PHARMACOGNOSY AND PHYTOCHEMISTRY

Dr. Galvina Pereira

Department Of Pharmaceutical Science and Technology

Institute Of Chemical Technology Mumbai

Week 4

Lecture 18

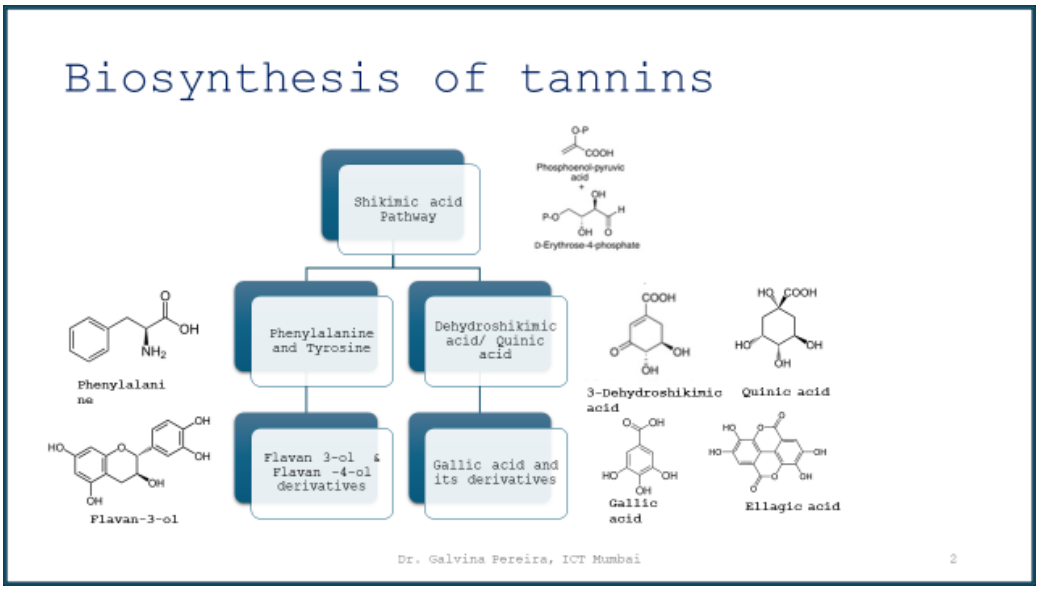
Biosynthesis, Distribution of tannins

Hello everyone, and welcome back to the NPTEL course on pharmacognosy and phytochemistry. In the previous sessions, we have seen what tannins are and the different classes of tannins. How do we check whether tannins are present in a given solution? In today's session, we will go through a few examples of tannin-containing drugs. We will understand the biosynthesis and also the applications of tannins.

So, tannins are biosynthesized in nature from a pathway called the shikimic acid pathway. Now, this pathway is unique to plants and microorganisms and does not occur in human beings. The shikimic acid pathway starts with two intermediates: the first one being phosphoenolpyruvic acid, which has its genesis in the Embden-Meyerhof pathway, and the second, D-erythrose 4-phosphate,

which has its genesis in the hexose monophosphate shunt. Now, phosphoenolpyruvic acid is a 3-carbon acid, whereas erythrose 4-phosphate is a 4-carbon compound. So, this 3- plus 4-carbon compound together find of cyclizes in themselves to give you a seven carbon compounds

which are called 3- dehydroshikmic acid and quinic acid. Tannins have their genesis from these key intermediates, principally quinic acid. So, if you see here, in quinic acid, when this hydroxyl group is removed and you have an aromatization of this ring, the structure mimics gallic acid.



Whereas in the previous session, we saw that two gallic acids mutually undergo a lactonization reaction to form what is called as ellagic acid. Now this gallic acid and ellagic acid are hydrolyzable tannins and have their genesis from dehydroshikmic and quinic acid. Now when we go to the condensed tannins you can see here the shikmik acid pathway also leads to amino acids called as phenylalanine, tyrosine and tryptophan.

