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MEDICINAL BEEKEEPING FOR BEEKEEPERS
MODULE 5

2021-1-TR01-KA220-VET-000034632

Beeswax and Bee Venom

In this module you will learn what beeswax and bee venom are, you will learn about their physical and chemical properties as well as methods of obtaining, preserving and storage them. In addition, this module will present the health-promoting properties of these bee products and the methods of their use in apitherapy.

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Beeswax and Bee Venom

TRAINING CONTENT:

1. Beeswax and bee venom – definition
2. General description of beeswax and bee venom
3. Physical and chemical characteristics of beeswax and bee venom
4. Pro-healthy properties of beeswax and bee venom
5. Beeswax and bee venom harvesting, preservation, processing and storage

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Beeswax - definition

BEESWAX - is a complex product secreted in liquid form by special eight wax glands in the abdomen of younger worker bees (aged between 12 and 18 days). In contact with the air, it solidifies in scales. Individual scales weigh from 0.8 to 2 mg, and are 0.2-0.5 μm thick. The wax plateles are scraped off by the bee, chewed and masticated into pliable pieces with the addition of saliva and enzymes. Beeswax is model with bees' jaws and used to build the honeycombs (after mixing with pollen and propolis).

Bee wax

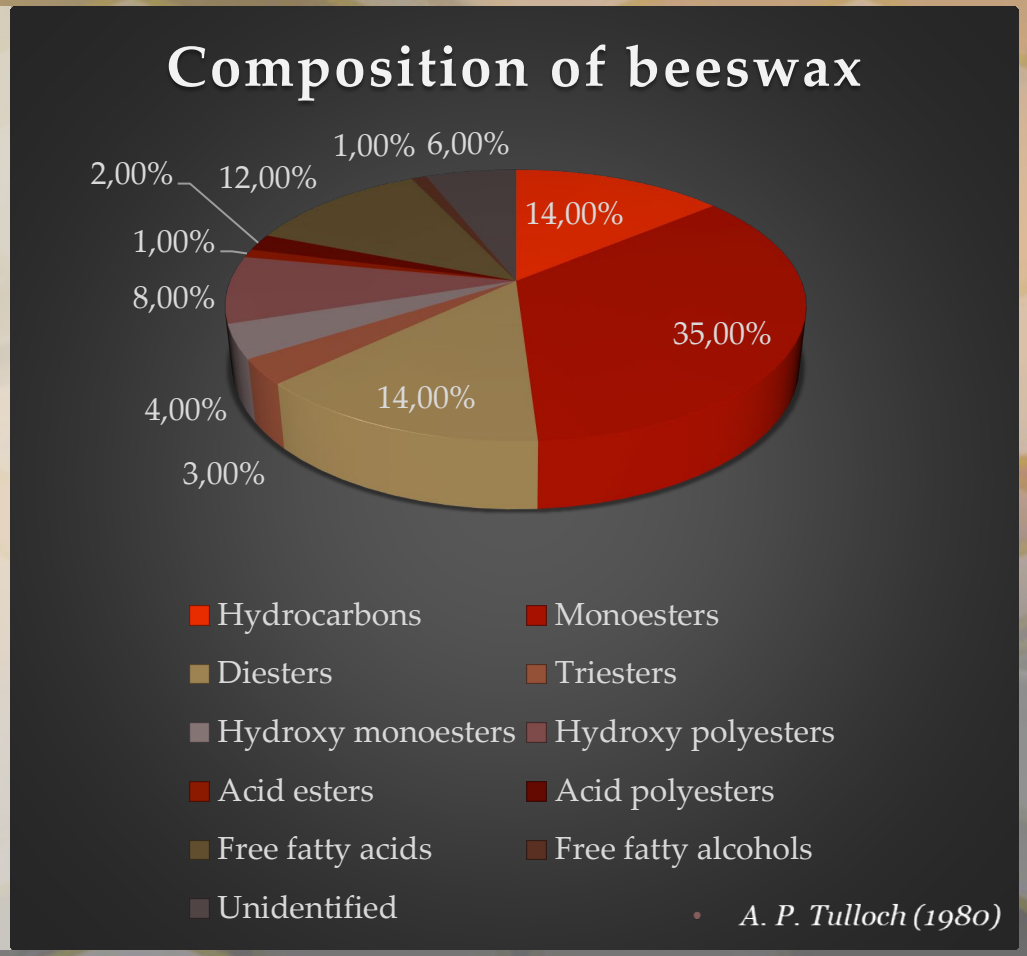
Beeswax - definition

Combs serves as food (honey, pollen) storage and provides infrastructure for rearing brood. About 1100 wax scales are needed to make a 1 g of wax. Beeswax has an important role in thermoregulation and colony waste management, and serves as a humidity buffer in honeybee nests. The wax is recovered as a by-product when honey is harvested and refined. Beeswax is widely used in pharmacy, cosmetics, and in food industry as food additive E901.

Bee wax

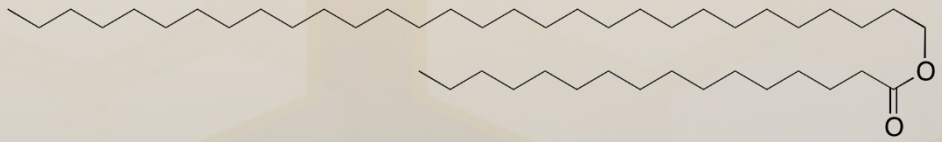
Beeswax – chemical composition

Beeswax is a complex mixture (more than 300 components). Chemically beeswax is a mixture of esters of fatty acids, hydrocarbons, free fatty acids, fatty alcohols and exogenous substances. Present in beeswax linear monoesters and hydroxymonoesters with chain lengths C40–C48, are mainly derivatives of palmitic, 15-hydroxypalmitic and oleic acids whereas complex wax esters containing 15-hydroxypalmitic acid or diols, which through their hydroxyl group, are linked to another fatty acid molecule.

