PHARMACOGNOSY AND PHYTOCHEMISTRY

Dr. Galvina Pereira

Department of Pharmaceutical Sciences and Technology

Institute of Chemical Technology, Mumbai

Week 10

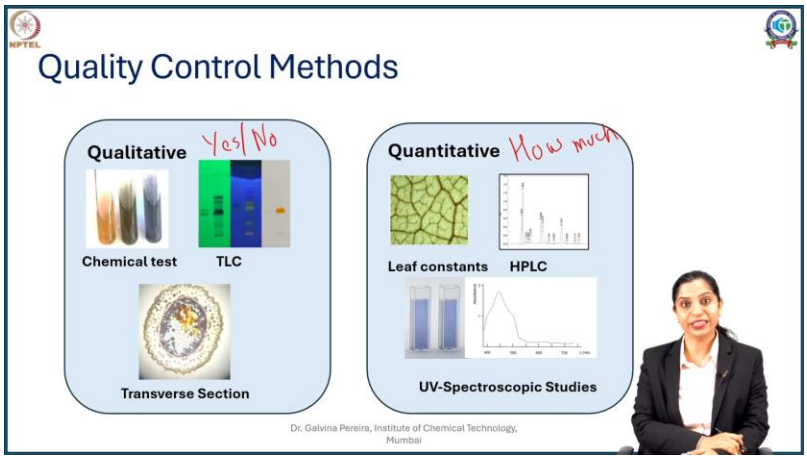
Lecture 47

Week 10: Lecture 47: Methods of Quality Control of Herbal Drugs

Thank you. Hello everyone, and welcome to session 2 of week 10 of the NPTEL course in pharmacognosy and phytochemistry. In the previous lecture, we learned what quality control is and why it is essential. So, just to recap, quality control is everything we do to ensure the identity and purity of a substance. These quality control methods will help you achieve what is called consistency, safety, and efficacy of a drug.

From this lecture onwards, we will delve a little into the different quality control methods we use or carry out to ensure consistency, purity, and efficacy. The quality control methods for herbal drugs can be broadly divided into two categories: qualitative methods of quality control and quantitative methods of quality control. So, what is the slight difference between qualitative and quantitative? Qualitative methods are the kind of methods that will tell me approximately whether the compounds are good or not. For example, does it contain tannins—yes or no? Whereas quantitative methods will tell me how much tannin it contains. So, qualitative methods are more of a yes-or-no approach, whereas quantitative methods are more about how much.

So, when I have to determine whether it contains something or not, is it passing or failing? Whereas if I have to determine how much it contains, what the exact range is, or what the concentration is, I will use a quantitative method. A few examples of qualitative methods are, perhaps in our tannins chapter—I have just taken the same image. We performed what is called the ferric chloride test. From that, we learned that this was your sample.



This was yours, if you remember your condensed tannins, and this was your hydrolyzable tannins. So from this test, I came to know that the tannins are present. The tannins are of the hydrolyzable type or the tannins are of the non-hydrolyzable type. Similarly, when you do the TLC, this is a TLC of black pepper. And it's going to tell me, based on the standard, whether your piperine is present or not.

Yes or no. Similarly, the transverse section will just tell me whether it is my ephedra drug—yes or no. So based on the section, I'll come to know: yes, it is ephedra; no, it is not ephedra. It cannot be 50% ephedra based on the transverse section. But when I go to quantitative methods, quantitative methods are more about what I can quantify in terms of numbers, especially. Say, for example, when we did carotenoids, you remember your Scoville index, your ASTA color index, or your concentration.

How much concentration of gallic acid does it contain? So that will depend on the intensity or absorbance. So you can do UV spectroscopic studies to see how much it is absorbing. If 10 micrograms is absorbing so much and my solution is absorbing, say, for example, 5 in terms of absorbance value. So I can just correlate how much is my approximate concentration.

Even HPLC or area under the curve will tell me how much quantity of my standard is in the sample or, similarly, on a smaller or microscopic scale, we also study what are called leaf constants, which also give us some numerical values to assess the purity of the drug. When we refer to WHO or pharmacopoeia standards, your quality control methods can be divided into many parts. This includes your organoleptic methods. Where you do a sensory

evaluation, macroscopic evaluation, where you visualize and feel the morphology, then you have microscopic evaluation, where you see the drug or its parts under a microscope and determine what kind of drug it is. You can also quantitatively determine how many compounds or starch grains are present or what the size of the starch grains is in the view. You can do physical evaluation, chemical evaluation, as well as biological evaluation.

