

PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 2

Lecture 9

Week 2: Lecture 9: Examples of lipid containing drugs karanja oil, wool fat, bees wax & carnauba wax

Hello everyone, and welcome to the NPTEL course in Pharmacognosy and Phytochemistry. In the previous session, we learned about different lipid drugs containing fixed oils. In this session, we will continue to see a few more examples of drugs containing lipids. In this session, we will be learning about a few drugs such as Karanja oil, wool fat, beeswax, and carnauba wax. To start with, let's discuss Karanja oil.

Karanja oil is actually obtained from the expression of kernels of *Pongamia pinnata*, often referred to as *Millettia pinnata*, a plant which belongs to the family Leguminosae. This plant is indigenous to the East Indies and grows in India as well as the Philippines. It grows abundantly in Maharashtra and Karnataka, from where Karanja oil is chiefly produced in India. If you see the tree, it is a medium-sized plant bearing white flowers and green-colored pods. These pods often mature and turn tan-colored, with a slightly elliptic, flattened shape.

Now, when you open these, you see a kernel inside. These kernels are basically what are used for the preparation of Karanja oil. So, these kernels are tiny and may be present as one or two per pod. What is done is these kernels are taken. Thrashed from the trees once they fall down, they are beaten with either sticks or hammers to crush them open and expose the kernels. So, you will see some kernels like this coming out. These kernels are collected; they have a thin, papery coating.

That is a kernel husk and then a whitish color in the endosperm layer. Now, this also contains the cotyledon. This is then pressed using mechanical expellers to obtain the Karanja oil. Karanja oil is slightly dark in color compared to other oils, with a disagreeable odor, and is mostly considered non-edible. Now, this Karanja contains numerous flavone derivatives, and if you see the structure, they contain mainly furanoflavone derivatives as well as flavones, which are often associated with phenylpropanoids or furan derivatives.

Karanja - Chemical Composition

Chemical Composition:
Flavonoid derivatives:

- Karanjin, pinnatin, pongagalabrone, pongamol, pongapin, and kanjone have been isolated from seeds.
- Soap made from crude oil tends to darken due to a component, Isolonchocarpin, which gives a wine red colour in the presence of alkali.

Lipids: The major lipid components are Oleic acid 71.3% Linoleic acid 10.8%

Karanjin

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Few of these flavonoids include Karanjin, which is the major compound, Pinnatin, Pongagalabrone, pongamol, pongapin, kanjone; all of these have been isolated from the seeds and also make their way into the oil. If you prepare or try preparing soap from the crude oil, because of a special flavonoid called Isolonchocarpin, it appears, or the soap takes on a reddish coloration in the presence of alkalis such as KOH or sodium hydroxide. The major lipid fraction of it is full of oleanolic acid, which makes up about 71.3% of the lipids.

And a small percentage of linoleic and other saturated fatty acids have also been found. Apart from this, a few other pigments and unsaponifiable matter also make up the composition of the Karanja oil. Now, Karanja oil, mostly which is non- expeller-prepared, that is, which is prepared using solvent extraction, goes into making biodiesel, and that's the chief application. But apart from that, Karanja oil or even leaves of the plant have been known for their insecticidal and pesticidal activity. The chief constituent of Karanja oil, Karanjin, is

the compound which is known to possess the insecticidal activity. Now because of its antibacterial activity and emollient activity owing to oleic and linoleic acid. It is used in treatment of eczema, psoriasis, skin ulcers, dandruff and also known to promote wound healing. So it's an oil often externally used for healing purpose and antimicrobial purpose as well as given for skin ailments and because of this it is used in body oil, body salves, lotions, medicated soaps, hair oils, shampoos and even garden sprays to get rid of insects and pests. In Ayurveda, Karanja oil carries a significance that is a small quantity of it mixed with other herbs is given as anthelmintic. In healing of wound, ulcers, itching, A-sides formation or enlargement of spleen or abdomen and in some cases when there are unwanted urinary discharges mostly owing to the infections. It is also reported in traditional medicine to cure biliousness, piles, leukoderma and numerous skin diseases. The other lipid containing drug is we discuss as wool fat.

