PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 4

Lecture 17

Different sources and evaluation of Tannins

Hello everyone, and welcome to the NPTEL online course in pharmacognosy and phytochemistry. In the previous session, we learned about what tannins are and the different classes of tannins. Today, we will delve a little deeper into that. We will learn about the different sources of tannins and the methods of evaluating tannins in different substances present in nature.

In nature, tannins are present in green tea. When you say green tea, it's the light green-colored concoction that you produce out of tea leaves, which contains epigallocatechin gallate and the very tart, very astringent amla. They contain low molecular weight pseudotannins such as gallic acid and epigallocatechin gallate. Apart from that, true tannins, which are high molecular weight tannins,

are present in the form of hydrolyzable and condensed tannins. The hydrolyzable tannins, gallotannins, are present in nutgalls. They are present in different fruits and berries, such as strawberries and raspberries, mostly in the form of pentagalloyl glucose as the smallest unit. They are in the form of ellagic tannins in pomegranate peels, in the form

of punicalagin, and in hardy fruits, they are present as chebulinic acid. When you go to non-hydrolyzable tannins, you have condensed tannins, the flavonoid type which are present in pale and black catechu and phlorotannin's which are present in sea kelp. Apart from that, complex tannins are found to occur in *Camellia japonica* in the form of camelliatannins A and B. So these are some sources of tannins.

You will find tannins occurring in numerous plants as they are protective molecules. So how do we evaluate them? The first step to evaluate tannins is to extract them in a suitable solvent and then subject them to different chemical methods of evaluation. The extraction of tannins is done in solvents which are polar in nature.

You can extract tannins with water. ethanol, acetone or mixture of them. That is you could use 50% water, 50% ethanol or 50% acetone and 50% water as a mixture to extract tannins. The solubility of tannins in this solvents depends upon their molecular weight. The higher the molecular weight the more is the resistance to its solubility in different solvents.

When you take tannins, it is best to take the drug in its fresh form. The reason being I just illustrated with the example of tea. When you take a fresh tea leaf, it contains tannins in a very low molecular weight form. But as you ferment, as you roast the coffee beans, the tannins polymerize in themselves and are converted into high molecular weight polymers.

This high molecular weight polymers are difficult to extract. Hence, in order to assess the tannins of a drug substance, it is best to extract them or keep the sample frozen till you need it for the chemical evaluation. Now, when you take a sample, sometimes there are certain samples which are extensively lipophilic. that means they contain oleaginous ingredients this includes your oils butters or waxes

when you have oils butters or waxes present in a plant they don't allow solvents such as water to imbibe deeply into the tissue and that is the reason when you want to evaluate such drugs for their tannin content the first step is to remove the waxy contents The waxy contents or the lipid contents or oils can be removed using organic solvents such as petroleum ether or hexane. They will dissolve this lipophilic compounds and now your tissue is free of them.

Once the tissue is free of all the lipid compounds, the water penetrates much much more easily and your compounds are extracted completely. The other problem is when your plant product is a leaf or a stem which contains pigments. Now these pigments can be something like your chlorophyll. Chlorophyll often interferes with color evaluation because inherently it is green in color.

To get rid of chlorophyll, it is advised that you extract the sample with chloroform or organic solvents in which your chlorophyll has a good solubility. Chloroform will dissolve the chlorophyll and then once the pigments are removed, the tannins can be easily assessed using colorimetric test. Now once you get the tannin extract you can further purify them by subjecting it to different chromatographic separation techniques.

Now let's see how we assess them. Once you have your extract of tannins ready you can apply a few general tests to see whether they contain tannins or not. The general tests for true tannins include gold bitters test, which uses a ox skin gelatin test which uses a gelatin solution lead acetate test and a phenazone test this test will tell you whether the two tannins are present in solution or not

Let's see them one by one so the gold bitter skin test is actually a test where you convert your animal skin into leather. Now this process is mimicking your tanning process. So what you take in for a tan or what you take in for an animal skin is actually an intestine of an ox. soaked in a hydrochloric acid solution. So we'll take a membrane or intestinal membrane of a ox which is untanned and unused

and then we will soak it in acid to get rid of the initial impurities and to make the skin more unable to tanning. Once that is done the acid is washed clearly and then what you can do is you add a tannin solution. This can be done by just taking a small container containing your tannin solution and you can just keep your skin or the ox skin immersed in it for a period of about 5 minutes. slowly imbibes all the tannins.

