these compounds include things like steroids. So numerous compounds such as flavonoids, terpenes, antibiotics, alkaloids in fungi, and aflatoxins are secondary metabolites that are produced. These will help provide all these functions to the organism.

Now, as I said, from a pharmacological or medicinal perspective, secondary metabolites are very vital to us. Now, where are these metabolites located? Suppose I want to explore a medicine, I want to extract the secondary metabolites from the plant. Where do I extract them from?

Now, the secondary metabolites have a little different location depending upon their polarity. So depending upon their polarity, you can divide the secondary metabolites into two categories. One is hydrophobic. That is a very lipophilic or lipid-like molecule. One is hydrophilic.

They are more water soluble or water loving compounds. Now, if you see hydrophobic waxy or lipid-like compounds, you will see them especially in the outer coatings. So if you see your leaf, your leaf doesn't get moist during the rainy season because they have that waxy coating. So plant waxes, terpenoidal or you know a very hydrophobic compound can be located in the outer cuticle that is the outer lining of the epidermis. They can be located on trichomes.

So if you know cotton. Cotton is an example of very long trichome which is present and very few of us know that this cotton is actually water repelling but if you somehow remove that waxy coating from cotton by a process called as defatting only then the cotton becomes absorbent so you will have your fats which are coating the cotton preventing it from soaking in the rain but when we want to use it we will de-fat it and make it more absorbent so the fat is there on the trichomes similarly you might have some terpenes which are located on trichomes say for example your charas ganja is a kind of resin It might contain terpenoidal compounds. It might contain phenylpropanoidal compounds.

The mixture of all of this is located inside the glandular trichomes, which are present on this cannabis plant. So you can just touch it, and you will get this transferred to your hand. Now, in some cases, they might occur in the resin ducts. There is something which we discussed in the previous session, which we called resin. Say, for example, asafoetida or even pine resin.

They are located in resiniferous ducts. So that might contain terpenes, flavonoids, phenols, or any derivatives. Not only that, there are certain cells inside the plant which are called oil cells. They specifically localize oil droplets, and these oil droplets are stored until you cut them. So, these are called oil cells, which are located in your chillies or in your gingers.

And sometimes, instead of a cell, it might be a huge pipe or a huge duct. So you might have or come across oil ducts. If you see umbelliferous fruits such as fennel or coriander, the oil is located not in cells but in the form of ducts. We call it vit A. So you might come across some phenylpropanoids or terpenoidal compounds which are present in the oil ducts of such fruits. Now, there are certain compounds called quinones, which are responsible for your electron transport chain, and they may be located in your plastid membrane.

So these are a few locations where you will find your lipophilic or hydrophobic molecules. Coming to your hydrophilic or water-soluble molecules, you will see the leaf mesophyll region or parenchymatous region, especially the vacuoles. Those are the places where you have abundant sap or water coming out. So there in the vacuoles, you will come across compounds such as saponins. Glycosides, which are nothing but molecules to which sugars have been attached.

Anthocyanins, which are beautiful pigments present in your flowers, responsible for the color of certain flowers such as your periwinkle. Now, some of them may be in the lactiferous ducts from where the latex oozes out. An example of that is your opium, or in some cases, your lactiferous ducts of papaya will contain numerous proteins, derivatives, and so on. Your cell walls, especially if you see the trees, the outer wall of the trees,

particularly the stem, is brown in color. And that brown color is due to the deposition of compounds called tannins.

So these are a few places where you'll find your hydrophilic metabolites. Now, coming to the biosynthesis of secondary metabolites, we have a biosynthetic pathway, especially in plants, which starts from something as simple as photosynthesis. So what happens in photosynthesis is you have your CO₂ + water taken up by the plant in the presence of sunlight. This process is called photosynthesis, whereby these add up to make your sugar. This simple sugar includes your glucose, but later on, there are numerous pathways called shunt pathways that help in developing other sugars as well.

So you know you have your ribose, sedoheptulose, pentoses and so on. So from photosynthesis you will get your glucose and via different shunt pathways you will get other saccharides also. Now this saccharides right from glucose to others will start polymerizing that is adding them. Now why does the plant do this is you know a process called osmosis. So if too many sugars accumulate in the plant it will have a very high osmotic pressure.