Now, wool fat is a pseudo fat. But because of its consistency and nature it is referred to as fat. Wool fat is often referred to as hydrous wool fat, lanolin or *adepts lanae*. Now this is something as the name indicates wool fat. It is actually the oleaginous layer which has been deposited on the surface of wool.

So if you take our hair shaft, you know it has a peculiar sheen. And that sheen is due to the presence of some oleaginous lipid or fatty mixture, which is secreted by the sebaceous glands. Similarly, in your sheep, *Ovis aries*, which belongs to the Bovidae family. This oleaginous mixture, which covers the wool, is what goes into making the wool fat. So it contains almost 25 to 30 percent of water, and the remaining composition is made up of lipids.




Wool fat: Preparation and Description

- Wool coverings = 31% wool sweat (K salts of FA) + 32% Earthly matter + 25% wool grease

(crude lanolin)

- Separated by sulphuric acid treatment (CRACKING)/ organic solvent/ soap solution, purified by centrifugation and bleached anhydrous lanolin or wool fat + 30% water = hydrous wool fat

Description

- Colour: whitish yellow; Odour: faint and characteristic; Taste Bland
- Forms ointment-like mass and separates in 2 layers on heating
- Practically insoluble in water and soluble in chloroform and solvent ether, with separation of water

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Now, this is chiefly found in Australia, the US, and India, where sheep rearing is abundant. If you go to see the wool covering or the sheen of the wool, in terms of composition, we can divide it into three parts. The three parts include the wool sweat. Now, what is sweat? When you see your sebaceous glands, they're going to secrete sebum and other fatty acids. As well as your cholesteryl and cholesterol esters.

So this makes up the majority of your wool sweat. So mostly, chiefly, what is here is your fatty acids. These fatty acids form soap with minerals secreted from the sweat, like sodium and potassium. So you have salts of fatty acids, which make up 31%. 32% is about earthly matter.

So it's dirt, grime and filth which coats your wool and then wool grease. Wool grease is the one which is rich in cholesterol and cholesteryl esters and that makes about 25% of the composition. So this is what your wool coverings are made up of and from this we try to make your wool fat. Now how this is done is your wool is taken. Now it is washed with a specially curated detergent.

Now this specially curated detergent will wash off everything that covers the wool. So it will kind of strip the wool of all its protective covering. You know that lipid layer is there to ensure that the wool stays dry and doesn't get moistened with water similar like to our

hair. So this special soap will remove everything out of it. Now, when everything is removed, these washings are collected and you will see that these washings separate to form an emulsion and then there is a top layer.

Now, what is done is to this emulsion and top layer, you add acid. Generally, sulfuric acid is added in this case and the process is called as cracking. What it does is it kind of separates out the fatty acids and other oleaginous matter and it starts floating out. Now, this floating layer or the lipid layer can be separated either by centrifugation or it can be separated by solvent extraction, that is dissolving in an organic solvent. This is done, but once this is done, it still contains certain dirt, grime or colored impurities, which we don't want.

In order to enrich the quality of that, it is bleached with certain bleaching agents, and what you get in the process is called anhydrous lanolin. Now, this is anhydrous because it's obtained from organic solvents. It has already separated water. So, the good part of this is that anhydrous lanolin has the ability to absorb almost 30% of water. So, this 30% water or 25 to 30% water is added, and what you get as a product is called hydrous wool fat.

This is something which is used in cosmetics as well as the pharmaceutical industry. If I have to describe wool fat, it is whitish or yellowish-white in color. It has a faint characteristic odor and a bland taste because it's a lipid generally. Now, this is a very sticky matter. You've seen this in the previous image.

So, this is how it forms. So, it's yellowish, sticky, and viscous. So, if you take it and heat it, it will separate out that 30% water, and you will get two layers. And again, on cooling, you can just stir it, and it will absorb back the water. Now, this is practically insoluble in water when it is anhydrous.