The tannins will react with the proteins present on the surface of skin and slowly the complex formation starts. Once the complex formation is done, after a period of 5 minutes, the tannin solution is discarded. The ox skin is nicely washed with water and then dipped with a solution which is called mordant. Mordant act as a fixative. So it will help intensify the color, convert the animal hide into a deep brown black color leather

and that you can easily assess by change in color of the or change in appearance of the ox skin. If that skin turns brown or black, we say that the solution contains tannins. So that's a positive gold bitters test. Now, if your solution contains pseudo tannins or low molecular

weight tannins, the intensity of color change is low or negligible. So this test is generally not indicative of pseudo tannins, but it is a test for the presence of true tannins.

The next test you can do is also a test that combines tannins with proteins, and that is your gelatin test. In this gelatin test, we use gelatin as a source of protein because gelatin comes from cartilage. It is the hydrolysis product of cartilage. So gelatin is taken. prepared in a salt solution saturated and to that when you add your tannins this tannins form complexes with gelatin

and slowly slowly precipitate as you can see here initially you have a very clear tannin solution but once this tannin solution is added a test tube containing gelatin with salt, you can see a deep-colored precipitate. This is what is called tanning—a tanned, blackish-brownish color complex has been formed. If you allow it to settle over a period of time, it will settle down, giving you a precipitate of the complex. The next test we can do

is a lead acetate test. In this test, Actually, the ability of tannins to form complexes with lead. Now, tannins have the ability to chelate metal ions, including lead. They form lead tannate. So when my tannin solution is added to a lead acetate solution, a yellowish or yellowish-whitish precipitate of lead tannate is formed, which

you can see here as indicated by turbidity. This turbidity, if allowed to mature over a period of time, will settle down. The presence of a whitish to yellowish precipitate is indicative of tannins present in your solution. Another test that is indicative of tannins is a phenazone test. So if you take a tannin solution, mix it with sodium acetate phosphate, boil it, filter it, and then cool it.

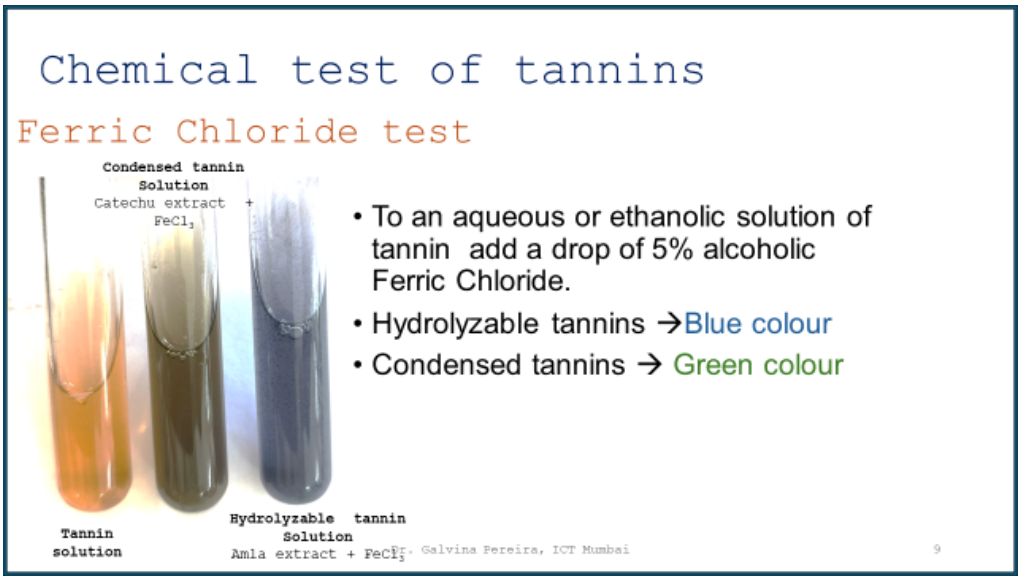
So after cooling, when you react with a 2% phenazone solution, you get a bulky precipitate. That is also indicative of the presence of tannins in your solution. Now with that, let's move on to some specific tests. So specific tests for tannins include the ferric chloride test. The ferric chloride test helps us distinguish between hydrolyzable and condensed tannins by the color formation.

The second test is the potassium iodate test, which is more specific for gallotannins and gallic acid. The nitrous acid test is specific for ellagitannins and ellagic acid, the vanillin

hydrochloric acid test which is given by flavanol derivatives, and the matchstick test which is again given by condensed tannins, the flavanol derivatives. Now let's see them one by one.