So in order to decrease it the plant kind of polymerizes it and precipitates it. whereby that osmotic pressure is no longer exerted in that manner and that's where you get your oligosaccharides and polysaccharides. So you will have your starches as polymers of glucose, you have your cellulose as polymers of glucose, you have your different oligosaccharides, polysaccharides as well as some of the sugars get converted into your polyols. So all of this is coming from your sugar pathway, that is your photosynthesis pathway. Not only that, if you take your glucose, this glucose will further undergo the Emden-Meierhoff pathway and then it will get converted into your pyruvic acid.

So if you recollect, your Emden-Meierhoff pathway was a pathway wherein your glucose gets converted into fructose and thereafter you split it into your pyruvic compounds and the main or the end product of your Emden-Meyerhof pathway is your pyruvate. Now this pyruvate further gets converted into acetyl-CoA. Your pyruvate and acetyl-CoA are important byproducts of your Emden-Meyerhof pathway and later on you will see that

your acetyl-CoA enters your citric acid pathway. Now before that acetyl-CoA may undergo a

Adhesion or polymerization to give you other secondary metabolites. This includes your fatty acids, waxes, terpenes, saponins, and quinones. So they all come from this particular pathway, or they kind of diverge from this particular pathway. Now, once your acetyl-CoA goes into the citric acid pathway, in your citric acid pathway, you know you have your malic, succinic, fumaric acid, and so on. Now, these acids or these organic acids will undergo a reaction called a transamination reaction, and this transamination reaction will convert these organic acids into amino acids.

Now, these amino acids are essential, and these amino acids can further be converted into the next set of secondary metabolites. This includes your alkaloids as well as antibiotics. So you will see that your basic pathways can just further extend and manipulate themselves to give secondary metabolite compounds. Now, let's see a few building blocks of secondary metabolites and let's focus on them one by one. Now, when you say your secondary metabolites, they include compounds, and in this case, we will be talking about organic compounds.

Now, when I am talking about organic compounds, they are made by the addition of numerous subunits. Like you are building a house brick by brick, a molecule is built brick by brick. Now, each of these bricks is called a building block, and depending on how I want my structure to be, I can choose my building block. So that is how your plant decides what is to be chosen in order to get a particular secondary metabolite. Now, starting with the simplest compound, the simplest compound or the simplest building block is C1.

So imagine a case when I want to add just one carbon. Now, this carbon generally occurs in the form of a methyl group. CH₃, and this methyl group is given by an amino acid called methionine. So, suppose you know your amino acid methionine. In this case, what happens is methionine is an amino acid wherein your methyl group is attached to sulfur. Now,

Adenosine is something which is generated or biosynthesized from numerous pathways. But the basic molecule here is ATP, adenosine triphosphate. ATP fuses with your methionine to give what is called S-adenosylmethionine. Now, what happens is this group carries a slight charge, and as a result, your methionine groups can easily be transferred. So, SAM or S-adenosylmethionine is an excellent methylating agent.

Now, this generally happens in the cytoplasm, but, The movement of SAM is done by SAM transporters, which help move S-adenosylmethionine to different organelles. This includes your mitochondria, chloroplasts, and numerous places wherever you want to carry out the methylation reaction. Now, where does methylation happen? The substrate for methylation can be an O, which will give rise to OCH₃.

The substrate can be an N, which will give rise to NCH₃. Or, in some cases or very rarely, C, which will give rise to CCH₃. So, this is very rare, but the first two cases are very common. Now, looking at the examples. So, suppose you see a molecule such as ferulic acid.

Now, I am focusing on CH₃. Why? Because I am seeing this building block. So, whenever you see a natural product with a methyl substitution of the nature O-CH₃ or N-CH₃. Can you spot it?

Here it is. So, in your ferulic acid, you can see an O-CH₃, and this CH₃ group has been contributed by S-adenosylmethionine. Similarly, in your eugenol, which is found in eucalyptus, again you can see an OCH₃, and this CH₃ group has been contributed. So, whenever you want to methylate anything or whenever you want to add a carbene, this is something that will help you do it, and that is your S-adenosylmethionine.

Now moving to complex molecules such as podophyllotoxin. In this case, you will also see O-CH₃, O-CH₃, O-CH₃. So there are 1, 2, 3. 3 molecules wherein your SAM has helped. Now let me tell you, it's not 3; it's specifically 4.

The 4th place is this methyl. So it is not just O-CH₃; it could be something like O-CH₂O also, in some cases or a few cases where your SAM also helps. So this is the fourth case where your S-adenosylmethionine has helped add one carbon. So whenever in plants you

see a unique isolated single carbon moiety, it has been contributed by your S-adenosylmethionine.