Wool fat

Synonyms: Hydrous wool fat, Lanolin, adeps lanae

Biological source: purified fat-like substance obtained from the wool of the sheep *Ovis aries*, Family: Bovidae
It contains 25 -30% water. It's the secretion of the sebaceous glands of sheep, deposited onto wool

Geographical source: Australia, USA and India

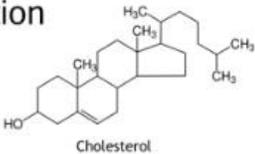


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You need stirring, but once you stir it well, it will absorb almost 30% water. Now this is a good water absorbing cream and it's a very good emollient for that purpose. Being lipid in nature, it is nicely soluble in chloroform, ether and certain organic solvents like benzene and carbon disulfide. Now coming to the chemical composition as we saw it contains fatty acids.

Wool fat –Chemical Composition

- Chemical constituents: esters and polyesters of 33 high mol wt alcohols and 36 fatty acids
- Hydrous wool fat contains esters of cholesterol, isocholesterol with carnaubic, oleic, myristic, palmitic, lanoceric and lanopalmitic acids. 50% water
- Aliphatic alcohols: cetyl, ceryl and carnubyl alcohol (BHT 200 PPM as antioxidant)



Cholesterol



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Now this fatty acids form esters with alcohols and most of this alcohols are cholesterol derivatives. So it can form esters with cholesterol or cholesterol like molecules. So if you see the composition, it's varied, containing thousands of different molecules. But the major ones include about 33 high molecular weight alcohols, which form esters with about 36 high molecular weight fatty acid. And that is the reason I said it's pseudo fat.

Because when we discuss your fixed oils and fats, we said that your fats are triglycerides. But if you see a wool fat, it is not triglyceride or it doesn't form a ester with glycerol in fact it forms ester with cholesterol and that's the reason when you see cholesterol and

ischolesterol esters though it has a fat like consistency it's still a ester derivative apart from that the other acids include your Carnaubic, oleic, myristic, palmitic, lanoceric as well as lanopalmitic acid and in some cases it might even absorb and take into about 50 percent of its weight of water depending upon the composition. Now if you see the aliphatic alcohols it might contain cetyl, sterile and carnaubyl as your aliphatic alcohols.

Now this is prone to rancidity and hence you add what is called as butylated hydroxy toluene about 200 ppm as an antioxidant to prevent it from becoming rancid because once it turns rancid it smells bad and it cannot be used in the cosmetic industry. As you see the standards, it has a melting point almost at body temperature. So at 30, between 34 to 44, it's a liquidity or it has a molten consistency. And that makes it a very nice emollient for our creams because it's going to melt on your skin. Now, acid value, if you see a pharmaceutical grade wool fat, the acid value should not be more than one.

Iodine value is about 18 to 36 in the mid-range and the saponification value is between 90 to 105. This rancidity is a critical parameter, so we check for the peroxide values which should not be more than 20. Now, how do you check for wool fat? Like we discussed, wool fat contains cholesterol and cholesterol derivatives. And one test what we can do for sterols is what is called as Liebermann-Burchard test.

So in this, you can take your sample about 0.5 gram of anhydrous wool fat, dissolve it or disperse it in chloroform till it forms a nice clear solution and add acetic anhydride. This helps in dehydrating And then you add H_2SO_4 . So it forms sulfate or disulfate derivatives of sterols. And slowly, slowly with acetic anhydride, this disulfate derivatives produces a deep green coloration with sterols.

So anything that contains sterols, especially wool fat, will show a green coloration with the Liebermann-Burchard reagent, which is acetic anhydride and sulfuric acid. Now, because of its properties, it is used as a water-absorbable ointment and cream base. It is used in water-soluble creams and cosmetics because it melts on the skin and is easily absorbable. Apart from that, a few official products of wool fat include wool fat BP. This is subjected to hydrogenation, and as a result, it becomes more solid or viscous, and wool alcohol is obtained by saponification of crude lanolin.

