

# **PHARMACOGNOSY AND PHYTOCHEMISTRY**

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**Week 4**

**Lecture 16**

## **Introduction and Classification of Tannins**

Hello everyone, and welcome to the NPTEL course on Pharmacognosy and Phytochemistry. Today, I Galvina Pereira, Assistant Professor, Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Mumbai, will take you through a set of very interesting compounds found in nature—those are tannins. Now, what are tannins?

Tannins derive their name from 'tanna.' 'Tanna' is a German word for oak, which was used in the tanning industry. Tanning converts animal hide into leather. This happens because tannins have the ability to precipitate animal hide proteins and convert them into stiff metabolites. These stiff metabolites prevented putrefaction.

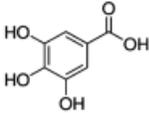
So, tannins are astringent. By their chemical nature, they are polymers of polyphenolic compounds, especially acids, biomolecules, and have a typical astringent effect. If you observe tannins, they have a huge molecular weight. The molecular weights of tannins range somewhere between 500 to 3,000 kilodaltons.

In some cases, when they form complexes or adducts with other metabolites, their molecular weight may be as high as 20,000 kilodaltons. Tannins in plants are generally born or biosynthesized through a unique pathway called the shikimic acid pathway. This pathway is unique for a reason: it is found in plants and microorganisms but not in humans.

A very simple example of a tannin derivative is this structure. You see here called gallic acid. It is trihydroxybenzoic acid. It is the simplest form and monomer of tannin. This may undergo polymerization to form a high-molecular-weight polymer of tannins.

## What are Tannins?

- Tannins- used for **tanning leather**.
- Astringent, polyphenolic biomolecules that precipitate proteins.
- Tannins are water-soluble phenolic compounds with molecular weights ranging from **500 to 3000 kDa**.
- Tannins are mostly biosynthetically derived from the **Shikimic acid pathway**



Gallic acid

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Now, let's see some day-to-day examples. Tannins are compounds that render a unique tan or brown color substance. Just to name a few: the tea you have daily, your chocolates, the deep brown color of dark chocolate, coffee, or even your club soda or nutmeg. All of them have a unique pigment, which is deep brown in color, and those are polymerized tannins.

When they are not polymerized, they are lighter in color, as you can see in amla, which has tannins but in a smaller molecular weight range. For example, when you consume amla, you get a typical tactile sensation termed astringency. This tactile sensation is often attributed to the presence of tannin compounds. These tannins mix with your saliva.

They precipitate compounds, especially proteins and enzymes, which are present in your saliva. And this colloidal precipitate in your mouth will give you a frictional, dry, rough feeling, often referred to as a puckering sensation. Now, this is what is called astringency. So these colloidal tannin precipitates then make your tongue slightly rough, and that sensation remains for a while until the tannins are digested by your saliva and then ingested by us.

So they form precipitates with salivary proteins. And if you observe this, the degree of salivation varies among individuals. So people producing more saliva will definitely have more proteins. They'll experience the sensation a little less. But in some cases, when your saliva production is lower, you

will get a very rough feeling after consuming astringent substances containing tannins. Now, why do plants synthesize tannins? Tannins play a very important role in plants. Now, let's take the example of our skin. The moment we step into the sun,

to prevent the deterioration of delicate molecules in our skin, the skin synthesizes protective molecules called melanin. The skin will synthesize protective molecules called melanin. This melanin serves to protect our skin from the sun's harsh radiation. Similarly, this is the role of tannins in plants. Whenever the tree is exposed to harsh sunlight, it will produce more of these polyphenolic compounds,

and as a result, you will find more tannins—especially in tea, for example. Those tea plants are often planted in very sunny, hilly areas where the exposure to sunlight is maximized. The second role it plays is preventing infections in plants. Now we know polyphenols such as cresol and other phenolic compounds have a very potent antibacterial or antifungal effect.

Plants produce similar polyphenols to get rid of bacteria and fungi. Whenever a tree is injured, what happens is it becomes prone to infestation by bacteria and fungi. To prevent that, the plant will synthesize these polyphenols and secrete them in the wounded region. As a result, the proteins of the bacteria and fungi are precipitated by the tannins, thereby killing them. But over time, many bacterial and fungal species have started producing an enzyme called tannase, which cuts or cleaves the tannins, making them resistant to the effect. Not only that, their astringency deters larger insects or animals from consuming them. Especially in the young stage, when the tannins are in a low molecular weight state, animals