In some pharmacopoeias, organoleptic, microscopic, macroscopic, and physical methods are all broadly categorized as physical methods. Because they fall into the category of the physical attributes of the material. So, what are these different quality control methods under these categories? Let's see them one by one. Let's first go to organoleptic.

When I mention organoleptic evaluation, as I said, I will assess the drug using my senses. I will use all five senses to evaluate the drug and determine whether it is genuine or not. Recall the previous examples we discussed when I asked you to get an orange from the market. You will visually inspect the orange. You might take a whiff of the orange's aroma.

You will feel, you will see the texture, you will feel how the pitted peel of the drug is. And then based on all your sensory evaluation, you will go to the market and try to search something very similar. That is basically what is called as organoleptic evaluation. So you might evaluate your drug by color. So say for example your nadgaals we saw in the tannins.

You see nutgalls from different category green to grey. The green being more raw ones whereas your grey being more matured and market ready ones. In terms of odour we come to know whether a spice is fresh or the spice is exhausted. Sometimes you come across samples which do not have any aroma at all. We say that probably it is too expensive.

Old or dated or probably everything in terms of its essential oil has been exhausted and extracted. So we do kind of evaluate most of the fragrant drugs by aroma and this include your spices. It can include your something like your sandalwood or it can even include your pine or certain resins which have aroma especially asafoetida. Taste, this is something which we do not recommend for all the drugs because there are certain drugs which are poisonous. So if you remember or recollect, we had certain drugs which are hallucinogen, opium.

We do not recommend you all taste it because tasting it will cause hallucinogenic effects. And as a result, there are certain attributes which can be felt by taste perception. This includes the bitterness of cinchona, principally due to quinine. The pungency of black pepper—you remember piperine was the principle which gave it the pungency—the astringency of your amla fruit owing to tannins, and the sweetness of stevia owing to your steviocytes. So you can just taste the drug and know if it's genuine or not. By just tasting, you can test the black pepper in the market and come to know whether it is exhausted, fresh, genuine, or adulterated with something. Similarly, you can feel the drug.

Like I said, if I give you an orange, you will feel the peel. You know it's pitted; the texture is somewhat rough. It's not very smooth, and I'll come to know what exactly the orange feels like. So whenever you feel a drug, it's referred to as the texture. So sometimes certain drug powders feel coarse.

Sometimes certain drugs, especially those having gums and mucilages—say, for example, your isabgol or even your senna leaflet. This senna leaflet, you will see that when soaked, they are mucilaginous, so they turn slightly slimy. So if the market replaces them with something which is not slimy, I come to know that this is not senna because the moment I add water, it's not turning slimy in nature. Not only that, I can feel the texture, and sometimes even just by feeling the texture, people come to know what variety of senna it is. Say, for example, your Cassia acutifolia—the leaflets are very thin to feel—whereas your Cassia angustifolia feels a little thick and papery in nature.

Now go into the next one. The next feature is your microscopy. Now in macroscopy, you will actually see and evaluate drugs, not in terms of organoleptics, but you can measure them. So measure the size and the shape of it. So you will come to know based on the size and shape.

You can even describe the margins. You remember we described the margins as ovate, lanceolate, acuminate. So depending upon the leaf, the leaf margin, the leaf shape, etc. Size, diameter, length—you can do the measurements, and then you can say microscopically. You know, imagine my normal orange sizes are 10 centimeters, and if in the market I'm just getting something which is like 2 to 3 centimeters, I know it's not going

to be the same quality. So in the same manner, this microscopic definition—or say, in the same manner, this macroscopic evaluation—will help me ensure the quality. Not only that, when I assess anything microscopically, I see visually: is the drug organized or is the drug unorganized?

Is it cellular? Is it not cellular? You need not put it under a microscope. Your oils and all—you can visually tell if it is an organized or an unorganized drug. Based on its visual appeal, you will come to know what plant part it is.

See, your leaf has a typical venation or arrangement. Your bark has a typical arrangement. So just by looking at it, you know what plant part it is. You need not go into an elaborate way. So somebody tells you that, you know, this is a leaf, and what is coming right in front of you is not a leaf.

It's actually a stem. We can macroscopically tell that it is a stem. You need not see it under a microscope. So this kind of evaluation is referred to as your macroscopic or gross evaluation. Moving to the next type of evaluation.