Now we move to the next one, which is beeswax. Beeswax comes from bees and is basically a purified wax obtained from honeycombs. That is the house of *Apis mellifera*, or what you call a bee. The Apidae family members all form honeycombs, and what you obtain from them is honey as well as wax. Now, this is generally sourced from France, Italy, Madagascar, West Africa, Jamaica, and India, where you see abundant

apiaries producing honey. This is one of the byproducts of honey-producing industries. So beeswax is actually produced by glands located on the abdominal scales of bees. They are secreted. The bees scrape them off onto their legs, especially the hind legs, transfer them to the middle legs, and then stick them.

It is said that the fresh beeswax is almost colorless, but as it ages, matures, it develops a little yellowness. And that's why most of your natural beeswax have the typically yellow coloration. And it has a typically agreeable odor. And because of the sugars, it has a nice honey like aroma. If you see the solubility because of its high molecular weight, beeswax do not dissolve in water, but it's soluble in hot alcohol, ether, organic solvents, cheaply like your chloroform, carbon tetrachloride, fixed oils, as well as volatile oils.

So if you want a stiffening agent for your creams, you might as well go for beeswax. Now how is it obtained? What is done is those beehives, the combs and cappings, once you are harvesting the honey, are all taken and what is done is the honey is drained. Sometimes the honey is centrifuged so that because of the centrifugal effect all the honey is gone and what is left is just the hive, combs, and cappings which is crushed. Now this crushed comb is kept in a gunny bag

with some weight inside to let that gunny bag sink and it's kept in boiling water now what happens is these wax or lipids as we know they are lighter than water so if you put something heavy in that gunny bag The beeswax in boiling water will melt. Its melting point is close to 65 degrees Celsius. And what happens is it will ooze through that gunny sack. It will melt and reach the top.

It will start floating. So once it boils and oozes out, it starts floating. You can stop the heat as the water cools. All the layer that is floating, as the temperature drops below the melting point (that is below 65 degrees Celsius), your beeswax is going to solidify. So this debris

is initially removed by scraping, and the slab of beeswax is taken. Now, this beeswax, like I said, if it is yellowish in color, is subjected to bleaching to get a good-quality pristine white beeswax. Now, beeswax, in terms of its chemical composition, is made up of higher molecular weight compounds.

Again, if you see, the chief compound or chief component of beeswax is myricyl. Which is made up of myricyl palmitate or often melissyl palmitate. If you see the structure here, it's basically an ester. You have your palmitic acid here, and you have your melissyl alcohol. So this is C30, and this is C16.

Beeswax- Chemical Composition

- Esters of straight chain monohydric alcohols, myricin (myricyl palmitate; melissyl palmitate, $C_{15}H_{31}COOC_{30}H_{61}$),
- Myricyl stearate, cerotic acid (C-26), melissic acid (C-30), and aromatic substance, cerolein
- Indian bees wax: low acid value

$$CH_3(CH_2)_{14}-\overset{\overset{O}{||}}{C}-O-(CH_2)_{29}CH_3$$

myricyl palmitate

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So all in all, you have a C46 compound, which is a huge high molecular weight compound. And that's the reason it is solid. Apart from that, the other components of it are your myricyl stearate. So instead of C16, you will have stearate, which is C18. You have cerotic acid, which is, imagine, a C26 compound.

so and melissic acid which is C30 so imagine you can have this as 18 you can have this as 26 you can have this as 30 and imagine how much the molecular weight the highest would be even C60 which would be making it a very waxy compound so it has all of this and if you see specifically the Indian beeswax it has low acid value Now you can check the beeswax with what is called as claud test. Claud test involves saponifying your beeswax with caustic soda for about 10 minutes. And if you know there's no turbidity, your beeswax forms a clear dispersion or clear solution with that. You know that it's your genuine beeswax sample.

Another thing what you can do or determine by titration is we saw ester value and acid value. The ratio of ester values to acid value of your natural beeswax comes to somewhere between 3.3 to 4.3. Now because of its stiffness and hardness, it is used in ointments as hardening agent. If you want some stiff ointments, not the soft ones, you can use this. It is also component of certain water repellent plasters, polishes.