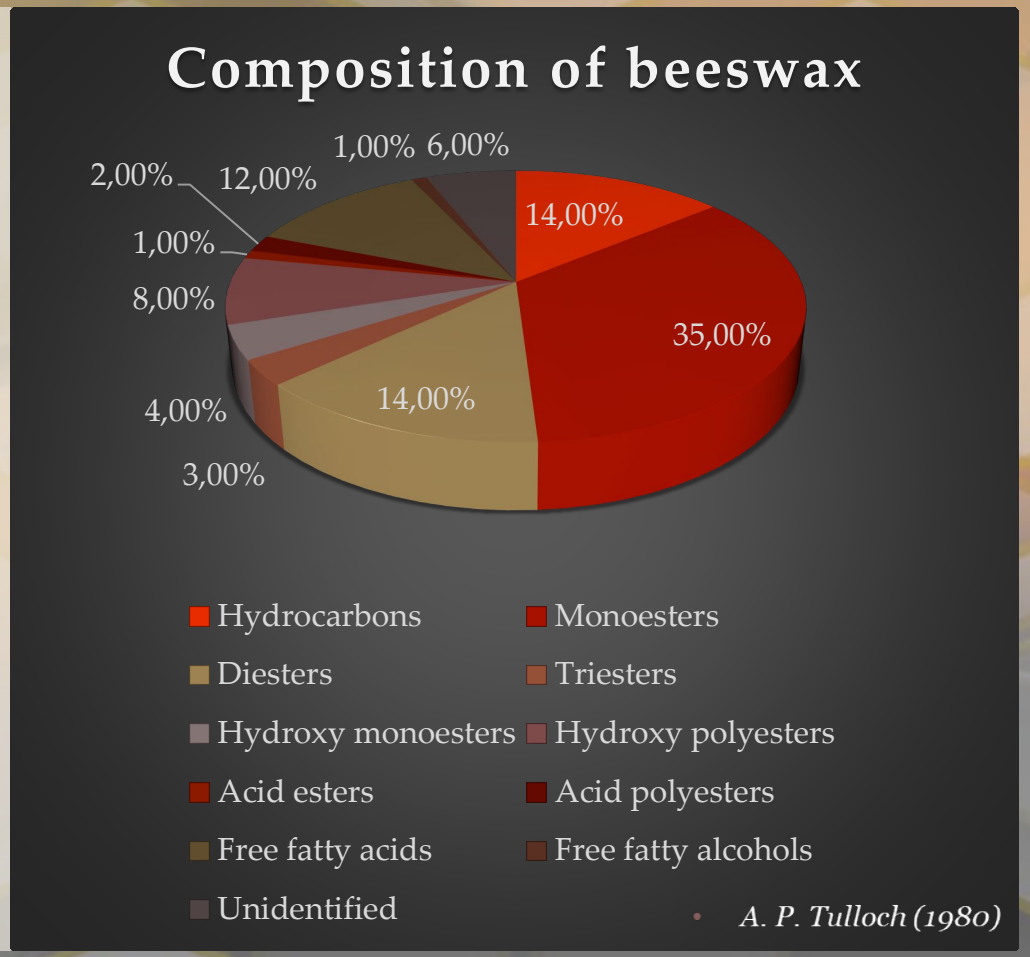


Beeswax – chemical composition

Among the hydrocarbons, with a chain length of C27–C33, the predominant ones are heptacosane, nanocosane, hentriacontane, pentacosanem and triacosane. Among the free saturated fatty acids, lignoceric acid is found most in beeswax. Exogenous substances that are mainly residues in beeswax are propolis, pollen, small pieces of floral component factors and pollution. Many pesticides used to control mites in the hive can contaminate beeswax.



Triacontanyl palmitate - a major component of beeswax.



Physical characteristic of beeswax

Among the organoleptic properties of beeswax, the following are distinguished: colour, structure, consistency, smell and taste. The colour of the wax produced by bees is white, but depending on the honey flow (pollen, bee bread, propolis) or as a result of its contamination with larval faeces, pupal skins it becomes light yellow, yellow, light brown and brown colour. The colour of the beeswax obtained can also change depending on how it is smelted, the longer and at a higher temperature the wax is smelted, the darker it becomes. White beeswax sold commercially consists of yellow beeswax that has been subjected to bleaching or has been filtered to remove impurities.

Colour	**yellow to yellow-brown**
Upon breakage	fine-granular, not crystalline
Odour	Honey-like
Consistency	should not stick upon cutting, workable with fingers, should not stick to them
Density	0.950-0.965 (0,927– 0,987 g/cm^3)
Melting point	61-65 °C (61–72°C)
Setting point	usually 2–3°C lower than melting point
Hardness	0.98–15 s/mm
Viscosity	22,0 mP
Solubility	benzene, ethyl alcohol, chloroform, ether

Physical characteristic of beeswax

At room temperature (20°C) the wax is a solid with a crystalline structure, and at the fracture its fine-grained structure and matte appearance are observed. As the temperature rises to 30–35°C, it softens and becomes plastic. In this form, when kneaded, it does not stick to the fingers, and when chewing, it does not stick to the teeth. At 46-47°C it loses its crystalline structure. The smell of wax resembles the smell of honey. It comes from honey flow (nectar and honeydew), as well as bee pollen and propolis. Its taste is delicate and pleasant. If it comes from a less valuable raw material, it can be sour-sweet and slightly bitter.

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Physical characteristic of beeswax

The basic physicochemical properties of beeswax include density, melting point, setting point, hardness, viscosity and solubility. Density at 20°C is in the range of 0.927–0.987 g/cm^3 (wax is lighter than water). This parameter is greatly influenced by impurities - the less there are, the lighter the wax. The melting point of the wax is 61-72°C. The purer it is, the higher its melting point. If beeswax is heated above 85 °C discoloration occurs. The setting point is usually 2–3°C lower and ranges from 60.5–70.5°C. The hardness of beeswax, expressed as a hardness coefficient, ranges from 0.98–15 s/mm and depends on its quality. The average viscosity of the beeswax product is 22.0 mP (millipoise).

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Physical characteristic of beeswax

Beeswax dissolves in hot benzene, ethyl alcohol, animal fats and vegetable oils, and only partially in chloroform (25%) and ether (50%). In order to completely dissolve the beeswax, the temperature must be increased beyond its melting point. However, it is insoluble in water, glycerin and most organic solvents.