So here again, in terms of macroscopic evaluation, I want to point out a few things. Now coming here to the macroscopic evaluation, a few things, say for example, even if I come to know bark, there are certain features of bark which will stand out. Say for example, your kurchi bark, it's a recaud. Recaud means if you see a cinnamon bark, cinnamon bark forms a quill. So you can see it like that.

Now, what is a recurved bark? Recurved bark will form an inverse quill. So the inner side is exposed, whereas in a normal quill, the inner side is covered. So kurchi has more of a recurved bark, whereas your cinnamon has more of a quilled or curved bark. Not only that, you can see the quality of the drug.

Certain times, you might end up with leaf samples that might have some fungus grown on them. Then you can take a call that, see, I can visually or macroscopically see fungus. I can macroscopically see the disease spots, the virus attacking the leaves by yellow spots. Some of them are black spots. Some of them have slightly formed cavities.

I know that my plant is diseased. In that case, it is safer not to take such a plant for preparation of your drug material. Now, sometimes there might be even scars or lenticels; you can see these tiny lines here. These lines are actually the lines where the plant can breathe easily. They are referred to as lenticels.

So even those scars are identification marks of Himalayan birch. So Himalayan birch is your bojh patra on which your ancient scripts are written. So for those kinds of drugs, you can also have unique identifying traits which can be assessed macroscopically. Now, moving to the microscopic part—in microscopy, you can divide it into two parts: qualitative microscopy as well as quantitative microscopy. If you recall, qualitative will tell you more of yes, no, what it is, whereas quantitative will tell you how much it is.

So, in qualitative microscopy, once we have a drug, plant material, or any other source, we will take it under the microscope and start studying it. Now, for studying it, what you can do is take transverse sections. A transverse section is when you have a drug of this type. stem, and then when you cut it like this, you call it a transverse section. Sometimes you just take a peel, like an orange peel or a leaf peel—you can just peel it and mount it. You can also take a petal and mount it. So, you can have epidermal peels. Sometimes people also do what is called histochemical localization, which is a mix of microscopy and chemistry. For example, my alkaloids are located in the epidermis, say for example here.

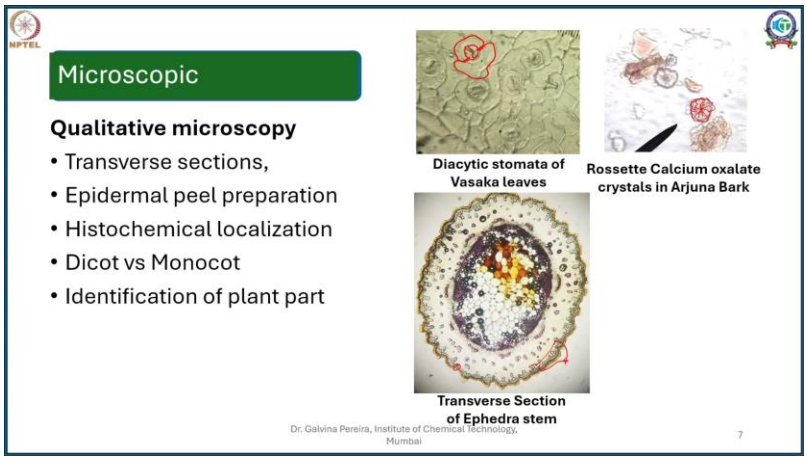
Now, if I stain it with a certain reagent like nitric acid or something, then suddenly the area which is rich in alkaloids becomes more yellowish. Take, for example, that happens with strychnine and brucine. In that case, you will also see that you can localize wherever your alkaloids are. So, that is called histochemical localization. Not only that, your transverse sections or your microscopy—when you see the plant under the microscope—you can identify whether it is monocot or dicot based on its anatomy, based on the arrangement of its vascular bundle, whether it is properly arranged as a dicot or whether it is scattered like a monocot. Not only that, there are certain attributes which will directly guide you to identify the plants. Because this feature is there, definitely this is the plant which I am seeing. So, microscopic evaluation allows you to see some identifying features.

This includes diacytic stomata, which you can carefully see here. So, you can see here. Diacytic means your stomata are there. This is the stomatal pore. And your stomatal pore, and if you see epidermal cells, the epidermal cells are 90 degrees to the long axis of the stomata.

So, this is diacytic stomata. There is a good chance that Wasaka has diacytic stomata. So, you know, this is going to be my drug. Similarly, when I see calcium oxalate, these are what are called rosette-shaped, flower-shaped, or rose-shaped. Can you see there's a nice flower shape?

