

# PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 8

Lecture 37

**Carotenoids containing drugs (Saffron, Annatto, Marigold)**

Hello everyone, and welcome to part 2 of week 8 session on the NPTEL course in pharmacognosy and phytochemistry. We are learning about a set of very beautiful compounds that occur in nature, and those are carotenoids. As we discussed in the previous lecture, carotenoids are compounds that impart yellow to red coloration to different plant parts as well as microorganisms. They are located in plastids in plants and sometimes might be esterified with fatty acids.

In this session, we will see examples of carotene-containing drugs such as saffron, marigold, and annatto. Saffron is something which is used in culinary and is a very expensive spice. Annatto is something which is used again in culinary for red to yellow coloration, and marigold is used as an insecticide. But did you know the carotenes in it are very good for the health of your eyes?

So let's start with saffron. Saffron consists of a flower part called the stigma. There is a flower called *Crocus sativus*, which belongs to the Iridaceae family and is an annual plant. It grows in India, especially in the Kashmir region, as well as in Spain and Iran.

So if you see, this is a kind of plant which is generally propagated by means of a comb. So this is an image of a comb which is very fibrous, and as you can see, it prefers very dry and arid soil. So it grows in very specific locations in the world and the The saffron, especially the Indian saffron, is very prized, especially for its carotenoid content and the flavor that it imparts. So what is done is during the monsoon season, that is during the July-August season, these combs are planted.

Now they are planted in raised soil which is well-drained, so there is absolutely no waterlogging, and what happens is a year subsequent to that, you will find them flowering. So the flowering season of saffron is generally between October and December, and you will see lots of fields of saffron with nice purple flowers. Now these flowers are very delicate, and they have to be collected very early in the morning, and

once they are collected, the stigmas are separated. Now the process of collection is done. They are done in baskets. They collect it, keep or pour all the flowers on a mattress so that they don't turn soggy. Once they have poured it on the mattress, each stigma—I mean, each flower—has about three such stigmas.

So each stigma is carefully separated by hand and dried. It is said that in order to get 1 kg of this spice, you might require about 1 lakh flowers or so, and that is the reason this spice is so expensive. Now once the stigmas are separated, they can be dried in a controlled environment like an oven at a lower temperature, like 40 to 50 degrees Celsius.

In some cases, the stigmas are even sun-dried. It is very essential that no moisture is retained; otherwise, they tend to catch fungus and deteriorate over time. Knowing the price of this spice, it's very valuable, and as a result, due care is taken to remove as much moisture as possible so that the stigma is maintained. The deep red color of saffron is attributed to a pigment called crocin.

Now, this crocin—I am just putting it in your perspective—is an apocarotenoid. As we discussed, apocarotenoids are a kind of pigment which are a shortened version or abbreviated version of carotenoids. So, in this case, let's take, for example, zeaxanthin. So, if you take zeaxanthin and carefully cut it over here and here.

So, I am just leaving methyl. So, 10, 10. What happens in the process is you get an intermediate which is very similar to this. Now, you have to do one thing: wherever you cut it, just put an O—that is, a ketone—and then put an alcohol. So, this becomes an acid now.

Now, this is something which is called crocetin. Crocetin, now when you add your sugars, it becomes a glycoside, and that is called crocin. So, from zeaxanthin, you get what is called a bi-apocarotenoid. Why? Because two cuts have been made, and from 40, you end up with a compound that is very small, like a C20 compound.

And a C20 diacid, to which your sugars have been esterified, is what makes your crocin. Now, if you observe carefully, there is one more thing that you will learn in the process:

this apocarotenoid, when it is cut, this unit is not wasted. This unit goes to make another molecule, which is present in saffron, and that's called picrocrocin. So, if you see carefully, you can see the dimethyl and hydroxy groups.

Dimethyl and hydroxy group. In addition to that, you will see this aldehyde group, which is here. So, you have dimethyl, you have an aldehyde, and you have, again, a methyl. So, dimethyl, aldehyde, and again a methyl, and this hydroxy group. Here, now it is conjugated with a sugar molecule to get what is called picrocrocin. Similarly, if you eliminate this

and a double bond is induced, you get a compound called safranol. So, that's the beauty of nature. Just take a zeaxanthin molecule, chop off 10 carbons from both ends, add sugars to it, you get your crocin. Now those ends which are chopped off are again converted into O-glycosides to get your picrocrocin. And from the toglycosides, if those elimination happens by inducing an unsaturation,

you get a compound called a saffronol. Now, all three of this have a slightly different attributes. So the color of saffron, which we saw the deep red color of saffron or sometimes when you put saffron in the milk or water, you see that saffron. It becomes orangish in color.