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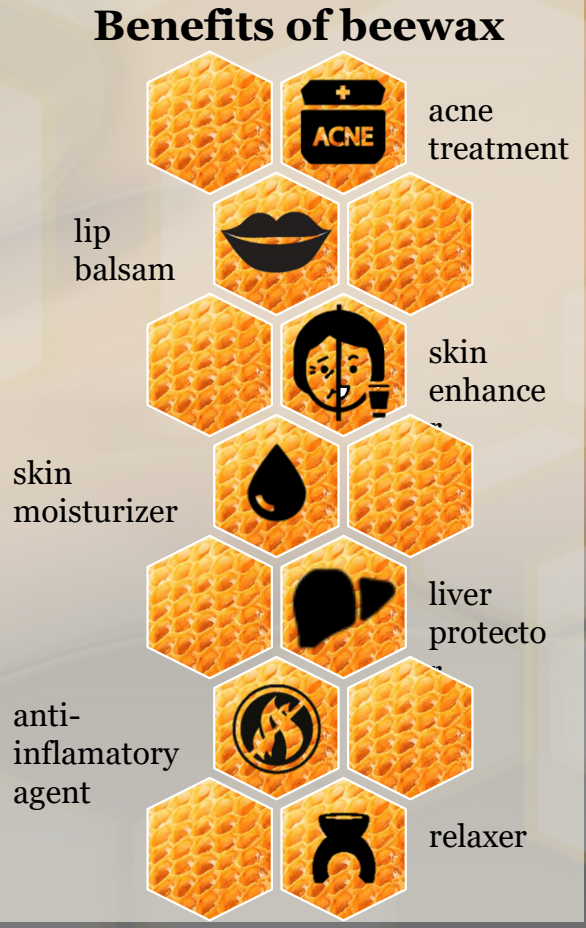
Pro-healthy properties of beeswax

In ancient Egypt, beeswax was the main ingredient of ointments used as preparations used to help pull plugs, to treat **burns and wounds** and to soothe **joint pain**. Hippocrates, recommended the use of beeswax in case of **purulent tonsillitis**. In ancient Rome beeswax as a component of ointment was recommended for the treatment of **burns**, **wounds**, **cuts**, **bruises** and **fractures**.



Pro-healthy properties of beeswax

For therapeutic purposes, only yellow wax (Cera flava) is used, which is not chemically processed. With its help, after enriching with various plant and animal origin substances, **skin diseases** such as **abscesses**, **acne**, **prurigo**, **boils**, **ichthyosis**, **vitiligo**, **mycosis**, **psoriasis**, **radiation burns**, **subcutaneous hemorrhages**, **neurodermatitis**, **leg ulcers**, **sun burns** are treated, and **ulcers** caused by malnutrition of the skin. For this purpose, wax preparations in the form of ointments, lotions, patches and hot compresses are used. Ointments with beeswax in their composition are also successfully used in the treatment of various types of **fungal infections**.

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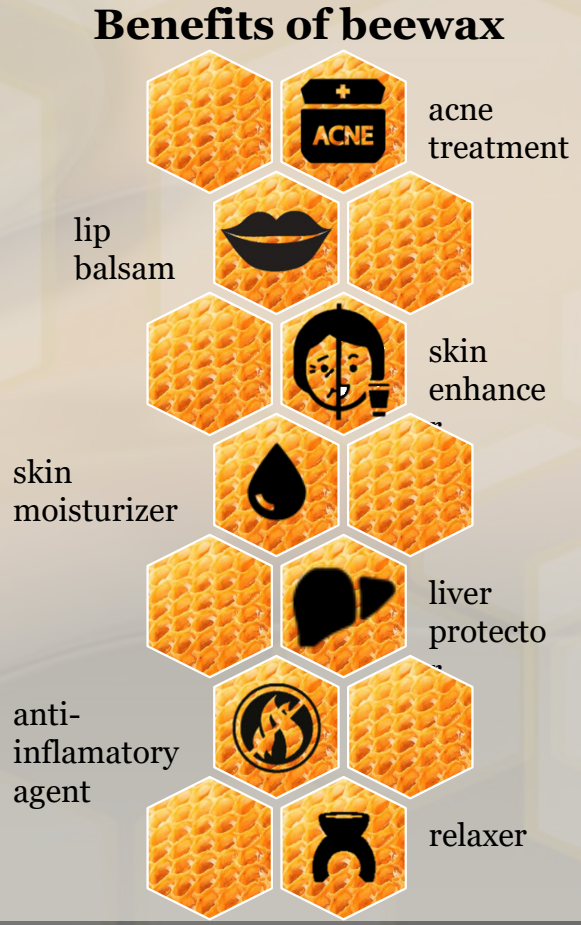
Pro-healthy properties of beeswax

Beeswax, especially in the form of sealing wax, is successfully used to treat the mucous membranes of the **oral cavity**, **gums, nose**, **throat**, **tonsils**, **bronchi** and **maxillary sinuses**. This product is also suitable for treating the mucous membranes of the anus. In the case of **haemorrhoids** and **anal fissures**, a liniment containing beeswax is used with good results. Beeswax after liquefaction at 50-60°C is used to treat many diseases of the **musculoskeletal system**. Treatment with beeswax hot compresses is used with good results in the case of **degenerative diseases**, **inflammation of the muscles, tendons** and **nerves**, **rheumatoid polyarthritis** and chronic **degenerative rheumatoid arthritis**.



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Pro-healthy properties of beeswax

Candles that have a therapeutic effect are also made of beeswax.
Candles that have a therapeutic effect are also made of beeswax.
Crude beeswax showed **antibacterial activity** against several bacterial strains and against the *Candida albicans*. The sample of beeswax was effective against both Gram-positive bacteria, in particular *Streptococcus aureus, Streptococcus epidermidis* and *Streptococcus pyogenes*, and against Gram-negative bacteria, in particular *Bacillus subtilis, Pseudomonas aeruginosa*, *Escherichia coli*. Beeswax is commonly used for cosmetics, and nowadays considerable interest is aroused by beeswax methanol and ethanol extracts as well.

Beeswax harvesting, preservation, processing and storage

Wax is usually removed from the capping during honey extraction. Old combs and capping serve as the raw materials for making wax. In order to turn old combs and wax bits into wax blocks, they should all be kept. Since newer combs produce wax of a higher quality, they should be rendered separately from older ones. Old combs range in price according to their age; the older the comb, the less wax it contains and the less valuable it is. The most expensive items are caps, which are made almost entirely of pure wax.