This is actually a cluster. So, there are numerous small calcium oxalates which are kind of aggregated to form a flower-like rose. So, rosette crystals you can see in powders of Arjuna bark. You can even see them in your rhubarb. But the thing is, the size of those is different.

So you can identify that you can do a transverse section, and based on the transverse section, what I can tell is there's a sunken stomata here. So probably the plant is xerophytic based on the arrangement and presence of pith. I can tell it's a stem, and overall, I can see unlignified fibers, wherein I can tell there's a good chance that this is ephedra as a drug. So my microscopic evaluation can tell me what drug it is. And whether it is pure or not.



Now, when you see powders, sometimes the raw material doesn't come to you as an entire drug. It might come to you as a powder. Even in that case, when you put these powders under a microscope with suitable staining reagents, the appearance of certain characteristic

features can help you in the identification of drugs. I've just shown you a few representative features here. See, for example, phloem fibers.

Phloem fibers are present in drugs such as cinnamon, and they are also present in drugs such as cinchona. So there is a good chance, and again, depending upon the size, I can tell which drug it is. You can see cork cells, and depending upon the cork cells, I can tell that it is a bigger tree which I am dealing with. And either the drug is a stem bark or a root bark—that is the outer covering. Then you can see calcium oxalate.

Here, typically, what is a prism or prismatic calcium oxalate, which is found in certain plants like kurchi. And then, at the last feature, what you are seeing are little tiny circles in here. About three of them. And this tells us that there is a drug which has oil cavities in it. This is an oil-containing drug.

So, probably something like cinnamon or clove could possibly be there, wherein oil cavities are seen. Now, these features or these microscopic studies are going to aid in, or these microscopic studies will help us in the identification and detection of adulteration. So, say, for example, I'm seeing clove as a drug. Clove by itself, even the powder of it, doesn't contain stone cells, but if it has a stem which is added to it, you will see the same stone cells because the stem has stone cells. Similarly, your bark will never have xylem, but if at all, when I'm putting a bark under a microscope, especially a bark powder, and then I see xylem, there's a good chance that it has been adulterated with a stem part of it. Now, going to the quantitative part of it.

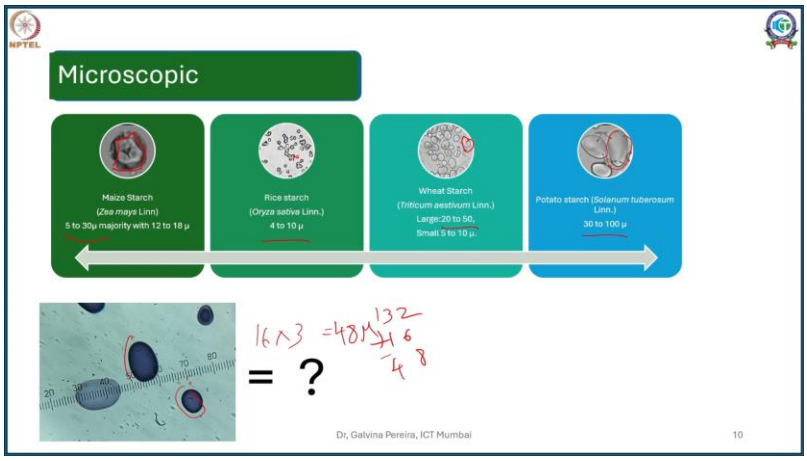
The quantitative part has more to do with the measurements. Here, you can measure, like I said, the calcium oxalate that is there in your rhubarb and arjuna. But depending upon the size, I can tell which drug it is. Similarly, you have other ergastic cell contents such as starch, proteins, resins, and so on. In terms of quantitative microscopy, you have your leaf constants, which will help you identify the drug and the quality of it, and also the lycopodium spore method, which will help you assess the purity of it.

So, let's start with quantitative microscopy. So, in terms of measurements, why are measurements important? So, when we did our carbohydrates chapter, we saw this particular table wherein we said that, you know, your maize starch is polygonal and sized

5 to 30 microns. Your rice starch is polyhedral, sized 4 to 10 microns. Your wheat is oval, sized 22 microns.

You know, 50 microns, and your potato starch is a little larger, sized 30 to 100 microns. Now, you can see what is mounted here, and based on that, what you can guess is, suppose if I say every reading of this is about 3 micrometers or so. You can just multiply it. So, you have like a drug which is more than 16. So, 16 into 3, 32 plus 16.