So that color is attributed to the pigment called as crocin. So this is what gives the color and this is a apocarotenoid. Now the flavor. Now if you see or sometimes when you go to buy a saffron, people ask you to check the genuineness of saffron by tasting it. Your saffron should taste bitter and that bitterness is attributed to picrocrocin.

The third thing is for saffron when you use it in cosmetics or any product or even food for that matter that is in your culinary you get a typical aroma of saffron and that aroma of saffron is due to compound called as saffronol. So all in all one zeaxanthin is sacrificed to give you all three of these compounds and all three of them have a slightly varied applications and properties. Now where all do you use saffron?

Now saffron is people often put it in their sweets or biryanis or even numerous culinary purposes have been attributed wherein the aroma and the color imparting properties of saffron. Saffron has been explored. But in addition to that, now that you know what carotenes are, they are very strong antioxidant compounds. Saffron is used nowadays as a sunscreen agent.

Now it is also used as an antioxidant, where saffron extracts have been proven to treat or at least, you know, manage the rashes and redness on the face, especially the burning sensation, and diminish the blemishes. Now, saffron can be, you know, added to attars. Like I said, that's where you have the aromatic properties of it. And saffron

to just get a little taste of sophistication that's added to your Western perfumes. Now it is there. It has a very mild, sweet aroma, but definitely a distinctive one. So these are all the uses of saffron. But yes, being a carotenoid, it can serve all the remaining functions also.

But being an apocarotenoid, remember, it cannot give you ionone or a retinol nucleus. The reason being is obvious: the retinol part, the cyclic part of your carotenoid, is already gone into making picrocrocin, and hence you cannot derive retinol from it. So this is not a good source of vitamin A, but it is definitely a very good antioxidant agent. So with that, let's move on to our next drug.

Our next drug is a beautiful pigment-containing plant called annatto. Now, this annatto consists of dried seeds. In the image, you can see the pods. When these pods open up, there are bright red-colored seeds. And if you take these red-colored seeds and press them in your hand, you get a lipophilic red pigment.

It is so lipophilic that it stains your skin instantaneously. In fact, some people also call this plant the lipstick plant. So it belongs to the family Bixaceae. It is seen growing predominantly in South American regions, in the Amazon regions, Brazil, as well as in Caribbean islands. And it's also cultivated in southern parts of India.

So there's a plant is exclusively used for its pigment so what is done is this plant generally flowers during the end of monsoons within two to three months, the fruits are ready. Once the fruits mature, you can see that they develop nice red seeds inside the capsule. Now, this is a tree.

So generally the initial flowering starts by second or third year and lasts till 15 to 20 years. So till 15 to 20 years, you can continuously get the crop from this plant. So it's very economic also. And when the fruits mature, these are harvested to collect the seeds. The seeds are used for its pigment.

Now the seeds contain as high as 4.5 to 5.5 percent of pigments which are carotenoids by nature. Now if you see the structure again you will see that they are not C40. So these are also apocarotenoids wherein the outer ring is kind of chopped off and you are left with

the diacid moiety. Now, these pigments, despite being diacids, you can carefully see there are two pigments which predominantly occur in your annatto.

The one which is little red in color is your bixin. The one which imparts little yellowish color is your norbixin. Now, a little difference between them is this methyl group. So the acid in Bixin, one of the acids is methylated. Now because of that acid being methylated that renders lipophilicity to the molecule

and that becomes more of a lipophilic or an oil soluble molecule whereas your Norbixin owing to its diacid nature is easily soluble in water. So what is done is if I want to separate the oil soluble and water soluble plant, the seeds are taken and soaked in water. The norbixin solubilizes in water whereas bixin remains attached to the seed. Now this bixin can then be removed or extracted by alkaline extraction.

But if you do alkaline extraction over a prolonged period of time or harsh alkaline extractions, what will happen is again this ester linkage will hydrolyze. So once the ester linkage hydrolyzes, you will get back your Norbixin. So you can get your Norbixin from Bixin by saponification also. And by saponification, all of this Bixin can be converted into your Norbixin.

Now, extraction of annatto pigment for commerce or for economics, what is used is the annatto pigment. The whole seed is not used in market. What you get is a crude pigment. So this annatto seeds is subjected to solvent extraction. Like I said, if you want the water soluble pigment, you might soak it in water for some time,

wherein all the water soluble pigment that is your Norbixin comes out. The bixin still remains in the seed. Now that bixin is extracted with organic solvents. Now these organic solvents can be something like your petroleum, ether, acetone or even ethyl acetate. Now they can be from mid polar to non-polar ranges.

