PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 7

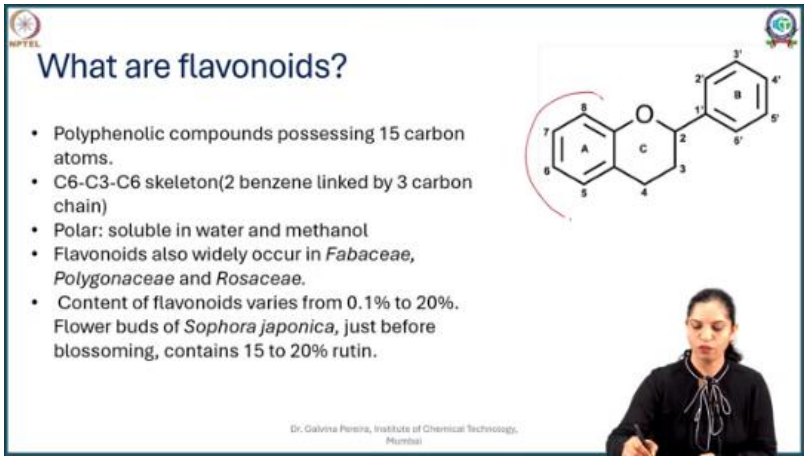
Lecture31

Week 7: Lecture 31: Introduction to flavonoids

Hello everyone, and welcome to the NPTEL course in Pharmacognosy and Phytochemistry. Welcome to another week of learning about beautiful natural sets of compounds. Now, whenever you take a walk through a garden, sometimes you see very beautiful flowers. Now, these flowers carry vivid colors, and sometimes these colors astonish you and make you think: what exactly does nature do to bring about such vivid plethora of colors that are present in plants?

How do they appear, and what are they? So this week, we are going to delve into a set of compounds called flavonoids, which also contribute to some of these natural pigments. So what are they? Let us study a little bit in detail. So flavonoids, by their chemical nature, are carbon-15 compounds; that is, they possess 15 carbon atoms, and this 15-carbon structure can be broken into a set of three: C6, C3, and C6.

How do you break this? So this is the first C6, then you have the C3, and the next one is C6. So together, you can call them C6, C3, C6 compounds, which occur in nature. Now, if you see them in nature, they are always hydroxylated, meaning they carry polyhydroxy groups across the rings, and that is why they are polyphenolic in nature.



So flavonoids can be defined as polyphenolic compounds having 15 carbons and carrying what is typically called a benzopyran nucleus. To this benzopyran nucleus, a phenyl ring is substituted, so chemically they are phenyl benzopyran compounds. Now, owing to their hydroxyl substitution, imagine many hydroxyl groups being attached. In some cases, at different positions, and as they are polyhydroxyphenolic compounds, these hydroxyl groups render them very polar in nature. As a result, you will find they have very good solubility in alcohol, which is a mid-polar solvent, because the phenyl rings bring their hydrophilicity toward a slightly mid-polar region. But the hydroxyl groups contribute to their polar nature.

So flavonoids are soluble in alcohol. On heating, they dissolve or are soluble in water as well. But on cooling, they tend to precipitate out sometimes. So depending on their molecular weight and nature, some flavonoid derivatives will be more soluble in alcohol. Some flavonoid derivatives will be more soluble in water.

Now they are present in different plant species, but the most common ones are the Leguminosae or Fabaceae family, Polygonaceae family, and the Rosaceae family. Many of the flower petals you see in gardens also owe their color to this beautiful set of pigments. 'Flava' naturally means yellow, and most flavonoid derivatives are white to yellow in color, bearing anthocyanins, which have a vivid color palette. Now, in terms of their occurrence, flavonoids have a slight UV-protective activity, and as a result, you will find them located on leaves or different flower petals. So when they are located in the

leaves, if you see the upper epidermis of the leaves, it is coated with waxy material to prevent water absorption.