So the first test we analyze here is the ferric chloride test. Now, why is this a distinguishing test? If you take a tannin solution, as you can see here, this is a tannin solution. Principally, tannins are buff to brown-colored compounds, so your extract appears something like this. Now, I have taken one extract which is rich in hydrolyzable tannins and one extract which is rich in condensed tannins. So what happens here is, the extract containing condensed tannins,

when I add a drop of ferric chloride to it, you will see a greenish coloration, whereas in the case of hydrolyzable tannins, you will see here a bluish coloration being formed. So this test helps you distinguish between blue or green color complexes, which are formed by reacting with hydrolyzable and condensed tannins, respectively. So let's see what happens exactly out here.



So in this process, you can see gallic acid as an example of hydrolyzable tannins or gallotannins. Now this gallic acid reacts and forms a coordination complex. So you can see here they form coordination complexes with iron and this chelate. gives you a typical coloration so you can see how the process of addition happens

this is a tube containing light a very dilute solution of tannins and i've added a drop of ferric chloride so at the interface when the reaction is happening you can see a blue color complex being formed Similarly, you can even check for the presence of specific hydrolyzable tannins such as gallotannins and ellagitanins. If we want to check for the presence of gallotannins, what we can do is take the solution containing gallotannins and react with potassium iodate solution.

If you get a presence of pink coloration, It is due to the presence of gallotannins. Now, instead of gallotannins, if we have pseudotannins like an individual monomeric gallic acid molecule, in that case, you will get an orange color. Now we can also check for the presence of ellagitannins.

Elagitannins form complexes with nitrous acid in an acidic pH that is in presence of acetic acid to give you a pink color. Now this test is obeyed both by ellagitannins also and monomeric ellagic acid also. So both of them will initially form a pink color complex. And gradually and transiently the color of this complex will change initially to purple and then to blue color. So presence of a transient pink to bluish coloration is an indication of ellagitannins being present.

Now moving to condensed tannins. Condensed tannins, especially the flavonol type, react with ferric chloride. to give a complex. Now, this is similar to gallic acid, but you can see the nature of the complex is slightly different. So here, chelation does happen—that is, the coordination complexes—and you can see

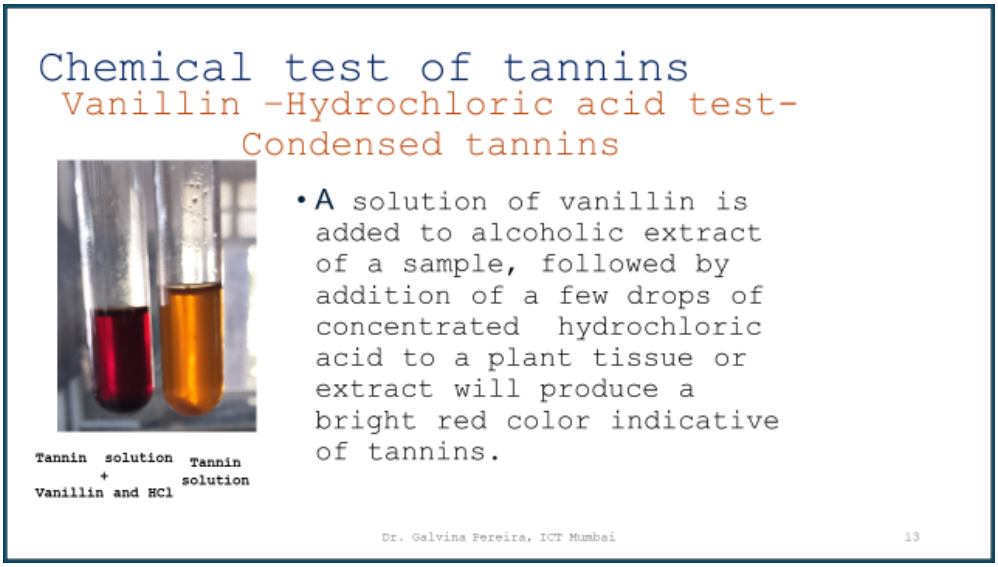
Now, because of the bulkiness and resonance of this kind of structure, instead of a blue coloration, you will get a green coloration being formed. So, you can see here at the interface, as compared to hydrolyzable tannins, condensed tannins give you a green color complex. Another test for condensed tannins, especially the flavanol type, is when you treat it with vanillin hydrochloric acid.

