

PHARMACOGNOSY AND PHYTOCHEMISTRY

Dr. Galvina Pereira

Department of Pharmaceutical Sciences and Technology

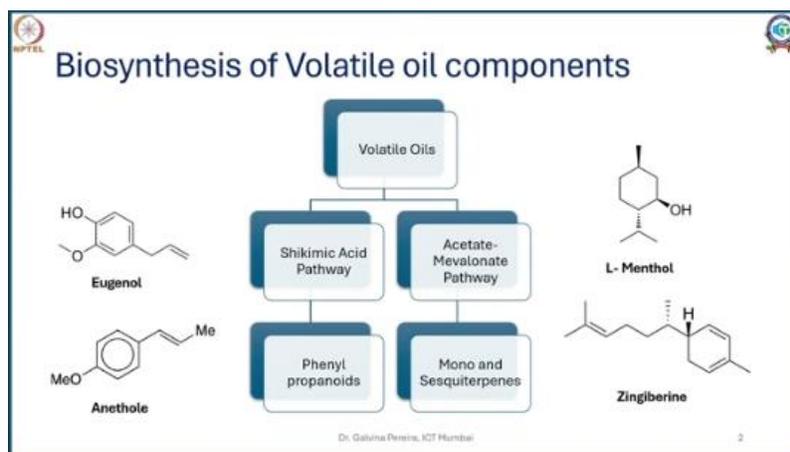
Institute of Chemical Technology, Mumbai

Week 2

Lecture10

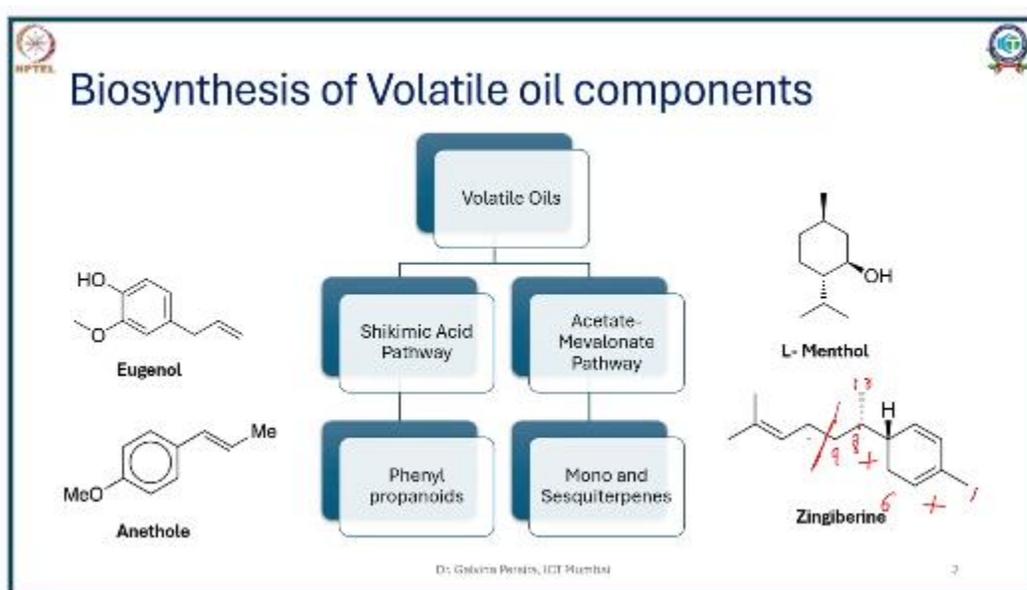
Week 2: Lecture 10: Volatile Oils: Properties, Extraction, Evaluation & Examples

Hello everyone, and welcome to the NPTEL course in Pharmacognosy and Phytochemistry. In the previous unit, we dealt with drugs that contain fixed oils, fats, and waxes. In this particular session, we will deal with a different set of drugs, which are essences or aromatic oils, also known as volatile oils. So, let's delve into the world of volatile oils. Volatile oils are made up of components that are chiefly essential or ethereal or low boiling compared to fixed oils.



If you examine their components, they can be derived from two basic biosynthetic pathways. The first biosynthetic pathway is the shikimic acid pathway. The shikimic acid pathway is responsible for amino acids in plants. It yields tyrosine, phenylalanine, as well as tryptophan. Essentially, these are your aromatic amino acids.