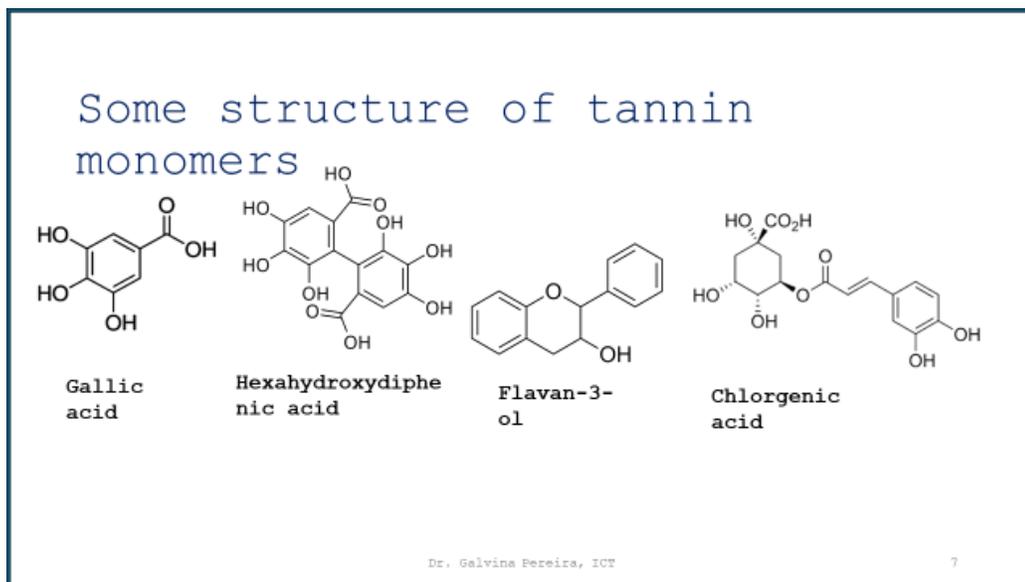
are deterred due to their astringent effects. So, properties of tannins. Now, tannins are amorphous in nature. So what happens here is that tannins are large molecular weight

compounds. They are polymers and if when you see a polymeric stage the degree of crystallinity reduces

and as a result most of the tannins are amorphous in nature. Now because of their large molecular weight and polyhydroxy groups, they are not soluble in organic solvents but they are rather polar and dissolve readily in water. With exception of very high molecular weight structures, many of the tannins are water soluble, ethanol soluble, acetone and glycerin.

They have a unique ability to combine with proteins and The hydroxy group of tannins combined with the amine group of proteins to form complexes. So the same lies true for gelatin which is an amine containing polymer. It also has the ability to complex with cellulose and pectin. Those are the sugar derivatives and have a unique ability to chelate with metals.

Now this chelation often leads to formation of colored complexes. So if you see tannins reacting with metals such as iron, it will give you an interesting blue-green color complex. In addition to that, being polyphenolic in nature, tannins show antioxidant properties. Here is an example of a few tannin monomers. Just like we discussed tannins are actually polymers.



But what are the building blocks of tannins? So the building blocks of tannins are monomers. And the simplest monomer we just discussed is a trihydroxybenzoic acid derivative referred to as gallic acid. The second monomer, if you see and if you carefully cut it from here. So if you just cut this molecule from here, you can identify two gallic acid molecules.

Okay, so here is the first gallic acid, and there is a second gallic acid residue which are joined to each other by a CC linkage. Now what happens is this is what is called hexahydroxy. There are six hydroxyl groups: 1, 2, 3, and there are 4, 5, 6, representative of gallic acid. So, hexahydroxy diphenic acid.

Eventually, what happens is this may undergo water elimination to form a lactone, and that lactone is called ellagic acid. Now, let me just quickly draw it for you. It's a bis compound, so there are two phenyl moieties, each representing a phenyl of gallic acid. Now

Trihydroxy, and this is the acid group, so I'm just keeping it as such. This is the acid group; I'm just keeping it as such, and let's put the hydroxies here. Okay, so what is going to happen in the process is when I draw this carboxylic acid like this. I have two gallic acid residues. They undergo water elimination to form what are called depside linkages.

Or lactone esters. So, these form lactone esters, and this is called ellagic acid. So, hexahydroxy diphenic acid undergoes lactonization to form ellagic acid, which is another monomer. The next set of monomers are your or benzopyrone derivatives, more specifically phenyl benzopyrone derivatives.

And the numbering goes like this: 1, 2, 3. So, they are called Flavan-3-ols. In some cases, an additional hydroxy group is found at the fourth position. In that case, they are called Flavan-3,4-diols. The last set of compounds you will find are actually called phenylpropanoids.

So, there's a phenyl ring. There's propane: 1, 2, 3. So, this is typically a cinnamic acid derivative, which forms an ester with quinic acid, and you get what is called chlorogenic acid. So, if you see tannin monomers, they are small molecular weight.

They may undergo polymerization to give what are called true tannin derivatives. So, how do you classify tannins? Those with small molecular weight monomers are called pseudotannins because they cannot bind to the animal hide and cause what is called the binding, stiffening, or tanning of leather. So they do not tan leather, and hence they are called pseudotannins.

