

# **PHARMACOGNOSY AND PHYTOCHEMISTRY**

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**Week 8**

**Lecture 36**

## **Introduction to Carotenoids**

Hello everyone, and welcome to week 8 of the NPTEL course in pharmacognosy and phytochemistry. This week, we are going to study a set of colored compounds called carotenoids. Interestingly, carotenoids derive their name from carrots. So, let's see what these carotenoids are. Now, carotenoids occur widely in nature; you will see them mostly in fruits and vegetables.

Also, in some cases, you will find them as part of flower petals. This gives them beautiful hues ranging from yellow to orange to red. And these are lipophilic in nature. Now, as compared to the class we have discussed previously, flavonoids—flavonoids were yellow-colored pigments, and some classes, specifically anthocyanins, class of flavonoids anthocyanins, gave you a whole range of colors from red to purple to blue. But when it comes to carotenoids, you get a slightly limited range, and that is yellow, orange, and red. But, as opposed to flavonoids and anthocyanins, these are lipophilic.

And their lipophilicity is attributed to a very long carbon chain. That is a 40-carbon background. So, carotenoids occur in plants. They occur in algae, bacteria, and fungi. In plants, they occur concurrently with chlorophyll.

So, you will find that in your green leaves. When you see it, it will be most predominantly chlorophyll. So, the carotenoids in such cases are not seen. But during the fall season, when the leaves start changing their color, when they no longer synthesize chlorophyll, you see beautiful orange, yellow, and red hues, especially in maple trees.

In that case, these hues are attributed to carotenoids. Now, carotenoids can also appear in the flesh of certain organisms. Certainly, you see your salmon or your shrimp, which are orange to Pink in coloration, and it is said that they acquire this coloration via

consumption of food rich in carotenoids, so they kind of absorb it, and that becomes part of their own meat.

So, if you see in terms of biosynthesis, we have done it previously. Now, since these are pigments present in your plastids, and in your plastids, we saw that plants have a different biosynthetic pathway called the methyl erythritol phosphate pathway. So your carotenoids, especially those present in your plant kingdoms, which are present in your plastids, are synthesized by the MEP pathway

and use what is called methyl erythritol phosphate as an intermediate compound. Now, if you see carotenoids—if you remember—these are C<sub>40</sub>, and they are made up of isoprene units. So, to understand C<sub>40</sub>, you can simply say C<sub>40</sub>. And divide by 5 because each isoprene unit has 5 carbons. And you will get 8.

So, ideally, we should have 8 isoprene units. They can be visualized if you remember. We can say it's a head-to-tail condensation. So, you have your 1, 2, 3, 4, and 5 carbons. Cut it here, and you will get your second isoprene unit.

1, 2, 3, 4, 5. Cut it here, and you will get your next one. 1, 2, 3, 4, 5. I am just putting the numbers for your convenience. So, wherever the phi is, there is your tail.

So, the next isoprene unit is 1, 2, 3 again, 4, 5. okay so you can see here one two three four five now what happens here is not a head to tail but a tail to tail condensation we did it in a way we started in this direction, so we had one, two, three, four, five again. Your one, two, three. four five again your one two three four five and so here eventually you get your four five and this is called as tail to tail condensation. symmetrically break it, I have my first, second, third, fourth isoprene unit here, and again in this case, I have my one, second.

So, 4 plus 4 accounts for the 8 isoprene units which go into making carotenoids. Now, understand one thing: these carotenoids also structurally have what is called a conjugated double bond system. So, if you see the structure carefully, they have what is called alternating double bonds. Now, these alternating double bonds help absorb UV radiation. And because they have many alternating double bonds, the wavelength they absorb goes into the visible range.

So, they absorb a good quantum of energy. a good wavelength and as a result compared to your other terpene derivatives, your carotenes, having a large number of unsaturated

carbons, will get what is called visible absorption, or you will get a color. Now, physical properties: because they are C40, they become extremely lipophilic.

If you see the structure here, you see it's plain hydrocarbon. Imagine a hydrocarbon with C40—it's extensively lipophilic. As a result, if you see carotenoids, they are soluble in organic solvents. Now, because of their conjugated system, you will see that they absorb somewhere in the region between 400 to 500 nanometers.