So let's take a case of phenylalanine. When you see a structure of phenylalanine, you can see here this is essentially a 6 carbon moiety. That is a phenyl moiety attached to 1, 2, 3. So, this class of compounds phenylalanine gives rise to are called as phenyl propanoids that is phenyl plus propane. In addition to that.

Apart from phenylalanine, phenylalanine and tyrosine just differ in the presence of hydroxy groups. So when a hydroxy group is added, you get tyrosine. Phenylalanine and tyrosine both give rise to phenylpropanoid moieties. Now, how does that go ahead? So if you see the structure of condensed tannins, I can just bring you the same molecule.

One, two, three. 4, 5, 6. So, this is phenyl and 1, 2, 3. So, this particular part of flavan-3-ol has their genesis from phenylalanine or tyrosine. Whereas this part, which is the other six-carbon moiety, has its genesis or biosynthesis from

acetyl-CoA as a biosynthetic intermediate. So, you can imagine that three molecules of acetyl-CoA. This is one. Acetyl-CoA has two carbons. So, one, two.

So, three acetyl-CoA molecules cyclize and then join this phenylpropanoid to give you a flavan-3-ol nucleus. Now, this flavan-3-ol nucleus undergoes mutual polymerization to form proanthocyanidins. We often refer to these as condensed tannins, the flavanol types. So, flavonoid-type condensed tannins have their genesis coming from the shikimic acid pathway through phenylalanine or tyrosine as key intermediates.

You can see tannins occur widely in nature. The reason is tannins are defensive molecules. They have protein-precipitating properties. As a result, they are very good antimicrobial agents. So they prevent infections in plants.

So you will see them principally in the bark of plants, where they give you a deep brownish coloration. Those are called your phlobaphenes and phlobatannins. They occur in cinchona, arjuna, acacia, oak, wild cherry, and numerous other barks. If you see the brownish coloration of the bark, that is principally attributed to the presence of tannins. Now when you go to the leaves, they may not be brown in color.

The reason being the molecular weight here is slightly less. The nature of condensation and the nature of tannins present vary. So in leaves, you can attribute it to a slightly astringent taste. So when you drink your tea, which is a decoction, when you have your hamamelis leaf, or when you taste your guava leaf, you get that

slight astringency, which is attributed to the presence of tannins. In fruits, you can have a tart or an astringent taste. Which is given by amla. Pomegranate peels specifically. And berries such as strawberry and raspberry.

In seeds you can see them in coffee. When you drink coffee you know it's astringent. It has a good pigment value. Cola. Now this cola seeds are also traditionally used to prepare your coca cola.

So those cola nitida or cola seeds. You have your buckwheat which is known as kuttu or kutti when you say your buckwheat or *phagopyrum esculantum*. Your supari or beetle nut are few examples of seeds containing tannins. In fruits we have amla which is your Indian gooseberry.