So, you have your 48. So, 48 is the micron size of this drug. Now, if I see more of this drug, more of this drug includes this starch grain. I'm starting to measure all of them, and the majority of them go in the range of 30 to 100 microns, as opposed to, you know, this was 48. And here it is 50, the max size where I can see the majority of them are either this or bigger than this.



One more thing I observe in microscopy here is that if you see wheat, wheat has striations. That means you see something like this. But here you can see. The striations are circular, which more closely resemble those of potato. So based on the size and appearance, microscopy can tell me that, of these four, there is a good chance this is potato starch.

Similarly, I can measure my calcium oxalates. For example, rosettes of calcium oxalate can be up to 200 microns. The Arjuna ones are slightly smaller in size. In rhubarb, they tend to form slightly larger clusters. In terms of quantity, rhubarb has a lesser amount of calcium oxalate rosettes compared to Arjuna.

So that will help me similarly identify your prismatic forms. I can measure the dimensions of prismatic calcium oxalate in kurchi or acicular raphides, which are needle-like calcium oxalate in squill. And I'll be able to identify what type of drug I'm dealing with. Now, moving on to leaf constants: leaf constants are values obtained by observing certain features on leaves. These features on leaves could include stomata.

They could be your palisades; they could be your simple veins. Based on that, quality control methods were established, and these are not just simple qualitative—these are quantitative methods. Now, a few of these quantitative methods include your stomatal number, stomatal index, vein islet and vein termination number, as well as palisade ratio. So, what are these? Let's just learn them one by one. So, when you focus your leaf under a microscope, you know your leaf breathes via organelles called stomata.

So, this is your stomatal pore, this is your stoma, and these are what are called epidermal cells. So, the stomatal index for a particular leaf is that value which indicates the ratio—or actually the proportion—of stomata to the total number of cells. Now, this total number of cells includes stomata as well as your epidermal cells. So, the stomatal index is actually a ratio that indicates the proportion of epidermal cells in that leaf which are actually the stomata, so it is stomata to the total number of cells

And what are the cells? Cells are actually epidermal cells plus stomata. Now, why is stomata counted again? Because they consider that stomata have originated from the epidermal cells, so each stoma is also counted as one epidermal cell. And when you multiply it by 100, you get a good percentage value.

So the stomatal index is ideally to be measured. This is the leaf. These measurements are done in the middle region, neither close to the vein nor too far from it. So in a region that is clearly focused where veins are not seen. But if you still want to have a good or improved version like the one we have put here, it is better to peel off or remove the epidermis and then mount only the epidermis.

In that case, it will give you clarity. So here are a few values you can see. Now, stomata or the stomatal index—before we see the values, you see something like UE or LE. Now, what is that? UE means upper epidermis, and LE means lower epidermis.

Now, why are UE and LE given as different values? So it is seen that the upper epidermis is the epidermis that is actually facing the sun. of a leaf, whereas the lower epidermis is something that is hiding from the sun, not exposed to the sun directly. So what happens is, if you observe the plants carefully, the stomata are more numerous on the lower side because The upper side is exposed to direct heat. There is a good chance of desiccation.

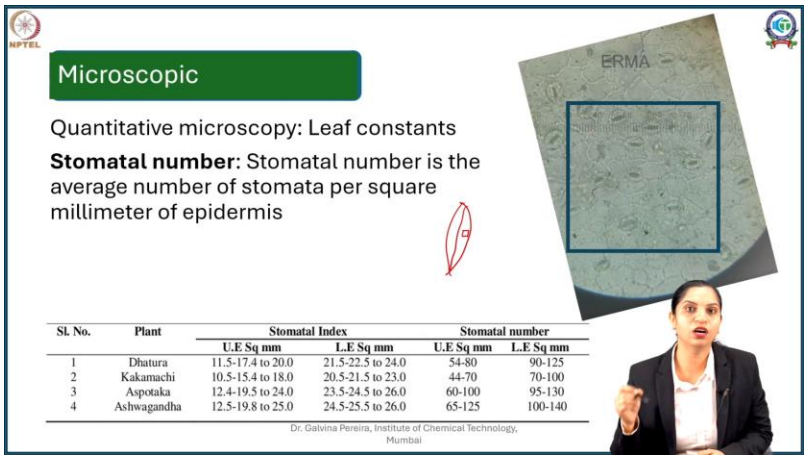
So water will transpire. It will dehydrate the leaf. For that reason, the upper epidermis has very limited stomata. Most stomata are located on the lower epidermis. So now if you compare, you will always see that the LE values,

or lower epidermis values, are much higher than your upper epidermis values. So for Datura, this range is somewhere between 11.5 to 17.4 to 20, depending again on the Datura species—you can see Kakamachi, Aspotaka, or even Ashwagandha. You have fixed values being attached. Now, these values are in a range. Why it is not a single value is because, despite being called a leaf constant, there is bound to be slight variation due to age, geography, climatic conditions, and the plant's own genetic makeup.