This is the visible light range, and what happens here is, because it's the visible light range, the color that you see is not the color they absorb. The color that you see is the color they reflect. So they absorb a very contrasting color, which is near to green-blue, and they reflect what is called the opposite color, and that absorbance wavelength is 400 to 500 nanometers.

Now, carotenoids, because of their unsaturation, are extremely unstable at higher temperatures, and they decompose rather than melt. So what happens is, because of those unsaturations, they tend to form free radicals, polymerize, and form a brownish-colored matter rather than melting sharply. In some cases, when you have purified compounds—say, for example, lycopene—some of

these compounds, like lycopene, do occur in their crystalline form. But what happens is a very interesting phenomenon. Like I said, they are unstable to light. So if you keep those crystals exposed to sunlight for a period of 7 to 8 days, you will see those crystals gradually turn colorless. They lose their form and become a very amorphous matter over a period of time.

So that is something which is a property that can help us identify carotenoids. Now, if you go to see the double bond backbone, it is generally inverted here, and this is what your tail-to-tail condensation is made up of, and because of this, we call it a flipping. So you say an inverted symmetry is observed past this point. So it's essentially the same, but what you get is a flip symmetry or inverted symmetry.

Now, this unsaturation is also, in a way, advantageous to us. Why? Because this unsaturation is one place where you will see that a lot of free radicals, which occur in nature, which occur in our body, or what we call oxidants, they will go on. You know, producing peroxides or free radicals, and for free radicals, it is very easy to attack a double bond.

So imagine you have something like this; it will attack a double bond and tend to form either a hydroxide or an epoxide, you know. By some reaction. So there is a good formation of hydroxides and epoxides, and these compounds tend to get oxidized fast. That is why when I said the crystals will lose their color over a period of time, it is basically due to oxidation and this.

In a way, because it gets oxidized, this set of compounds are very good antioxidants. You remember, for patients, especially those suffering from inflammatory disorders or those suffering from cancers, they have been told to have a diet rich in antioxidants. So your carotenoids meet that requirement. So when you eat your colored fruits, especially those which are yellow or red, which are rich in carotenoids, your free radicals,

whatever are produced during the disease state, are kind of reacting with lycopene your lycopene act as antioxidant why bit because it itself undergoes oxidation so it will prevent your free radicals from attacking other vital organelles or other vital cell organelles. So because of this, antioxidant action is kind of very preventive in disease conditions such as cancer and also helpful as a good antioxidant

agent. Now, not only that, if you see it in plants, in plants also there are pigments such as chlorophyll. And chlorophyll, when it is excessively exposed to sunlight, it kind of becomes a photosensitizer. So a photosensitizing agent is something which will start emitting chlorophyll. Light but slightly in a different wavelength, but that light can cause damage to the cell organelles.

So for such tissues, what the plant does is, wherever there is chlorophyll, it will produce some carotenoids in its way to curb that. You know, the radiation which is coming from the photosensitizer molecule. So it works as a kind of insulation. It works as a kind of antioxidant molecule, and it protects the organism from that.

Now, because of this, it is highly sensitive to light. It is sensitive to heat. Now, what happens here is, you will see that your lycopene or most of the carotenoids in their most stable form are all trans forms. But the moment you cook them, the moment you process them,

what is going to happen is some of them will get converted into cis form. Not that all cis forms are harmful, but cis forms are unstable. They tend to react easily. They tend to form degradation products. And as a result, the effect, the antioxidant effect, is not as good as the all-trans isomers.

Now, if you have seen or if you have heard your parents or your teachers say that carotenoids are good for vision—eat carrots for your eyes— They are right because there is science behind it. And the science is, if you see carotenoid derivatives, especially something like your beta-carotene, Now in nature, so far, more than 600 carotene derivatives have been documented, and

they are still in process because they are at different stages of oxidation. You know, they do undergo oxidation. So you have alpha carotene, beta carotene, beta cryptoxanthin, lutein, lycopene, and zeaxanthin. These are the major ones. So major that they account for 90% of all the carotenes we consume in our diet.

So take one case, which is your beta carotene. Now, beta carotene is an example of a bicyclic carotene. So we just saw here what has happened: if you open the chain from here, it becomes what is called—just see here. But if I close my chain, I will see here that it becomes your beta carotene. Now, this beta carotene has what is called iononuclease in it.