This gives you phenylpropanoid compounds, and a few examples of phenylpropanoids are eugenol, which contains a phenyl group and, as you can see here, 1, 2, 3 propane, as well as anethol. Again, you can see a phenyl group as well as a propane. These phenylpropanoid derivatives—eugenol is present in clove, and anethol is present in fennel fruit. The other pathway is the acetate mevalonate pathway. This pathway, if you recall from biochemistry, is responsible for sterol synthesis and has an intermediate called isopentyl pyrophosphate or dimethylallyl pyrophosphate.



These are C5 compounds, often referred to as hemiterpenes. They dimerize to form C10 compounds, which are called monoterpenes. An example of a monoterpene derivative present in your volatile oil is menthol. As you can see here, the component is a 6-carbon nucleus. And then you have your 7th carbon, and you have your 8th, 9th, and 10th.

So, 7 plus 6 plus 1 plus 3 makes up 10 compounds. So, this C10 is called monoterpenes. If you add one more component of isopentyl pyrophosphate or dimethylallyl pyrophosphate, you get C15 compounds, and C15 compounds are called sesquiterpenes. A good example of an aromatic compound, which is a sesquiterpene, is zingiberene, which is present in ginger. So, as you can see here, this is very similar.

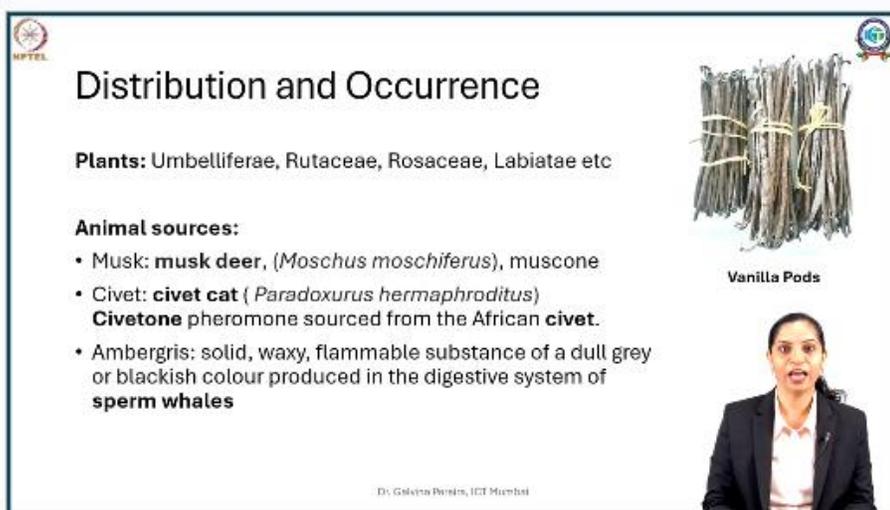
So, if you can cut it from here. It is almost similar to menthol. The only thing menthol had is a non-aromatic six-membered ring. But you can count here. This is your 10th.

So this is 1 plus 6 plus 7. You have your 8th. You have your 9th. You have your 10th. So this is your 10-carbon, which is very similar to your menthol, plus 1, 2, 3, 4, 5.

So 10 plus 5 is your C15, called sesquiterpene. Now, in distribution, you will find aromatic compounds. Now, most of the things we see or feel in nature have some aroma or another. Some of them have a pleasant aroma; some are unpleasant. If you look at pleasant aromas, if you see the distribution of this,

They are generally found in plants in the form of fruits, extracts, or leaves. They have a typical aroma associated with them. So this beautiful aroma can be found in the Apiaceae family, which includes most of your spices: cumin, caraway, fennel, coriander; the Rutaceae family, which includes all your citrus family members; Rosaceae, the floral family members; and Lamiaceae, which is your mint family members. Apart from that, you might have come across something very fragrant, such as vanilla.

Right here is a picture of vanilla, which is a very expensive spice. If you see, it has a compound called vanillin. Coming to animal sources, even animals are associated with aroma. Some of them are prized for it. The ones which are really prized are musk, which comes from the musk deer, *Moschus moschiferus*, and the compound is muscone.