Now let's move on to the second building block, and that is a slightly bigger brick. Instead of C1, we have a C2. Now, C2 comes from a 2-carbon unit. So where does this 2-carbon unit come from, or what is the precursor of this 2-carbon unit? The precursor of the 2-carbon unit is generally acetyl-CoA.

This is your C1, C2. But if you see in terms of reactivity, your acetyl-CoA is a little less reactive. So what happens in plants is this acetyl-CoA, in the presence of biotin, carbon dioxide, and ATP, as well as an enzyme called acetyl-CoA carboxylase, will get converted into malonyl-CoA. Malonyl-CoA is a more reactive substrate compared to acetyl-CoA but will still give you the same two-carbon addition. What happens is this particular group gets eliminated when it goes on adding.

Now, imagine something like a big fatty acid. Which is an even-chain fatty acid. Now, stearic acid is basically a C18 fatty acid. So, I can just break it as C2. So, ideally I should have 9 cuts.

So, this is C2, 2, 3, 4, 5, 6, 7, 8, and this is the 9th one. So, 1, 2, 3, 4, 5, 6, 7, 8, 9. So, 9 C2, so 9 into 2 carbons equals 18 carbons. is stearic acid.

So similarly, you have your lauric acid, you have your palmitic acid, C12, C14, even C22, C30. You can see the number of carbons, and you can just go on multiplying. You will know how much acetyl CoA is going to be required for that particular fatty acid just by dividing it by two. So if it is C30, you are going to require 15 acetyl CoA. Suppose it's a C20, you are going to require 10 acetyl CoA in order to produce it. So what happens here is it gets converted into malonyl CoA and gets added, but every time it gets added, this carboxylate is eliminated. So you might have fatty acids, you might have polyketides, and you might have phenolics.

Now you might ask, what are polyketides? Now when I say acetyl-CoA, I can also represent it in this manner. What is generally happening in case of fatty acid synthesis is

all my ketone groups here are getting reduced. So imagine a case where they don't get reduced at all. You get compounds which are called polyketides.

So polyketides are very common in marine scenarios. Polyketides often fold to give you compounds called quinones. Sometimes polyketides condense to give you phenolics, and these phenolics—a good example of that is one of the rings in flavonoids—is coming from a folding of acetyl-CoA. So you can give rise to fatty acids, polyketides, or phenolics with the help of acetyl-CoA, which is C2 as a building block. But the simplest example is your fatty acids.

So now the next set of building blocks is we have the C5 building block. Now, if you go to see the building blocks between C3 and C4, they are not as abundant, but C3 generally comes from pyruvate or glyceraldehyde 3-phosphate, whereas C4 generally comes from erythrose or succinic acid units. The abundant one is the C5 unit, which leads to the formation of steroids, terpenoids, and many other compounds such as essential oils. Now, if you go to see the C5 unit, the C5 unit consists of a monomer which is referred to as isoprene. Now, isoprene is a C5 monomer, and it has a genesis from two molecules or two key biosynthetic intermediates.

These include mevalonic acid as well as 1-deoxyxylulose 5-phosphate. Now, mevalonic acid as well as deoxyxylulose phosphate come from different pathways. Whereas your mevalonic acid comes from your acetate mevalonate pathway. Your deoxyxylulose phosphate comes from the methyl erythritol phosphate pathway. Now, both of these pathways occur differently in different sets of organisms.

And what happens is they give a very similar result. intermediate. This intermediate includes your isopentyl pyrophosphate and dimethylallyl pyrophosphate, and this is the basis for all the isoprene units. Now, these isoprene units give rise to a lot of terpene derivatives. Now, to understand terpene derivatives, let's just put some numbers to this isoprene so you can relate to it.

Now I'm just putting numbers like 1, 2, 3 to call this as a head and then 4, 5. I am numbering the carbon atoms. So, here we have 5 carbon atoms representing the 5 carbon units. Now, if I see my first molecule geranyl, geranyl is something which is giving you a

nice rose like aroma, you have it in rose, you have your geranyl, you have your geranyl acetate and so on.

So, I have picked up geranyl acetate as one of the molecules and see if you can correlate your isoprene unit here. Now I am just putting the numbers in a very similar manner. 1, 2, 3 that's the head and you have your 4 and 5. Just cut this molecule here.