We will discuss that shortly. But when it comes to nomenclature, this is the 6 carbon leaving the methyl. So this is what is put as 7, 8. This is put as 9, 10. This is 11, 12, 13, 14.

14. This is 15. And because there is an inverse symmetry, this is something like your 15-dash, 14-dash, and so on. So you can go there. So basically, in your beta carotene, what happens is you have your 15, 15-dash coming out.

Now imagine a case where your 15, 15-dash breaks down. If this bond breaks down, what happens as a result when your 15-15 breaks? Usually, the enzyme which catalyzes this is a 15-15-dioxygenase. So it will add oxygen to it, and in the process, it will convert it into aldehydes. So beta carotene breaks at 15-15-dash in the presence of an enzyme called 15-15-dash dioxygenase to give

you a compound called retinol. Now, this retinol is a precursor to vitamin A, and your aldehyde just has to be reduced to alcohol. So this is done with an enzyme called dehydrogenase. So you will have your retinol dehydrogenase, which will reduce it. It will convert this aldehyde into alcohol, and you will see or get your retinol.

Your retinol is nothing but your vitamin A. So your vitamin A A is chiefly derived from carotenes that you're going to consume, and it is very vital for good vision. And that is the reason they say you have to have carotenes in your diet, especially rich in compounds

such as alpha-carotene, beta-carotene, xanthines, luteins, and lycopenes, so that you get your retinol prepared from them. Another set of analogs derived from carotenes are compounds called ionones.

Now, ionones are used in perfumery, and they have a very sweet, beautiful scent. Now, ionones can be cut off again. So, if you remember, we just numbered this as 7, 8, and this was your 9. 10, okay? So, if you just carefully see, there's a very similar symmetry here. Okay, now what is done is you're cutting it at 10. So, because you're cutting it at 10, you will get what is called—

10 dash apo beta 10 carotenol so again after cutting it's a oxidative degradation or the presence of an enzyme called carotenoid cleavage dioxygenase. Basically, the purpose of dioxygenase is to oxygenate it, and the end product is an aldehyde, which comes out of breaking that. Now, if you carefully see, what is done is it's cut here. And because it's cut here, what you get in the process is the very same structure.

The very same double bond—7, 8—at 9, you will see this is the 9. At 9, you will see a CH<sub>3</sub>, which is this, and this double bond, which is now cut, is oxidized to a ketone, which is a beta-ionone. So, carotenoids are deliberately—or sometimes enzymatically—oxidized and broken down into ionones, and these ionones are used in perfumery as scents. Now, this apocarotenoid or what is called as the breakdown product of carotenoid

used for the production of another ionone moiety, or it can again go into a more degradative pathway to give you retinol. Now, provitamin B—sorry, provitamin A. That is your retinol. If you have to produce you have to have a very accurate structure.

When I say accurate structure you have to have a typically what is called as a beta ion ring structure. You cannot have it say for in this case you cannot have it like this that the bond is shifted here. Then in that case, you will not get your beta ionone. So this alignment of conjugated double bond should be correct. The placement of methyl should be correct.

And always the nature of unsaturation decides if it can be converted to ionones, if it can be converted to retinol and so on and so forth. Now classification of carotenoids. Carotenoids basically are hydrocarbons. They are tetra terpene derivatives.

That means they are C<sub>40</sub>. But what happens is in certain cases this C<sub>40</sub> after their synthesis break down. So imagine a case if I am just breaking this down here. So I break this here lutein and I break this lutein here. Now what has happened here is I put 10 carbons away in both cases.

If you want to do the counting, let's do the counting. The phenyl makes it 6. This will be the 7, 8, 9, and this is 10. So, you have 6 of phenyl. You have your 7, 8, 9, which are methyl, and the 10th, which is cut off here.

In a similar manner, your 10 carbons are cut off here. So, from 40, if I am cutting 10 and 10, then from 40, I am deducting 20, so I have what are called as you know, little shortened carotenoids, and those are called apocarotenoids. So, broadly, you will have your carotene derivatives, which are C40, and you will have your apocarotenes, which are shortened versions.

A good example of that is crocin. In crocin, what has happened is these are sugars. But if you ignore the sugar part, the chief middle molecule is made up of C20. So, this happens basically via the mechanism I had shown, that is, your lutein or your carotene breaks at the 10th position, okay?