So when these flavonoids are in the upper layer, they are mostly aglycons, as we studied, and do not have sugars. But when they are located in the mesophyll or the internal regions, they are generally more polar, and many of these hydroxyl groups are then substituted by sugars. So you will have flavonoid glycosides in the sap region or in the mesophyll region, whereas in the outer coat or the lipophilic region, you will have more of these aglycons occurring. Now, there is a plant called *Sophora japonica,* and if you see the flower buds of this plant, they have as high as 15 to 20 percent of a flavonoid called rutin.

Now, what functions do they serve in plants? Flavonoids are very important for maintaining the structural integrity of plants. Some of these plants also use flavonoids as a kind of messenger. They are used in signaling or communication. With adjacent plants—not only with adjacent plants—they also use it as a way to communicate with insects and microbes.

Now, this mode can be used in a very easy way; in the case of color, they can act as attractants. Sometimes these flavonoids form derivatives that can act as insect repellents. Sometimes these polyphenolic derivatives have antimicrobial activity. That is, they have the ability to kill bacteria, fungi, and some other microorganisms. In that case, they are categorized as phytoalexins.

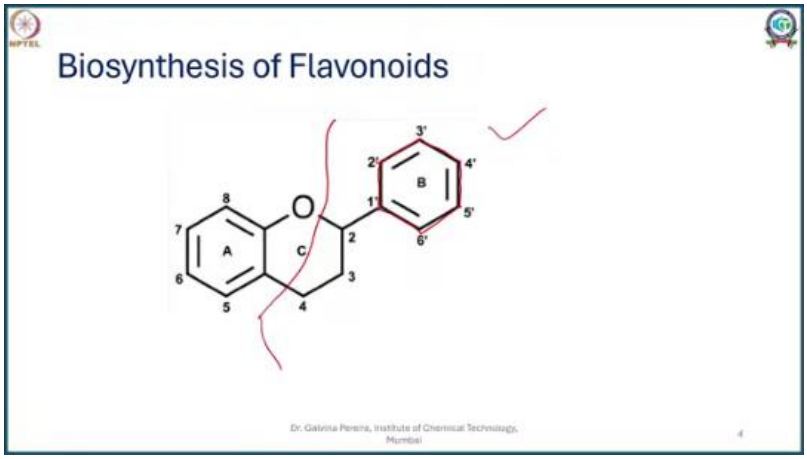
Now, if you see some leguminous plants where these flavonoids occur, especially in the root region or in the underground region, they have an important role to play. That is, they associate with nitrogen-fixing rhizobacteria species and form symbiotic associations with them. This is something which helps the plant thrive well. Now, not only that—if you know flavonoids, you must have seen when people advocate green tea or colored fruits. They say that they are antioxidant free-radical scavengers. Yes, because of their polyphenolic nature, flavonoids and flavonoid derivatives possess what is called stress-protection activity.

They do this especially by scavenging free radicals, acting as antioxidant molecules themselves. Not only are they antioxidants, but they also have a good ability to form complexes with metal ions. So, they chelate metals as well and decrease the toxicity of metals in us. Not only that—if you see carefully, these flavonoids, they have an aromatic ring structure, and this aromatic ring structure absorbs UV.

So, they act as good UV filters as well for the plants, as well as—you will see nowadays in cosmetic applications—some flavonoids have been used for different UV-protective effects. Not only that, there are certain classes of flavonoids, which we will be studying subsequently, that have phytoestrogen-like activity. Now, coming to biosynthesis: how are they originated, or from where does the plant produce them? Now, you will see—or you will think—that whenever a plant is there, it is mostly greenish in color, owing to the presence of chlorophyll.