Bee wax

Beeswax harvesting, preservation, processing and storage

Propolis and cocoons are found in dark combs, which reduce the wax's quality. To avoid potential fermentation and mold growth, honey should be taken out of the storage combs. Old combs that are free of sugar feed and honey should be placed in plastic bags. Combs but not pure beeswax, are highly susceptible to damage by the Greater wax moth *Galleria melonella L.*

Bee wax

Beeswax harvesting, preservation, processing and storage

Beeswax production techniques have a significant impact on the product's quality. **Melting** and **chemical extraction** are the two available wax extraction techniques. The process that is most usually utilized is melting. Wax can be melted using steam, hot water, electricity, or solar power. Only in a laboratory, where small-scale wax production is required, is chemical extraction by solvents practical (with gasoline and xylene as solvents). The drawback of this approach is that it dissolves all organic wax impurities. As a result, wax quality may be decreased.

Bee wax

Beeswax harvesting, preservation, processing and storage

To get high-quality beeswax, remember not to use too high a temperature and not to melt the wax for too long, because it destroys the structure of the wax and causes it to darken; do not use steel, aluminium, zinc and copper vessels when melting wax; do not use combs with fermented honey as it negatively affects the smell of the obtained wax.

Bee wax

Beeswax harvesting, preservation, processing and storage

Beeswax can be obtained dry and wet. It is obtained dry using **solar or electric melters**. Solar melters that use solar energy are economical and easy to use. Under the influence of sunlight, the inside of the melter heats up and melted wax flows into a container with water, where it solidifies. Large impurities are collected on a special mesh placed in the path of the flowing wax. In electric melters, the wax raw material is placed on a perforated electrically heated plate. In steam smelting machines, the wax raw material is placed in a special basket to which steam is supplied. The melted product is collected in the lower part of the device.

Electeric melter of bee wax

https://www. https://www.ipszczelnictwo.pl/tygiel-do-topienia-wosku-230-v-2-5-kw-12-l?virtuemart_currency_id=114&gclid

Solar melter of bee wax

https://www.abelo.co.uk/shop/wax-melting/solar-wax-melter/

Beeswax harvesting, preservation, processing and storage

Wax from grains (a residue after the processing of bee wax raw material with impurities containing large amounts of wax up to 50%) is recovered by soaking or overcooking in water, and then it is centrifuged or extruded. The wax obtained as a result of melting contains impurities of various sizes. Mechanical and chemical methods are used to remove them. Wax can be cleaned by clarifying – keeping it liquid for a long time – during this time heavier pollution sinks to the bottom, and smaller ones float to the surface. Important elements of this process are the quality of water, its ratio to the amount of wax (1:10) as well as the cooling time of the purified product.

Grains of bee wax with impurities
https://www.beyondthechickencoop.com/rendering-beeswax/

Beeswax harvesting, preservation, processing and storage

Impurities on the surface of the mixture are collected and the residue is strained through fine sieves or a dense mesh into appropriate vessels. The containers are protected with insulating material and left to cool (2-6 days). The resulting clarified wax is cleaned of impurities collected on the underside using a knife or apiary chisel. After melting and cleaning, beeswax normally has a beautiful yellow colour. If it is dark for any reason (overheating, presence of metals) it can be brightened by exposing it to the sun or by chemical means.On an industrial scale, beeswax is purified by filtration and centrifugation, using cotton fabrics, canvas or filter paper.

Grains of bee wax with impurities
https://www.beyondthechickencoop.com/rendering-beeswax/

Beeswax harvesting, preservation, processing and storage

Filtration of liquid wax, using plate or frame presses, is carried out under pressure. The cleaned product should be stored in clean, dry and airy rooms away from pungent odors. The temperature at the storage site should be below 10°C and the air humidity below 40%. These conditions limit the possibility of development of wax pests and mold. Lumps of wax can lie loose, in piles, on the floor, shelves or in boxes. For best preservation of colour and aroma, they can be stored in wrapping-paper or in containers made of stainless steel, glass or plastic. They should be protected from contact with oxidizing materials. It must not be stored together with wax raw material or slopes.

Grains of bee wax with impurities
https://www.beyondthechickencoop.com/rendering-beeswax/

Beeswax quality control

Beeswax is a natural product, and no additives are allowed. Examination of the organoleptic properties (e.g., odor and color) of beeswax allows for quick and easy quality control. Wax adulteration can be detected by different methods. Pharmacopoeial determination of **organoleptic and physicochemical** properties does not guarantee that waxes have not been adulterated, although in some cases they may give indications of possible adulterance.

Beeswax quality control

Parameter	Requirements
Sensory and physical characteristics	
Colour	yellow to yellow-brown
Upon breakage*	fine-granular, blunt, not crystalline
Odour	honey-like
Consistency*	Should not stick upon cutting
Melting point	61–65 °C
Density	0.950–0.965
Refractive index (at 75 °C)	1.440–1.445
Physicochemical properties	
Acidic number	18–23
Esterification number	70–80
Peroxide number	at least 8
Authenticity*	no adulterants
Contamination*	according to requirements or as low as possible

* excepted

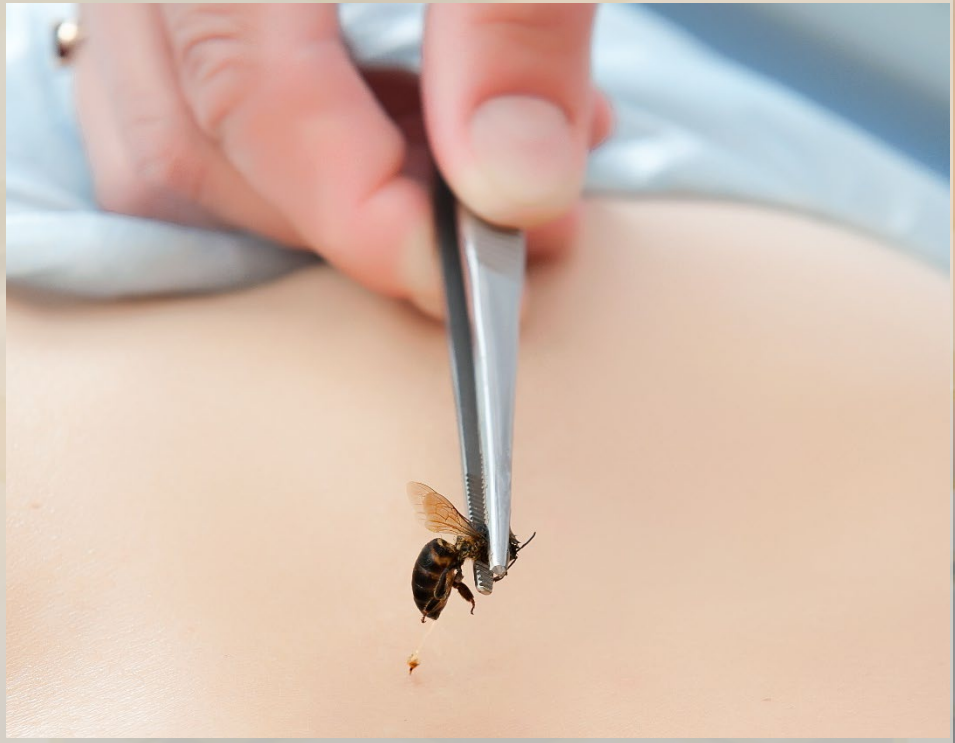
Properties and quality criteria for beeswax according to the Pharmacopoeia