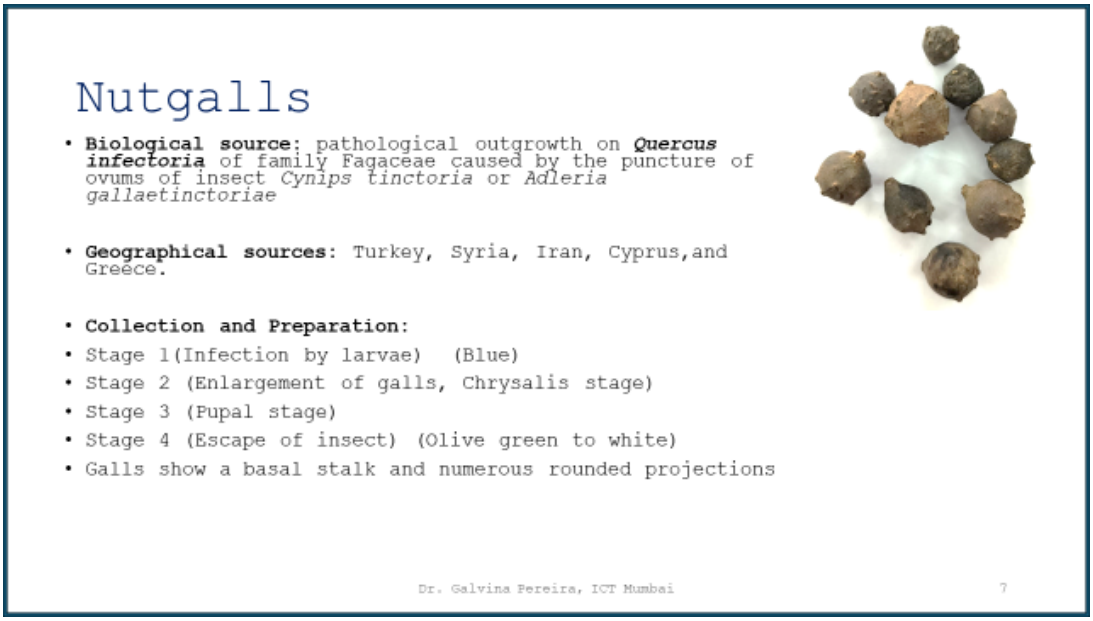
We have harada and behda. All three of these fruits put together come in an Ayurvedic formulation. We call it as Triphala churna. Triphala means three fruits. The three fruits of Triphala are your amla, harda and behda which are rich in tannins which are used to treat GI disorders.

Certain extracts or concentrates are also rich in tannins. You can take the example of your pale and dark catechu. The black catechu is nothing but your katha that you apply on paan and kino, which is your *pterocarpus marsupium* plant extract. Let's go to a few examples. Now we'll study detailed examples of drugs containing tannins.

We're going to go into the details of nut gall, amla, pomegranate peel, myrobalans, especially your harda and behda, and catechu, the pale and black one. If you see the classification of this, the hydrolyzable tannin-containing drugs in this are your nut galls, amla, pomegranate, and myrobalan. All of these contain tannin derivatives

which are chiefly gallotannins or ellagitannins, which can be easily hydrolyzed by the enzyme tannase and hence come in the category of hydrolyzable. Whereas, if you see condensed tannins, in that category we have a few examples such as your pale catechu and black catechu. Now let's start with nut gall as an example.

So what is nut gall? So you can see this circular object. That's a nut gall. That's actually a pathological object. Growth like a tumorous growth, which is found on a very young twig of what is called Dyer's oak.



Now, Dyer's oak, botanically, is *Quercus infectoria* or referred to as the oak tree. Now, this oak tree is a plant that grows up to two meters in height, and what happens here is you will see tiny insects. Now, these insects lay their eggs. The insects that lay their eggs on this oak are Synapse Tinctoria and Adleria Gallatingtoria. So when these insects lay their eggs, they give rise to tumorous outgrowths.

They are called galls. And those are then dried and separated. Now, geographically, this oak tree is found in Turkey, and Turkish galls are the most common ones in the market. But they're also found in Syria, Iran, Cyprus, and Greece. Apart from that, if you see the nut galls market, you also come across certain Japanese and Chinese nut galls.

So what are they? We'll discuss this in the later part of the section. But let's just try to understand what galls are. So when this Dyer's oak or *Quercus infectoria*, this plant is very susceptible to the insects. So they will lay their eggs, and for the nutrition, they tend to lay their eggs on the twig.

That is the young part because the young part is more permeable. to the eggs. So as the eggs mature and little larvae come out, the larvae start infecting and feeding on these young twigs. Now, when they start feeding on these young twigs, what happens is that in those

young twigs, you will have starch, and this starch is utilized by these insects through the production of something like an amylase.