And sorry, it breaks, leaving 10 carbons. So, once these 10 carbons are removed, you will get your apocarotenoid compounds present. So, carotenes are the ones which are pure hydrocarbon compounds having C40. Xanthophylls are oxygenated derivatives.

So, if you see this as compared to your beta-carotene, lutein has a very similar structure, but it has hydroxy groups attached. Now, this hydroxylation makes this compound slightly polar. Not only does it make it slightly polar, but it also makes it slightly yellowish as compared to your native carotenes, which are red to orange.

So, the amount of oxygenated or hydroxy groups or any oxygens added to the carotene nucleus will impart more yellowness to the compound. And if you look at apocarotenoids, they are kind of abbreviated or shortened carotenoid derivatives. So, there is a slight difference between carotenes and xanthophylls, and let's understand them. So, the first point of difference, as I said, is that carotenes are pure hydrocarbon compounds.

They are tetraterpenes; they contain only C and H. So, they are only C and H, meaning you will have only a plain hydrocarbon nucleus. Whereas, if you see xanthophylls, they are oxygenated derivatives. We just saw the example of lutein, which had hydroxy groups. But in some cases, it might be an epoxy group.

In some cases, it might be a keto group, a methoxy group, or a carboxylic group, like you saw in your crocin. It was a COOH. So you might have a compound with diacids also.

Now, knowing that these oxygenated compounds are going to be more polar and hydrocarbon compounds are going to be relatively non-polar.

So if you check the solubility, carotenes are more soluble in extensively non-polar solvents such as hexane or petroleum ether. So the preferable solvents for having them—or rather, the preferable solvents for extracting them—are petroleum ether or hexane. Whereas if you want to extract xanthophylls, you can even do it with mid-polar solvents such as alcohol or acetone. Now, oxygenation also changes the color, whereas your plain hydrocarbons with a series of

what are called conjugated double bonds give you red to orange color. You will see your xanthophylls, which are more orange to yellow in color. A good example of such compounds that contain carotenes are carrots—definitely—and your tomatoes' lycopene. So if you see in your ketchup, Or if you have seen your tomatoes when they are very ripe, that intense red coloration is due to the presence of lycopene.

Whereas during your Dasara time, you see something called your marigold flowers. The petals of marigold flowers contain lutein. Whereas, if you see your corn, in your corn kernels, there is a slight yellow coloration. And that yellow coloration is attributed to zeaxanthin. Now, how do you extract it?

Now, knowing that these carotenes are very beneficial in the market nowadays, you are getting a lot of carotene supplements or carotene capsules. You might have something like your astaxanthin capsules, or you might have something called lutein capsules. They contain them in a liquid, semi-solid, or sometimes they are converted into solid form for ease of handling. So, how do you extract them from plants?

Now, if you see this, these are pigments. These are located in plastids of plants and are also located somewhere in the vacuoles of microorganisms, stored in a very lipophilic area. You have to note that when the compound is stored in the lipophilic area, your carotenoids, especially the xanthophyll derivatives, because they have hydroxy groups, are esterified with fatty acids. So, instead of having your waxes, what is done is your fatty acid,

plus your carotene alcohol, forms an ester, and that is how they stay in the plant. Now, if I am to extract this compound out of that, what I have to do is hydrolyze it. So, the first step is saponification. Saponification has many advantages. When you perform saponification, compounds such as chlorophyll,

and your fatty acids get saponified. Now, once they get saponified, your carotenes don't get saponified, and that's the advantage. So these two compounds become polar—that is, your chlorophyll and lipids—because of saponification. Whereas your carotenoids stay non-polar and can be extracted in an organic solvent. So immediately after the saponification reaction is done, now one thing is in most of the cases

the literature, you will see the saponification reaction is done at room temperature to prevent deterioration, done in glass vessels to prevent any reaction, because it is seen that even when you do it in plastic, the carotenes do react, and some leachates from the plastic can interfere. And it is done in an extensively clean apparatus so that there are no acids present or any other compounds, such as

oxidants, which can hamper your carotenoid composition. So if you take the due diligence of these things, you can nicely have it where you read the literature, in your organic solvent using supercritical fluid extraction, which happens with supercritical carbon dioxide at almost room temperature. the carotenes do react, and some leachates from the plastic can interfere.