Beeswax quality control

Adultery is mainly detected by gas chromatography (GC) or liquid chromatography. In the specific case of blending with carnauba wax, a simple bioassay may also be used. The main contaminants of beeswax are chemicals used in beekeeping (mostly acaricides, paradichlorbenzene). Another potential problem for the quality of beeswax used for beekeeping is the presence of American foulbrood (*Penibacillus larvae larvae*) spores.

Bee venom - definition

BEE VENOM (APITOXIN) – physically is a clear, odourless, watery liquid a bitter taste and basic pH (4.5 to 5.5). When coming into contact with mucous membranes or eyes, it causes considerable burning and irritation. Dried venom takes on a light yellow colour and some commercial preparations are brown, thought to be due to oxidation of some of the venom proteins. Bee venom is synthesized in the venom glands of worker bees and queen. Venom is produced by two glands associated with the sting apparatus of worker bees and is stored in the venom sac.



Bee venom therapy

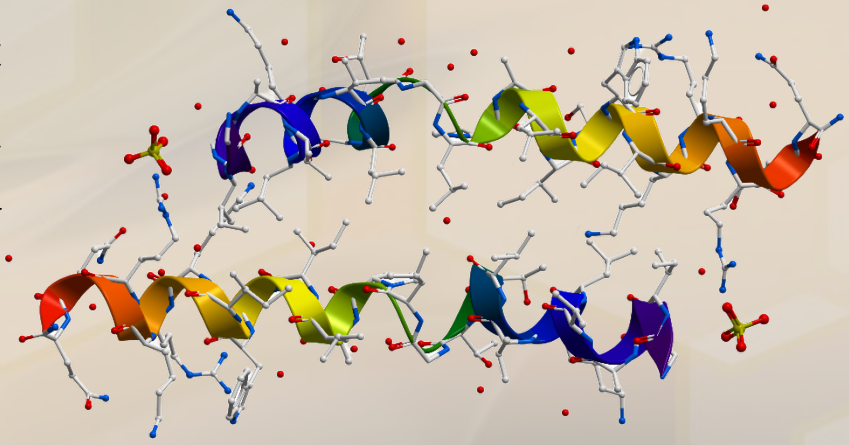
Bee venom– chemical composition

There are more than 60 identifiable components in bee venom. The honeybee venom consists of enzymes, proteins, peptides, and a variety of smaller molecules (amino acids, catecholamines, sugars, and minerals). Most types of venom induce immediate pain because they contain phospholipases, hyaluronidase, and other enzymes.

Class of molecules	Component	% of dry bee venom
Enzymes	Phospholipase A2 Hyaluronidase Acid phosphomonoesterase Lysophospholipase a -glucosidase	10-12 1,5-2,0 1,0 1,0 0,6
Proteins and peptides	Melittin Pamine Mast Cell Degranulating Peptide (MCD) Secapine, Procamine Adolapin Protease inhibitor	40-50 3,0 2,0 0,5 1,4 1,0 0,8
Physiologically active amines	Histamine Dopamine (DA) Noradrenaline	0,5-1,6 0,13-1,0 0,1-0,7

Bee venom– chemical composition

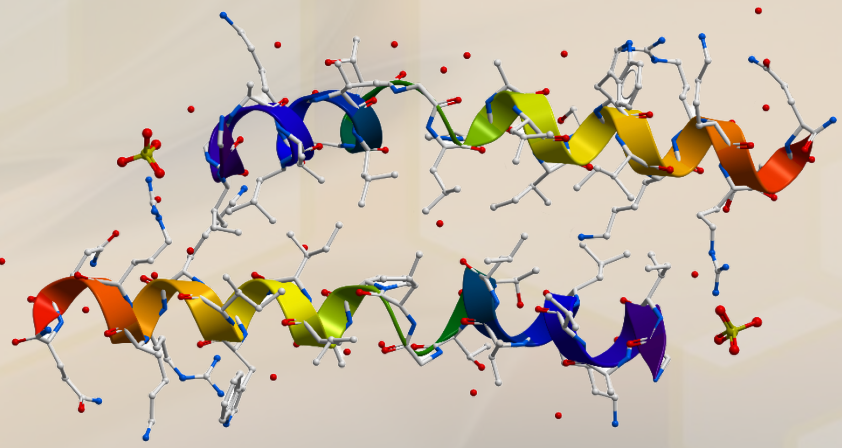
MELITTIN is the main bee venom component (approximately 40–50% of the venom dry weight) and it has many positive biological effects and relatively low toxicity. Chemilally, it is cytolytic linear peptide with a molecular weight of 2.8kDa and contains 26 amino acid residues. Its chemical formula: $C_{131}H_{229}N_{39}O_{31}$.