Distribution and Occurrence

Plants: Umbelliferae, Rutaceae, Rosaceae, Labiatae etc

Animal sources:

- Musk: **musk deer**, (*Moschus moschiferus*), muscone
- Civet: **civet cat** (*Paradoxurus hermaphroditus*)
Civetone pheromone sourced from the African **civet**.
- Ambergris: solid, waxy, flammable substance of a dull grey or blackish colour produced in the digestive system of **sperm whales**

Vanilla Pods

D: Galaxya Perinis, IIT Mumbai

Then you have civet, which is obtained from the civet cat, *Paradoxurus hermaphroditus*. Civetone is a pheromone which is present in or sourced from the African civet. Then you

have whale vomit, which is actually vomit but considered very fragrant and aromatic. The more it is aged, the more aromatic it is. And that's ambergris, often referred to as floating gold, obtained from the sea.

It's actually obtained from the digestive systems of sperm whales. Now, the chemistry of volatile oil: we can classify volatile oil into two components—the components that are solid and the components that are liquid. The components that are solid are termed as stearoptenes. This portion or the solids which are dissolved in the oil, owing to their lipid solubility or lipophilicity, as one may call it, generally separate once you start cooling the oil.

So the oil is no longer able to hold it because decrease in temperature will lead to decrease in solubility and the solid components then precipitate out. Mostly there are oxygenated derivatives. A good example of solids which are dissolved in volatile oil are your menthol and thymol. Now, the second component of volatile oil is your eleoptene. Eleoptene are liquids and these liquids are attributed to the aroma component of the volatile oil.

It is generally said that as compared to stearoptene, your eleoptene are more fragrant. And they develop a good flavor. Example of this is from your citrus. You can think of limonene from your clove. You can think of eugenol or when you're consuming coriander fruit, you can think of the linalool flavor that is generally therein.

Now, this volatile oils can be extracted, but their extraction methods differ as compared to your fixed oils and waxes. Fixed oils were generally obtained by expression. fats and waxes were generally obtained by expression and melting sometimes solvent extraction now volatile oils owing to their volatility or owing to their vapor pressure a different set of methods can be employed for purifying it few of them are discussed here first one is steam distillation in this method you can just imagine there is a big pot

And from this pot, you will get steam being generated. Imagine a perforated basket, and on this basket, whatever drugs or leaves—say, for example, mint leaves, eucalyptus leaves—whatever oil you want to obtain, all these leaves are kept here. Now, this vessel is closed so that whatever oil is evaporated along with the steam rises up and is then connected to a condenser. Now, this condenser is thoroughly cooled, and through this condenser—or

sometimes it is supported by a wet cloth— All the steam, which contains volatile oil, is condensed in a vessel.

On cooling, what happens is the steam will separate into a water phase, and the volatile oil will separate into an oil phase. This oil phase is then collected. And that is how you get your steam distillation. In hydro distillation, this water is not kept separate. You can see here your water as well as your drug.

Water as well as your drug is kept as it is. Now, what happens is the same thing. Your steam as well as your essential oil will both rise. They will pass through this, rise up to the condenser, and then come down. Now, as it cools down, it will form water and oil.

So this is the water phase, and this is the oil phase. Now, I am presuming your essential oil is lighter than water. There are exceptions, such as clove, where the oil is heavier than water. But let's study a case where oil is lighter than water. This is what is called a Clevenger apparatus.

So what is going to happen as more and more of the solution condenses? This is going to overflow, and the excess water will be recycled back into the vessel. Over a period of time, more and more oil will be collected, and you will get more oil. Whereas the water will always be recycled back. For oils which are heavier than water, you can remove this bend.

So what happens in this process when this bend is removed? We call it a Dean-Stark assembly. So your heavier oil will settle or remain at the bottom, whereas your water will always recycle from the top. Another method to do it is enfleurage, where you can keep a waxy layer, especially for plants such as delicate petals, rose petals, or flower petals that cannot be exposed to intense or harsh heat.

In that case, you just keep it in a fat layer. You know, like dissolves like, so it will dissolve in the oleaginous matter, including the essence. Rasping involves putting it in a bag or placing it in an assembly where it can be squeezed. Generally, horsehair bags are used for this purpose because horsehair is non-absorbent. So you squeeze it and remove as much oil as possible. In some cases, even sponges are used to absorb it from the layer of the horsehair. Now, pneumatic extraction is a method where you use an oven. In this oven, you

have trays, and on one side of the oven, a hot air fan is applied. As the fan blows, you will see hot air moving, and this hot air will evaporate all the essential oil from the drug.