The starch is converted into glucose. Now, when the plant senses more and more glucose is being produced in a particular cell by hydrolysis of starch, in that region, a pathology changes and abnormal cell division happens, something like a tumorous outgrowth. So this outgrowth continues, and what happens is as the insect is still consuming these juices, which are rich in glucose,

the insect tends to get entrapped inside the galls. So you have an insect in the center and a tumor-like gall growing around it. Slowly, initially at the stage of infection, which we call stage one, you will see that this outgrowth is blue or slightly bluish-green in coloration. and it starts maturing. It starts enlarging, and as it starts enlarging, you can see here there are certain projections also coming out. So this is the basal stalk—stalk

means the place from which it is attached to the plant. You can see some stalk here. So there's the stalk region. These are all stalk regions. And then you can see numerous circular projections. So here, the irregular outgrowth of the tumor starts.

All in all, if you see *Quercus infectoria* galls, they are generally circular in nature. So they will start growing, enlarging till you get a stage 2, which is called your chrysalis stage. In this, the insect inside is in the chrysalis stage, and you will see the color is still blue or bluish-green in coloration. Now, stage 3 comes in where your insect still enlarges and goes to a pupal stage. At this pupal stage, it creates and starts digging in the cavity.

The reason being, it now wants to escape from this gall. It no longer wants to remain encased in it and starts boring numerous cavities inside the outgrowth. Now, slowly, what will happen is the insect succeeds in boring a cavity, and once it does that, it can escape out of the gall. Once it escapes, you will see some cavities occurring.

So during the gradation stage, we can say that initially the gall is blue. Later on, when the insect is mature inside, you will see it growing to slightly olive green. And later on, when the insect escapes by making a cavity, it slowly starts becoming grayish or whitish in

appearance. Now, when you see the pathology of it, initially what happened was you had a lot of starch.

This starch was converted into glucose. Then this glucose is utilized by the insect. But when the plant senses it, it converts and prepares glucose. A lot of derivatives or secondary metabolites, we refer to as tannins. So tannins start accumulating in an attempt to kill the insect.

So the inner layer of this gall becomes very rich in tannins. What develops or what deposits inside this is your gallic acid. Chiefly, this gallic acid goes on polymerizing. So you can see here. I'll just show you with a pen.

So you can see here; this is our gallic acid. Esterified with another molecule of gallic acid, and these are all attached to a central glucose moiety. So all these glucose moieties, which the insect has produced, are now esterified with the help of tannins, especially gallic acid, so you get a lot of gallotannic acid. If you see the chemical composition of galls, approximately 50 to 70 percent of the chemical composition of gall is chiefly gallotannic acid.

Then you have a few monomeric gallic acids, up to 2 to 4%. Ellagic acid, starch along with it. Inside the fruit, you will have deposits of rosette to prismatic calcium oxalate and very characteristic lignin bodies because they are very hard. Stiff lignin starts depositing in them, and you can easily identify that by staining it with Phloroglucinol water. So if you see the appearance, what is going to happen is you can go—this is like a stalk of it.

Numerous projections come out, and if you see the internal cavity, this internal cavity is a large layer of parenchyma. So you have a parenchymatous cell layer out here, which is thin-walled, and then you might have a single or double layer of sclerenchymatous tissue. Inside the sclerenchymatous tissue is another inner layer of parenchymatous tissue. So I'm just putting here as sclerenchyma and another layer of parenchyma,

but the internal parenchymatous layers are thick-walled and they contain a lot of this starch, a lot of tannins, and this cavity. Now, within this cavity, you might have your insect. If the

insect has not escaped or if the insect has escaped, it appears like an empty cavity here. A few other compounds also start to be present here. They are little lipophilic ingredients,

that is, your betulic acid methyl ester and your oleanolic acid methyl ester. So methyl betulate and methyl oleanolate are present along with sitosterol. Now, since the plant is undergoing pathogenesis, it will secrete acidic compounds to get rid of the insect. A few of these acidic compounds which the insect secretes are your nyctanthic acid, rubric acid, and syringic acid.

So lot of these are present. So if you want to check if the galls are genuine or not, what you can do is just take this galls and dip it in water. The first thing is they should sink. Second thing, if you cut the galls, you should see a cavity inside. And if you add a ferric chloride or any reagent to check for tannins, it should give you a tannin positive test.