Once that is done, this is filtered in. Now, what you will get is a deep red-colored filtrate. Why? Because all your Bixin, which is deep red in color, has now solubilized in that. Now, the filter paper will retain the insoluble residue.

Now, if you use very organic solvents like petroleum ether to extract it—apart from that, you know, seeds are oleaginous in nature. So, apart from your pigments, they will also have your fatty acids. So, these fatty acids are generally precipitated either by concentrating or by using another non-solvent such as acetone.

Now, once this is done, you can remove the solvent, and then the Bixin or the crude Bixin is subjected to crystallization. After crystallization, this is filtered and dried, and this pigment is sold in the market as annatto pigment. It is also sold as your Bixin, but it is not 100% pure. So, depending upon the purity, it is labeled as annatto pigment containing. It is standardized to like 90%, 80%, and so on, and thereupon you get your different pigments.

Now, remember that carotene pigments are not heat-stable. So, anytime you are using an extraction process, you have to be very careful and diligent that you are not using excessive heat. Otherwise, your carotenes will undergo isomerization. They are all trans. They will get converted into cis.

And the next thing that they can undergo is oxidation. So without using much heat, with mild conditions, all this extraction is done. But in some cases, the spray-drying processes are also done, which gives a quick flash of heat with sudden cooling. Where do we use annatto? Annatto is chiefly used for food coloring purposes.

So if you see your Amul butter, ideally, your butters are supposed to be colorless. But that yellowness of butter is imparted by adding some natural colorants. And the natural colorants in your butter, mostly in the case of Amul butter, if you see the package carefully, is annatto. Apart from that, if you want to see cheese, different grades of cheese show different levels of yellowness. If you want to see an aged cheese, it has to have a higher degree of yellowness.

So for such purposes where you want to show a little age on the cheese, where you want to show a little yellowness, in that case, also for your cheese-based products, your annatto is used as a dye. In some cases where you want colored rice, In that case, also your Norbixin can be used as a pigment. Not only that, like I said, the seed which was used was initially known as a lipstick seed

because it imparted a very prominent stain which could not be washed off easily. So it is used regularly. In lipsticks and in blushes and it gives you a nice tint. Annatto is not only just for cosmetics or for food. It also has therapeutic effects and this therapeutic effects include, you know, most of its antioxidant properties.

Due to its antioxidant properties, it is good in management of ailments such as diabetes, diabetes. diarrhea, eye health and some skin problems especially when you apply it topically its antioxidant effect is good for the skin. Now, in some cases or some

communities, it is seen that they use this seed extract. They kind of press it, get a good extract out of it. And then they mask their bodies with that extract like body paintings as a ritual and also as a sunscreen.

So this is your annatto. This is something which we see in our cheeses, in our butters, but fail to recognize that there's a natural pigment in there. Now with that moving on to a plant which is very common to India and that's your marigold. Now marigold is something we use in our festivities for decoration purposes. Now, marigold flowers are flowers of a plant called *Tagetes erecta* and belong to the family Compositae.

Now, if you have seen the marigold flower, you know that it comes in different hues. Some marigolds are a little bright yellow, some are orange, and some are even reddish in coloration. So, the hue of this marigold varies depending upon the variety of *Tagetes erecta* that you use. they are central you have the south American variety you have the African variety it is cultivated in Spain as well as in India We use it for most of the festivities to make garlands, so it is a part, and there is a reason behind it. The reason

why we use this particular flower only in the garland is attributed to its insect-repellent activities. The aroma of marigold is known to deter certain species of insects, and that is the reason, along with mango, which is also known to repel insects. The combination of mango and marigold is used in conjunction as an insect-repellent garland, which is used in your festivities. So, what does this marigold contain?

Let's learn that. So, the chemical composition of marigold, if you go to see in terms of carotene derivatives, it contains xanthophylls. If you remember, xanthophylls are nothing but the oxidized versions of carotene. So here, what you see is nothing but your alpha and beta carotene, and to that, if you attach a hydroxy, you will get your lutein and zeaxanthin now in terms of lutein and zeaxanthin the structure appears almost the same

until you see the position of this double bond. Like I said, they are derivatives of alpha and beta carotene, and that's the reason they are just dihydroxy derivatives of alpha and beta carotene. Because of their hydroxylation, your alpha and beta carotene are red in color, whereas if you see your marigold flowers, they are more of yellow to orange in color, attributing to these two pigments. Now, apart from that, you will see your flavonoids, quercetin, rutin, your triterpenoids, essential oils, and other compounds.