On the other side, when they themselves polymerize all these monomers, they give what are called high molecular weight complexes. They acquire the ability to tan leather, and in that case, they are called true tannins. Now, true tannins can be classified into many classes depending on their susceptibility to hydrolysis. The simplest ones are hydrolyzable. Now, hydrolyzable, as the name indicates, the ester linkages in them can be hydrolyzed

by acids, by bases, or by enzymes, as we just saw, that is your tannase. Depending on which monomer is involved, if it is a hexahydroxy diphenic acid or ellagic acid, we call such tannins ellagitannins. If it is a gallic acid monomer, we call it gallotannin. If it is a mixture of both, that is ellagic plus gallic acid together.

That is, if it is a mixture of both ellagic acid and gallic acid put together, we call it a combined or both tannins. Now, if you see the hydrolysis, there is another category called non-hydrolyzable tannins. Now, these aren't susceptible to hydrolysis, the reason being there are no ester linkages, but most of them have what are called carbon-carbon linkages. In this, there are two categories: the condensed type, which have a flavan nucleus,

and phlorotannins, which have a phloroglucinol often linked to C-C linkages or carbon-oxygen-carbon, what are called your ether linkages. The last one, complex tannins, is a mixture of hydrolyzable and non-hydrolyzable tannins. So, let's see the properties. If you see pseudotannins, they are easily water-soluble because they are small. They do not tan leather, but because they have a small molecular weight, they

can bind to your tongue, salivary proteins, and produce a very good astringent effect. On the other side, true tannins, because of their large molecular weight, have decreasing solubility as the molecular weight increases. They have a good ability to tan leather, and as they are high molecular weight, their binding ability to proteins decreases, and so does their astringency.

So, giving you a few examples of pseudotannins: the first one is chlorogenic acid, others being gallic acid, ellagic acid, and so on. Whereas the same monomers may polymerize. So, as you can see in this case, there is a central sugar moiety. To which your gallic acid residues are attached.

So, I'll just show you gallic acid. You can see here there's one monomer of gallic acid esterifying with another monomer of gallic acid, which in turn esterifies with sugars. Now, this may polymerize in nature. I just remove this edge and add one more gallic acid residue to it, and you can see the reaction can go

endlessly. So, this is one gallic acid that I added. Again, I can remove one of the hydrogens and cause further esterification with an additional gallic acid group. So, in the same way, they polymerize in nature to give you what are called the polymers of gallic or ellagic acid. The hydrolyzable tannins can be easily distinguished by the presence of a central sugar, generally which is a glucose multi.

The hydroxyl groups of the sugar are complexed with monomers such as your gallic or ellagic acid and, in some cases, hexahydroxy diphenic acid. They are highly susceptible to hydrolysis because of the ester linkage. So, they are susceptible to acids, bases, and the enzyme tannase. Now, an interesting application of the enzyme tannase is if you see these tannins, as the molecular weight increases,

you will see their solubility decrease, and then they tend to form colloidal mixtures. So, imagine your tea, imagine your coffee, or imagine your red wine or beer. All of these examples are of extracts or water extracts containing tannins. So when you boil the tea the tannins dissolve but as the tea cools down like if you want to sell cold tea or cold coffee decrease their solubility with temperature and, as a result, form colloidal suspensions.

Now, as a marketer, if I want to sell this tea, my tea is no longer transparent. So, if I have to render it transparent, some of these food industries add tannase to this colloidal suspension. The tannase will cleave all the polymers, convert them into small molecular weight compounds, and the clarity of your tea, coffee, wine, or beer can be improved.

So, this is an interesting application of tannase. Now, if I have to distill it or dry distill it on dry distillation, all of these compounds give rise to what are called as pyrogallol. So pyrogallol is when you just cut off from this ester, you get a trihydroxy derivative, which is called pyrogallol. Now, hydrolyzable tannins generally give pyrogallol on dry distillation. Two examples or two classes of hydrolyzable tannins are ellagitannins and gallotannins.

In ellagitannin, the building block is hexahydroxy diphenic acid, whereas in gallotannins, the building block is gallic acid. So on hydrolysis, they'll give you the same monomers. Ellagitannins, you will see actually gallic acid molecules are there but they are joined by CC linkages. That is difficult to cut but once they form the ester linkages, the ester linkages are easily cleaved. So gallic acid groups are present but they are joined by CC linkages and hence cannot be broken.

Whereas if you see gallotannins, there are gallic acid moieties or monomers which are joined to each other by ester linkages so they eventually break and give you gallic acid. A few examples of ellagitannins are harda, which is a common fruit found in your Ayurvedic preparation triphala, which contains chebulagic acid. And another example is your pomegranate peel, which contains an ellagitannin called as punicalagin.