This is then collected into a collecting assembly and condensed. This condensate, upon cooling, will give you a nice quality oil. Another method, but these are non-specific methods. They might extract components other than volatile oils as well, such as solvent extraction. You can just take your drug, such as cinnamon or clove, dip it in petrol or any lipophilic solvent to dissolve the essential oil.

But it will also dissolve other lipophilic components, and then slowly evaporate the solvent. As the solvent evaporates, you are left with the residue, and the majority of that residue is your essential oil. Also used in industry nowadays is something called supercritical fluid extraction, where supercritical carbon dioxide is used as a solvent for the extraction of your oils. Now, for chemical tests, if I have to assess for the oils, I can either check it on the drug through a section or I can check it on the powder by performing a chemical test. Now, microchemical tests are generally non-specific, meaning they can detect.

Either volatile oil or fixed oil. So, let's take an example. Say, for instance, you have a club, and in that club, on this particular club, you have your oil glands. This is filled with volatile oils. What you do is, on your club, you add your Sudan red solution. What will happen? Sudan red is an oil-soluble dye.

So, it will dissolve and be solubilized, turning red in color, filling all your oil cavities. The Sudan red will disperse uniformly, giving you a red-colored stain. But the problem with this test is that even fixed oils dissolve the Sudan dye. So, even if the drug contains fixed oil, you will see the same phenomenon happening. Similarly, like Sudan red, there is another dye called Alkanna, which contains naphthaquinone's alkannin.

They have good oil solubility and perform or behave in the same manner. But rather than a red color, you will observe a maroon to magenta coloration. Now, this is exhibited by both fixed oils as well as volatile oils. Now, coming to specific tests, what you can do is a chemical test. So, take your drug, which you suspect contains essential oil, extract it in a lipophilic solvent such as chloroform, and then

You carry out a staining test on filter paper. So drop or put a few drops of it on the filter paper. If the stain disappears along with the solvent, there is a good chance that your drug contains volatile oil. But as the solvent disappears, if the stain still remains, that means your solvent has dissolved a fixed oil. The next test you can do is the same chloroform layer; you can treat it with vanillin and sulfuric acid.

It is said that terpenes react with vanillin-sulfuric acid to give you a reddish-colored complex, and that is one confirmatory test you can do to check for volatile oils. Now, coming to examples, let's discuss an example of clove here. Clove is one of the most commonly used spices. It is referred to as *Syzygium aromaticum* or *Eugenia caryophyllus*, belonging to the family Myrtaceae. What is used from this plant is the flower bud.

You can see a flower bud here. So this is a typical stalk. We call it the hypanthium. This is the sepal, and this is the corolla part, the petal part of it. But because it is unopened, you can see each petal here.

You can see a nice border here. So this is the petal. If you take this out, this is called the crown. If you take out the crown, you will see anthers below it. So these unopened buds are generally what is used as drugs.

Now, this grows mostly in tropical regions of Africa, chiefly the islands. Such as Zanzibar, Sumatra, you might find it in Madagascar, Mauritius, West Indies, in India, and also Sri Lanka. So these are the chief producers of clove as well. Clove is generally taken out or harvested from plants which are about six years old. And as they age, they keep on yielding until they are as much as 70 years old.

So if you see clove oil, Clove contains almost 14 to 20 percent oil, which is a very high content, and it's present both in the stalk, that is the hypanthium, in the corolla, in the sepals, and everywhere. And that's why we take the whole plant here. It contains 14 to 21 percent, of which the chief component is eugenol. If you see eugenol, this is again a phenylpropanoid derivative. And then, which is almost in some cases 90%, but on average, it is 60 to 90%. Most of the analgesic properties or painkiller properties of clove are attributed to eugenol.



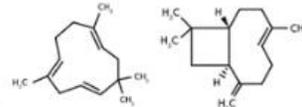
Chemical constituents

Clove contains 14–21% of volatile oil.

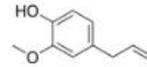
The other constituents present are the eugenol, acetyl eugenol, gallic acid

two crystalline principles; α - and β -caryophyllenes (12–17%), methyl furfural, gum, resin, and fibre.

Clove oil has 60–90% eugenol, which is the cause of its anesthetic and antiseptic properties



α - and β -caryophyllenes



Eugenol



In addition to it, it contains other sesquiterpene derivatives. And these are your alpha and beta-caryophyllin, chiefly your beta-caryophyllin. Now, this is your beta-caryophyllin, which is the major. Sesquiterpenes means it contains 15 carbons.