Now, vanillin in the presence of acidic pH reacts with flavan, especially the meta-substituted flavan derivatives, to give you a reddish color complex. Now, this is an electrophilic reaction where you form or where you get a vanillin adduct. Now, this vanillin

adduct is again not selective. So, even if you have a plain Flavan-3-ol derivative like an isolated catechin molecule in monomeric form, it

will still give you a red color complex. So, the vanillin hydrochloric acid test is a test which is used to check flavanol derivatives, which gives you red color complexes. Interestingly, this test is also used histochemically. So, what you need to do is take your section—that is, a transverse section—in that transverse section, just add a drop of your vanillin solution, that is, alcoholic vanillin, let it dry off, and then you

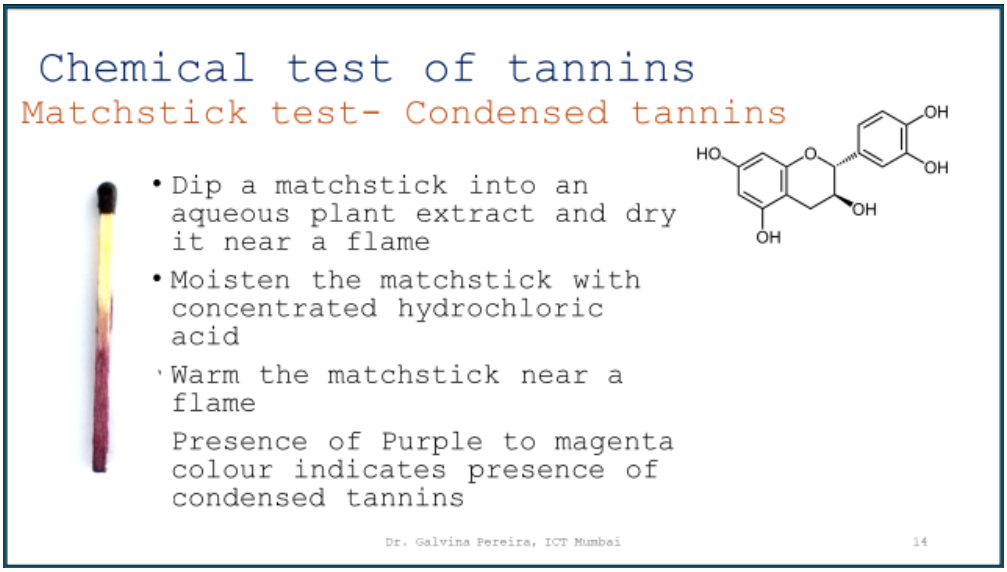
add a drop of concentrated hydrochloric acid. What will happen is wherever your condensed tannins are present in the section, that section will tend—or that area of the section—will tend to form a reddish zone. The presence of a reddish zone in your section indicates that your condensed tannins, especially the Flavan-3-ol derivatives, are present there.



Another test for tannins, especially the condensed flavon-3-ol derivatives, is the matchstick test. Now, this is a very interesting test. If you see the matchstick, it comes from wood,

which is rich in lignin. These lignin derivatives have cinnamaldehyde groups, and the cinnamaldehyde groups react with the catechin or flavan-3-ol derivatives.

Now, here is an example of a catechin molecule. So, what happens is when I dip my



matchstick into a solution of catechin or condensed tannins and bring it near a flame, it will start reacting with the cinnamaldehyde functionality. As it reacts, if I add a concentrated acid such as hydrochloric acid—just by

dipping the matchstick in concentrated hydrochloric acid—slowly, this molecule breaks down to give you what is called phloroglucinol. Now this phloroglucinol has a tendency to react with the lignin to give you a purple color complex. So lignins react with this free phloroglucinol moiety and I get a presence of purple magenta shade. You can see it here.

Initially the matchstick is a nice yellow in color but after I dip it in catechu you can see the color is brownish. Here, the region where it is dipped in HCl and brought near a flame, you get a purple-to-magenta color, indicating the presence of condensed tannins. Now, apart from that, we can even quantify tannins. The general methods for quantifying tannins are based on the reaction of tannins with proteins.

So, they might react with blood components to give you the hemolyzed blood method. They might react with proteins in bovine serum to give you a bovine serum albumin

method. They might react with the proteins when present in animal hide. To give you an animal hide method. So how does this work?