Now this tannins. in galls chiefly are used for pharmaceutical purpose so in market you will get two type of galls turkish galls and i said earlier you have chinese or the japanese galls the difference between them is the host plant in your turkish galls the host plant is quercus infectoria

and the insect which generally causes this pathology is your *adlerian galtingtoria* or synapse tinctoria Whereas in Chinese galls it is rust chinensis the plant which is generally infected by aphids that is *Schlechtendelia chinensis*. Now in terms of chemical differences here you will find the tannic acid derivative mostly as hepta or hexagalloyl glucose and in this case you will find mostly as octa or nonagalloyl glucose. Now tannic acid is a molecule which has no fixed structure.

You can attach as many gallic acid as possible. So in the market you get numerous tannic acid derivatives varying in their molecular weight. So by the nature of tannic acid you can come to know what is the source of this tannic acid. If you see the appearance of Turkish galla, they are more smooth and circular. The photos which I showed you previously were Turkish galls.

Whereas the Chinese galls are little irregular, naughty. That is they show not like appearance or bumps. Irregular and they are soft. That is they break easily. Whereas the Turkish ones are little stiff in nature.

Talking about the applications, the main applications of the galls happen in the pharmaceutical industry because they give you a good quality compound called tannic acid. Now, this tannic acid has strong astringent properties. This strong astringent properties will help in protein precipitation and hence it can take care of your wounds. It will close the wounds, stop bleeding, stop oozing.

So it can be used as a styptic. It can be used to treat minor ulcers, even in hemorrhoids or bleeding hemorrhoids. They are given as suppositories to, you know, kind of seal the skin and stop the bleeding. Now, interestingly, one more attribute of tannins is their ability to chelate metals. So initially when there was metal poisoning

a tannic acid solution was administered which could easily chelate the metal ions and then they could be removed from the system. Also tannins have an ability to form complexes with nitrogen containing compounds such as proteins and alkaloids. So even in the case of alkaloidal poisoning, you will see that nut galls are beneficial as they tend to form complexes and precipitates. Now, this has been discontinued lately.

The reason being that it was found out that despite forming metal complexes, some amount of this tannin still gets metabolized in our gut and gets absorbed into our system. Once they get absorbed into our system, they were found to have some adverse effects associated with the liver, and that was not observed.

And as a result, what you see now is their declined use in systemic applications. But in terms of topical applications, they are used for mouth ulcers, bed sores, topical creams, and suppositories. Yes. And to a minor extent, they are used in tanning leather. Not the Turkish galls, but the Chinese galls have also been used in tanning leather and dyeing.

Now, along with it, numerous allied drugs are present. We discussed the Chinese and Japanese galls. Then there are crowned Aleppo galls. The interesting feature of this is that

rather than those projections occurring everywhere, they occur at the apex. That is the top of the fruit.

And that is the reason they give you an appearance like a crown. And that's the reason they are called crown galls. And another type is Hungarian galls, which are now more commonly cultivated in Yugoslavia. They also contain or are rich in tannins and are an important source of tannin-containing drugs. So, we move on to the next drug, the Indian gooseberry, also referred to as amla. This is a common plant found in

India. It consists of a fruit. It can be used fresh or, you know, even as dried fruit in India, where it is coated or laced with sugar as a mukhwas. The plant was previously known as *Phyllanthus emblica*.

Now it is known as *Emblica officinalis*, belonging to the Euphorbiaceae family. This is a plant you can identify by its occurrence of pinnately compound leaves, with tiny leaflets that occur in pairs. and these are little tiny leaflets which occur in pairs. You can also see an insect coming out there. This plant is native to the deciduous forests of India, Sri Lanka, and Myanmar.

This plant is commonly cultivated, but the majority of the harvest still comes from widely grown plants. So cultivation is done by seeds, cuttings, or budding. This plant produces separate male and female flowers. It is observed that male flowers generally occur in the leaf axils, whereas female flowers mostly occur on the stem.