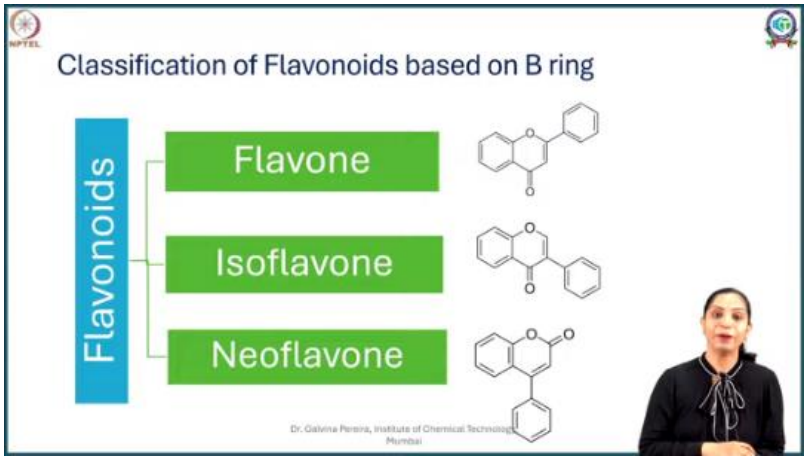
But the moment that beautiful flower bud develops, it gets those intense red, blue, and purple hues. Now, from where does this happen? So in plants, we have previously studied that there is a pathway called the shikimic acid pathway, and the shikimic acid pathway gives rise to different amino acids. One such amino acid is phenylalanine. This phenylalanine gives rise to a phenylpropanoid nucleus.

And if you can see, when I divide this molecule into two parts, your phenylpropanoid can be seen located here. Let me just help you with it. So this is your phenyl. And this is your propane. So here you have carbon number one.



Two and carbon number three. So this is your phenylpropane, and this has its genesis from your shikimic acid pathway. Now, coming to this side of the chain, especially the A-ring part of it, the A-ring part is unique in that it is found or formed by the union of C2 building blocks, which are acetyl-CoA building blocks. This acetyl-CoA forms malonyl-CoA. This undergoes elimination, and in such a manner, you will find three acetyl-CoA contributing.

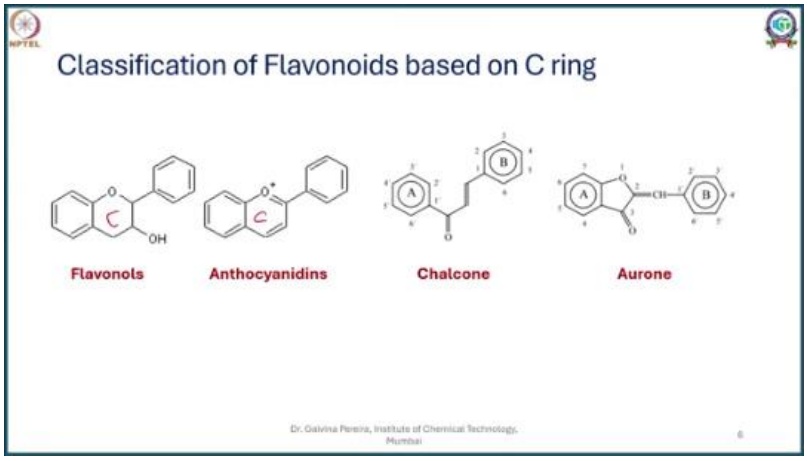
So this is one acetyl CoA, this is second acetyl CoA and this is third acetyl CoA. Now acetyl CoA you can draw it like this also so when I want to do it I can convert it into a ketone and now you can see that acetyl CoA you have three hydroxies. So alternating oxygens and these oxygens later on or these ketones later on get reduced to hydroxy. So if you see a typical flavonoid the substitution pattern remains almost the same, that is you will see the majority of the flavonoids have their hydroxy in 5th position as well as 7th position and this is because of their biosynthetic origin. So, acetyl CoA condenses it in a way that the oxygen is located at 5th and 7th position and subsequently as you see more and more of these natural flavonoid derivatives, you will reinforce that the hydroxy is typically present at the 5th and the 7th places. So, this comes from what is called acetate Now you can classify flavonoids depending upon their ring structure. So what we have seen here so far is you could put this as ring A, you could put this as ring B and then the ring C. The reason the ring C gets a middle ring Position is because eventually ring A is biosynthesized differently, ring B is biosynthesized differently, they fuse and in the end ring C is formed. So, in terms of numbering starting from hetero group you have like this 1, 2, 3, 4, 5, 6, 7, 8 and then your B ring gets 1 dash, 2 dash and so on. So, if you see your flavones now, your flavonoids can be classified depending upon where your B-ring joins. So, depending upon where your B-ring joins, you can have them as flavones, isoflavones or neoflavones. So, again I will just write the numbering for you.