Microwave you to be little careful because microwave may increase the temperature, increase the temperature, and ultrasound, which again does produce a little localized heat, is a comparatively quicker and easier option to disrupt the tissues and get the carotene out. So you can determine or analyze carotenes. They are very easy.

The reason being, they are colored, and anything that is colored can easily be assayed by what is called colorimetric determination. So I can carry out colorimetric determination of my carotenoids at a wavelength somewhere between 400 to 500 nanometers. Now this is more specific to them and depending upon which carotene I have beta carotene, lycopene, lutein, a little yellowish, or lycopene, which is reddish—the wavelength may vary, but it

is generally between the 400 to 500 nanometer range. Now, there's an interesting thing: if you see this picture, this is of what is called king chili, which is a really spicy, pungent chili and um If you see the color of it, it's intensely dark. So in your chilies, you will see carotenoids, and these carotene derivatives vary by species.

So when you're preparing masala or when you're preparing colors out of it, because of the species variation among the chilies, your color— spice traders often found lot of issues pertaining to color because the color of masala or the spice blend used to change change.

So they fixed it, or they have something called ASTA, which is the American Spice Trade Association. What they do is take this chili powder, extract it in acetone, and check the UV absorbance.

So for every variety of chili, they have their ASTA values. Even for chili extracts, they have their ASTA values. So if I am broadly using chilies as such, I should have my ASTA values somewhere between 65 to 180. And if I have oleoresin, which is more of a concentrated extract containing my chili pigments, my ASTA values go as high as 500 to 3500. Now, these values are basically how much dilution you need to do in order to get a specific standard value for chilies.

Now, carotenes can also be analyzed by treating the extract, especially with what is called acidified pyridine. So, if you have a carotene extract and treat it with acidified pyridine, the color of the extract gets transferred into the acidified pyridine layer. So, this is a complexation that happens. The

reaction happens between the carotenes and the acidified pyridine, and eventually, the end product is your acidified pyridine layer turning orange to red in color. Now, if that happens, there is a sure certainty that your powder contains carotenes. So this is a little determination of it. Now let's see the application.

How are carotenes useful to us? Carotenes have been used as natural colors in foods. You will see them in ketchups. You will see them in certain, basically not so heat-treated, but certain blends or sausages where carotene is used. They are stored at cold temperatures, and for that reason, you can safely use them.

They are also used nowadays in natural cosmetics. They are used as dietary supplements because of their antioxidant effects. They show health benefits in CVS disorders as well as cancers. Carotenes are a good dietary source of vitamin A, so we have to consume them for good vision. Now, it is seen that in carotenes, there is a phenomenon called macular degeneration.

There is a region called macula lutea, which is very close to the retina and is related to color perception. So, how we perceive colors. Now, if you are deficient in carotenes, you will see that the macular region also becomes deficient in carotenes. And these carotenes are essential for perception of colors.

So what happens is gradually as you age, the degeneration happens. So, if you are having a diet which is rich in carotenes, it will replenish it. At the same time, the antioxidant

activity of carotenoids will help prevent in its deterioration. So, carotenoids are definitely beneficial and curative when it comes to age-related macular degeneration.

Now, it is also important found that in your poultry products or in your fish you want that intense pink or red color in your sausages in your salmon and you want that intense orange color in egg yolk if you know your egg yolk as compared to the broiler chicken the orange or the country chicken egg fetches a high price so what this farmers do is they give the poultry a feed which is rich in carotenes

Now they are extensively lipophilic. So when your chicken eats a diet which is rich in carotenoids, it is naturally secreted and becomes a part of the egg yolk. And as a result, more carotenes your chicken consume, more the yolk becomes orange. So that's also an advantage as well as a disadvantage the way that you use it. But definitely it is used for enhancing the pigmentation of egg yolk, fish and poultry.

Now because of its antioxidant effects, you might see herbs such as your sea buckthorns or certain carrot extracts which have been used as an anti-aging and skin care to prevent oxidation and oxidative damage. So these are few applications of carotenoids. If you wish to read more about carotenoid derivatives, here are a few references. So, thank you everyone for your patient listening.

Thank you.