So these are almost similar. The only thing is this is cyclized. In addition to that, it contains tannins such as gallic acid. It contains acetyl eugenol. It contains furfural, methyl furfural, little amount of gum, resins and lot of fibers.

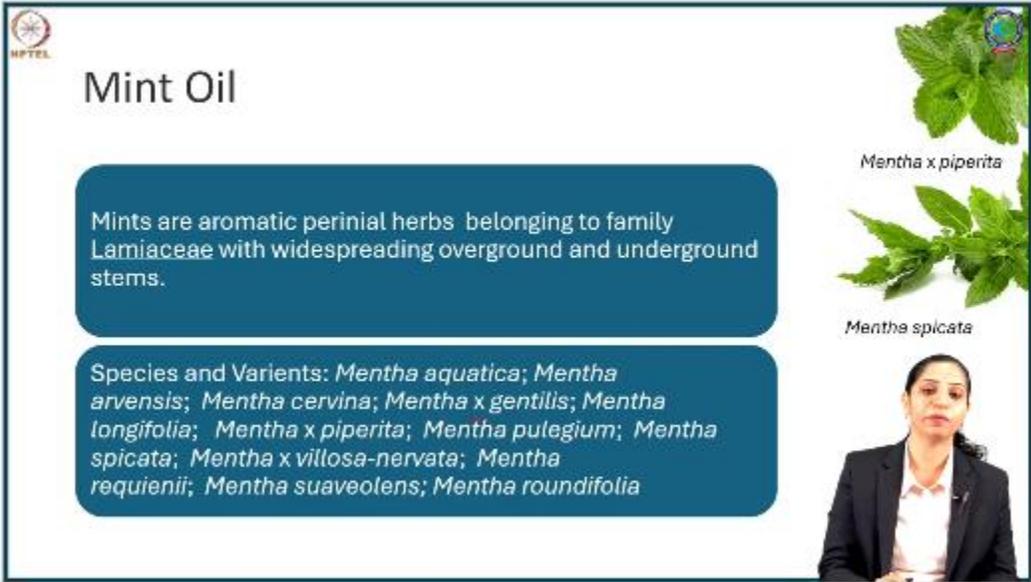
So this all in all gives you antiseptic properties. If you see a way to evaluate clove, if it is good, you can just press the hypanthium. You can see some oil oozing it or you can take a section and add some potassium hydroxide into it. You will see that this potassium hydroxide will convert the eugenol into potassium eugenate and you will see the needles in the oil cavities. If you want to do a chemical test, what you can do is you can take the clove oil, dissolve it in alcohol and then add a drop of ferric chloride.

With alcoholic ferric chloride, you will see a blue coloration, which is again attributed to the phenolic group of eugenol. Coming to the uses, clove has numerous applications, from traditional to modern-day. It is used as an antiseptic, stimulant, carminative, aromatic, as well as a flavoring agent, a condiment, and a spice. It is traditionally used for stopping toothaches. People generally take a clove and keep it between their teeth if they have a toothache, as a precautionary measure, or as an anodyne.

It's also anti-emetic. Dentists also use clove oil, especially for root canal treatments, when they need to disinfect the area, as it also acts as a local anesthetic. It works two ways. It kills parasites. It has good antimicrobial and antifungal properties.

So, it's also used in internal applications, such as the treatment of diarrhea and worms. It is also used to manage local, or what are known as, digestive ailments. It is a strong germicide, and people often give children a drop of clove oil as an expectorant to deal with bronchial issues. So, these are a few applications. But if you see modern-day literature, you might come across more and more uses of clove.

Now, moving to the next herb, the next herb is mint. Now, mint is not a single herb. It is actually a collection of plants or perennial herbs belonging to the family Lamiaceae. Now, these are widespread, as you can say, with overground stems, and the chief ones in the market are peppermint and spearmint.



Mint Oil

Mints are aromatic perennial herbs belonging to family Lamiaceae with widespread overground and underground stems.

Species and Variants: *Mentha aquatica*; *Mentha arvensis*; *Mentha cervina*; *Mentha x gentilis*; *Mentha longifolia*; *Mentha x piperita*; *Mentha pulegium*; *Mentha spicata*; *Mentha x villosa-nervata*; *Mentha requienii*; *Mentha suaveolens*; *Mentha roundifolia*

Mentha x piperita

Mentha spicata

The slide features a logo in the top left corner, a small globe icon in the top right, and a photograph of a woman in a dark blazer and white shirt in the bottom right corner.