So these three methods are basically complexes of tannins plus proteins. So just let's try to understand them. In the hemolyzed blood method, what is done is blood is treated with a tannin sample. The proteins in the blood will settle or form complexes with the tannins. The sample is centrifuged, and the amount of deposit is quantified gravimetrically.

The same is done for bovine serum albumin, but the method is reversed. So first, we quantify how much bovine serum albumin is present, then you add a tannin solution. Some amount of this bovine serum albumin will react with the tannins. They form a precipitate. The tannin removes the amount of serum albumin equivalent to its moles

and that much amount of bovine serum albumin is now gone from the solution. Again, when we quantify the bovine serum albumin, the difference in weight will tell me how much tannin is present. The same applies to animal hide powder. You can prepare a suspension of animal hide powder by boiling it,

making it slightly hydrated, and then reacting it with tannin. The amount of precipitate will tell you how much tannin is present. The last method is a non-specific method. It is used for determination of polyphenols in general.

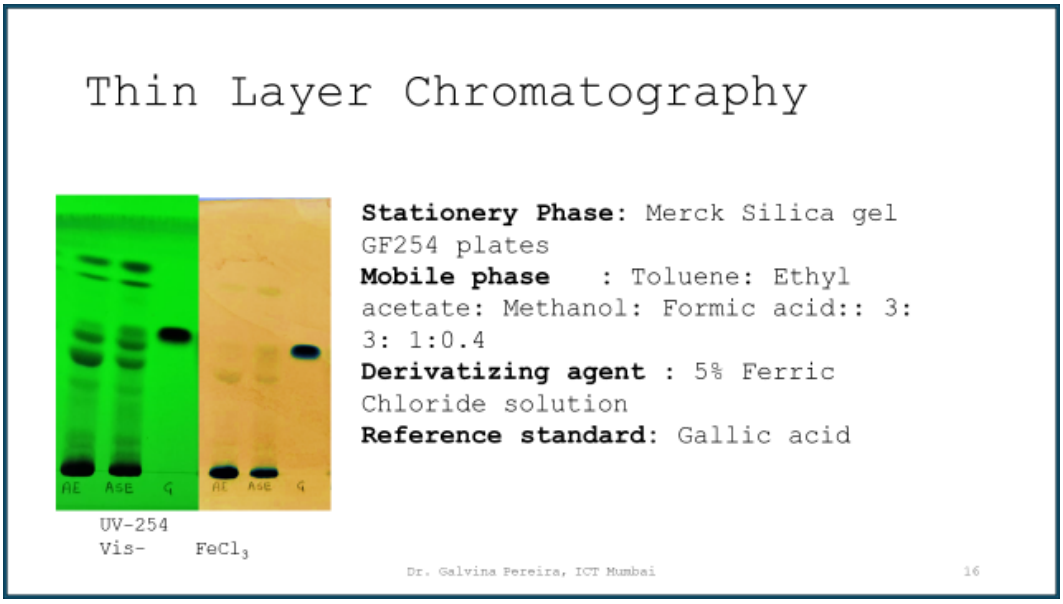
So, fallen CO cal 2 reagent consists of phosphotungstic and phosphomolybdic acid. They form a adducts blue color complexes with tannins under basic pH. So, this blue color complexes, intensity of this blue color complexes is directly proportional to the intensity of polyphenolic compounds being present.

Now, since tannins are also polyphenolic, we can use the Folin Ciocaltue method to quantify tannins using the UV-VIS spectroscopic method. One more method for quantification and analysis is your thin layer chromatographic method. This method uses your silica as a stationary phase and organic solvents such as toluene, ethyl acetate, methanol formic acid, a combined phase

which we call the mobile phase for running this. The principle is in this case your stationary phase is polar and your mobile phase is nonpolar. So tannins, depending upon their affinity

to the stationary versus mobile phase, will start running on the plate and they run to a specific distance. We call it a retention factor RF.

So if you can see here, we have a plant extract being applied. These are two different plant



extracts, and here G stands for gallic acid. So, if you see, gallic acid travels a certain distance, and you can simultaneously see a corresponding band in the plant extract. What does this indicate? This indicates that my plant extract also contains gallic acid.

TLC, along with a scanner called photo documentation, can also be used to quantify the amount of tannins present based on their RF and intensity. Other methods that have been used for tannins are HPLC, size exclusion, and a few also use GC, but those are beyond the scope of this.

So, thank you everyone for your patient listening. Here are a few references, just in case you wish to know more about this. Thank you.