I can again restart 1, 2, 3. That's the head and again 4, 5. And this moiety, you know, comes from your acetyl CoA. So this is a different unit. And here you have two isoprene units joined.

This I call it as head and this I call it as tail. So joined in head to tail manner. to each other and these are called as condensation products now this may be polymeric in nature now take for example lycopene lycopene is a very red pigment which is present in your tomatoes so if you see that red coloration of your tomatoes now what happens it's very big and

seeing the number of double bonds it absorbs in the uv region so can i figure out my isoprene in that definitely yes so i'm just putting it randomly for you again 1, 2, 3, 4, 5. Now you can correlate very easily. 1, 2, 3, 4, 5.

Again cut it. 1, 2, 3, 4, 5. So you see this pattern being repeated almost. 1, 2, 3, 4, 5. Now what happens here is now again I don't see a head but if I just start from the reverse side.

So let me start from here: 2, 3, 4, 5. Again, 1, 2, 3, 4, 5. So here you can see a nice head-to-tail again. And I am cutting it here: 1, 2, 3, 4, 5. Now, what happens in this case is, in most cases, your 1, 2, 3 is joined to the 5—that is, your head is joined to the tail. But when you talk or see this molecule here, you see 5 is attached to 5. This is what is called tail-to-tail condensation. So in molecules like lycopene, you will see 4 molecules joined head-to-tail. Four molecules of isoprene joined head-to-tail, and this $4 + 4$ —that is, $4 * 5 = 20$ —plus 20 carbons are joined to each other. Now, going to the next one, we have our next building block, which is your phenylpropanoids.

You might have come across the phenylpropanoid unit. Now, this phenylpropanoid unit comes from building blocks such as phenylalanine and tyrosine. They are synthesized

from pyruvic acid and erythrose 4-phosphate, which come from your Emden-Meyerhof pathway as well as the hexose monophosphate shunt, respectively. Now, this gives rise to shikimic acid and thereafter gives rise to phenylalanine and tyrosine. Now, during this process, they undergo deamination and are left with just phenyl and propane groups.

So these are your phenylpropane derivatives. Now, if you see phenylpropane derivatives in nature, there are abundant examples of phenylpropane derivatives. We just saw an example of eugenol, where we said this methoxy is contributed by SAM—that is, S-adenosylmethionine—especially the methyl group. But the other part of it is clearly phenylpropane. So here you can see one phenylpropane.

You can see phenylpropane in your coumarin. So, you can imagine here again you have your phenyl and 1, 2, 3. What has happened is cyclization. You can see phenylpropane in molecules such as lignans, which are dimers of phenylpropane. And again, you have phenyl.

This methoxy is contributed by SAM, and again you have your 1, 2, 3. Similarly, in this case, you have your 1, 2, and 3. So, these lignans are nothing but dimers. Now, what happens if they polymerize? So, in plants, you have something called lignified tissue.

What happens here is imagine a lot of your phenylpropanoids just joining to each other. via either an ether linkage or a C-C linkage. Now, what happens is this polymerization—you can see the monomer is still a phenylpropanoid. So, all of these phenylpropanoids will fuse with each other to give rise to a compound called lignin. Now, this lignin deposits with cellulose and makes your tissues lignified.

The lignification strengthens the stem, and as a result, the bark or the wood is no longer soft due to lignin deposition. You can easily identify lignin deposits by staining them with reagents such as fluoroglucinol and hydrochloric acid, which stain the lignin or the phenylpropanoid moiety in it. Now, moving to the other functionalities, apart from that, your phenylalanine and tyrosine, in the case of nitrogen elimination, may undergo carbon elimination. In that case, they are left with what are called C₆ elements. C₂, that is 1, 2, but instead of 3 here, I am saying N. So it is a C₆, C₂, N, or called a phenylethylamine

derivative. Now, you will come across some phenylethylamine derivatives in nature that are hallucinogenic or extensively CNS stimulants.

Or either analgesic in nature. An example of a CNS stimulant is mescaline, which occurs in certain cactus species such as peyote. A good example of an analgesic agent is the alkaloid morphine. Now, if you see, you can again observe here that the methoxy group is nothing but a contribution of SAM. But this is not a phenylpropanoid.

This is phenylethylamine. Whereas in this case, it's a little difficult to visualize, but let us try. So here you have your phenyl. Let's put your carbon number one. This is your one.