But if you see the species and variants of it, mainly you'll find *mentha aquatica* and *arvensis*. When I see this one, these are the pure ones: *mentha cervina*. But if you see X out here, it means it's a hybrid. So *mentha gentilis*, *mentha longifolia*, *mentha piperita*—that is your peppermint—is a hybrid. It's actually a hybrid between *mentha spicata*, which is your spearmint, and *mentha aquatica*.

So you will have *mentha pulegium*, *mentha spicata*—again a hybrid—*mentha villosa*, *Mentha requienii*, and numerous others. So there are numerous *mentha* species, but let's discuss two here: peppermint and spearmint. Now, coming to peppermint, it is obtained—or mint peppermint oil is obtained—by distilling the leaves, which are obtained from *mentha piperita*, belonging to the family Lamiaceae. Now, mints have been cultivated in Europe, the US, as well as India.

Peppermint, you will find it abundantly growing and nowadays it's cultivated because of its food application cheaply. So these are very easy to propagate. In fact, it's a very aggressive plant. So what you need to do is just dig the runners in the early spring and just lay them off. little away that's like three two or three feet apart and then you will find that during summer they'll all spread they'll be full bloom and when they are ready you can or when they are in the flowering stage you can cut them off

it is also observed that during the dry season the oil content is higher so that is also an advantage and reason why it is harvested during the summer season because moisture is less the oil is more so the yield is more So once you obtain this, this is generally done by steam distillation. As discussed earlier, you might get a yellowish or a greenish color liquid depending upon the method of preparation. It has an intense penetrating odor and if you go to consume menthol, it has a burning sensation. And this burning sensation is followed by a very stinging cool flavor and that's attributed to its chemical compounds which is your menthol.

Your L-menthol is the natural element which is present in your mint oil. So peppermint oil contains chiefly L-menthol with other menthol derivatives, menthone, isomenthone, menthyl acetate, isovalerate, cineole, limonene and other compounds. Now menthol is a stereopting. That means it's going to be a solid. So if you take the mint oil and start cooling it,

Once the temperature goes below minus 22 degrees Celsius you will see on the sides of the flask menthol starts depositing as tiny crystals or tiny white powder. So you can separate it out by taking the temperature further down and what you get is a separated menthol and

a separated oil. This oil is called dementholized oil. This is used as a flavor. Menthol is chiefly used as a cooling agent.

So if you think your peppermints, especially your polo or puddin hara, you might have consumed it. That has a stinging sensation or cooling sensation. That's because of menthol that has been added. So The flavoring properties are due to both esters as well as alcohols. This is alcoholic whereas menthyl acetate is an ester whereas the medicinal value if you go to see is more attributed to alcohols.

Chemical constituents

- The chief constituent of Peppermint oil is L-Menthol, along with other constituents like menthyl acetate, isovalerate, menthone, cineole, limonene etc.
- Menthol separates on cooling it to a low temperature (-22°C).
- The flavouring properties of the oil are due to both the ester and alcoholic constituents, whereas the medicinal value is attributed only due to the alcoholic components.
- The English oil contains 60 to 70% of Menthol, the Japanese oil containing 85%, and the American has only about 50%.

L-Menthol

C[C@H]1CC[C@@H](C)[C@H](O)C1

Now, there are different variants of mentha. The English oil, if you go to see, contains 60 to 70% menthol. The Japanese one, generally called as the mentha arvensis, gives you as high as 70 to 85%. Whereas American oil contains little less, about 50% menthol. If you want to separate it, the Japanese one is the best source in all.

Now, coming to applications, the most common application is flavoring agent. And you can see here, I've just put a few emphasis. If you see, this is your natural L-menthol. Now, menthol is also obtained synthetic. But what happens is when you do synthetic, you often end up getting a racemic mixture




Applications

- It is stimulant, stomachic, carminative, flatulence, and colic; in some dyspepsia, sudden pains, for cramp in the abdomen and also in cholera and diarrhoea.
- Oil of peppermint allays sickness and nausea.
- Peppermint is good to aid in raising internal heat and inducing perspiration.
- It is also used as flavoring agent and in cosmetics as coolant.