Melittin molecule – main component of bee venom

Bee venom– chemical composition

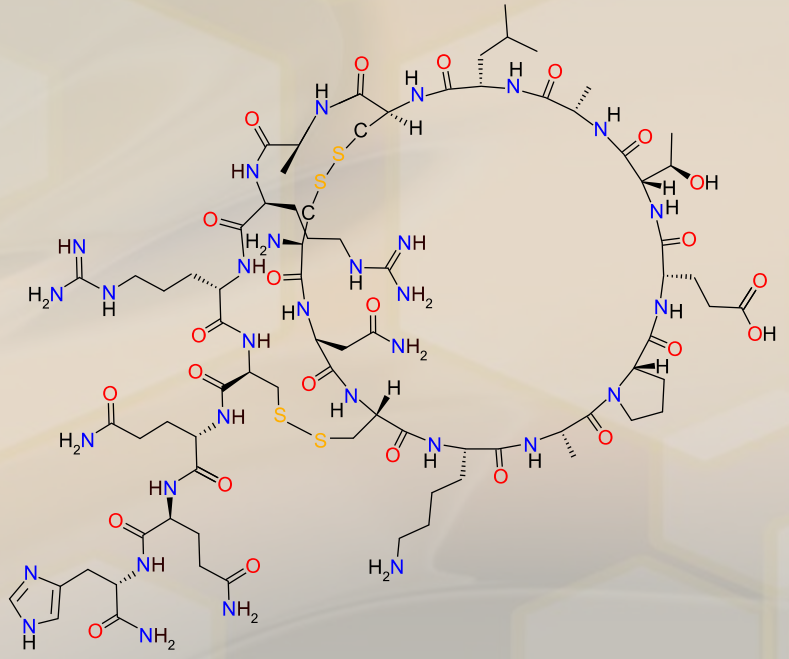
Melittin is a surfactant, causes hemolysis of erythrocytes, releases histamine from mast cells, increases the fluidity of the phospholipid matrix of the membranes (change in the activity of many membrane-bound enzymes). The principal function of melittin as a component of bee venom is to cause pain and destruction of tissue of intruders. As was stated in many experiments this pepride has anti-inflammatory, anticancer, antibacterial, antifungal and antiviral activities.



Melittin molecule – main component of bee venom

Bee venom– chemical composition

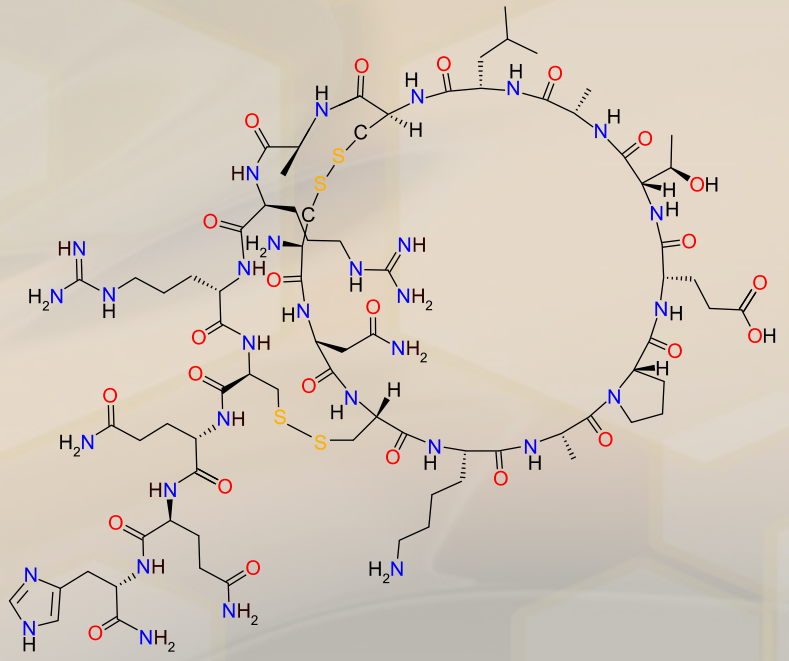
APAMIN is the minor active component of bee venom, is a low molecular weight peptide containing 18 amino acid residues of which 4 are half-cystines. Apamin has excitatory neurotoxic effects on the central nervous system and when lethal or sublethal doses are intravenously injected into mice it causes extreme uncoordinated hypermotility, clonic convulsions, followed by respiratory distress and death.



Apamin molecule – bee venom component

Bee venom– chemical composition

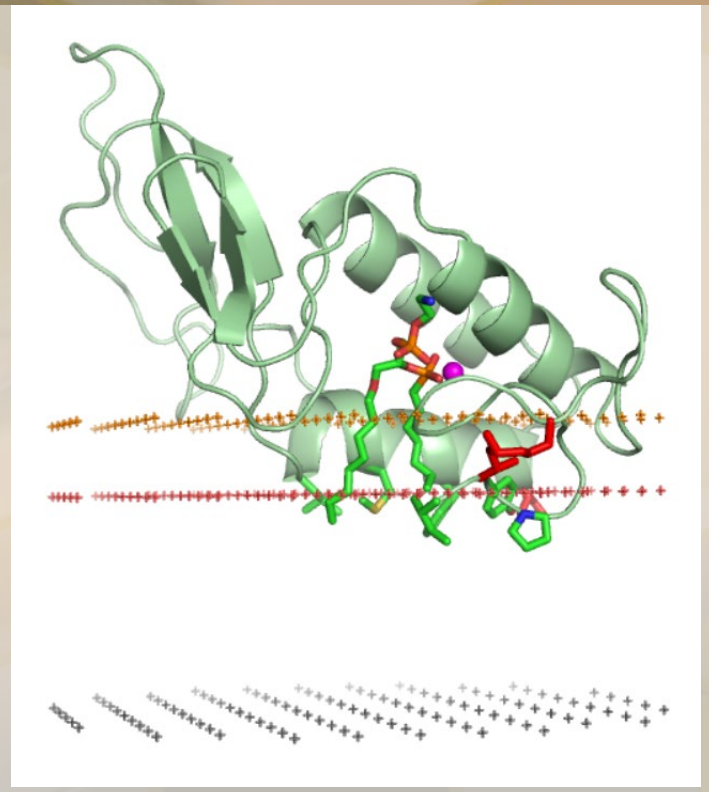
Apamin the LD50 is in the range of 4-5 mg/kg of body weight. Apamin reaches its target organ, the central nervous system and inhibits small-conductance Ca2+ activated K+ channels (SK channels) in neurons. SK channel blockers, as apamin, may have a therapeutic effect on Parkinson's disease, epilepsy, emotional disorders and schizophrenia.



Apamin molecule – bee venom component

Bee venom– chemical composition

PHOSPHOLIPASE A2 (FLA2) is a calcium-dependent enzyme. The enzyme has a molecular weight of 14.6kDa and consists of 129 amino acid residues, of which 12 are cysteine, which enter the disulfide bridges. Bee venom phospholipase A2 is the main allergen in the bee sting allergy. It is also held to be responsible for some systemic anaphylactic reactions in bee venom sensitized individuals. Of all the components of bee venom, phospholipase is the strongest antigenic and allergenic protein. In the presence of melittin, the phospholipase becomes even more active and toxic.