Here, if you see gallotannins example, they are found in nutgalls, they are found in clove, rhubarb. Below is the structure of tannic acid which is commonly found in your nutgalls and which is used for tanning purposes. Now coming to condensed tannins. Condensed tannins have a monomer which is a flavan type, and you can see the simplest monomer is a catechin. Now, these monomers may form adducts.

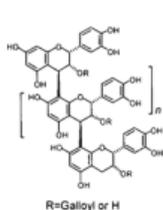
So, imagine three molecules joined to each other. They form polymers, and imagine this going on for about 2 to 50 such residues. They form a complex which is called proanthocyanidins or condensed tannins. Now, because the linkages here are C-C linkages, they are not susceptible to hydrolysis. Often, if you see the molecular weight, when they go up to 50 monomers joined together, they form huge

molecular weight complexes. In that case, their solubility is very poor. So, few classes or subclasses of condensed tannins are A-type or B-type. If you see A-type, they are polymers,

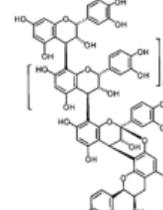
but in addition to that, you will see some linkages which are about C2 to C7. Okay, so this is carbon number 1, which is O, and this is carbon number 2.

**Condensed Tannins**

- The A-type : linkage between C-2 → C-7 of the basic flavan-3-ol units.
- B-type : linkage between C-4 → C-8 or C-4 → C-6 (although it is less frequent)
- polymers based on the presence of flavan-3-ols or phloroglucinol



B-type Proanthocyanidin



A-type Proanthocyanidin

R=Galloyl or H

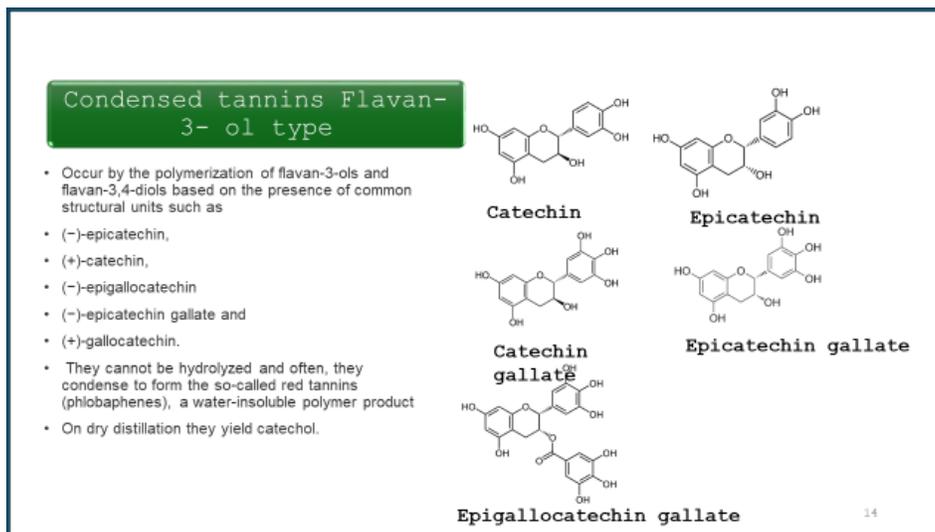
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So, from 2, it has formed a linkage with C7. Whereas, if you see B-type, in B-type the linkages are between C4 (this is carbon number 4) and C8. So, your condensed tannins can be classified as A-type or B-type depending upon the linkages. The condensed flavan-type nuclei can be further subclassified into many categories. The simple one is catechin, which we just saw.

If you see catechin, you can focus on these three hydroxy groups of catechin; they lie ahead of the plane. Whereas if you see epicatechin, the next monomer involved in the formation of condensed tannins, the hydroxy group goes behind the plane. So that's a slight optical difference between catechin and epicatechin. Now, if you add one hydroxy group here, you get what is called catechin gallate.

The same applies to epicatechin. Adding a hydroxy group here gives you what is called epicatechin gallate. In some cases, this 3-hydroxy group is involved in an esterification

reaction with a gallic acid moiety. In that case, it is called epigallocatechin gallate. This is found in tea and is extensively investigated for its anti-cancer effects.



On dry distillation, they yield catechol moieties. The last category of these are your phlorotannins. Phlorotannins are actually polymers of phloroglucinol. So you can see here phloroglucinol is a dihydroxyphenol derivative. These monomers may form adducts via either ether linkages or C-C linkages.

So they undergo polymerization. This class of compounds is not commonly found in plants, but they are generally found in algae, especially sea kelps or some brown and red algae. Good examples of these are E. coli and Fucoflorethol. The polymers of this range weigh somewhere between 126 Daltons to 650 kiloDaltons.

Thank you, everyone, for your interest. Here are a few references if you want to enhance your reading on this topic. Thank you.