Now the compound which is responsible I just mentioned for a mosquito repellent or an insect repellent activity is What is called as alpha-terthinyl. Now alpha-terthinyl gives

you that typical aroma of marigold which is not favored by many of the insects and that's how it becomes an insect repellent. Now when you come to your carotene derivatives, your lutein and zeaxanthin are very similar varying the position.

of the double bond and if you see the molecular weight wise they are almost identical now this two pigments that is lutein and zeaxanthin occurs in this flowers as esters now what happens is because you have hydroxy groups and you know that your carotenes often associate or often stay in the lipophilic regions of the plant so what happens is your fatty acids carboxylic acid group and the hydroxy of your carotene

is involved in esterification same thing happens here and what you will get is two fatty acids esterifying with one molecule of lutein and two fatty acids esterifying with one molecule of zeaxanthin now once they are esterified you remember the esterification if the depending upon the molecular weight of the fatty acid they become extremely lipophilic and are very difficult to extract So if I want to extract my lutein or zeaxanthin as compared to extracting your Bixin which was just a solvent extraction.

For extraction of lutein and zeaxanthin it is always advisable that you treat it with alkali. The moment you treat it with alkali the ester bond breaks and you get your free lutein. And now this free lutein and free zeaxanthin are much much easier to purify and separate. So generally depending upon how the complexation is occur either for after hydrolysis you get fatty acid and lutein in the ratio of 1 is to 1 or you might get 1 molecule of lutein and 2 molecules of fatty acid.

So this is there and that's the reason why you will see that when industries are preparing lutein extracts, we know that lutevit or there are many carotene supplements for which they require the marigold extracts. Now, these companies, they generally carry out saponification. They do the extracts in appropriate solvent and then concentrate the extracts and then load it in their medications. Now, because of their pigment, one more interesting application is if you go to see, especially in the poultry industry,

when you have your broiler chicken and your country chicken, the eggs of country chicken are very much priced owing to their intense orange-colored yolk as compared to your broiler chicken, which has a yellowish-colored yolk. So people associate country chicken with being more organic and healthy, and definitely it is. So what is done is broilers are now fed a diet that is rich in lutein. As a result, what happens is when the broilers eat lutein,

enhanced levels of lutein that somehow through their body passes down to the egg yolk as a result, the eggs of these chickens, which have been fed with a lutein-rich diet, turn out to be orange in color. So that's one application and also a way of fooling the market, but that's done, and definitely, the higher lutein in your egg yolk is going to benefit you health-wise. Now, coming to the applications. If you see the applications of lutein in the area near your retina, there is a region called as macula lutea

and this macula lutea not only contains vitamin A but also specifically molecules like lutein and zeaxanthin. So from your diet, whatever you consume, molecules of lutein and zeaxanthin are taken up by your blood and specifically deposited in the macula lutea region. They are very vital for the functioning of the eye, and if these lutein and zeaxanthin molecules are deprived from this region, your macular degeneration starts.

So it is very important; they are antioxidants and also help you in the perception of color. So what is done is people who have poor vision, especially poor color vision, have often been advised to take lutein supplements. Now, taking lutein supplements, it has been clinically found that if you take lutein,

more and more of your carotenoid-based diet or if you take a supplementation of lutein, the risk of age-related macular degeneration decreases. Now, this is more particular for elderly people, plus 50, also when your macula starts degrading. You can have a diet that is richer in lutein and zeaxanthin to prevent that from happening. Also, it is seen that consuming a lutein-rich diet decreases the risk of cataract development, which

opacifies your lens and makes it difficult to see clearly. Now, there's one more finding which states that if you consume more than 2.4 mg of lutein daily, there is a significant decrease in nuclear lens opacities in such patients. Now, coming to veterinary applications, it is said that people nowadays even prefer carotenoids for their pets. In poultry, it is definitely used for the yolk,

but for animals, it is for their good health, antioxidant activity, and it is also associated with, you know, the playfulness or good health of animals. Now, Ayurveda also recognizes the significance of this flower, and marigold flower in Ayurveda is reported to possess properties such as carminative, astringent, and stomachic. It is used to treat liver problems and fever.

The plant is used to treat epileptic fits and even conditions like scabies. This is attributed to the fact that the whole plant contains sulfurous compounds or thiophene derivatives.

These thiophene derivatives not only kill microorganisms but also help treat infections. As a result, it is also seen that marigold plant extracts can be used as an insecticide. Moreover, if you focus on the flower part of it, it is very good for your plant.

The health of the eye and age-related macular degeneration are also concerns it addresses. So here are a few references if you wish to read more about this beautiful compound called carotenoids, and thank you everyone for your patient listening. Thank you.