Phospholipase A2 (FLA2) – bee venom component

By Biophys - self-made by Biophys, CC BY 2.5, https://commons.wikimedia.org/w/index.php?curid=1643383

Physical characteristic of bee venom

The newly extracted bee venom is a clear, **syrup-like, pale yellow liquid**. It is characterized by a **bitter, burning taste** and a **strong, pungent odor reminiscent of honey**.

Density of bee venom is 1.085-1.131 g/cm3.

The acidity of fresh product is very low and ranges **from 0.38 to 0.56 pH**, while the aqueous solution of this product is 4.5 to 5.5.

As it dries, the venom loses all its aromatics and some volatile fatty acids, and then hardens into a transparent residue.

Dried venom is pale yellow, and some commercial preparations are brown, which is thought to be due to oxidation of certain venom proteins.

It is soluble in water and insoluble in alcohol and ammonium sulfate. Bee venom contains several volatile compounds, which are easily lost when collected.

Pro-healthy properties of bee venom

Many years of observations of apitherapists using bee stings and bee venom preparations indicate that many diseases can be treated with this bee product, especially those that are difficult to respond to classical therapy.

Diseases of the musculoskeletal system	Diseases of the peripheral system nervous	Post-traumatic pain syndromes	Cardiovascular diseases	Other diseases
rheumatic disease, rheumatoid arthritis, ankylosing arthritis spine, osteochondrosis, muscle diseases (myalgia and myopathy).	neuralgia (including sciatic nerve, lumbar, trigeminal); polyneuritis, radiculitis of spinal nerves; epilepsy.	discopathies, peripheral nerve paralysis, osteoarticular injuries.	myocarditis, hypertensive disease, ischemic heart disease, thrombophlebitis	atopic bronchial asthma, hard-to-heal wounds, trophic ulcers, endometritis, frostbite and postoperative scars, impotence, alopecia, hyperthyroidism

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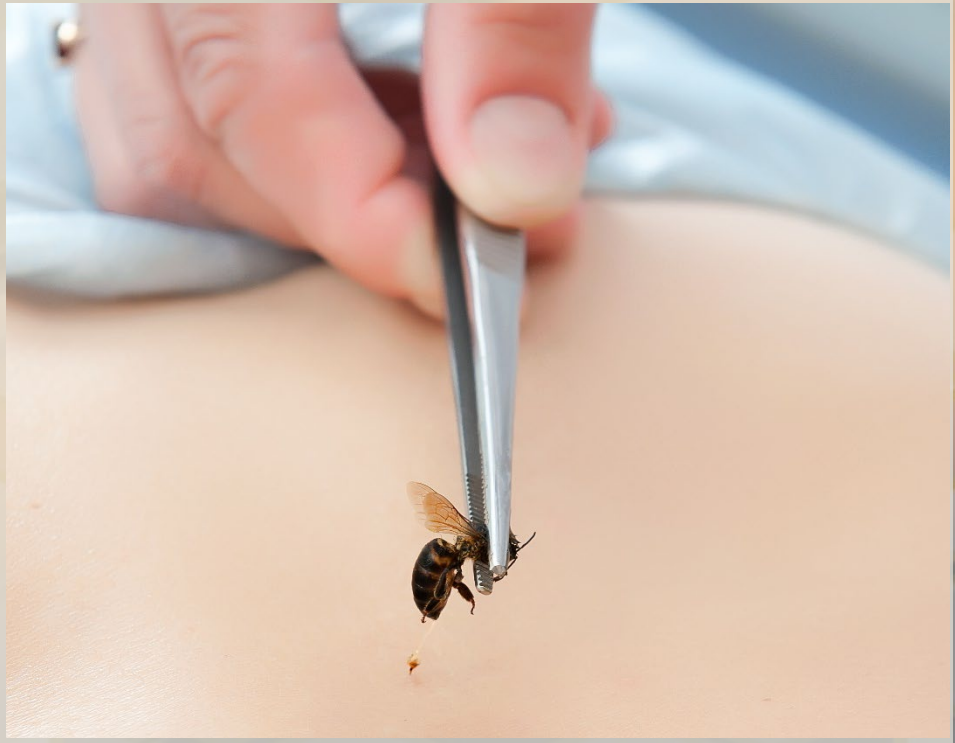
Pro-healthy properties of bee venom

In classical medicine, bee venom is used to treat chronic inflammatory disorders because they have various effects such as anti-arthritis, anti-cancer, and analgesic. In bee sting therapy, the bees go directly to the target point through the stinger, while in bee venom therapy, lyophilized venom (taken from the bee and then freeze-dried) is injected directly with different doses.



Bee venom therapy

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Bee venom harvesting, preservation, processing and storage

Bee venom is usually extracted using low voltage electrical stimulation. Beekeepers use a collection frame that has wire electrodes installed that have a low electrical current running through them on a glass base. These frames are installed in honey hives and bees that come into contact with the wire electrodes will receive a small electrical shock. This causes bees to sting the glass, releasing the venom without losing their barbed sting.

Honey bee venom collector
https://beevee-collector.com/

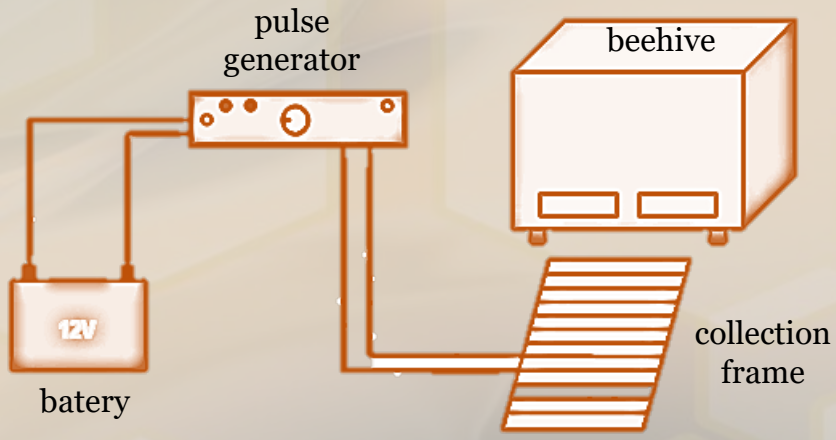
Bee venom harvesting, preservation, processing and storage

The main problem in the collection of bee venom is to limit the loss of valuable volatile compounds that occurs when the bee venom dries out. Therefore, it is proposed that standard venom collection devices should be equipped with a cooling system that will reduce the evaporation of volatile compounds. No bees are harmed during the bee venom collection process. Under the influence of an electrical impulse, one bee secretes an average of 50 µg of venom. The venom is obtained in spring or summer and the cycle of its acquisition lasts 12-15 days, during which time you can collect about 1 g of bee venom. Up to 4 g of bee venom can be collected in 3 cycles during the season.