So male flowers occur as groups or bunches whereas the female flowers occur as solitary or single and once they fertilize the fertilization rate is about like 30 to 40 percent you will see a amla mostly occurring here somewhere because the female flowers are located here on the stem axis now the flowering season is generally summers and post that the fruiting takes a little while

about a three months time so you get that in winter seasons so chemically amla is a very rich source of vitamin c it is said that amla contains almost 160 times vitamin c as compared to your apples by weight it contains almost more than 30 percent 30 to 40 percent sugars about 14 percent gum and cellulosic that is a good source of fiber

In terms of a tannin content, it contains chiefly gallic acid. You remember the video we saw in the previous session where we found a blue color complex with ferric chloride that was the amla solution. So it gives you a nice bluish color complex owing to the presence of high content of gallic acid. It contains ellagic acid and also phylambin as tannins.

It also contains alkaloid that is nitrogen containing compounds such as phylantidine. and phyllanthin. Now amla is commonly taken for diuresis. That is you know whenever your body swells or there is a water retention in your body consuming amla helps you to get rid of water especially through urine and that's the reason it's called as a diuretic.

It's cooling and for a body now having a rich tannin content it can address hemorrhage that is bleeding and it's also used in diarrhea because of its antimicrobial activity we saw that it's protein precipitating and it's because of its protein precipitating activity it binds to microorganisms and can kind of effectively

So in Indian traditional medicine, especially in the Ayurvedic medicine, you can see Amla being used in Triphala Churna, which is there for GI disorders. And it is also an immune booster because of its vitamin C content. And that's the reason you find it being used in Chavanprash. So moving on to the next drug.

The next drug is a very simple pomegranate peel. Now, we all have consumed pomegranate, but just in case, when we take a little bite of this peel or those feathery remains of the fruit, you get a slightly bitter taste, and that's attributed to the presence of polyphenolic compounds, especially tannins. So, pomegranate peel is medicinal.

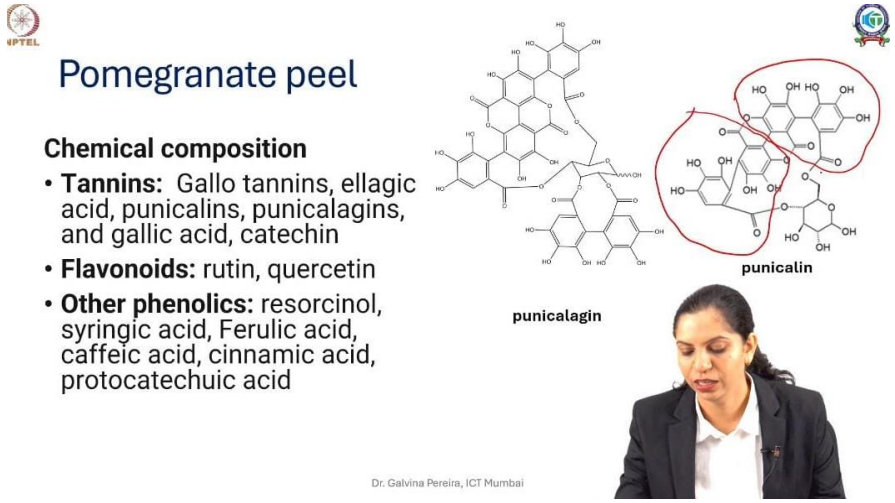
It consists of dried peels of *Punica granatum*. That's your pomegranate, belonging to the family Punicaceae. Now, this is a tropical plant that grows in the Middle East, extensively in India, and in the Mediterranean region, where it is commonly consumed. Now, this is a plant that produces fruit or flowers throughout the year.