These are your two. And this is your nitrogen. So you have your phenyl. This is one, this is two, and nitrogen phenyl ethylamine. And again, this methyl can be contributed by your S-adenosylmethionine.

So in a similar manner, you can still have slightly longer functionalities, that is indole plus C₂N. Now, what happens out of the shikimic acid pathway is you get one more amino acid, which is tryptophan. And tryptophan contributes not only to the indole, that is your C₆, C₂N. But in addition to that, you can still retain two carbons and a nitrogen. So, imagine a decarboxylation happening.

What I am getting is an indole plus a C₂N. So, indole plus C₂N is carbon. You can see a very big functionality, which is present in certain alkaloids. Now, these alkaloids are very big in their structure. To give you an example of such an alkaloid which contains indole C₂N or tryptophan unit, the two common ones are reserpine and vincristine.

So, you will see in this case, this is—I am just drawing it for you so that it is more convenient for you to visualize. So this is my indole: carbon number 1, carbon number 2, and N. So I have my C₆, C₂, N. That is indole plus a C₂, N. Again, if you see a complex molecule such as your vinca alkaloids—vincristine, vinblastine—in the same way, they have the same indole moiety. And you can carefully see: this is carbon 1, this is carbon 2, and N. So its indole C₂N. Not only that, because this is a dimer, there is one more place where you can localize this. 1, 2, and N.

So in vincristine, it occurs, or the tryptophan moiety is substituted or added twice. Now, you will go on to more nitrogen-containing compounds, which are also derived from amino acids. See, for example, you have the amino acid ornithine. Suppose it undergoes decarboxylation and then deamination. The moment it undergoes decarboxylation, it gets converted into a compound called putrescine.

This putrescine will further undergo deamination, and what will happen is when this amine is also lost, you are left with nitrogen and 1, 2, 3, 4. 4 carbons. So I am just putting nitrogen plus 1, 2, 3, 4. That is, you get your pyrrolidine nucleus. Now, this pyrrolidine nucleus is involved in the biosynthesis of numerous alkaloids, which include your hygrine, nicotine—especially this functionality of your nicotine—and atropine.

In atropine, it is difficult to visualize, but let's try. So this is your 1. This is your 2, this is your 3, this is your 4, and this is your 5-membered ring system. So this is a fusion of a 5-membered plus a 6-membered ring system, wherein your 5-membered ring system is contributed by an amino acid, ornithine. Now, instead of pyrrolidine, what happens if you want a piperidine ring?

In that case, you have to increase the amino acid by one carbon, and that's the amino acid lysine. So, the amino acid lysine is responsible for giving you a piperidine kind of nucleus. So, the amino acid lysine has 1, 2, 3, 4, 5 carbons and 2 nitrogens. Again, it will undergo decarboxylation to form what is called cadaverine.

Then it will undergo deamination and then cyclization to have all these 5 carbons. So, you will have 1, 2, 3, 4, 5, 5 carbons and a nitrogen, which is your piperidine. Now, this piperidine is present in alkaloids, such as, for example, piperine, which is found in black pepper. It's an amide alkaloid, but the piperidine ring is there.

Then in your lobelia leaves here, you see an anabasin. In both cases, as well as previously, if you see the other ring of your nicotine, that is your pyridine ring of nicotine, which has also been contributed by your piperidine. So these are all the building blocks that have been involved. So just quickly going through it, we started with C1, that is your S-adenosylmethionine, then C2, which is brought in by acetyl-CoA, then you have your C5, that is your isoprene unit, which is brought in by mevalonic acid or

deoxyxylulose 5-phosphate, then you have your phenylpropanoids, which have been given by your phenylalanine and tyrosine.

Phenylalanine and tyrosine also give you what are called phenylethylamines, which give rise to compounds such as your mescaline and morphine. After that, you have what are called tryptophan derivatives, which are indole plus C₂N derivatives, which are present in your reserpine and vincristine. Now you have heterocyclic compounds, which are your pyrrolidine. And pyrrolidine is given by the amino acid ornithine after decarboxylation and deamination, as well as the amino acid lysine, which gives rise to piperidine. Now, these are a few examples.

Secondary metabolites, but these are not all. So thank you, everyone, for your patient listening. Here are a few references if you wish to go through the important parts or detailed discussion on how these biosynthetic precursors contribute to the genesis of secondary metabolites. So thank you, everyone. Thank you.