Honey bee venom collector
https://beevee-collector.com/

Bee venom harvesting, preservation, processing and storage

Different extraction or collection methods result in different components of the final product. Venom collected from surgically removed venom sacs showed different protein content than those collected by electroshock method. Main problem in venom collection is how to protect volatile substances against their evaporation. Venom collected under water seems to yield the most potent venom as well as use a cooling system with the standard electro-shock collecting apparatus in order to preserve more of the volatile compounds.



Bee venom collector pulse generator system
https://www.proelektronic.hu/termek/bvc-02-bee-venom-collector-pulse-generator?language=en

Bee venom harvesting, preservation, processing and storage

The dried venom forming a "transparent film" is hygroscopic. It dissolves in water, water solutions of glycerin and vegetable oils, and forms suspensions with ethanol. Oxidizing substances and digestive enzymes lead to the loss of biological activity of bee venom.

Dried bee venom
https://www.sciencedirect.com/topics/chemistry/bee-venom

Bee venom harvesting, preservation, processing and storage

When assessing bee venom quality, it should be borne in mind that it is a mixture of many groups of biologically active substances. Microbiological, cytological, pharmacological and chemical methods are used to assess bee venom. The microbiological method of bee venom standardization determines the lowest concentrations of bee venom that inhibits the development of *Staphylococcus aureus* ATCC 6538P. The MIC of fresh bee venom is 4-8 µg/ml.

Bee venom harvesting, preservation, processing and storage

The cytological method uses the protozoan *Paramecium bursaria* and determines the dilution that causes damage to approximately 50% of the cells of this microorganism (LD50). The most active are considered bee venom samples causing cytolysis in the range of 0.5-16 µg/ml.

Bee venom harvesting, preservation, processing and storage

Dried bee venom is durable and packaged in tight, moisture- and light-proof glass packaging, it can be stored at room temperature without changing its biological properties. Dried bee venom can also be lyophilized and stored at low temperatures **(-15 to - 20°C)** up to 5 years

During its storage, it should be **protected from sunlight and temperatures above 40°C**, because it decomposes in these conditions.

Bee venom harvesting, preservation, processing and storage

Bee venom **is sensitive to strong acids and bases as well as oxidizing agents and ethyl alcohol**.

Bee venom, due to the activity of microorganisms, is unstable in aqueous solutions.

Since bee venom does not need to be handled, it can be prepared anywhere that bee venom therapy finds sufficient support. Producing in small quantities is easy, as long as strict hygiene controls and sterile working conditions can be provided.

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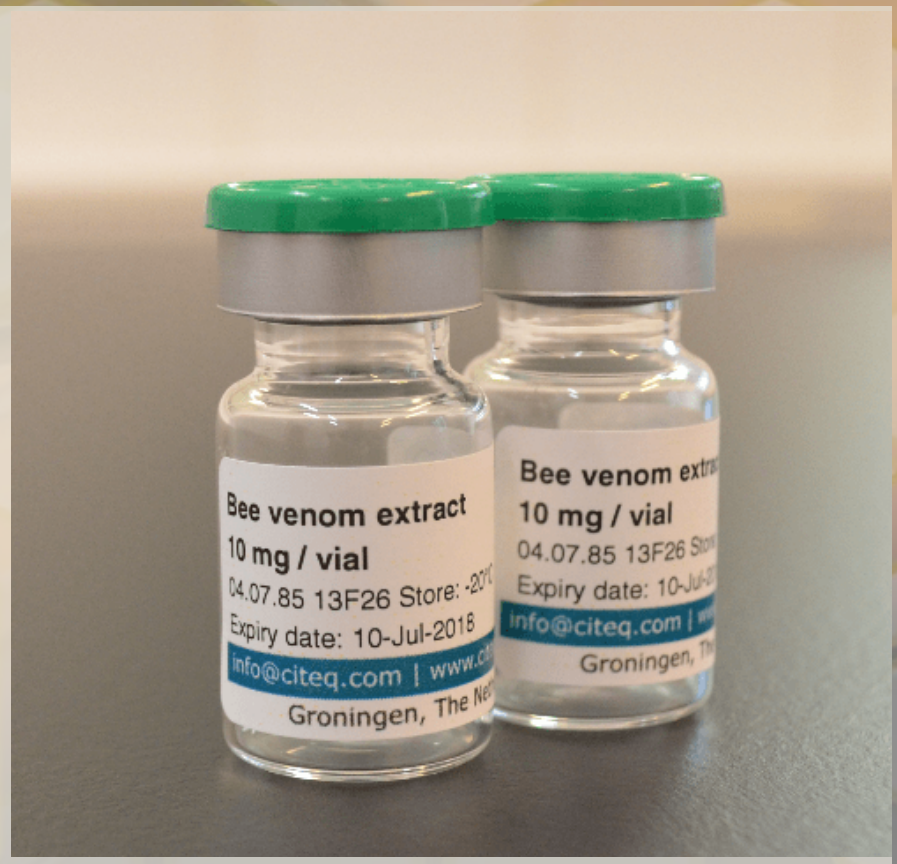
Exceptional hygiene conditions must be kept during the collection of bee venom. When handling dry venom, laboratory gowns, gloves and face masks should be worn to avoid getting venom dust into the eyes and lungs. Using bee venom injections, bee venom solutions are prepared with sterile water, some salts or oils, which are stored in special ampoules. Such ampoules are prepared only by certified pharmaceutical laboratories, due to the need to prepare strictly defined doses of bee venom and to maintain rigorous aseptic conditions.



Bee venom ampules
https://citeqbiologics.com/product/bee-venom-extract/

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https://www.medibeeb.eu/

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TÜRKİYE ULUSAL AJANSI
TURKISH NATIONAL AGENCY

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