It might vary due to certain reasons, and that is why it is always in a range. But if it falls outside this range as well, there is a good chance you are dealing with some other drug. Similarly, for the stomatal index, you have what is called the stomatal number. Now, what is stomatal number? Stomatal index was how many stomata there are per epidermal cell, precisely.

Whereas stomatal number refers to how many stomata there are per square millimeter. So if this is a leaf. The leaf will have numerous stomata on it. As you can see, we have focused on a microscopic view. Now, from that leaf, I cut a 1 mm by 1 mm square.

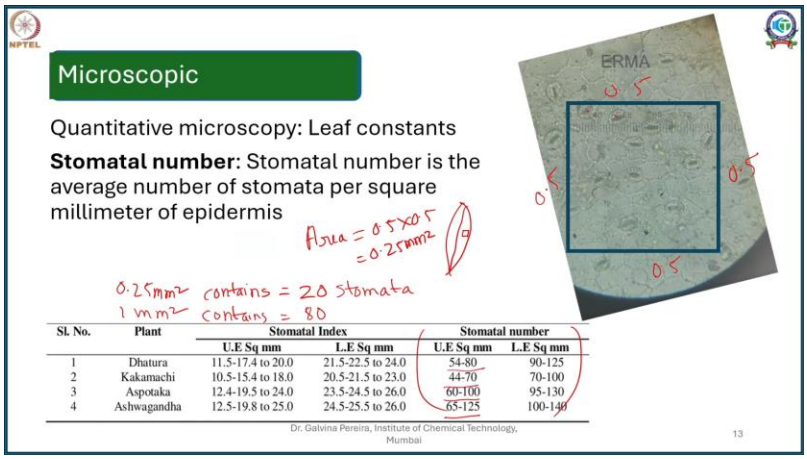
1 mm square. The number of stomata visible in that 1 mm square is referred to as the stomatal number. So how do you do that? That's very simple to do. What you do is use a microscope that has been well calibrated.



You focus on your stomata. Now I have put an arbitrary box. Now, say for example, this box is 0.5 mm by 0.5 mm. And in this case, what is going to happen is, say for example, the number of stomata here is 20. I am just counting them: 1, 2, 3, 4, and so on.

So let's put it as now the area of this is going to be 0.5 into 0.5 is equal to 0.25 mm square. Now this 0.25 mm square contains, I am just putting it for convenience, 20 stomata. So 1 mm square will contain how much? So you know it is going to be 4 times.

That's why I said I am just making it for convenience. It is going to be 20 stomata. So it will be 80. So the stomatal number of this leaf, if it has 20 stomata inside, is going to be 80. per mm square. So similarly, you have these values for Datura. Now you see these values, that is, your stomatal number for Datura is somewhere between 54 to 80. For your Kakamachi, it's 44 to 70. Aspotaka is between 60 to 100, and Ashwagandha is between 65 to 125. So it varies from plant to plant. There will be an overlapping region, but the range is slightly broad and varies.



Now again, you will see the upper epidermis and lower epidermis, and as predicted, since the number of stomata is more on the lower epidermis, this number is going to be higher on the lower epidermis and lower on the upper epidermis. So here you will see on the lower epidermis for Datura, it is 90 to 125. You have your Kakamachi at 70 to 100, Aspotaka at about 90 to 130, and Ashwagandha at 100 to 140. So that is how you do your stomatal number. Now moving on to the next leaf constant, we have what is called vein islet and vein termination.

So whenever you see leaves, you have something called venation. some of you might have even seen those people leaves where people dry and just take a fine mesh or make a fine mesh of that venation so this venation what happens is this veins further subdivide form a meshwork where in every cell should get its nutrition like how in us the blood vessel network is so good that every blood every cell in our body receives blood similar way the veins are connected to all the cells in the plant and they form a venous network and this venous network sometimes creates what is called as vein islet now vein islet is a condition wherein Say for example this veins are going to be surrounding this much area throughout. Now this area is surrounded from all side by vein.

We call it as an island area or an islet area. So this is called as vein islet. Then what is vein termination? In certain cases now inside this what will happen is To get the nutrition inside this there will be a vein going inside it and it will end out here.