Traditionally, it has been used to make candles or even moulds. Because of its high melting point. It is also found application in dental and electronic industries. But now a days synthetic waxes are replacing it. Still with the urge of natural it makes its way in lipsticks, face creams or even paraffin ointments.

Despite this, it is adulterated with colophony or synthetic lipids such as paraffin, stearic acid, and those obtained from other plants such as Japan wax, artificial spermaceti, and carnauba wax. But you can easily detect them by checking the solubility. You can do the Claud test or you can do the ratio. That is the ester-to-acid ratio, and you will come to know whether it is genuine or not. The bleached pristine white beeswax is not fit for ophthalmic use.

Going to the next one, and this is carnauba wax. It is obtained from a palm tree called *Copernicia prunifera* or *Copernicia cerifera*. Now, these have palmae-like leaves, and if you see the leaf stems or the petioles part of it, they are covered with a waxy layer. This waxy layer, or this plant, is found chiefly in the South American continent. So what is done is the whole big leaves, like palmae, have big leaves.

So these leaves are cut off, dried, and then threshed. Now what you can do is you can either brush the wax from the surface or you can beat it such that it separates out. So either by brushing or by beating, the waxy layer is separated and then put into a vessel to melt it. It's molten. Whatever impurities are there, they are kind of strained off.

And then this wax is poured into a mold to give you a yellowish-green color wax, which is the carnauba wax. So far, carnauba wax is known to be the wax, you know, which contains huge or high molecular weight compounds. Again, as we know, the waxes are esters. So this wax is chiefly an ester of melissyl cerotate. So you will see cerotic acid, which is C26, and melissyl alcohol, which is your C30, same like your bees.




Carnauba wax - Chemical Composition

- Aliphatic esters contain monocarboxylic acids and monohydric alcohols with a medium chain length of C-26 and C-32, respectively.
- 90% of the esters are ω -hydroxy acids (C-26) and mono alcohols (C-32).
- E.g. carnaubic / cerotic acid (C-26) and melissyl cerotate
- 10% of them are mono-carboxylic acids (C-28) and α, ω -diols (C-30).
- The monomeric units of these esters are diesters of cinnamic acid with mono and polyhydric alcohols, as well as ω -hydroxycarboxylic acids

$$\text{CH}_3(\text{CH}_2)_{24}\text{C}(=\text{O})\text{O}(\text{CH}_2)_{29}\text{CH}_3$$

melissyl cerotate

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So this again, a 46-carbon compound or ester, is the major component of it. Apart from that, you will find In terms of its composition, 90% of the esters contain what are called omega hydroxy acids. So they are there. Then you have your mono alcohols, mostly about C32 ones.

You have your carnaubic and cerotic acids, which are C26 acids. You have melissyl cerotate, which is the ester, the primary component. Now, since the majority of them are your esters of omega hydroxy acids and mono alcohols, some of them are also diols. So in this case, you can imagine instead of this being an alcohol, you can imagine this as a diol that contains hydroxy on alpha as well as omega. Now, when you have diols, especially your C31, imagine this can be as high as 30-carbon ones. Now, these tend to form esters with two acids, so one part can form an ester with cerotic acid, and the second part generally forms esters with your cinnamic acid and other derivatives. So in this carnauba wax, you will see monoesters and diesters also due to the presence of diols.

Now, this is one of the hardest waxes with a melting point of about 82 to 86 degrees Celsius. And because of that, if you see numerous fruits nowadays, people are applying paraffin wax on them, especially on apples. Why? To increase the shelf life and to preserve the food. This is something that has been authorized by the European Union.

You can see it has been given an E number, E903. So, you can use it as a coating agent to increase the shelf life of food, and it is considered safe. In addition to that, it is used for making films. It is used to coat flavors so that they don't volatilize or easily spread out. You can have hydrophobic, biodegradable packaging material.

It has been used in cosmetic products and depilatories because of its stiffness. It is used in roll-ons and deodorant sticks. It is also used for tablet coating when you want a highly hydrophobic coating. It is a part of polishes and automobile waxes. So, these are a few lipid examples that we come across in this chapter.

For further reading, you can refer to these references. And thank you, everyone, for your patient listening.