But what farmers do is cultivate it in a way that you get seasonal yields. So, you can curtail water supply to the plant, and in that way, whenever you want flowering, you could, you know, give it abundant fertilizer, provide ample water, and you get three seasons of it. In India, we call it Ambe Bahar, which is the summer flowering season, generally occurring

close to summer. January or February. Now, when it flowers in this season, the fruiting generally happens in summer,

and that's the one that gives you the highest yield, so this is preferred by farmers all over India. The next one is Mrug, or the little rainy season, when the flowering season is in June or July, and the fruiting generally occurs in September. The Hastabahar is generally post-rainy season flowering, and the fruiting happens much later. So, of the three, the summer flowering is much preferred due to increased yields.

Once in the juice industry, the internal fruit—that is, the pulpy seeds—are removed. The peel is a waste product. Now, this waste product has been valued for its tannin content. So it contains two important tannin derivatives. What you can see here is Punicalin.



Now, Punicalin is a dimer. Now, just carefully see, I am kind of dissecting this. But you can see two molecules of hexahydroxy diphenic acid. So, these two molecules of hexahydroxy diphenic acid form ester linkages with a sugar moiety to give you punicalin.

Now, this punicalin further adds one more molecule of hexahydroxy diphenic acid to give you punicalagin. So, punicalin and punicalagin are the chief tannin derivatives found in pomegranate. They further polymerize to give you large molecular weight compounds also. So, in pomegranate peel, you will get your gallotannins, you will get your ellagitannins, ellagic acid, gallic acid, punicalin, and punicalagin.

So, apart from that, you get flavonoids such as rutin and quercetin, and it is rich in acids such as ferulic acid, syringic, caffeic, turmeric, and protocatechuic acid, which are also antioxidants. So, this pomegranate peel, if you take it and boil it in water, it's a very rich elixir of antioxidant compounds. And that's the reason it has numerous applications. People have been taking pomegranates.

Pomegranate peel extracts for its anti-diarrheal effect for its wound healing effect because it's a tannin rich one because it's rich in antioxidants it is known to be good for heart it is anti-inflammatory cocktail you can say. Tannins make it a very good antimicrobial preparation and lot of studies have now been focused towards anti-cancer effect. So pomegranate peel extract nowadays is also proving as a good aid for people with cancers

because this provides antioxidant compounds and decreases what are called as free radicals in the body. Now this one more interesting finding about pomegranate is once this pomegranate peel extract is consumed it is converted by our gut microbiomes in a compound called as urolithins. I just have one structure here and that's the structure of your urolithin A but numerous such urolithins have been produced. You can see it is chiefly if you just put a ring to it you can

Imagine your ellagic acid. So it's just kind of a derivative produced by microorganisms coming out of ellagic acid metabolism. Now, this urolithin, interestingly, has good. amount of mitocytic activity like you know in cases of a malfunctioning mitochondria it undergoes you know it pushes it to undergo a phagy and your mitochondria which are unwanted which are not working well

are done away with by the body you can have a anti-aging property because of this One more thing is urolithin is also linked to production of energy. So in people, those who want to slim, it is a good source of burning. ATP molecules and a good energy burner. So it gives you improved muscle strength.

It helps in weight management. The antioxidant property of urolithin is also linked to a protective effect on the brain, especially in people with Parkinsonism, who have been administered ellagic acids and urolithin. So if you see or go to some common e-websites or e-retail sites, you can see a number of urolithin supplements that have

come out. But a natural source of urolithin is just taking a concoction or a peel extract of pomegranate that has all the goodness of it. The pomegranate peel has also ventured into the cosmetic industry because of its very good antioxidant content. It is known for its anti-aging effects. It is known as a sunscreen agent because of the flavonoids and derivatives it carries.

It also has other tannin derivatives, which decrease acne. The microorganisms responsible for the production of acne are controlled or curtailed with it. So that's also one way to address it. It also addresses the issue of hyperpigmentation, and that's how your pomegranate peel is gaining popularity. You can find in your markets face sheets, face masks, or products containing pomegranate extracts

that are loaded with antioxidants. So, these are a few examples that we will cover. In the next sessions, we will be covering catechu and myrobalan. So, thank you, everyone, and here are a few references for extensive reading on this topic. Thank you once again.