This part is what is called as vein termination. So my vein ends there. So vein islets are island like regions created by veins. So you can see you can create islets and then these are terminations. So there are terminations which will supply inside those islets as well.

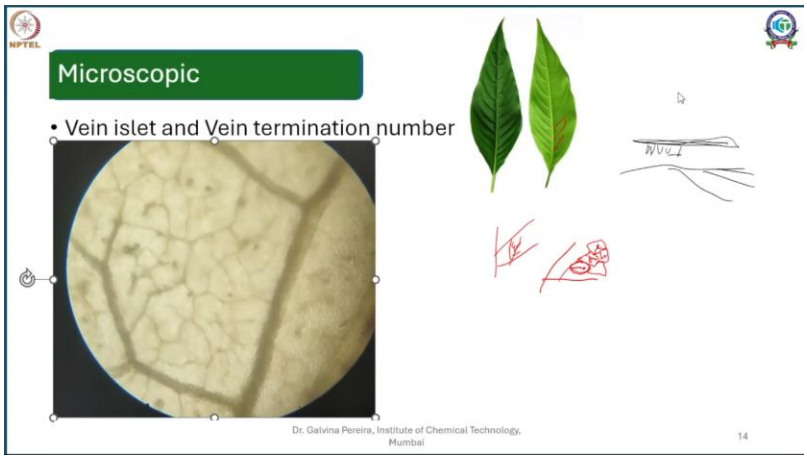
So let's see a microscopic video of that and that will make it more clear for you. So this is the Senna leaflet focused under 10X. That is eyepiece 10X and your objective 10X. So this is seen under 100X magnification and this is your senna leaflet. Now as we are focusing, the first layer that you will see is the epidermis.

After epidermis, you will see a little layer of palisade which is not clear because it's just under a very low magnification and after that are your actual veins. So you see an initial epidermal tissue, then little bit of your palisade and then you see a venation. So let's see it once again. So you can see I'll just stop at the point when we start seeing the stomata. So you can see here there is a faint epidermal texture to it.

So these are all your epidermal cells. And you can start because this leaf has been clarified. It has been made transparent. The venation is visible. But if you mount a fresh green leaf, no way it is possible to see the vein terminations inside it.

So for this experiment, you require a clarified leaf. So after you focus on the stomata, you go a little deeper. And there you can see some terminations. You can see these are the bigger or the larger veins, and these are the smaller veins. So we will just stop at this point and let's observe it.

So here, what is happening is you can see this whole thing is a vein islet, whereas these are terminations. So you can see here, there is this whole one big islet, and then there are tiny, tiny terminations. So we can see in per millimeter square how many islets are present and how many terminations are present are fixed. Now since these are intermediate, like I said, Here you have your epidermis.



There you have your veins in between, like your cheese slice, and your epidermis is like your bread slices. So whether you see it from the upper side or you see it from the bottom side, your cheese slice is going to appear in a very similar manner. And as a result, you will see here also your venation from the top or from the bottom; it will be the same. And as a result, the value of that will not change. Similarly, your vein terminations per mm square are called your vein termination number.

So you have your vein islet number, which is the number of vein islets surrounding your cells per mm square, and vein termination number, which is how many veins are terminating in that particular 1 mm square area. Another important leaf constant that you can see is the palisade ratio. Now, as we discussed, when you are observing palisades, here you have your epidermis. Below the epidermis, there are layers of palisade cells. Now, this is a transverse section view.

Then we see here are your veins. This is the mesophyll region. Again, you will have your palisade cells here. And your lower epidermis. Now, each of these epidermis layers will have epidermal cells as well as stomata.

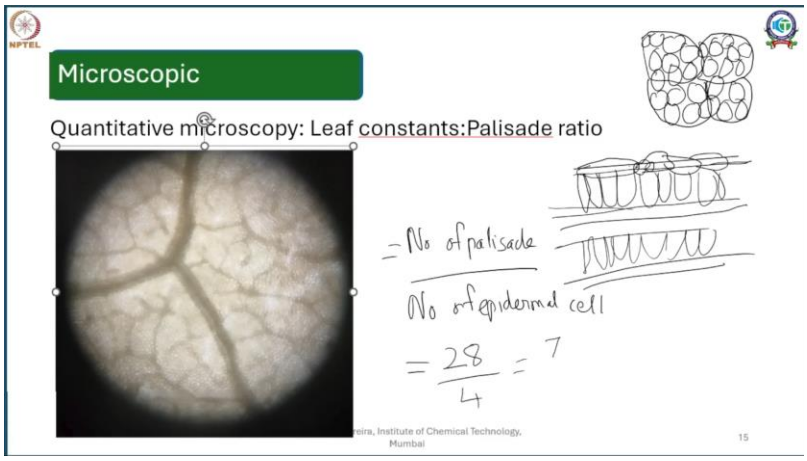
So all your epidermal cells are located here. Now, what is the palisade ratio? The palisade ratio is actually the ratio of the number of palisade cells to the number of epidermal cells. So it is the ratio of the number of palisade cells beneath each epidermal cell. Now, if I just change this view for convenience, say, for example, I have about four epidermal cells.