So, 1, 2, 3, and 4 if you remember, and this is your 5, 6, 7, 8. So, if my B-ring joins to the C-ring at position number 2, I call it a flavone. If my B-ring joins to the C-ring at position number 3, I call it an isoflavone, and isoflavones are the compounds that have phytoestrogen-like activity. Now, what happens here is

So, typically, instead of your 2 or 3, sometimes your ring B joins here at the 4th position. So, you get a neoflavone. When your ring B joins to ring C via carbon number 4, you call this compound a neoflavone. Now, what happens here is, if you see your flavone and isoflavone, they are typically benzogamma pyrone, that is, alpha beta gamma pyrone.

But in this case, because the gamma position is substituted by the B-ring, your ketone moves here, and you get a lactone-like structure. So, this typically mimics your coumarin structure. You can also classify the flavonoids based on the C-ring. So, if you remember, this is what we were discussing as the C-ring system. So, depending upon the C-ring, if the C-ring is closed to form a pyrane, you call it a flavan.



So, this is a typical flavonoid nucleus. So, this is flavan. In some cases, you will encounter a pyrone or a ketone out here. Now, what happens is, in some cases—especially in flower petals or in the pigments we were discussing—your oxygen gains an additional valency.

So, oxygen is generally divalent, and as you can see here, it is shown as trivalent, and as a result, it carries a charge. Now, this charge moiety is what is called a flavylium ion. The flavylium ion is the core of most plant pigments, and they are categorized as anthocyanidins. So, anthocyanidins are responsible for the different colors of the petals. And they are very unstable.

The reason is that they carry a flavylium ion on which the oxygen carries a positive charge. Not only that, because of this charge—if you see your kokum juice, for example—you will notice that on heating or just keeping it for a day or two, they will quickly polymerize and disintegrate to form brown-colored adducts. So, they are not very stable because of the charge. Now, in some cases, the C ring totally opens up, and you get what are called chalcones. So, here in this case, the C ring is not cyclized at all.

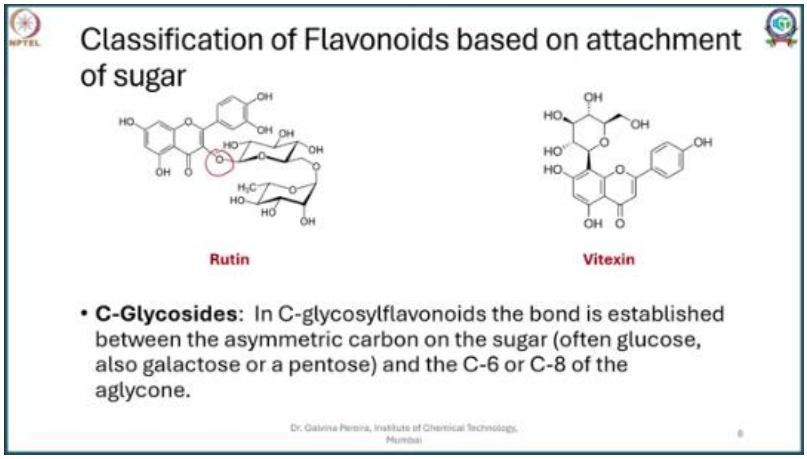
In that case, you will catch such derivatives as chalcones. In the fourth case, instead of forming a six-membered pyran ring, here you will get a furan kind of ring structure, and such compounds are called aurones. The carbon is there—that extra carbon which was making this six-ring is there—but it has been pushed out of the ring and forms a bridge molecule between your B ring and C ring. Such compounds or such flavonoid derivatives are called aurones.