CC1=C(C)CC(O)C1 L-Menthol CC1=C(C)CC(NC(=O)N)C1 WS-3
CC1=C(C)CC(NC(=O)Nc2ccc(N)cc2)C1 Evercool 180

Semisynthetic analogs of Menthol



and racemic mixture has slightly dull flavor as compared to the natural one now you can see here there are numerous attempts to increase the cooling sensation and increase the residual time of cooling this has been done by using amide linkages so to that menthol amides have been prepared And few derivatives such as Wilkinson's WS3 derivative or Evercool 180, which have many fold cooling properties as compared to menthol are already in market. If you see the pure mint oil, it's used as a stimulant, stomachic, carminative.

It's used to manage flatulence, colic pains, in some cases dyspepsia. sudden intestinal pains or cramps in abdomen and also in cases of cholera and diarrhea. Now peppermint in some cases for people those who feel nauseous or have motion sickness, peppermint oil is known to allay this. It is also good for people who want to raise the internal heat and induce perspiration. And like I said, majority also goes in your food industry.

And in cosmetics nowadays, you find soaps and deodorants containing menthol for giving you that cooling sensation. Now, the other species which is mostly used in your chewing gum is spearmint, and that is obtained from another species of mentha called mentha spicata or also known as mentha viridis. This is also a Lamiaceae family member and is most abundantly found in Europe and southern temperate Asia. It's also cultivated in regions from Ireland to China. Now, the difference here is that we mentioned our discuss species, which had a very high content of menthol.

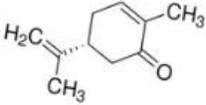



Mentha spicata

Biological Source: *Mentha spicata*, also called *Mentha viridis*, is a medicinal plant of the Lamiaceae family

Geographical Source: Europe and southern temperate Asia, extending from Ireland to southern China.

Chemical composition : carvone 50% to 80%. , carvacrol, trans-carveol, piperitone oxide, limonene, 1,8-cineole, camphene, p-cymene, dihydrocarvone, pulegone, β -caryophyllene, germacrene D, menthone, α -pinene, and linalool.



(R)-(-)-Carvone



Here, if you see your spearmint, instead of menthol, the active component is carvone. It's a ketone, not an alcohol. And the composition is as high as 50 to 80 percent. So it almost replaces the menthol from mentha piperita with carvone. And that's the reason your chewing gums don't have an intense stinging flavor, but they have a very mild, sweet flavor.

And that's not menthol. That's your carvone. Apart from that, it has trans-carveol. Piperitone, limonene, cineole, camphene, paracymene, dihydroxycarvone, pulegone, caryophyllene, germacrene—which is also being explored nowadays—menthone, alpha-pinene, and linalool, which also contribute to the sweet aroma. Now, all of this, once you prepare or get the dementholized oil—demytholized oil also has numerous applications.

So it's used in food. Like I said, if you take menthol, it has a stinging, cooling sensation. If that is gone, now it is more of a sweet, cooling sensation. And that's the reason dementholized peppermint oil has been used in pharmaceuticals, cosmetics, foods, and beverages also. Ayurvedic medicines, because in some cases, people are sensitive to menthol.

So if you consume or if they consume menthol products, that leads to sneezing or irritated mucosa. So dementholized oil offers a better alternative for treatment of common cold, cough, and flu. It is nowadays also used in soaps, body lotions, deodorants, creams, and also in shampoos because the dose of menthol is minimal. So the irritancy, itching, or stinging sensation is not there. Apart from that, it's also used as a mild agent in food and flavoring, especially in the beverage industry.

Now, what we do is, in some cases, the essential oils we saw that lipids and fats rancidify. But volatile oils polymerize, and they form resins, and as a result, they lead to loss of aroma. In order to enhance the shelf life, we prepare what are called terpene-less volatile oils. This has been obtained by fractionation, wherein the low-boiling fractions have been removed. So most of the hydrocarbons from this oil are removed, and as a result, these are richer in oxygenated products.

So once they become rich in oxygenated products, they become more stable. They are highly soluble in alcohol, and their aroma profile increases. So terpene-less volatile oils are better concentrates than the native essential oils to be used in perfumery, to be used in foods for flavoring purposes, and in the pharmaceutical industry. So here are a few references that you might want to read for additional knowledge. And thank you, everyone, for your patient listening.

Thank you.