And beneath these 4 epidermal cells, I am just drawing 5, 6, 7, 8 cells. 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26. So, for example, Fill it full: 27, 28. So let's put it as 28 palisade cells and 4 epidermal cells. So I have just made it more rational and easy to calculate.

So you have your 28 cells divided by 4. So you get 7. So your palisade ratio is 7. So let's see this under the microscope now. So this is again your focused leaf.

And you can see here these tiny circular matrices are your palisades. So here you have your epidermal cells. These epidermal cells—I just focused it further. A little down. Here.

As I go a little down, I can see palisade cells, and if I go further down, I will see the venation. So, this is because all three of these cannot be brought into the same focus. What needs to be done is you capture how many epidermal cells, then you capture how many palisade cells, you superimpose the images, and then what you get is the ratio of palisade, that is your palisade ratio. So, this is how it is determined. The third microscopic method Which is also used for quantitative microscopy is the lycopodium spore method.



Now, this lycopodium spore method uses lycopodium, which is a spore. For a commonly occurring club moss, which is found near springs or river springs. So, these are well-defined particles. They are used as a standard. The reason being they are uniform in thickness, almost close to a 25-micron size, and the characteristic number of particles per milligram is also fixed.

So, since these two are very fixed quantities, traditionally it was used to assess purity. Say, suppose I know that about You know, 1 mg of this has, for example, 94,000 characteristic particles. So, I say 1 mg of lycopodium is equal to 94,000 spores. So, this quantity was fixed, and this quantity will remain fixed.

So if I know my 1 mg of pyrethrum is going to give me 1000 pollens. I can use this ratio to check the purity. So if I weigh 1 mg of my lycopodium, I weigh 1 mg of my pyrethrum. For every 94,000 spores I see, I should be able to find the 1000 amount of pollens. Now you will ask, microscopically, am I supposed to count 94,000?

No. The reason is, in the formula, it becomes easy. If I use the same weight, For every 94,000 particles, I should get 1000 particles of pyrethrum. What does that also imply?

For every 94 particles of my lycopodium, I should get 1 particle or 1 pollen of pyrethrum. Similarly, wheat starch or ginger starch, some particles which are fixed for a mg of substance, I can use it in terms of ratio. Then I can dilute it and quantify it. So this method is used for particles which have a single-layer thickness or uniform thickness, or for particles which have a characteristic number of contents, such as starch or fibers per milligram of substance. So let's see it in terms of microscopy.

Say, for example, I am quantifying the purity of ginger. For ginger, I know each milligram of ginger gives me almost 286,000 starch grains. And 1 milligram of my lycopodium, as we discussed, gives me 94,000. So, I am going to mix both of them. Take some glycerin, and I am going to count per field.

Now, I will not count all 94,000. I will just count in 25 views and total it. So, after 25 views, I will total all the lycopodium particles that I came across and all the starch grains that I came across. And then, I just have to put it in this formula. Here, you have your N multiplied by W multiplied by 94,000, which is the characteristic number of particles of your lycopodium, and multiplied by 100, divided by SMP.

Now, what is this? This N is actually the number of characteristic structures. So, this is for the starch. Whereas this is for the lycopodium. So, how many starch grains do I see in 25 views?

How much lycopodium have I seen in 25 years? Again, this is the weight of lycopodium taken. This is the weight of your ginger taken. These are the characteristic particles of your lycopodium. These are the characteristic particles of your ginger.

So, if I put it in this formula, I get exactly the percentage purity of the sample. So, all in all, in this session, we have discussed mostly the microscopic and macroscopic methods for the evaluation of drugs. This includes your leaf constants, lycopodium methods, organoleptic evaluation, as well as microscopic evaluation. So here are a few references if you wish to learn more about these particular techniques, and thank you everyone for your patient listening.