So you have flavonoids if the C-ring is intact, anthocyanidins if the oxygen carries an additional charge, chalcones if the C-ring is open, and aurones if the C-ring is a five-membered ring. Now you can also classify the flavonoids based on the nature of substitution. Now observe carefully. Now between This is your 1, 2, 3, 4.

So whenever there is an unsaturation between 2 and 3, you call such a compound as flavo. It is vo. But whenever this unsaturation is removed, these compounds are called flava. So the name of the flavonoid itself will tell. If I am talking about flavonoids, I should understand that the double bond between 2 and 3 is intact.

Whereas, if I am discussing a flavone, that means the bond between the two is gone and a reduction has happened. Now, depending upon that, if any alcohol is attached, because the double bond is there, I have a flavone. And all of this is represented by a flavonol. And in this case also, if you see carefully, there are biflavonoids, that is, two flavonoids which are attached to each other. But because there is unsaturation, they are flavonoids.

Had it been saturated, it would have been called biflavonoid derivatives. Now, you can also classify flavonoids based on the sugars present. Now, this is similar to glycosides. So, if the linkage is an oxygen group, you call it flavonoid O-glycosides. If the linkage is a carbon group, you call it flavonoid C-glycosides.



Rutin is a classical example of flavonoid O-glycosides, whereas vitexin is an example of flavonoid C-glycosides. So, buckwheat is a common source of rutin, whereas vitex is a common source of vitexin. Now, here, one more thing you will observe is, as we said, the

hydroxyl groups are present on 7, 5, and 3 most commonly, and as a result, if you see O-glycosides, the O-glycosidation will happen when I am talking about ring A and ring C on the 3, 5, and 7 groups, whereas if I am talking about ring B, mostly it will happen on the 3' and 4' groups. Whereas, whenever I am talking about C-glycosides, because the other groups are hydroxylated, you will see mostly the C-glycosylation will happen on the 6th and 8th positions.

These positions generally do not have hydroxyl groups. So when I talk about a C-glycoside, it will mostly be at positions 6 or 8. When I talk about O-glycosides, they will be at positions 3, 5, 7, 3', or 4' most commonly. Now, how do you extract flavonoids? Flavonoids, as I said, may be mid-polar to polar.

So whenever they have sugars attached, that is, when they are in the glycoside form, they are more polar. In that case, they can easily be extracted with water or a mixture of water and alcohol. That is your hydroalcoholic solution. Whereas when you have native flavonoids, the aglycones or aglycons per se, In that case, you can use slightly non-polar solvents ranging from alcohol, ether, ethyl acetate, butanol, or even chloroform.

Now, how do you evaluate or check for the presence of flavonoids? To check the presence of flavonoids, there are a few common tests. The first one is the Shinoda test. There is the alkaline reagent test, lead acetate test, and ferric chloride test. So let's discuss them one by one.

So the Shinoda test is one of the most common tests which is used. Now, the Shinoda test is an extension of what you call Clemmensen's reduction. In this case, your flavonoids get reduced to anthocyanins in the presence of MnHCl rather than zinc HCl. So to perform this reaction, what you need to do is take your plant, extract it in alcohol, take the ethanolic extract of the plant, and to that, you will add your magnesium turnings. Be careful not to add excessive amounts, otherwise those magnesium turnings will make the solution appear much grayer because of the metal.

So just add three to four pieces of magnesium filings and add your concentrated hydrochloric acid. Now, interestingly, you will see the reaction happening. The HCl will

react with the turnings, and hydrogen is released. So you can see the hydrogen gas bubbling out. And in the process, the reduction starts.

As the reduction proceeds, it might take a little time, like two to three minutes. And you will see, compared to your extract, your solution now starts turning a little pink to red in color. Now, if it is an extract, you will observe a very soft color change, but if it is a pure flavonoid, the color change will be much more intense. So depending upon what compounds are present, you might get a slight difference in color. For example, if you have flavones, you might get an orange to red color.

If you have flavonoid derivatives in your pure form, you will get red to crimson color and deep magenta if you have flavonoid derivatives. Now, if you see chalcones or auron-type derivatives, they don't clearly answer this test. So for chalcones, what you can do is perform a test called the antimony pentachloride test. Take an extract of your chalcones, especially an alcoholic extract of your chalcones.

To that, add antimony pentachloride and carbon tetrachloride. You will see that gradually a reddish-purple color or reddish-violet color develops. That indicates that you have chalcones present in the solution. Now, another test is the alkaline reagent test. This test was discovered through keen observation.

Whenever you take a flavonoid-containing test tube and wash it with detergent, you will see the test tube turn bright yellow. This gave rise to the test called the alkaline reagent test. If you have a flavonoid or alcoholic extract of flavonoid and treat it with any alkali, especially NaOH, you will see the flavonoid turn bright yellow or yellow-orange in color. What happens here is that your ring C opens up. So if you remember your flavonoids.

So now this is what is going to happen. Your ring C opens up, and as a result, you get an intense yellow coloration because it forms a sodium salt. Depending also upon the other hydroxyl groups, they will also form metal chelates with sodium salt, and as a result, you get a nice yellow shade. Now, this shade shows a nice UV absorption maxima, one between 259 and another around 362 nanometers. The next test you can carry out is the lead acetate test.

Now, the lead acetate test is a test to see whether polyhydroxy groups are present in that. So this is true for polyphenolic compounds. So what happens is your lead forms complexes with the polyphenolic groups. It forms chelates, and with flavonoids, it forms yellow-colored chelates. Later on, this precipitates out, and the formation of a yellow precipitate indicates the presence of flavonoids.

Another test is one we also saw in tannins. When you see condensed tannins, catechin-type, they have a good resemblance to flavonoids in terms of biosynthesis and structure as well. So they perform or give the same test as that for the condensed tannins. So the ethanolic extract, if you treat it with 5% ferric chloride, will give a nice greenish or deep green color complex, which indicates the presence of flavonoids. Now, how do you quantify?

Now, if you want to see this, you can do a TLC test. Or you can do an HPTLC to quantify. So, TLC is a technique where you have your stationary phase, which is polar silica in our case. And in some cases, the silica is coated with a fluorescent dye, which gives you good fluorescence at UV 254. Now, whenever your flavonoids run on this plate with a very polar mobile phase, you will see some bands coming out.

So, what is going to happen is, because these flavonoids have double bonds, they tend to quench the fluorescence. That means they tend to pull back the fluorescence. As a result, this will appear black. And what happens here is, the remaining plate will show or continue to show a green fluorescence. Now, in some cases, there are certain reagents which will help give you good fluorescence.

themselves, but at a slightly larger wavelength, that is about UV 365 nanometers. One such reagent is often referred to as the natural product PEG reagent, which is a 1% solution of diphenyl boroxyethylamine. Now, this is subsequently treated with PEG. That is polyethylene glycol 4000 grade. And if you see this plate under UV 365, you will see different colors.

Now, these different colors are also indicative of flavonoids like apigenin, which will give you a yellowish-green color. Rutin will give you an orange color. So not only are they derivatives, but their color will also tell you the type of flavonoids. You can non-

specifically check these compounds, especially the double-bond compounds, with iodine vapors. Wherever the double bonds are, the iodine will add up and show you a dark band.

You can also dip it in ferric chloride. It will give you greenish, blue, or blackish color bands. Another way you can quantify is colorimetry. They form good metal complexes. So you can reactivate your flavonoid solution with aluminum chloride. They will form a good color complex, which shows UV maxima at 415 nanometers. So react it with AlCl3 in the presence of a sodium acetate buffer and then quantify it. It will also form colored metal complexes with different metals, attributing to their polyphenolic nature, like iron, aluminum, antimony, zinc, and so on. Now, modern methods use chemical detection or chemical detectors, or even HPTLC or HPLC with a good quantification system like a PDA system, photodiode array system, or a UV-Vis detector, to help you detect the flavonoids.

So, these are different ways in which you can quantify the flavonoids present in plants. So, here are a few references if you wish to know more about this set of compounds, and thank you everyone for your patient listening. Thank you.